



Psyche

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Volume 2010

Psyche: A Journal of Entomology

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Volume 2010

ISSN: 0033-2615 (Print), ISSN: 1687-7438 (Online), DOI: 10.1155/6152

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Research Article

Ambient Air Temperature Does Not Predict whether Small or Large Workers Forage in Bumble Bees (*Bombus impatiens*)

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Received 12 August 2009; Accepted 6 October 2009

Academic Editor: James C. Nieh

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Bumble bees are important pollinators of crops and other plants. However, many aspects of their basic biology remain relatively unexplored. For example, one important and unusual natural history feature in bumble bees is the massive size variation seen between workers of the same nest. This size polymorphism may be an adaptation for division of labor, colony economics, or be nonadaptive. It was also suggested that perhaps this variation allows for niche specialization in workers foraging at different temperatures: larger bees might be better suited to forage at cooler temperatures and smaller bees might be better suited to forage at warmer temperatures. This we tested here using a large, enclosed growth chamber, where we were able to regulate the ambient temperature. We found no significant effect of ambient or nest temperature on the average size of bees flying to and foraging from a suspended feeder. Instead, bees of all sizes successfully flew and foraged between 16°C and 36°C. Thus, large bees foraged even at very hot temperatures, which we thought might cause overheating. Size variation therefore could not be explained in terms of niche specialization for foragers at different temperatures.

1. Introduction

Although a plant might be fertilized by a variety of organisms, bumble bees (*Bombus* spp.) possess many features to make them one of the most essential of crop pollinators [1–3]. Like some other bees, developing larvae are fed pollen as a protein source [4, 5], which necessitates their foragers visiting a large number of flowers to collect resources. Bumble bee foragers exchange information at flowers to improve foraging efficiency [6–10] and can also recruit nestmates to profitable types of food sources by transferring information about presence [11–14], quality [15, 16], and scent [14, 17] in the nest, although contrary to honey bees [18], the location of food sources is not communicated [14]. However, in contrast to many other bee species, bumble bees, with their larger size and plentiful insulation, are much hardier and faster pollinators [3, 19–21] and able to fly even in cold and wet conditions, down to temperatures of 5°C [22] or even lower (*Bombus polaris*, where workers are quite large, is capable of foraging at near freezing temperatures:

[23–26]). Lastly, bumble bees are a relatively large genus compared to honey bees, thus providing many different types and sizes of foragers, able to handle a variety of floral styles and shapes [3, 27]. All of this results in bumble bees visiting many flowers, facilitating the effective transfer of pollen. However, despite their economic importance, bumble bees remain a relatively unstudied insect pollinator compared to honey bees.

One important and unusual feature in bumble bees is that highly related worker sisters from the same colony will display as much as a 10-fold difference in mass (Figure 1; [3, 28]). Larger workers emerge from the center of the nest, where larvae receive more intense care due to high density of nurses [29, 30]. Body size predicts worker task allocation: larger bees tend to forage and smaller bees tend to be nurses [29, 31–33]. Is worker polymorphism therefore an adaptation for division of labor? Larger bees do perform better as foragers [34, 35], also reviewed in [32, 36]. However, specialization is generally weak in bumble bees [37], and it is not clear that small bees are particularly suited as nurses



FIGURE 1: Bumble bees (here: *Bombus impatiens*) may display as much as 10-fold difference in mass between workers in the same nest, even though they are full sisters.

[38] (and Dornhaus, unpublished data). On the other hand, smaller workers may require less investment to produce and may be more robust to starvation [36]. The production of polymorphic workers may thus be a colony-level adaptation to increase colony efficiency or robustness. In addition, having workers of different sizes may also be an adaptation to foraging, akin to niche partitioning. For example, workers of different sizes may be ideally suited for flowers of differing corolla depths [39]. Alternatively, workers of different sizes may be suited to different temperatures at which the colony needs to forage. This is what we test here.

Bumble bees are cold-hardy foragers, especially compared to most bee species that are smaller. Their native range includes temperate, alpine, and even arctic zones [3, 40]. Nevertheless, thermoregulation is important even in most ectotherms [41], and a bumble bee's flight muscles must be warmed to at least 30°C before flight is possible [3, 42–44]. Bumble bees, like some other insects, are capable of a type of endothermy that is achieved by rapid muscle contractions [19, 45–48]. Bumble bee body temperature may also be influenced by external factors like the ambient temperature and the quality of their food [42]. If ambient temperature is less than body temperature, the bumble bee will be susceptible to heat loss. Since body size affects the surface area-to-volume ratio, to which heat loss is related, this may prevent smaller bees from achieving flight temperature in colder weather [49–51]. On the other hand, while larger bees are better thermoregulators [52], they may be susceptible to overheating during flight [53]. This is because metabolic heat is not transferred to the environment as quickly in larger organisms. The maximum thoracic temperature that bumble bees can tolerate is 42°–44°C [43, 54]. The dramatic intracolony variation in worker body size may thus be linked to different bees' abilities to forage at variable temperatures. If bumble bee size variation is an adaptation for foraging that allows for specialization, then larger workers should specialize in foraging at cooler temperatures and smaller workers should fly at hotter temperatures. Indeed, if workers

from different bumble bee species are compared, those from colder climates are often larger [50]. However, in the same study, it was also shown that both “large” and “small” workers of *Bombus terrestris* (exact body sizes were not measured) could be collected in the field at all temperatures between 18° and 33°C [50].

Here we test whether ambient and nest temperatures determine which workers are allocated to foraging in the bumble bee (*Bombus impatiens*). To test this hypothesis, we systematically manipulated ambient temperatures in a large, enclosed flight chamber and observed marked foragers of known size who accessed a suspended feeder. We predicted that larger foragers would tend to forage in cooler temperatures and, conversely, smaller foragers would tend to forage in warmer temperatures.

2. Materials and Methods

2.1. Study Organism and Experimental Setup. We obtained 2 bumble bee colonies (*B. impatiens*; colonies 1 and 2) from Koppert Biological Systems (Romulus, MI). At the start of the experiment, colonies were queenright with typically 20–30 workers with brood; over the course of the experiment, colonies grew to a size of over 100 workers. We housed colonies in Plexiglas boxes (22 × 22 × 11 cm) with screened ventilation holes and an opening over the top through which we directly delivered pollen each day of the experiment. The nest boxes were placed inside a large drink cooler (61 × 33 × 37 cm) to simulate typical ground nesting (i.e., insulated) conditions. In this way, the nest box was kept in the dark; however, the foraging arena was on a 12 : 12 light: dark cycle. A petri dish of water, which we refilled daily, was placed inside the nest box as well. Each nest box was then connected to a separate foraging arena (58 × 36 × 40 cm) by plastic tubing. Inside the foraging arena, feeders were placed on platforms (8 × 8 × 10 cm) suspended from the mesh top of the arena, which required the bees to fly instead of walk to the food. We placed the entire experimental setup inside a growth chamber, which allowed us to regulate precisely the ambient temperature, while also measuring the nest temperature.

2.2. Data Collection. Before the experiment began, we marked a subset of worker bees by gluing unique number identification tags (“Opalithplättchen”) to their thorax. Although tagged workers were chosen at random, we pulled bees from the foraging area of the nest box to assure that tagged bees were potential foragers. We continued to tag bees throughout the experiment to maintain a population of tagged bees for observation. The tags did not interfere with normal bee behavior or flight. Data were collected 5 days a week. Each morning, we would make sure that at least 25% of the honeypots in the nest contained honey; this provided a standardization of worker motivation and recruitment [16]. If less than 25% of the pots were full, we would fill some of the pots with sugary solution (“BeeHappy”) by syringe. The growth chamber temperature was set to the experimental ambient temperature setting for that day at 9:00 hours. In this way, the chamber was heated or cooled to the specified

temperature, which we verified by thermometers set both inside and outside the nest. Typically the nest box, insulated by the cooler, would not heat or cool as much as the room itself, which simulates natural conditions. The experimental ambient temperature was set between 16°C and 36°C. We randomized the order of experimental temperatures in 5°C increments (16, 21, 26, 31, 36°C). Colonies were monitored for stress at extreme high and low temperatures, even though the cooler provided a measure of insulation. We chose 36° as the maximum temperature because above it colonies showed signs of high stress, with many workers fanning and beginning to abandon brood. Below 16°C, very few or no bees foraged in our setup.

At 12:30, the chamber had always reached the desired temperature, and we placed feeders on the suspended platforms. Feeders were filled with sugary solution, which was always of the same concentration and quality (BeeHappy, Koppert Biological Systems, 1 : 1 diluted with water), as these factors influence the thoracic temperature of the bees [42]. We began data collection at 13:00. This allowed time for the bees to discover the food and initiate foraging [16]. For 90 minutes, we recorded the identity of any bee who successfully foraged (extended proboscis) at the feeders. Foraging at the suspended platforms required flight, which required sufficient heat with which to activate flight muscles. On 8 days, we additionally recorded for how many trips each foraging bee returned to the feeder.

At 14:30, we stopped data collection and fed each colony a teaspoon of pollen. Honeypots were verified as 25% full. The growth chamber temperature was set back to 26°C, and any dead bees were removed and stored in the freezer. After the experiment, we measured the thorax width of all the worker bees with digital calipers to the nearest 0.01 mm. Thorax width is a typical measurement of size in bumble bees [3].

3. Results

Overall, we found that all forager body sizes were measured at all temperatures (Figure 2). The number of trips made per observation period decreased at higher temperatures and was on average lower in larger foragers (defined here as foragers over 4.75 mm thorax width, Figure 3; ANOVA, $df = 9$, $R^2 = 0.86$, ambient temperature $P = .002$, body size $P = .028$, interaction $P = .61$). It is not clear why larger bees made fewer trips; perhaps because they needed longer to fill their crop on each visit. This result is the same if, instead of the average number of trips across bees in the respective category, each bee's number of trips is entered in the analysis separately ($df = 338$, $R^2 = 0.07$, ambient temperature $P = .004$, body size $P = .0002$, interaction $P = .43$).

3.1. Worker Body Size Did Not Predict Average Temperature at Which She Foraged. Averaging the temperatures for the days on which each bee foraged, we found no effect of body size on foraging temperature, although there was a significant effect of colony as well as its interaction with body size (ANOVA, $df = 80$, $R^2 = 0.15$, thorax width $P = .57$, colony $P = .003$, interaction $P = .046$; Figure 4(a)). Similarly, there was no

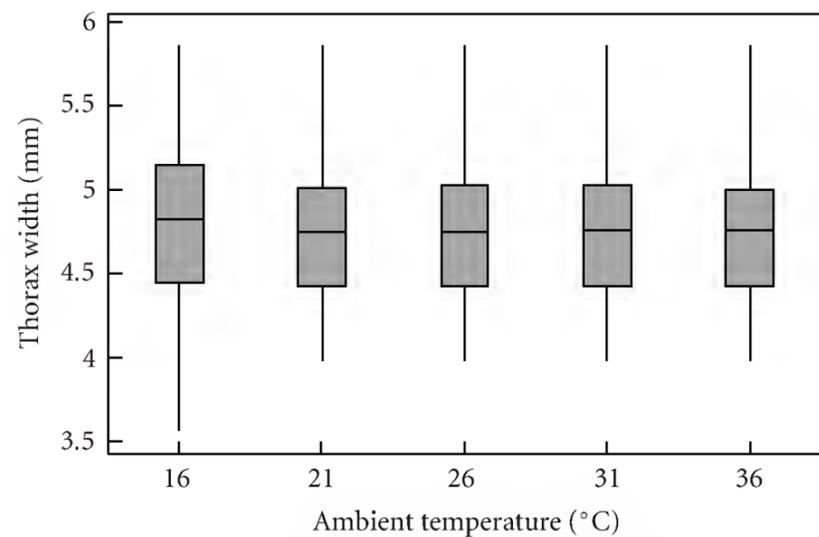


FIGURE 2: Bees of a wide range of body sizes were found to forage at all ambient air temperatures. Data are pooled here for both colonies; shown are medians (lines), quartiles (boxes), and ranges (whiskers) ($n = 70$ bees for 16°, $n = 65$ for 21°, $n = 67$ for 26°, $n = 71$ for 31°, $n = 73$ for 36°).

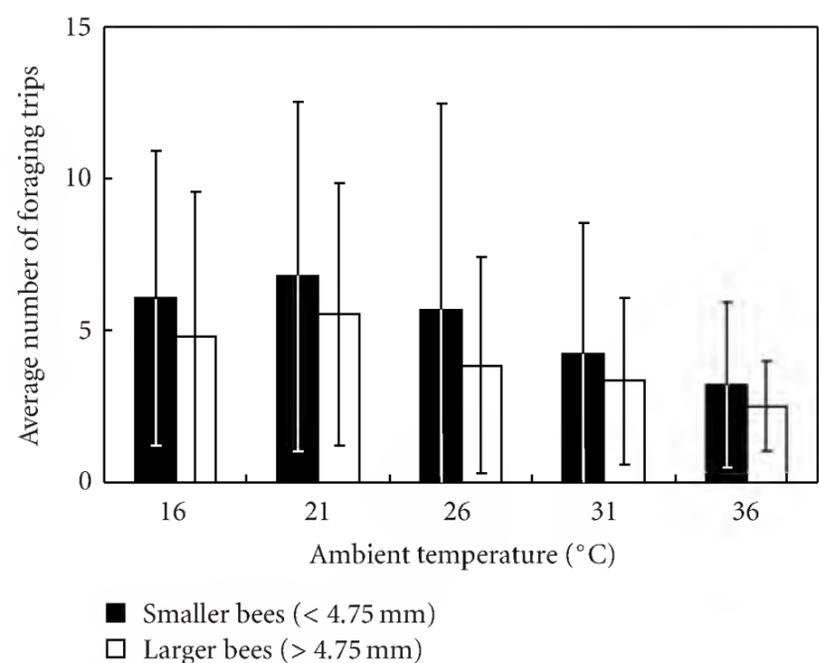


FIGURE 3: Foraging activity, measured as the number of trips per bee in one observation period, declined at higher temperatures. Shown is the average (with standard deviation) of all bees in the respective category across all days with the respective temperature (total $N = 339$ bees* days).

relationship with average temperature measured in the nest when bees of different sizes foraged ($R^2 = 0.86$, thorax width $P = .35$, colony $P < .0001$, interaction $P = .035$; Figure 4(b)).

3.2. Worker Body Size Did Not Correlate with Maximum Foraging Temperature. The maximum temperature, out of the temperatures tested by us, at which a worker would forage seemed at first predicted by body size, with larger bees foraging at higher maximal ambient temperatures (ANOVA, $df = 80$, $R^2 = 0.25$, thorax width $P = .0009$, colony $P = .016$, interaction $P = .0003$; Figure 5(a)). However, there was the single outlier of one bee that was only seen foraging once, at 16°C (Figure 5(a)). Since this single trip entered the analysis as a maximum foraging temperature of 16°C, it strongly affected the results. If that bee is removed from the analysis

of maximum foraging temperature, there is no remaining effect of body size ($R^2 = 0.25$, thorax width $P = .48$, colony $P = .11$, interaction $P = .26$). The same was true for the relationship between body size and maximal in-nest temperature at which the bee foraged, although there was always an effect of colony on in-nest temperature (with the outlier: $R^2 = 0.42$, thorax width $P = .006$, colony $P < .0001$, interaction $P = .003$; without the outlier: $R^2 = 0.37$, thorax width $P = .80$, colony $P < .0001$, interaction $P = .64$; Figure 5(b)).

3.3. Worker Body Size Did Not Correlate with Minimum Foraging Temperature. Neither minimum ambient nor minimum in-nest temperature at which a worker foraged was predicted by its body size (ANOVA, $df = 80$, ambient: $R^2 = 0.03$, thorax width $P = .29$, colony $P = .28$, interaction $P = .85$; in-nest: $R^2 = 0.77$, thorax width $P = .21$, colony $P < .0001$, interaction $P = .80$; Figures 5(a) and 5(b)).

4. Discussion

We found no significant effect of ambient or nest temperature on the average size of foragers flying to a suspended feeder. Instead, bees of all sizes successfully flew and foraged at ambient temperatures between 16°C and 36°C. These results lead us to reject the hypothesis that producing small workers may be a colony-level adaptation to foraging at warmer temperatures in bumble bees.

Larger animals are often thought to be more prone to overheating, because of their smaller surface to volume ratio; on the other hand, smaller animals may suffer detrimental heat loss. This temperature-body size relationship is thought to pose constraints on the evolution of very large animals, such as dinosaurs [55, 56], but it also is thought to affect distribution and evolution of a variety of other taxa (e.g., mammals: [57]; birds: [58]; reptiles: [59]; insects: [41, 60, 61]). In dinosaurs specifically, larger body size was likely associated with reduction in loss of metabolic heat as well as heat from solar radiation to such a degree that overheating became a risk and increased blood flow to the skin and other adaptations to increase heat loss became necessary [56, 59], although this may only apply strongly at body sizes of over 10 kg [56]. As a consequence of this, it has been hypothesized that larger species tend to be found in cooler climates, and this relationship is known as “Bergmann’s rule” [60, 62–66]. Species may also evolve different body sizes in response to climatic change [57]. However, evidence for “Bergmann’s rule” remains contradictory [67, 68]. Body size-temperature relationships are idiosyncratic in different taxa and may be caused by indirect effects, such as precipitation or seasonality correlating with temperature, and affecting body size across taxa [57]. In addition, developmental regulation of body size can be complex and vary even among related taxa [69]. Also, body cooling by wind can significantly increase heat loss [56], an effect that should be even more relevant in flying bees.

We were curious to see whether thermal ecology would also affect the evolution of size polymorphism within a species, namely, among workers of bumble bee colonies. Across species, thermal ecology may result in niche separa-

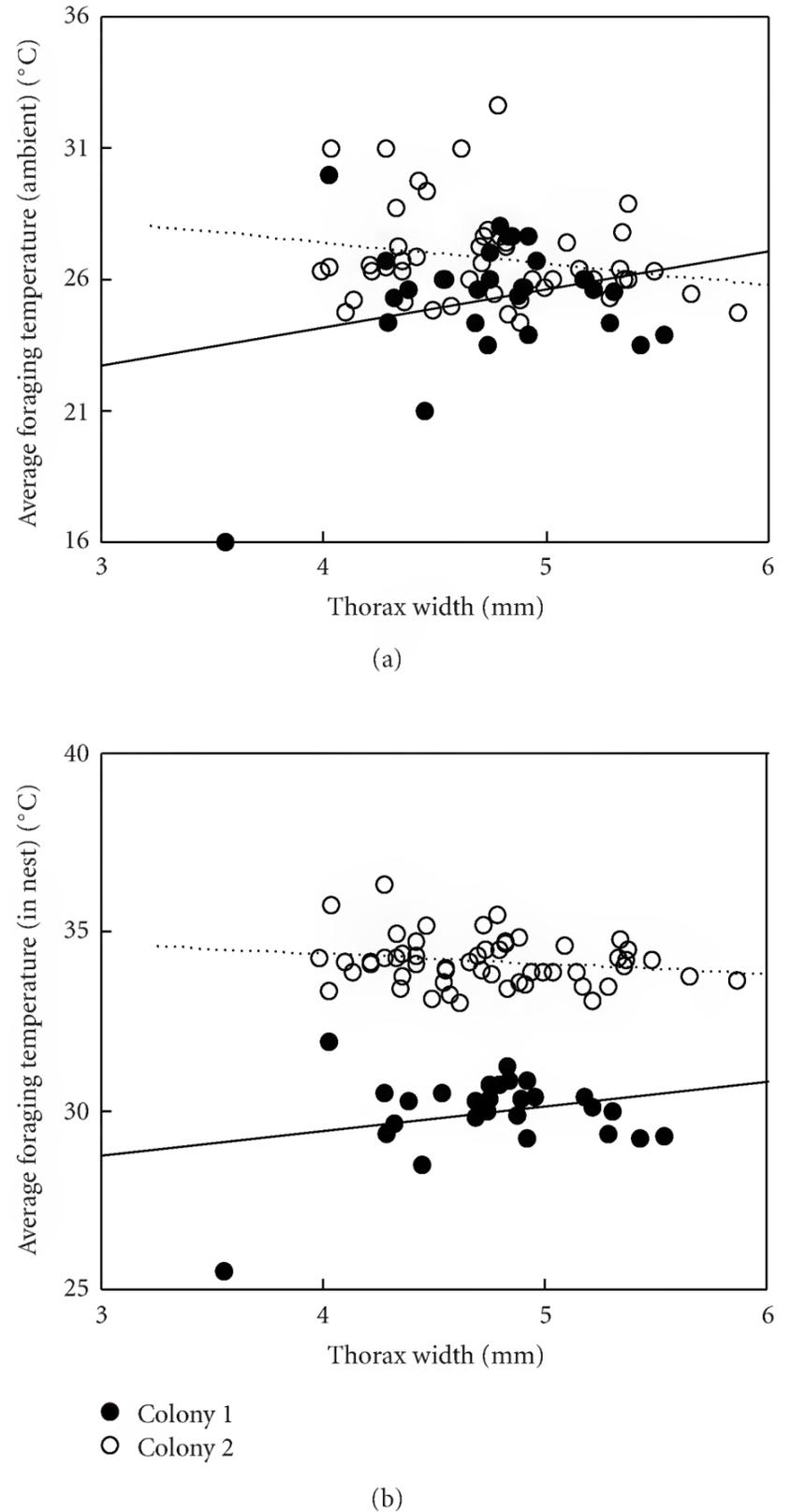


FIGURE 4: Foragers of different body sizes did not significantly differ in the average temperatures at which they foraged (although linear fits are shown, slopes are not significantly different from zero). However, colonies differed significantly from each other. Shown are (a) ambient temperature and (b) temperature measured in nest; each data point is the average temperature across all days on which that bee foraged (each bee foraged on average on 13.9 days), and in total, 81 bees are shown.

tion: for example, the ability of many bumble bee species (*B. terrestris*, *B. pascuorum*, and *B. hortorum*) to fly at much cooler temperatures than honey bees can result in temporal separation between the two families [22]; thermal niches may also exist among ant species [70, 71]. Within individual bumble bee colonies, larger bees were proposed to fly at lower temperatures, so they would be expected to fly earlier and later in the day and on colder days, whereas smaller bees might have been more resistant to

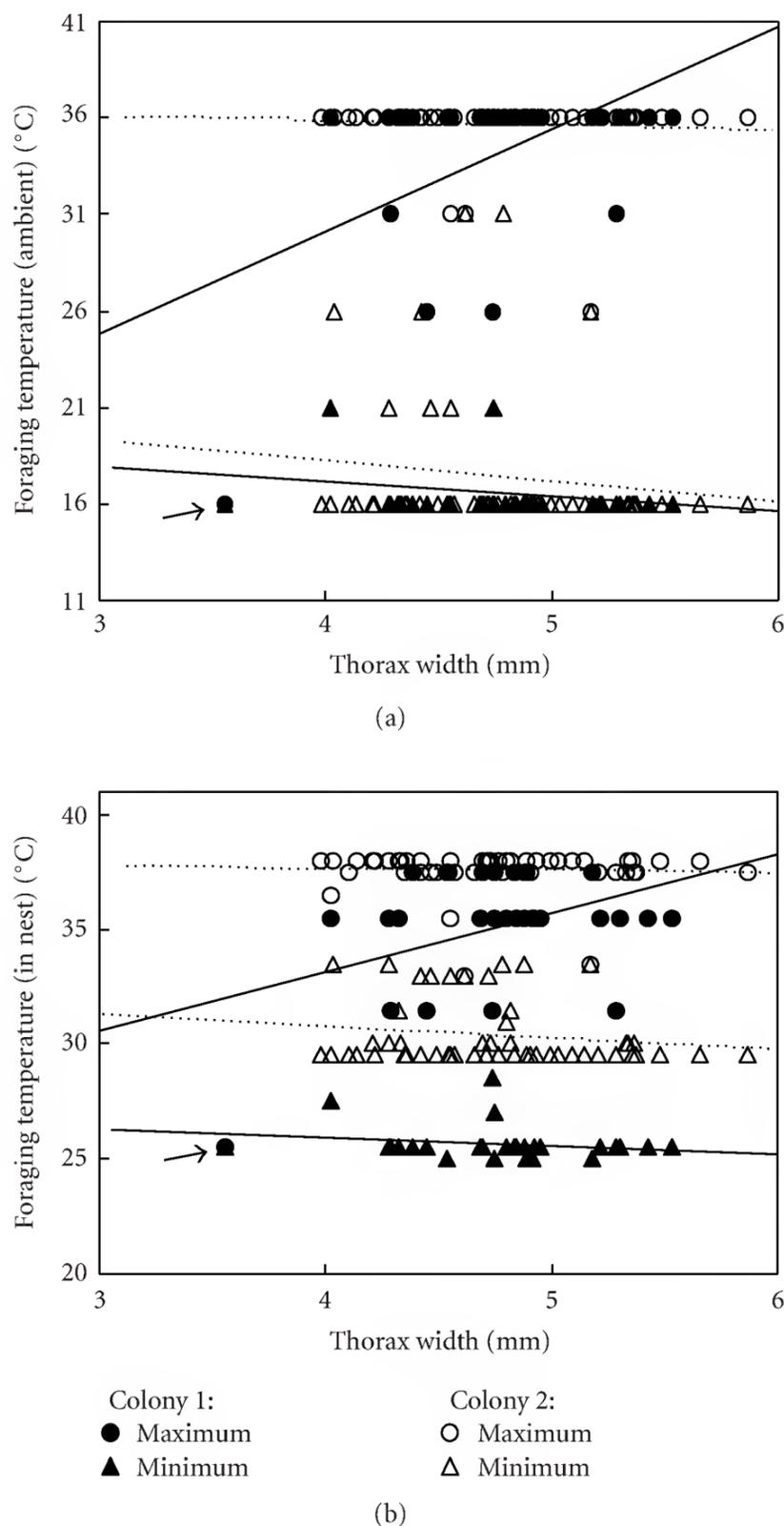


FIGURE 5: Foragers of different body sizes had similar maximum and minimum foraging temperatures within the temperature range studied here (up to 36°C ambient temperature). The relationship of thorax width and maximum foraging temperature was only significant if the outlier (marked with an arrow) of one bee which only made a single trip in the whole study was included. Shown are (a) ambient temperature and (b) temperature measured in nest; each data point is the maximum or minimum of all observations for that bee. In total 81 bees are shown.

overheating, therefore flying at midday and on warmer days [3]. This idea that bumble bee worker body size may predict foraging temperature has been proposed a number of times [3, 47, 72]. However, while it is still possible that only larger bees can forage at extremely low temperatures (<16°C), our study shows that small bees do not have a higher maximal temperature tolerance, as bees of all sizes still forage at >36°C. This result is consistent with a study by Peat et al.,

who also found no evidence that ambient temperature affected the activity of workers of different sizes [50].

In summary, it is likely that overheating does not constrain foraging activity for large bumble bees as long as outside temperatures remain within the tolerable limits. Flying bees may not overheat easily because of their overall small size, cooling effects of air movement while flying, and distance to the ground. It is also possible that bees would have been more susceptible to overheating had they been forced to fly longer distances than in our study. In future studies, it would be interesting to see whether flight distance affects forager susceptibility to overheating, and also whether individual experience will affect the temperatures at which bumble bee workers decide to forage. We also found significant colony differences in the average temperature at which workers foraged. There may thus be colony variation in worker temperature preferences or in how well colonies regulate in-nest temperature. However, there was no significant interaction between colony and the body size-maximum/minimum foraging temperature, indicating that in neither colony large and small workers differed in the range of temperatures at which they foraged.

In our experiment, foraging activity decreased at the highest temperatures but had not yet completely ceased, even when nest temperatures reached >38°C. At these temperatures, many bees are fanning the brood in the nest to cool the developing larvae, which may have lower heat tolerance [73–78]. Foraging activity may thus have decreased because foragers were occupied with nest thermoregulation more than because they were unable to fly at high outside temperatures. The fact that temperatures in the nest reached higher values than those outside opens up other interesting questions: clearly overheating and lack of effective shedding of metabolic heat may not be problematic at the individual level, but may be problematic at the colony level in spite of behaviors that regulate nest temperature in bumble bees [79–88]. It would be interesting for future research to compare the ventilation structures and other thermal adaptations of nests of tropical and temperate bumble bees and of larger and smaller colonies (as in other social insects: [89–91]).

Our study is one of a growing list of studies showing that in bumble bees, larger workers outperform smaller workers at many tasks, or perform equally well [32, 34, 35, 38], although see [36, 39] for how smaller workers might possess adaptive advantages. It is thus possible that small workers, rather than being adapted to particular conditions or tasks, are produced because they are less costly (both in production and maintenance), yielding a better gain per investment for some tasks compared to larger workers.

Acknowledgments

The authors would like to thank Michelle Mistelske and Isabel Rivera for their help with data collection. This work was funded by a Grant from the NIH (Postdoctoral Excellence in Research and Teaching-PERT) to MJC and by the Department of Ecology and Evolutionary Biology at the University of Arizona.

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Research Article

Trade-Off between Foraging Activity and Infestation by Nest Parasites in the Primitively Eusocial Bee *Halictus scabiosae*

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Received 10 August 2009; Accepted 12 November 2009

Academic Editor: James C. Nieh

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Diurnal activities of *Halictus scabiosae* bees and their nest parasites (major bee-flies, cuckoo wasps, ichneumon wasps, *Sphecodes* bees, and velvet ants) were investigated at a study site with 159 nests in Eastern Austria. Foraging activity correlated with ambient temperature only before midday and decreased in the afternoon. The activity of nest-infesting parasites increased during the day and correlated with ambient temperature. The match factor fm between the ratios of the foraging activities of *H. scabiosae* and the ratios of aspects of morning temperature was assessed on three consecutive days with different weather. The activity patterns of halictine bees and their nest parasites differed: the parasites exhibited only small time windows in which their activities were synchronised with those of their hosts. The bees exhibited an anticyclic behaviour and collected food in times of low parasite pressure and decreased foraging activity when parasite pressure increased.

1. Introduction

The way in which animals may alter their foraging behaviour under predator or parasite threat is a large and well-researched area of behavioural ecology [1, 2]. The interdependence of activity patterns between predators and their prey, as well as between parasites and their hosts, is affected by a diversity of factors. It has been observed for mammals and birds [3] in particular, that foragers cease feeding when the benefit of harvesting no longer outweighs the cost of foraging. Marginal costs of foraging include the risk of predation, also while the animals are carrying food items to their protective cover [4], and they also include the infestation of potential hosts by nest parasites. This paper reports on the principles of trade-off between foraging and the risk of infestation by parasites, using *Halictus scabiosae* bees, which need to juggle between keeping their nest entrances open to facilitate foraging traffic and closing the entrances in order to reduce parasite impact.

Halictus is a large genus of Halictidae, which is divided into 15 subgenera with well over 300 species, primarily in the Northern Hemisphere. Many species in the genus are

eusocial [5–7], with colony sizes ranging from very small (2–4 bees) to large (>200), showing division of labor and castes, and guards for defense. Nests are typically underground burrows [8], with several ovoid cells in which a mix of pollen and nectar is stored as food for the developing larvae. The cells may be arranged in clusters resembling a honeycomb, but constructed of soil rather than beeswax. The nests have a main entrance. The duct widens below the entrance, allowing the bypassing of a forager aside a guard bee, and reaches 20 to 30 cm into the soil. It is ramified by lateral ducts, cells, and emergency outlets. Due to the long flight season from the end of April until October [9, 10], halictine bees have evolved as a polylectic species. In the summer, they develop smaller sterile summer females with a low rate of production of males [8]. Queen females copulate in autumn and hibernate in polygyne associations [9]. Two to five summer bees stay in the maternal nest and provide food for the brood [10]. Females hibernate in the maternal nests and reuse them to rear the next generation. After the hibernation period the polygyne society turns into *semisocial*, and the egg-laying female, the queen, is engaged as a guard bee [10]. Later on, the queen chases off the other females [9] and lives with her



FIGURE 1: *Halictus scabiosae*. (a) Homing forager bee with pollen on its coxae and femurs reopening the nest entrance hole that had been previously closed by the queen. (b) Guard bee at the nest entrance.

filial generation turning the society to *primitive eusocial*. The females that have been chased away by the queen may found their own nests in the vicinity of their original home nest by usurping nests of other bees such as *Lasioglossum nigripes* [11, 12]. In this way, *Halictus scabiosae* societies are regularly observed in nest aggregations.

In halictine bees activity patterns of nectar and pollen foraging are determined by a set of major factors [13–15]. Microclimatic conditions primarily affect the life of the bees regarding flying ability, mating behavior, and the development and survival of larvae [16]. Ground-nesting insects, like many wasps, bees, and ants, likely depend more on soil temperature than on air temperature with regard to nest-site selection, daily activity patterns, foraging success, and sex allocation [16, 17]. This is also true for the social ground-nesting halictine bees with an annual life cycle. Soil temperature controls the duration of the development of helpers and the rate of provisioning [18], affecting the number of broods that can be produced during the limited flight season, and therefore also the colony size and the level of social complexity [17]. Furthermore, the amount of time a bee forages per day is associated with the amount of pollen she can gather. More specifically, the daily rate of foraging trips is correlated with the minimum temperature over daytime [19], with the shortage of food for their offspring [15], and with the quality and quantity of floral rewards available [13, 14].

Finally, foraging bees have to cope with insect predators and parasitoids. In general, the level of compatibility of a particular host-parasite combination depends not only on unsuitability but also on active resistance by the host against the parasite which implies a cost for the host [20, 21]. There are at least three main strategies that bees have evolved against nest parasites. (a) Many halictine species keep the nest entrances open during the foraging period during the day but seal them with soil daily after foraging activity ceases [10]. Pleometrotic species such as *Halictus scabiosae* with more intense flight traffic keep their entrances open for longer than haplometrotic species. In addition, social species close their nests after the provision of the larvae has been

terminated. In this case the entrances are not reopened again until the new generation hatches. (b) Sociality facilitates foraging activities while the nest remains guarded. This aspect is considered an important factor in the establishment of social behaviour [22], in particular in the evolution of larger social units among the halictine bees. On the other hand, there are arguments which make it unlikely that the protection against predators or parasites bestows any significant advantage to pleometrotic nests [23–25] (c) *Halictus scabiosae* societies are regularly observed in nest aggregations which may improve mating efficiency and nesting success. On one hand, a higher nest density may provide a visual stimulus for further nesting in a given locality by social facilitation [17, 26] but also allows females to enter foreign nests, along with their general tendency to guard nest entrances. On the other hand, a nest aggregation considerably increases parasite and predator pressures because aggregated hosts can be found more easily [22].

The paper reports on activities recorded for a batch of 28 nests of an aggregation of 159 *Halictus scabiosae* nests that have been monitored continuously over three days during daytime. We first measured the foraging activity and nest-sealing behaviour of the *Halictus scabiosae* nest members, and second, we assessed the activity of parasites, which tried to infest the bee nests under observation. We gathered evidence for the hypothesis that the foraging activity of the worker bees in *Halictus scabiosae* is anticyclic to the diurnal activity of nest parasites. A positive proof of this hypothesis would suggest that halictine bees minimize the parasite impact on their nests by decreasing their foraging activities when parasite pressure is high.

2. Material and Methods

2.1. Species and Study Site. We observed an aggregation of nests of *Halictus scabiosae* (Figure 1) distributed along the edge of a forest in an area of 5×24 m (120 m^2) in Krobotek ($46^\circ 58' \text{N}$, $16^\circ 11' \text{E}$) in southern Burgenland, Austria, at an altitude of 300 m sea level (Figure 2). The nesting area was



FIGURE 2: Study site. A strip of 5×24 m (orange bar in the middle image and orange arrows) near the edge of a forest in Krobotek ($46^{\circ}58'N$, $16^{\circ}11'E$) in southern Burgenland (Austria) at an altitude of 300 m sea level was selected as the study site. An aggregation of 159 nests of *Halictus scabiosae* was found in a meadow with thermophilic vegetation on sandy-loamy soil. Yellow scale bars represent 100 m in length. The orange area and the arrows mark the study patch. The bottom image shows the four camcorders on tripods filming the activities at four selected batches as defined in Figure 3.

located on a slope that was slightly inclined to the south, with thermophilic vegetation on sandy-loamy soil. We counted 159 entrance holes of 4 to 6 mm diameter and 36 slightly bigger (7 to 10 mm) holes (Figure 3). All holes were active and used by outgoing and incoming bees, by guard bees or other bees that closed or reopened the holes, but no traffic was observed at or around the 36 bigger holes. The fact that the holes remained still open let us assume that they belonged to the nests in close vicinity. Possibly, these were “emergency outlets” which are known from *Halictus* nests [8].

2.2. Videotaping and Recording of Meteorological Data. The strip selected for observation was situated along the edge of a forest as documented in Figure 2. Here, four cameras on tripods were positioned to trace four sample batches with a total of 28 nests: (a) 22×22 cm with four nests; (b) 28×31 cm with eight nests; (c) 36×32 cm with twelve nests; (d)

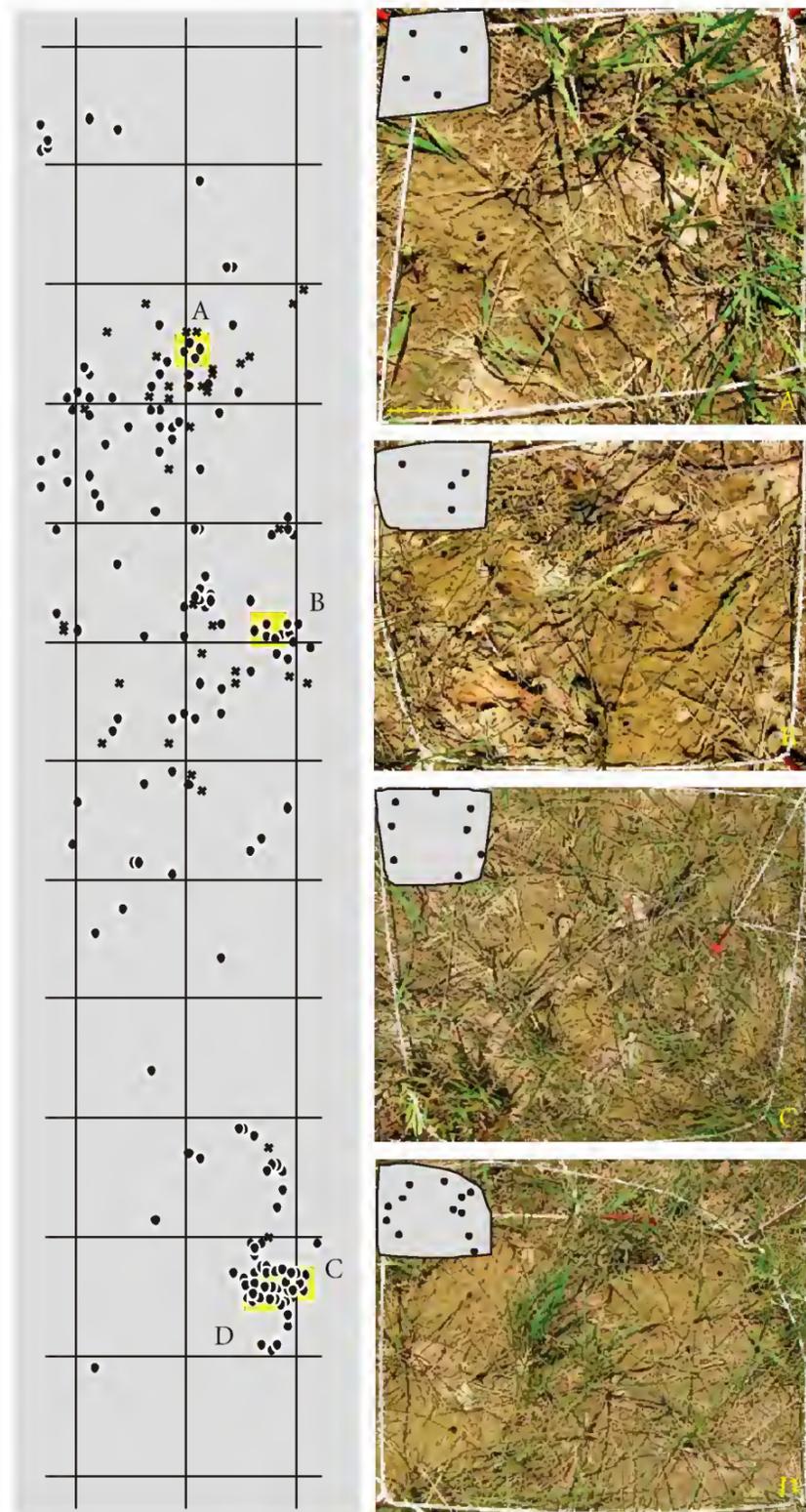


FIGURE 3: Mapping of the nests of *Halictus scabiosae* at the study site. Left: strip of 5×24 m (see Figure 2) with the four batches of nests (A–D, marked by yellow areas) where the activities of *Halictus scabiosae* and their parasites were videotaped. The grid indicates $2 \text{ m} \times 2 \text{ m}$. Right: batches of active nests; the insets show the batches on a smaller scale with the nest entrances shown as black full circles.

28×22 cm with four nests. The entrances of these sample nests were videotaped on 3 days in July 2008 during daytime (between 08:30 and 15:00 h). Recording was only interrupted for few minutes when it was necessary to replace the used tape by a new one. The pauses were logged and considered in the assessment of the rate of nest activities.

Ambient temperature, irradiance, and relative humidity were recorded every 10 seconds using a HOBO data logger.

2.3. Assessment of Nest Activities. Typically, foraging bees flew off the nest within seconds. Guard bees only appeared with their heads at the nest entrance and stayed there. When

foragers came back and tried to enter the holes, the guards retreated and let the incoming forager pass [27]; afterwards the guards immediately reappeared at the entrance hole. The frequency of outgoing (A_{out}) and incoming (A_{in}) activities was assessed from the videos. The kind of activity, its time, and the nest concerned were recorded.

2.4. Assessment of the Impact of Parasites on the Nests of *Halictus scabiosae*. The presence of five groups of nest parasites was observed: the major bee-fly (*Bombylius major*, Asilomorpha, Bombyliidae), the cuckoo or gold wasps (subfamily Chrysidinae, family Chrysididae), ichneumon wasps, members of the genus *Sphcodes*, and velvet ants (Mutillidae). Each parasite that patrolled around the nests was logged and identified from the video tapes. The five nest parasites were grouped for statistical analysis. However, there were typically two categories of parasite impact (I_p). First, if parasites scanned unspecifically around the nests, equal impact values were assigned to all active nests in each batch. For example, if there were four nests, each of the nests was assigned an impact value of 0.25 for a single observation of a scanning parasite. Second, when a parasite visited the nest area specifically at the nest entrance, a parasite impact value of 1.0 was assigned to the visited nest.

2.5. Basic Statistics. Means and mean errors of all data were calculated. The data of nest activities (A_{out} , A_{in} , I_p) were normalized per nest and per observation period of 30 minutes. For that, nest entrances were selected on the first day of experimentation, which were active with regard to outflying and incoming frequency and guarding. Inactive nest holes were not considered in the normalizing of the rates of bee activities or parasite impact. For description of diurnal activity patterns and for comparison of the rates of nest parasites and bee activities the means of the normalized data were used to calculate the corresponding regression polynomials which were tested for significance using Sigmaplot.

2.6. Calculation of the Probability of the Match between Nest Activities and the Aspects of Morning Temperature. The question was investigated of whether and how the temperature conditions in the morning affect the activities of bees and their parasites later in the day. The morning temperature conditions could first, trigger the decision of the bee to start foraging and second, it may influence the rate of foraging throughout daytime. Similarly, the nest parasite could be influenced in its starting time to infest the nests of the hosts and in the infestation rates throughout daytime. In our model, we take into account that bees and parasites have to sense the following critical morning temperature aspects prior to their first bout. These include the temperature inside the nest (T_n), the ambient air temperature (T_a) outside the nest, the difference between both aspects (ΔT_{a-n}), and lastly, the change in ambient temperature within a given initial time interval [t_0 , t_1].

For calculating these morning aspects of temperature we considered the time interval between $t_0 = 8:50$ to $t_1 = 9:50$ h

in the three experimental days (d_1 , d_2 , d_3). The mean change in ambient temperature in this initial hour of experiment (ΔT_a) was calculated by averaging the changes in steps of 10 seconds. The initial temperature inside the nest T_n was mathematically assumed with a virtual range between 12° and 19°C. This allowed us to calculate a usable measure of the morning aspect of temperature according to the equations $\Delta T_m[d_i] = (T_a - T_n) + \Delta T_a$ and $r\Delta T_m[d_i] = \Delta T_m[d_i]/\Sigma\Delta T_m[d_{1+2+3}] * 100$ (for $i = 1$ to 3 experimental days). This relative value includes all crucial temperature aspects which could be important for the bee or the parasite to decide to start the first bout in the morning and is a useful measure for the correlation of behavioral traits of bees and parasites on sequential days under varying weather conditions, provided that similar mathematical procedures are applied. The outflyer rate A_{out} of the bees was taken as an estimate for the aspect of foraging activity A_{out} , and the impact rate of parasites I_p for the aspect of infestation. Both measures were normalized per nest and per 30 minutes observation time and related to the sum of the respective time interval of the three experimental days according to

$$\begin{aligned} rA_{out}[k, d_i] &= A_{out}[k, d_i]/\Sigma A_{out}[d_{1+2+3}] * 100, \\ rI_p[k, d_i] &= I_p[k, d_i]/\Sigma I_p[k, d_{1+2+3}] * 100 \end{aligned} \quad (1)$$

for each time interval k of observation of the day d_i (for $i = 1$ to 3).

We then compared the ratios of the relative aspects of morning temperature $r\Delta T_m[d_1] : r\Delta T_m[d_2] : r\Delta T_m[d_3]$ with the ratios of the relative aspects of foraging activity of the bees $rA_{out}[k, d_1] : rA_{out}[k, d_2] : rA_{out}[k, d_3]$, and that of the infesting activity of their parasites $rI_p[k, d_1] : rI_p[k, d_2] : rI_p[k, d_3]$ regarding the time intervals of observation on the three experimental days. The chi-square test and the F -test were used to estimate the probability with which the daytime-dependent relations of the behavioural activities of each host and parasites match the morning aspect of temperature. In this paper, this probability of matching P_m is taken as the crucial measure to estimate whether and how behavioural activities remain linked to the morning aspect of temperature throughout daytime. P_m values of $>.05$ signify that the ratios of foraging activities of the bees and the infesting activities of the parasites match the morning aspect of temperature, whereas P_m values smaller than $.05$ document a contrast between the ratios of behaviors and the morning aspect of temperature.

3. Results

3.1. Diurnal Activity Patterns of Foraging. We recorded the activities of four batches of *H. scabiosae* nests at the study site over three days and assessed the number of bees departing (F_{out}) or homing (F_{in}). Although the weather conditions differed considerably from day to day, the activity patterns were uniform insofar as they peaked between 10 and 12 am and declined in the early afternoon, mostly before the daily ambient temperatures reached their maximum values (Figure 4(b)). Day 1 was representative for this tendency; the

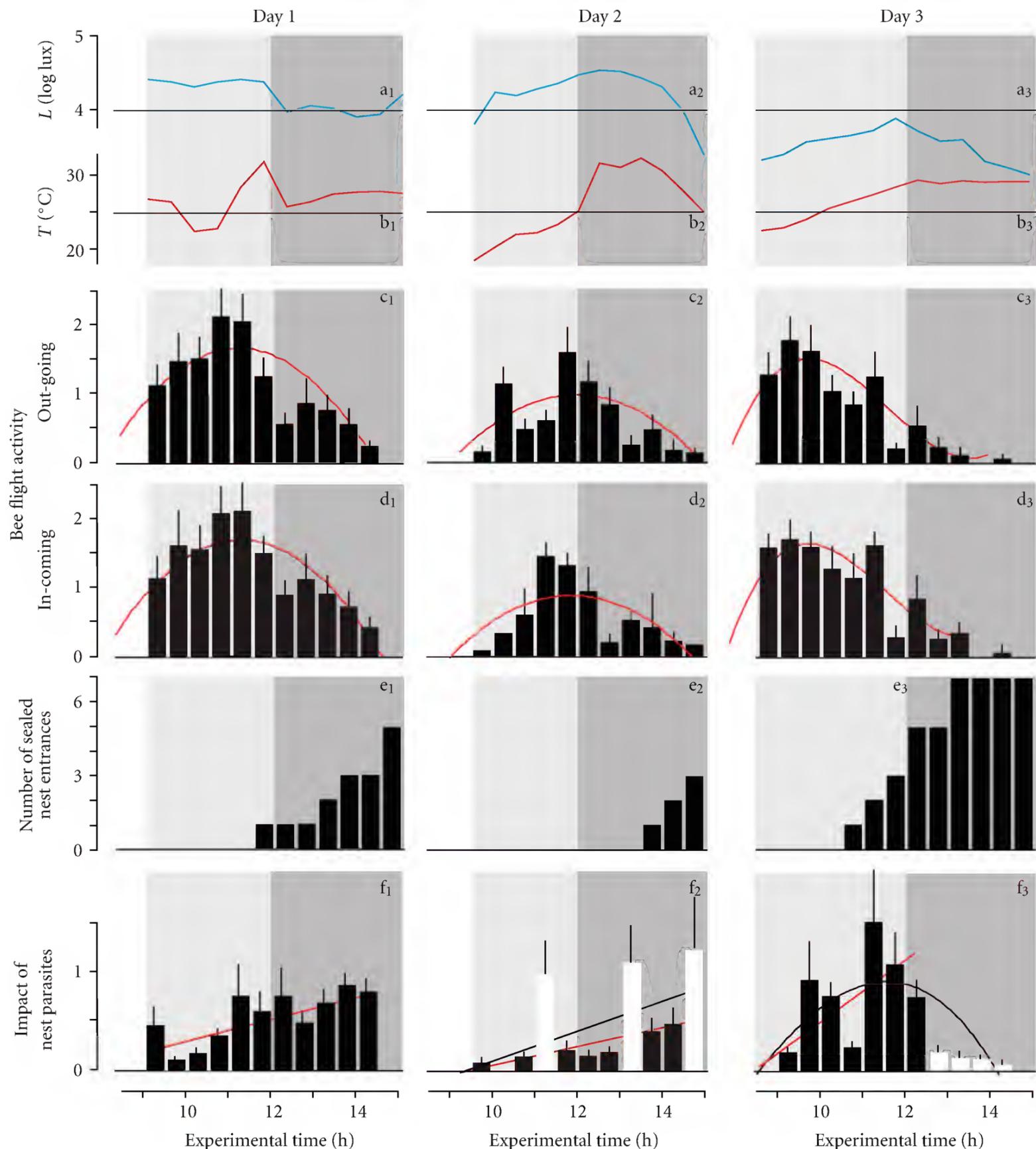


FIGURE 4: Activities observed at 28 nests (four batches) of *Halictus scabiosae* nests over three days (1–3). (a) Irradiance in lux and (b) ambient temperature in °C measured in the shade. (c) Out-flying and (d) incoming activity pooled over all 28 nests; data were normalized per nest and 30 minutes of observation time; red curves show the regression functions, which allow to extrapolate the flight rates before the start of the observation sessions (which are coded by the grey background; am, bright grey; pm, dark grey); (e) sealing activity at the study site, given by the number of sealed nests in steps of 30 minutes; (f) the impact of nest parasites (for measurement, see Methods) on the experimental nests of *H. scabiosae*, given by the number of parasites per nest and 30 minutes of observation time. The regression curves (red) refer to all means coded in black columns which are used for further analysis (for equations of the regression functions see Tables 1 and 2 and text).

foraging activities reached the maximum level of 2 individuals per nest and 30 minutes observation time (Figures 4(c₁) and 4(d₁)). The activity patterns on all experimental days are displayed in Figures 4(c) and 4(d) and their regressions are defined in Table 1 (line 1–6).

The daily activities of the bees reflect, to some extent, the weather situation. For instance, the morning temperature prior to experimentation triggered the emergence of the bees for their first bouts. Correspondingly, the lower temperatures in the morning of the second day (Figure 4(b₂)) triggered

TABLE 1: Regression functions of foraging activities of *Halictus scabiosae*. F_{out} and F_{in} : outgoing and incoming foraging activity (per nest and per 30 minutes observation session); $F = (F_{\text{out}} + F_{\text{in}})/2$; t : daytime in hours; T_{amb} , ambient temperature in °C; a–d: regression coefficients, r : correlation coefficient. *Additional virtual values (marked with +) were introduced for the calculation of the regression function.

Line	<i>Halictus scabiosae</i> reference	Regression	Day	a	b	c	d	r	n	P
1	Figure 4(c ₁)	$F_{\text{out}} = a * t^2 + b * t + c$	1	-0.145	3.230	-16.517		0.844	12	.004
2	Figure 4(d ₁)	$F_{\text{in}} = a * t^2 + b * t + c$	1	-0.159	3.576	-18.486		0.901	12+2	<.001
3	Figure 4(c ₂)	$F_{\text{out}} = a * t^2 + b * t + c$	2	-0.110	2.531	-13.601		0.742	13	.033
4	Figure 4(d ₂)	$F_{\text{in}} = a * t^2 + b * t + c$	2	-0.108	2.556	-14.339		0.742	13	.033
5	Figure 4(c ₃)	$F_{\text{out}} = a * t^2 + b * t + c$	3	0.053	-1.809	20.100	-71.450	0.761	12 + 3*	.014
6	Figure 4(d ₃)	$F_{\text{in}} = a * t^2 + b * t + c$	3	0.058	-1.965	21.777	-77.358	0.669	13 + 2*	.048
7	Figure 5(a)	$F = a * t^3 + b * t^2 + c * t + d$	1–3	0.0022	-0.077	0.704	-0.743	0.940	15	<.001
8		$F_{\text{out}} = a * T_{\text{amb}} + b$	1	0.020	0.592			0.087	11	.800
9		$F_{\text{out}} = a * T_{\text{amb}} + b$	2	0.005	0.488			0.045	11	.893
10		$F_{\text{out}} = a * T_{\text{amb}} + b$	3	-0.079	2.914			0.363	11 + 2*	.223
11	all day	$F_{\text{out}} = a * T_{\text{amb}} + b$	1–3	0.024	0.1089			0.139	39	.396
12	pre-noon	$F_{\text{out}} = a * T_{\text{amb}} + b$	1–3	0.107	-1.4523			0.615	15	.015
13		$T_{\text{amb}} = a * t + b$	1–3	1.239	12.392			0.680	39	<.001
14		$F_{\text{in}} = a * F_{\text{out}} + b$	1–3	0.152	0.878			0.826	988	<.001

foraging flights later and at a lower rate (Figures 4(c₂) and 4(d₂)). On the third day, the moderately warm temperature caused earlier foraging activity which reached levels higher than that on the previous day, in spite of lower irradiance due to overcast sky (Figure 4(a₃)). This higher foraging level on the third day was probably caused by a shortage in food provisioning for the larvae due to the lower temperatures on the day before.

The rates of outgoing (F_{out}) and incoming bees (F_{in}) correlated significantly ($r = 0.826$, $P < .001$; Table 1: line 14), and there was no time lag between the patterns of outgoing and incoming rates. This indicates that the departing individuals were identical with the homing bees, and that the foraging flights were on average shorter than 30 minutes.

In Figure 5(a) the activities of all 28 nests at the study site were pooled for a more integrative view. The regression of the mean values of this distribution ($r = 0.940$; $P < .001$; Table 1: line 7) confirms that there was a peak in foraging activity between 10 and 12 am. However, this summarization does not consider the weather conditions which changed during daytime and from day to day. The question is how the foraging activities in *H. scabiosae* were affected by the weather conditions; the most important measure for weather appeared to be ambient temperature, which is discussed in the next section.

3.2. Do Diurnal Foraging Patterns Correlate with Ambient Temperature? We correlated the foraging activities of all experimental days with the ambient temperature over the entire experimental time when halictine bees were actively foraging (9:00–15:00 h). The resulting match ($r = 0.139$; $P = .396$; Table 1, line 8–10, 11) was much lower than the correlation of the ambient temperature with the daytime ($r = 0.680$; $P < .001$; Table 1: line 13). These data, together

with Figures 4(c) and 4(d), strongly suggest that foraging in halictine bees is not dependent on ambient temperature alone. The bees are affected by daytime, which could, at least partly, be independent of ambient temperature. We therefore investigated whether a time window exists in which the bees would have decided their foraging strategy of the day, for example, when to emerge first and at which rate foraging should proceed. A possible answer comes from the correlation of the foraging rate with the ambient temperature, if merely the time intervals before noon are considered. In this time period, the foraging rate is directly correlated with ambient temperature ($r = 0.615$; $P = .015$; Table 1: line 12). After the midday foraging peaks, the bees apparently organized themselves according to other principles. Most of the colonies decreased their foraging activities and retreated to the nest; some nests were closed (Figure 4(e)).

In a second approach, we investigated if the weather conditions in the morning are crucial for the bees to control the consecutive foraging later in the day. The question is to which extent this *aspect of morning temperature* (for definition, see Methods) correlates with the subsequent foraging activity of the same day. The large differences of the three experimental days regarding weather and foraging compromised the feasibility of the analysis of mean behavioural processes. This variability of the experimental conditions, however, allowed us to compare the ratios of the *aspects of morning temperature* with the ratios of the foraging activities of the bees and the infestation activity of parasites. The respective probability of matching P_m evaluates this comparison of all three experimental days (see Methods) under two surmises (Figure 6): first, the worker bees compare, in particular before they emerge for their first foraging bout, the current temperature inside the nest (defined virtually in the model in the range from 12° to 19°C) with the ambient temperature outside of the nest.

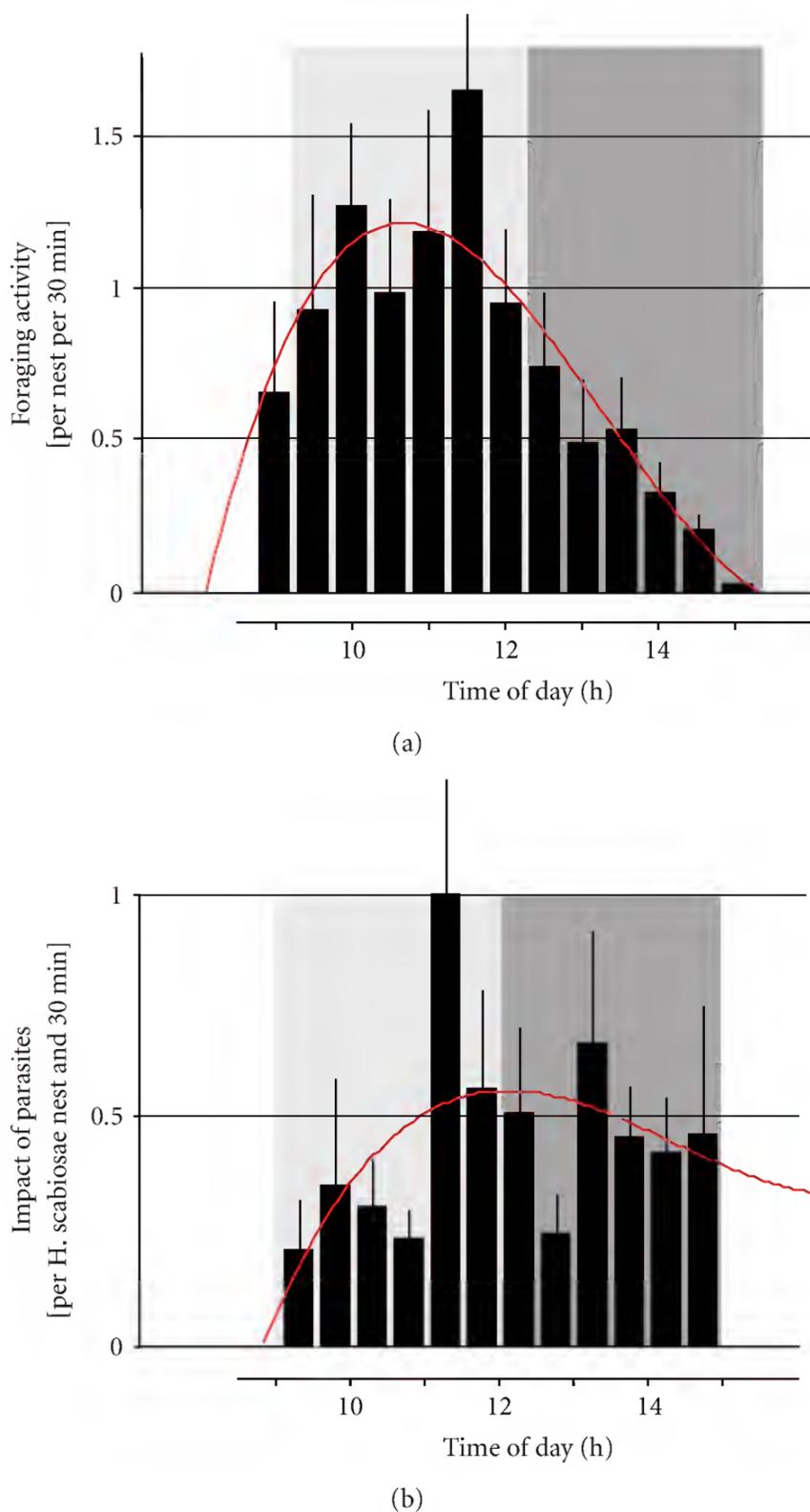


FIGURE 5: Summary of (a) mean foraging activities of 28 nests over three days and (b) mean impact rate of parasites as assessed at the experimental nests of the study site. Red curves, regression functions of the means (for equations, see Tables 1 and 2 and text).

Second, the bees remain affected by this *aspect of morning temperature* while they continue foraging. This surmise could be important if the morning temperature conditions were not sufficiently appropriate to predict the weather later in the day. In fact, the probability P_m with which the temperature conditions in the morning (T_m) would allow a prediction of the ambient temperature between 10:00 h and 14:00 h on the experimental days d_1 – d_3 decreased over daytime and was lower than 0.05 for nest temperatures greater than 15°C (Figure 6(d)), which turns the morning aspect of temperature into an unreliable predictor of ambient temperature relations later in the day.

Figure 6(a) shows the probability value P_m between the aspect of morning temperature and the foraging activity. The probability P_m was calculated for each time step of 30 minutes of the entire observation time and detects matched ($P_m > .05$, chi-square test) or significantly diverse ($P_m < .05$) relations. There were two peaks in the P_m curves for the foraging bees; the first prominent peak occurred before noon when the foraging activities also peaked. The second peak in the P_m curves occurred after midday, between 13:00 and 14:00 h, and was weaker and shorter than the first one. The results let us assume that the aspect of morning temperature could be important for a bee's decision on her consecutive foraging activity at least in her pre-noon foraging activity.

3.3. Nest Closing and Guarding. In halictine bee colonies the nest entrances are usually closed (Figure 7(a)) as soon as the flight activity is terminated for the day. During nighttime or rainy weather the nests were also kept closed [10]. The nest entrance normally ends at the surface as a funnel with elevated rims, because some of the material gained by digging the nest tubes accumulates around the entrance hole. The females take material from the inside of the tube and close the entrance hole with their abdomen. Females that arrived after their home nests had already been sealed off were able to reopen it by digging at the right place (Figure 7(b)). For that, the homing foragers circled around with their body and removed soil parts with their mandibles. We counted the number of entrances that were initially open and also those that were sealed later in the day. In Figure 4(e), the cumulative numbers of closed entrances on each of the experimental days are shown. In the four batches of 28 nests, we counted five closing activities on day 1, three on day 2, and seven on day 3. These ratios of closing fit with the ratios of ambient temperatures on all experimental days in the time before midday ($P > .05$, F test); later, the data match at ($P < .05$, F test). Therefore, it is likely that the temperature aspect before noon determines the activity of closing the nest entrances.

Generally, all active nests provided guarding at least at certain periods of the day. Guard bees were initially present at the entrance, in particular before midday. Later on in the afternoon, they only appeared at the entrance hole, as soon as a parasite had approached. We did not observe any guard bee that flew out during her guarding. It happened several times that homing bees came to the nest entrance and competed against the guard bee which kept the entrance closed with her body. Sometimes the arriving forager bee succeeded to drag the guard out of the entrance hole, but some seconds later the guard bee had returned to her place.

3.4. Nest Parasites. The presence of five groups of nest parasites was observed. (1) The major bee-fly (*Bombylius major*, Asilomorpha, Bombyliidae) generally feeds on nectar and pollen in the adult stage; thus they are pollinators. The larval stages are predators or parasitoids of other insect eggs and larvae. We observed adult females that scanned around the nest sites and deposited eggs in the vicinity of the open entrances. *Bombylius major* has been described

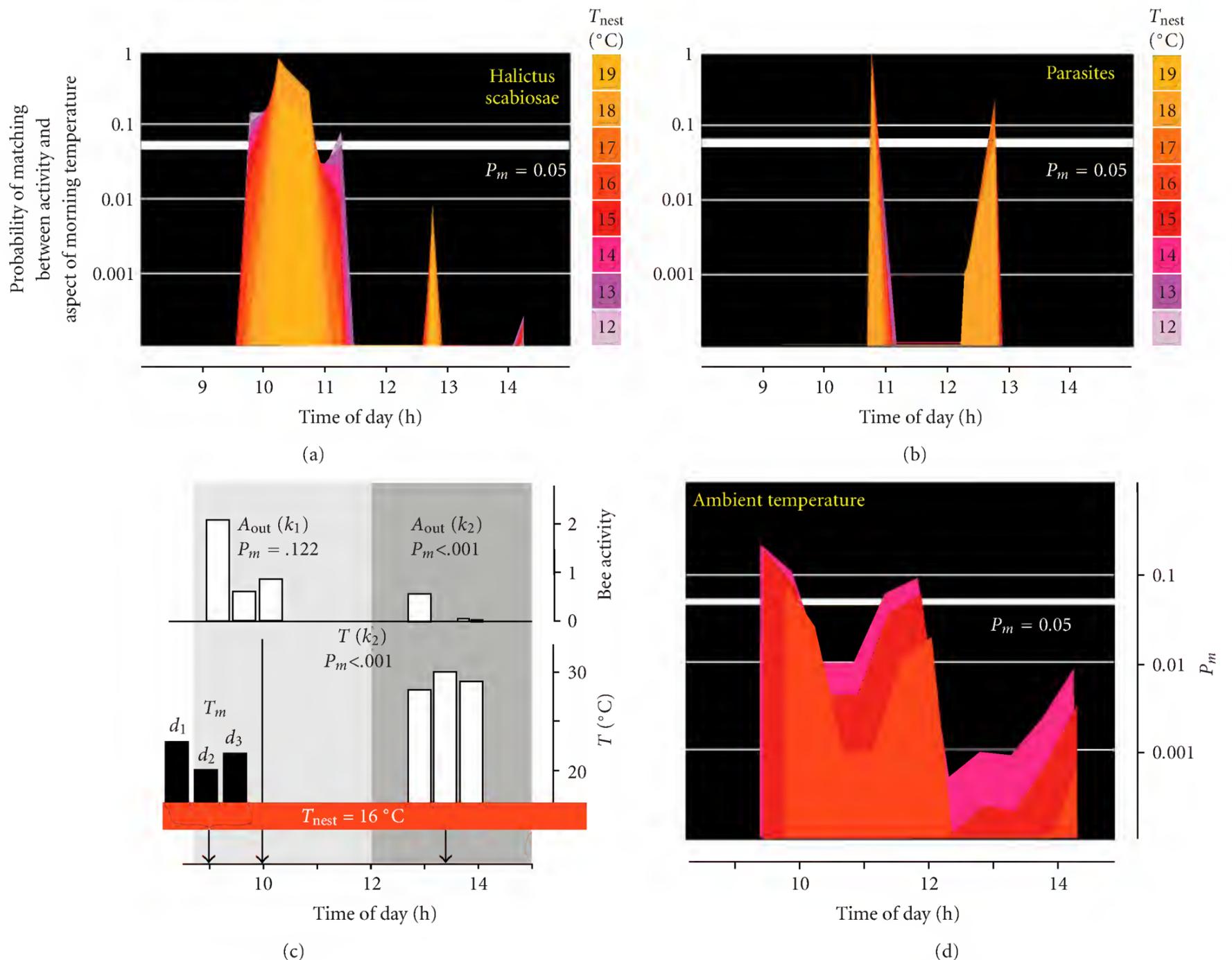


FIGURE 6: Support for the hypothesis that the aspect of morning temperature (for definition see Methods) determines the foraging activity of *Halictus scabiosae* and partially also the impact of nest parasites at the *H. scabiosae* nest over daytime. The abscissa gives the time of the day in hours and the ordinates show the probability of the match (P_m) with which the morning temperature conditions affect (a) the foraging activity rate in *Halictus scabiosae*, and (b) the ratio of the impact rates of parasites at *Halictus scabiosae* nests. The temperature conditions in the morning for the bees or parasites (for definition, see Methods) refer to the ambient temperature which was measured outside of the nest as well as to the temperature inside of the nest, which has been introduced into the model as a virtual parameter in the range from 12°C (blue violet) to 19°C (bright orange). The probability P_m was calculated by chi-square tests for each time step of 30 minutes of the entire observation time. (c) explains the assessment of the probability P_m ; $P_m > .05$ (chi-square test) signifies similarity, and $P_m < .05$ signifies contrast. T_m : morning aspect of temperature on the three successive experimental days (d_1, d_2, d_3) defined for the initial hour of experiments; $A_{\text{out}}(k_1)$ and $A_{\text{out}}(k_2)$: the relations of outflyer activities in the observation intervals k_1 (9:30–10:00 h) and k_2 (13:30–14:00 h) on the three successive experimental days (d_1, d_2, d_3) with the $P_m(k_1)$ -values 0.122 and $P_m(k_2) < .001$. $T(k_2)$: the temperature relations in the early afternoon in the observation interval k_2 with $P_m < .001$; the examples refer to a virtual nest temperature of $T_{\text{nest}} = 16^\circ\text{C}$ (d) gives the probability P_m by comparing the morning aspect of temperature on the days d_1 – d_3 with the ambient temperature later in the day. The graph shows that the match is decreasing over the day with $P_m < .05$ for nest temperatures greater than 15°C.

for *Andrena*, *Colletes*, *Osmia*, and *Megachile* spp. but not for *Halictus* sp. [8, 11]. (2) The cuckoo or gold wasps (subfamily Chrysidinae, family Chrysididae) are typically associated with solitary bees and are generally cleptoparasites [11], laying their eggs in host nests where their young larvae consume the host eggs or larvae, later also consuming the provisions. (3) Some species of ichneumon wasps lay their eggs in the ground, but most inject them directly into a host's

body, typically into a larva or pupa of solitary bees [28]. At the study site, ichneumon wasps were observed scanning around the nests of *Halictus scabiosae*. (4) Members of the genus *Sphecodes* are solitary parasitic bees; the larvae of this species are parasites of other solitary bees [8, 11]. (5) The velvet ants (Mutillidae) are a family of wasps whose wingless females resemble ants. The male wasp flies around searching for females. After mating, the female searches for a suitable



FIGURE 7: Nest closing and reopening. (a) Closing of an entrance hole from the inside of the nest by an egg-laying female; the sealing itself took 8 seconds while the whole closing activity continued over a further five minutes; images a_1 to a_6 were taken every seconds. (b) Opening of the nest entrance by circling movements by a worker bee from outside. This process took 8 minutes. The nest entrance was closed up at 11:20 h; at 11:28 h the worker bee in the image reopened the nest from outside. Four minutes later, the entrance hole was closed again. The images b_1 to b_6 were taken in the last minute before the forager bee succeeded to enter the nest.

host, typically a bee's nest, and lays her eggs near the larvae or pupae. The mutillid larvae are idiobiont ectoparasitoids that eventually kill and eat their immobile host.

The activities of nest parasites at the nests of *H. scabiosae* are displayed in Figure 4(f). The first experimental day

appeared to be representative regarding the impact of nest parasites. The rates with which parasites appeared at the nest per 30 minutes interval increased steadily over time and did not peak before 15:00 h when the observation session was terminated. A similar tendency was also observed on day 2,

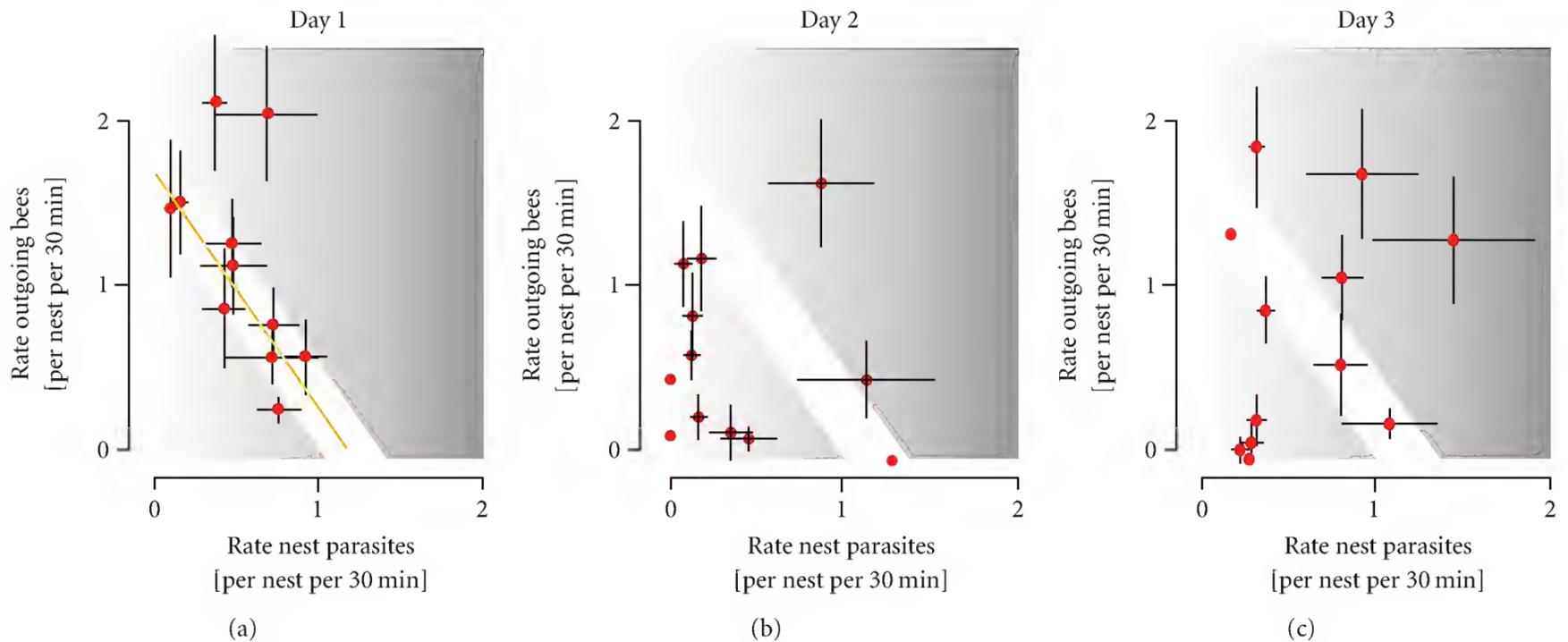


FIGURE 8: Correlation between the foraging rates and the impact rates of nest parasites on three successive days with different weather conditions. Values were normalized to nest and 30 minutes of observation time. On day 1, the bees obeyed the simple rule that foraging activity decreases with increasing parasite impact (regression function: $F_{out} = -0.4903 * I_p + 1.6834$; $n = 9$; $r = 0.892$; $P < .001$; shown by a white stripe in the correlations graphs of all experimental days). The graphs indicate that averaging of the daily rates of bee and parasite activities is crucial, in particular due to the massive influence of weather conditions.

but at lower level, whereas on day 3 the activity of parasites, after having developed in a similar way as on the previous days, was reduced due to the worsened weather conditions in the early afternoon. Conforming to previous reports [29] the impact of parasites showed a general trend to increase over daytime ($P = .008$) and with ambient temperature ($P < .001$; Table 2, lines 7–10); this was assessed on each of the experimental days (Figure 4(f); Table 2: lines 1–5) and averaged over the whole session (Figure 5(b); Table 2: line 6).

If it was true that the infestation of halictine nests by nest parasites is controlled by daytime rather than ambient temperature, then it can be expected that the aspects of morning temperature are not essential for the parasites, which would contrast with the foraging activity of their host. Indeed, the match factor f_m (Figure 6(b)) for the impact of nest parasites showed only a weak correlation in two short time windows. The first match with $P_m > .05$ occurred before noon at the same time when the bees were maximally active in foraging. A second peak with $P_m > .05$ occurred in the early afternoon, which corresponded also to a small peak in the P_m -values of their hosts. This finding let us assume that the parasites' activities match the foraging activity of their host, either by specific adaptation to the morning temperature conditions on their own or by pursuing their hosts. In any case, the diurnal activity cycles of nest parasites of *H. scabiosae* differ strongly from that of their host.

A second, much simpler, but very gross way to assess the interrelation between bees and their nest parasites is to directly correlate the activities of both (Figure 8). As would be expected, the correlation graphs differ strongly due to the different weather conditions. Again, the first experimental day was representative of the diurnal pattern of the foraging activity of bees and of the impact of parasites: the bees obeyed

the rule that they decreased their foraging activity with increasing parasite impact ($P < .001$), with the exception of the time when foraging activity peaked. At this point, the bees were more active than predicted by the regression function. This regression line has been marked as a white stripe for comparison with the graphs of day 2 and 3. On day 2, the basic activity of the parasites was low; only a single individual of *Sphcodes albilabris* visited a limited number of nests directly at their entrances for at least two times and caused two of the singular activity peaks (cf. Figure 4(f₂)). On day 3, the activities of both host and parasites were high throughout the first half of the day; afterwards the activities strongly decreased due to the cloudy and windy weather in the early afternoon. Thus, the graphs in Figure 8 show that averaging of the daily rates of bee and parasite activities for correlation could be crucial, in particular due to the strong influence of the weather.

4. Discussion

4.1. Diurnal Foraging Activity Patterns of *H. scabiosae*. The daily pattern of the pollen- and nectar-collecting activities of *H. scabiosae* is very similar to those of other halictine species. In *Halictus ligatus* [19, 23, 28], the first foraging flights were observed before 9:00 h. Within one nest, each worker may start independently or may be influenced by her nest mates. In the latter case, the rate of activation is a linear function of the number of workers waiting in the nest [30]. It is an open question [5, 22] whether bees nesting in aggregations in close proximity to each other, such as at our study site, stimulate each other to forage. The foraging activities in *Halictus ligatus* showed a diurnal peak activity around 10:30 h for queens and 10:30–13:00 h for workers

TABLE 2: Regression functions for the activities of parasites infesting *Halictus scabiosae* nests. ^{[b][r]}: black and red curves (see text). For other details see legend of Table 1.

Number	Parasites reference	Regression	Day	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>r</i>	<i>n</i>	<i>P</i>
1	Figure 4(<i>f</i> ₁)	$Ip = a * t + b$	1	0.120	-0.928			0.744	10	.014
2	Figure 4(<i>f</i> ₂) ^[b]	$Ip = a * t + b$	2	0.0393	-0.757			0.549	11	.081
3	Figure 4(<i>f</i> ₂) ^[r]	$Ip = a * t + b$	2	0.060	-0.592			0.904	9	<.001
4	Figure 4(<i>f</i> ₃) ^[b]	$Ip = a.t^3 + b.t^2 + c.t + d$	3	-0.008	0.150	-0.124	-5.326	0.730	11	.065
5	Figure 4(<i>f</i> ₃) ^[r]	$Ip = a * t + b$	3	0.279	-2.458			0.633	8	.092
6	Figure 5(b)	$Ip = a.t^3 + b.t^2 + c.t + d$	1-3	0.001	-0.023	0.238	-0.207	0.445	34	.008
7		$Ip = a * T_{amb} + b$	1	0.006	11.606			0.543	13	.055
8		$Ip = a * T_{amb} + b$	2	0.039	-0.756			0.513	13	.073
9		$Ip = a * Ln(T_{amb}) + b$	3	2.343	-7.248			0.503	13	.080
10		$Ip = a * T_{amb} + b$	1-3	0.058	-1.174			0.550	34	<.001

[19, 31]; some of the workers continued foraging as late as 18:30 h. In July, the worker foraging period of *H. ligatus* started and peaked after two weeks. It is known [19, 31] that foragers undertake fewer, longer foraging bouts or more numerous, shorter ones. Average handling time, flight time, and round trip time were consistently negatively correlated with the number of bouts per day. Foragers that have a higher foraging rate tended to take shorter bouts [19, 31].

In the pleometrotic halictine species such as *H. scabiosae* foraging is achieved by the smaller females, the auxiliaries, while the bigger egg-laying female (Figure 1(b)), the queen, generally guards the nest at the entrance [10, 12]; this may occur in up to 75% of the nests, even in the matrilineal phase. We frequently observed at *H. scabiosae* nests that the guard bee blocked the entrance against homing individuals which potentially could have been own nest members [10, 12]. Such aggressiveness displayed by queens is supposed to increase with parasite pressure and leads to nest founding by the smaller females which may successively usurp nests of unrelated halictine species in close vicinity [10].

4.2. The Trade-off between Foraging and Becoming Infested. It is a common view that insects use temperature to determine the “time of season” [32]. Temperature is an important factor for bees to control activity rates, but it does not control all aspects of life; different species, of both foragers and food sources, are geared differently to rising temperatures [33]. Regarding the decision of the bees to emerge in the morning for the first foraging flight, it has been demonstrated [17] for halictide (*H. rubicundus*) and anthophoride bees (*Anthophora plumipes*) that they depend on a certain nest tunnel temperature just inside the entrance. Similarly, the foragers of other halictine bees (e.g., *H. confusus* and *H. ligatus*) opened their burrows soon after sunrise, when the soil temperature 2.5 cm below the surface was about 18°C [34]. In this paper we investigated how temperature controls activity rates of *H. scabiosae*. We tested whether foraging rates over daytime obeyed one of the following two rules: Rule A defines that the current ambient temperature

conditions trigger the current foraging activities. The other, nonalternative, rule B defines that the foraging activities over daytime are determined by the temperature conditions in the morning. The correlation between ambient temperature and foraging activity in the course of the experiment with different weather conditions resulted in a weak correlation (Table 1: line 11). This clearly demonstrates that foraging activity is apparently too complex to propose a mere dependency of ambient temperature according to rule A. However, the pre-noon aspect of foraging, when considered independently of the foraging activity later on the day, correlated significantly with ambient temperature (Table 1: line 12). Pre-noon foraging activity was strongly correlated with the initial morning temperatures (Figure 6(a)), thus obeying rule B. However, this is restricted particularly to a single time window before noon in which the foraging rate complied with the morning temperature aspect (Figure 6), in the same time period when foraging activity was the greatest. The important point here is that in *H. scabiosae* this match between the aspect of morning temperature and foraging was even stronger than the match between the aspect of morning temperature and the ambient temperature at any time of the day. With other words, the morning temperature aspect was a rather weak predictor of the temperature over the day, because in the model the correlation did not exceed in the model the critical level of $P_m = .05$ after 10:00 h (Figure 6(d)), at least not for nest temperatures above 15°C. This finding let us assume that the bees control their daily activity cycle by the temperature relations in the morning, in particular in the main foraging period in the morning and not later. Interestingly, the model was rather robust to changes of the virtually defined reference, which was the temperature inside the nest, and delivered similar results for the range of nest temperatures between 12°C up to 19°C (Figures 6(a) and 6(b)).

The question arises of why *H. scabiosae* reduces foraging in the early afternoon. We observed that the match between foraging activity and the aspect of morning temperature became less significant just after the peak of foraging before

noon. This means that the bees control their afternoon activities by other principles. One of the proximate aspects of reducing foraging is the fact that food sources may degrade in the course of the day. The pollen offer could diminish over daytime [35] although it hardly changes in its residual attractiveness throughout the day. It is more likely that the foraging bees become conditioned to terminating their foraging bouts by a possible shortage of nectar secretion in the afternoon. The second proximate cause for the pre-noon foraging peak activity could be linked with the fact that the bees have to cope with their nest parasites. Following this argument, this temporal activity pattern would constitute a further line of defence [21] against parasite pressure. This surmise is strongly supported by the fact that the bees not only guard their nest as good as they can throughout the day (Figure 1) but start to close up their entrances just in the period after their midday foraging peak (Figures 4(e) and 7(a)). Therefore, it is plausible to consider the decrease in the foraging rate in the early afternoon (Figures 4(c), 4(d), and 6(a)) as a response not only to degrading nectar sources but also to increasing parasite pressure.

4.3. The Parasite View: When Is the Best Time for Infesting the Host? In this paper the activities of nest parasites are compared with those of their hosts, *H. scabiosae*. Their tendency to infest bee nests was estimated by their impact per nest and 30 minutes observation interval. The activity patterns of the nest parasites showed significant correlations with daytime and with the current ambient temperature (Table 2, Figure 5(b)), which corresponds to rule A that the current ambient temperature conditions trigger the current activities. Additionally, they exhibit two short peaks with a probability of matching of $P_m > .05$ (Figure 6(b)). The first peak occurred before noon, in the same period when the bees were maximally active in foraging, and the second peak was observed in the early afternoon when the bees had already terminated their foraging, and some of the nests of the experimental batches had already closed their entrance holes. Thus, it seems that the nest parasites also correspond with rule B, although much weaker than *H. scabiosae* did.

4.4. Anticyclicity between Host and Parasite Activity. In summary, the diurnal activity patterns of bees and their nest parasites display different strategies. The bees control their foraging activity by aspects of the morning temperature, while they decrease their activities outside of the nest in the early afternoon, independent of the rules A or B. The nest parasites' activities are primarily controlled by daytime and ambient temperature, which confirms to rule A. They additionally displayed two short-time windows in which they acted as if guided by rule B; in the first, shorter, activity window they obviously synchronized themselves with the activities of their hosts; the second window in the noon is seemingly broader. However, the data cannot indicate whether the parasites follow rule B on their own or through pursuing the pre-noon activity patterns of the bees. In any case, the findings confirm that the strategies of nest parasites differ from those of their hosts. The nest parasites strive

to increase the infestation rate over the day. In response to parasite pressure, the bees mainly tend to decrease their activities outside of the nest and to close the nests in the early afternoon. The nest parasites would still have the chance to visit a few open and unguarded nests of *H. scabiosae* in the afternoon, but they also experience that even in one and the same habitat the formation of host chains changes during daytime [36].

Acknowledgments

The authors thank Mr. Alois and Hans Gerencser, farmers in Krobotek, Burgenland (Austria) who gave permission to conduct the experiments on their land for their hospitality.

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Research Article

Impact of Bee Species and Plant Density on Alfalfa Pollination and Potential for Gene Flow

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Received 15 September 2009; Accepted 1 December 2009

Academic Editor: James C. Nieh

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In outcrossing crops like alfalfa, various bee species can contribute to pollination and gene flow in seed production fields. With the increasing use of transgenic crops, it becomes important to determine the role of these distinct pollinators on alfalfa pollination and gene flow. The current study examines the relative contribution of honeybees, three bumble bee species, and three solitary bee species to pollination and gene flow in alfalfa. Two wild solitary bee species and one wild bumble bee species were best at tripping flowers, while the two managed pollinators commonly used in alfalfa seed production, honeybees and leaf cutting bees, had the lowest tripping rate. Honeybees had the greatest potential for gene flow and risk of transgene escape relative to the other pollinators. For honeybees, gene flow and risk of transgene escape were not affected by plant density although for the three bumble bee species gene flow and risk of transgene escape were the greatest in high-density fields.

1. Introduction

Different pollinators are expected to vary with respect to their relative role in the pollination of specific crops. Although managed pollinators are used for the pollination of large seed production fields of outcross bee-pollinated crops such as alfalfa, a number of wild pollinators also visit the flowers and participate in pollination [1–4]. Different insect pollinators have been shown to vary in how effectively they deposit and remove pollen from individual flowers [5, 6]. For example, the tripping rate varies between bee species visiting alfalfa racemes [1, 7, 8] and distinct species deposit different quantities of pollen on cranberry flowers during a single visit to a flower [2]. Such differences in tripping rates and pollen deposition can be influenced by whether a pollinator forages for pollen or for nectar [9] and has been shown to influence fruit and seed set [2, 7].

Pollinator type and landscape features can affect gene flow. Besides influencing pollination, distinct insect pollinators can also differentially affect gene flow or how genes are moved around the landscape [10]. In the Rocky Mountain

columbine, pollen carried by bumble bees was more likely to sire seeds when it moved shorter distances but this was not the case for pollen carried by hawkmoths [10]. In addition to pollinator type, different features of the landscape can affect gene flow. For example, increasing plant density has been shown to reduce gene flow as pollinators respond to locally abundant floral resources and shorten their flight distances [11–13]. The pollen load carried by a pollinator between pollen donors and recipients is expected to turn over more rapidly as a pollinator visits a greater number of plants per unit distance traveled. This in turn reduces gene flow. In alfalfa, gene flow has been shown to be less in larger commercial fields relative to smaller experimental fields [14]. However, we know little about whether distinct insect pollinators react differentially to these various landscape attributes and whether these different responses affect pollen dispersal.

As commercial use of transgenic crops increases, it becomes important to determine the impact of distinct insect pollinators on gene flow, especially in outcrossing crops like alfalfa where various wild insect pollinators can contribute

to the pollination and movement of alfalfa pollen [1, 4, 15]. Cresswell et al. [16] have developed a model to predict gene flow between transgenic and conventional fields in agriculture. Their model predicts that the number of flowers that a bee visits in a patch during one foraging bout, that is, the mean residence, estimates the extent to which transgenic pollen is diluted by conventional pollen [16]. In fact, the relative amount of transgenic pollen on the conventional field's flowers is inversely proportional to the total amount of pollen delivered by each bee during a bout of foraging in the conventional field [17]. Therefore the more flowers that a given pollinator visits per foraging bout, the smaller the relative amount of transgenic pollen and proportion of fruits set from transgenic pollen by that pollinator [17].

In the current study, we examine the impact of pollinator species and plant density on pollination and potential for gene flow in alfalfa, *Medicago sativa*. We compare the behavior of honeybees, three species of bumble bees, and three species of solitary bees visiting alfalfa flowers in patches planted at two different densities. As a measure of pollination, we compare the number of flowers visited per raceme, the number of flowers tripped per raceme, and the percentage of open flowers visited and tripped by the different pollinator types in patches planted at two densities. As a measure of the potential for gene flow, we compare the mean residence for each pollinator at the two planting densities. This study examines the relative contribution of distinct bee species to pollination and gene flow in alfalfa and determines whether and how distinct bee species are affected by plant density, a feature of the landscape known to influence gene flow.

2. Materials and Methods

2.1. Study Species. *Medicago sativa* (Fabaceae) is a perennial herb cultivated throughout the world mainly as a forage crop. In the United States, seed production occurs in California using honeybees (*Apis mellifera*) as managed pollinators and in the Pacific Northwest using mainly alfalfa leafcutting bees (*Megachile rotundata*). An alfalfa plant produces racemes of small perfect flowers typically ranging in color from pale to dark purple. The flowers require bee visitation for pollination. When a bee opens the keel petals, the enclosed stamen and pistil snap forward, forcefully striking the bee. Honeybees foraging for nectar soon learn to avoid this mechanism by approaching the flower from the side and inserting their proboscides through the petals near the base of the flower to reach the nectar (nectar thieves).

2.2. Experimental Setup and Data Collection. This study was conducted at West Madison Agricultural Research Station in Madison, WI, USA. In the spring of 2008, eight patches of 121 alfalfa plants were planted with four replicates at each of two densities: one plant every 0.3 m (3 m × 3 m plot), and one plant every 0.9 m (9 m × 9 m plot). Patches were laid out linearly with 3 m between patches, alternating between high and low density patches for a total of 968 alfalfa plants in the experiment. These densities represent 1/2 and 2

times the density typically used for commercial alfalfa seed production.

In mid-June 2009, a hive of bumblebees (*Bombus impatiens*) and a hive of honeybees (*Apis mellifera*) were set up near the alfalfa plots. Five nesting blocks for alfalfa leafcutting bees (*Megachile rotundata*) were placed throughout the field, and alfalfa leafcutting bees were released periodically throughout the study period. When many alfalfa plants were in bloom, from late June to late July, one to three observers examined the behavior of the diverse bee species visiting alfalfa flowers. The observers noted plant density in the patch and recorded the insect species at a raceme, the number of open flowers visited and the number of flowers tripped at a raceme and counted the total number of open flowers on the raceme. A two-way analysis of variance with bee species and plant density as main factors and their two-way interaction (proc GLM, SAS, version 9.2) helped to determine the impact of bee species and plant density on the number of flowers visited per raceme, the number of flowers tripped per raceme, the proportion of open flowers visited per raceme, and the proportion of visited flowers that were tripped. Differences within a main factor were examined using Duncan's multiple range tests. Proportions were arcsine transformed prior to analyses to stabilize the variance. Graphs were drawn from the untransformed values.

To examine the potential impact of bumble bees and honeybees on gene flow, the border of a patch was observed: when a bumble bee or honeybee was spotted entering the patch, the bee was followed until it left the patch and the number of flowers it visited in the patch was tallied. Solitary bees proved too difficult to follow through the patch and therefore data on the number of flowers visited per patch could not be gathered for these bee species. The number of flowers visited per patch was recorded for the various bee species in the different patches and the impact of bee species and plant density on the number of flowers visited per patch during a foraging bout was examined using a two-way analysis of variance with bee species and plant density as main factors and their two-way interaction (proc GLM, SAS, version 9.2).

3. Results

3.1. Insect Visitors. Besides *B. impatiens*, *A. mellifera*, and *M. rotundata*, we commonly observed two species of wild bumble bees, *B. griseocollis* and *B. auricomus*, and two species of wild solitary bees, *Halictus rubicundus* and *Andrena asteris* visiting alfalfa flowers.

3.2. Effects of Bee Species and Plant Density on Pollination. The number of flowers visited per raceme, the proportion of open flowers visited on a raceme, the number of flowers tripped on a raceme, and the proportion of visited flowers that were tripped (tripping rate), were each affected by bee species ($P < .05$) but not by plant density (Table 1). Thus, bee species but not plant density affected the potential for pollination. However, the relative ranking of the different

TABLE 1: Analysis of variance with pollinator species and plant density on (a) the number of flowers visited per raceme; (b) the number of flowers tripped per raceme; (c) the proportion of open flowers that were visited per raceme; (d) the proportion of visited flowers that were tripped per raceme.

(a) Number of flowers visited per raceme					
Source	d.f.	Sum-of-squares	MS	F-ratio	P
Bee species	6	229.20	38.20	9.77	<.0001
Plant density	1	9.89	9.89	2.53	.11
Bee* Density	6	17.10	2.85	0.73	.63
(b) Number of flowers tripped per raceme					
Source	d.f.	Sum-of-squares	MS	F-ratio	P
Bee species	6	170.47	28.41	13.01	<.0001
Plant density	1	4.99	4.99	2.29	.13
Bee* Density	6	17.40	2.9	1.33	.24
(c) Proportion of open flowers that were visited					
Source	d.f.	Sum-of-squares	MS	F-ratio	P
Bee species	6	3.10	0.52	3.86	.0009
Plant density	1	0.0007	0.0007	0.01	.94
Bee* Density	6	0.84	0.14	1.05	.39
(d) Proportion of visited flowers that were tripped					
Source	d.f.	Sum-of-squares	MS	F-ratio	P
Bee species	6	49.94	8.32	24.27	<.0001
Plant density	1	0.14	0.14	0.42	.52
Bee* Density	6	3.28	0.55	1.59	.15

TABLE 2: The relative rank of Bee species with respect to the number of flowers visited per raceme; the number of flowers tripped per raceme; the proportion of open flowers that were visited per raceme and the proportion of visited flowers that were tripped per raceme.

Bee species	No. of flowers visited	No. of flowers tripped	% open flowers visited	% visited flowers tripped
<i>B. griseocollis</i>	1	5	2	7
<i>B. auricomus</i>	2	1	1	3
<i>B. impatiens</i>	3	4	4	4
<i>A. asteris</i>	4	2	7	1
<i>H. rubicundus</i>	5	3	5	2
<i>A. mellifera</i>	6	7	6	6
<i>M. rotundata</i>	7	6	3	5

bee species depended on the specific variable under consideration (Table 2). For example, the three bumble bee species visited the most flowers per raceme and visited significantly ($P < .05$) more flowers relative to the solitary bees and the honeybees (Figure 1(a)). On the other hand, the proportion of flowers visited per raceme was not as distinct between bee species (Figure 1(b)); most bee species visited fewer than half of the open flowers on a raceme on average (33–49%) and *B. auricomus* visited 58% of the open flowers (Figure 1(b)). One of the wild bumble bee species, *B. auricomus*, and the two wild solitary bee species, *A. asteris* and *H. rubicundus*, tripped significantly more flowers than the other bee species (Figure 1(c)). Interestingly, the wild solitary bee species tripped on average over 80% of the flowers visited while the leaf cutting bees and honeybees only tripped 25% or fewer of the flowers visited per raceme in this study (Figure 1(d)). The number of flowers tripped per raceme depended on both the number of flowers visited per

raceme and the proportion of these visited flowers that were tripped by the pollinators. Although they did not visit quite as many flowers per raceme as some of the other bee species, the tripping rate of the two wild solitary bee species was the highest and therefore together with *B. auricomus*, these two bee species tripped more flowers per raceme relative to the remaining pollinators.

3.3. Effects of Bee Species and Plant Density on Potential for Gene Flow. Bee species, plant density, and their interaction all significantly affected the number of flowers visited per patch during one foraging bout (Table 3). All three species of bumble bees visited more flowers in low relative to high density patches, while honeybees were not influenced by plant density (Figure 2). Bumble bees visited more flowers per patch relative to honeybees and *B. impatiens* visited fewer flowers per patch relative to the other two bumble bee species, especially in low density patches (Figure 2).

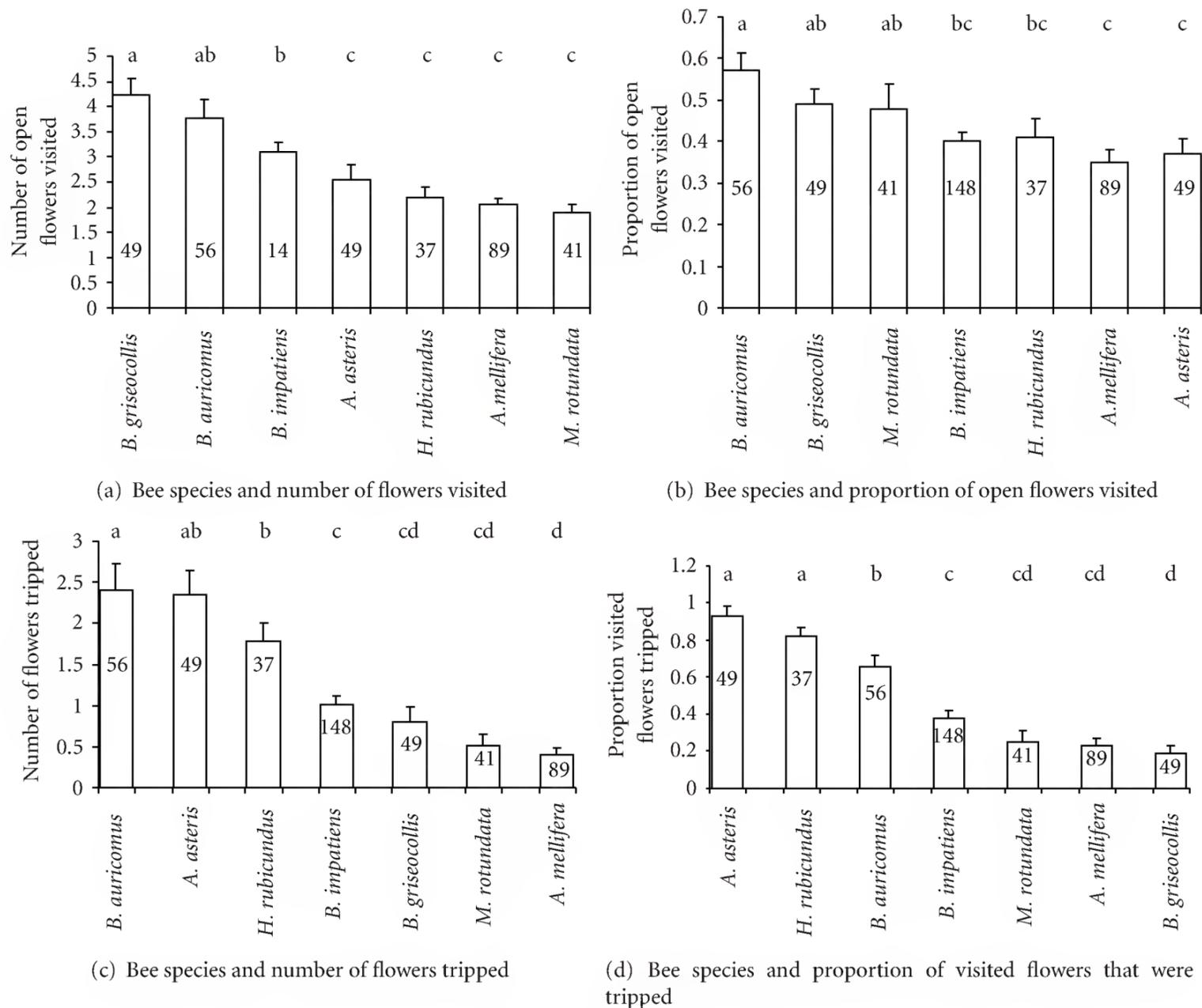


FIGURE 1: The impact of bee species on (a) the number of flowers visited per raceme; (b) the number of flowers tripped per raceme; (c) the proportion of open flowers that were visited per raceme; (d) the proportion of visited flowers that were tripped per raceme. The numbers inside each column represent the sample sizes while different letters indicate a statistically significant difference between bee species as determined by the Duncan's multiple range test.

TABLE 3: Analysis of variance with pollinator species and plant density on the number of flowers visited per patch during one foraging bout by bumble bees and honeybees.

Source	d.f.	Sum-of-squares	MS	F-ratio	P
Bee species	3	258071.2	86023.7	3.92	.009
Plant density	1	321828.9	321828.9	14.68	.0002
Bee* Density	3	197478.8	65826.3	3.00	.0307

4. Discussion

4.1. Effects of Bee Species on Pollination. The distinct bee species differentially affected pollination. The two wild solitary bees, *A. asteris* and *H. rubicundus*, had the highest tripping rate followed by one wild bumble bee species, *B. auricomus*. Together these three bee species tripped the most flowers per raceme. The leaf cutting bees, honeybees, and one of the wild bumble bee species, *B. griseocollis*, had the lowest tripping rate and tripped the lowest number of flowers per raceme. Variation in tripping rate between bee species

visiting alfalfa flowers has been reported previously [1, 7, 8]. In caged enclosures or in the greenhouse, female leaf cutting bees and alkali bees, *Nomia melanderi*, have tripped close to 80% of the visited flowers [7, 8]. Lower tripping rates (51%) have been associated with male leaf cutting bees [7]. However, under field conditions, the tripping rate of leaf cutting bees can vary over the growing season [18]. In Oregon, tripping rates of 10% were detected during the first three weeks of alfalfa blooming and this rate increased sharply to over 80% later in the season, presumably as females became fully established (nesting females) in the

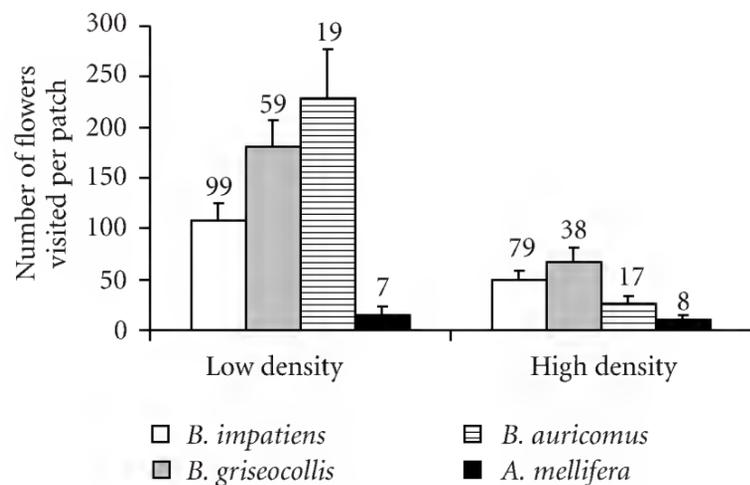


FIGURE 2: The impact of bee species and plant density on the number of flowers visited per patch during one foraging bout.

field [18]. In the current experiment, leaf cutting bees were released in the field throughout the alfalfa flowering period. The presence of males in the samples and the cooler temperatures in Madison in summer 2009 may help explain the low tripping rates observed for leaf cutting bees in this study because higher temperatures are known to ease the tripping of flowers by bees [1, 7, 10, 19]. Tripping rates of honeybees have been well studied and tend to be low (reported between 2 and 22%) unless temperatures are very high [1, 7]. The low tripping rate of honeybees results from the fact that honeybees quickly learn to work a flower from the side in order to avoid the tripping mechanism [20]. In this study, two of the pollinators that are used on a commercial scale to pollinate alfalfa flowers, honeybees and leaf cutting bees, had some of the lowest tripping rates and number of flowers tripped per visit to a raceme.

The differences in tripping rates and number of flowers tripped per raceme between the three bumble bee species were not related to tongue length where longer tongues would allow the species to collect nectar without tripping the flowers [1]. The species *B. griseocollis* and *B. impatiens* have similar tongue lengths [21] and shorter tongues relative to *B. auricomus*. All three species were observed collecting nectar and had pollen sacs which suggest that both nectar and pollen were being collected from alfalfa flowers. The efficiency of wild solitary bees at tripping alfalfa flowers has been reported in other studies (summarized in [1]). Although we only examined tripping rates and did not collect corresponding data on subsequent fruit and seed set, Cane [7] has shown that tripping rate was a reliable relative measure of pollination when comparing different bee species. In his study, Cane [7] demonstrated that bee species and sexes did not differ in the proportions of tripped flowers that set pods and that on average 45–54% of pollinated flowers set fruits in alfalfa.

Tripping rate is only one of the variables affecting the efficiency of bee species as pollinators of alfalfa flowers. The number of flowers tripped per visit to a raceme is also important and depends on the number of flowers visited per raceme, a variable that has been shown to vary between bee species in the current study. However, increasing the number of flowers visited per raceme may also increase the

level of geitonogamous selfing (selfing among flowers on a plant) [22, 23]. Because inbreeding depression is significant in alfalfa (selfed progenies have decreased vigor and seed productivity [1]), a bee species that visits a large number of flowers per plant could negatively impact yield. In addition, visited flowers that have not been tripped but whose nectar has been depleted may lower subsequent visitation rate and pollination success while also increasing the cost of pollination in term of nectar production to the flowers. Nectar robbing has been shown to decrease fruit and seed set of plants in some plant species [24, 25] although the impact on plant reproductive success is not always negative [26]. It would be interesting in future studies to determine how the proportion of nectar thieves in a field (pollinators that collect nectar but do not trip the flowers) affects nectar production, visitation rates to alfalfa flowers, and fruit and seed set (yield).

In the current study, we measured the impact of a pollinator visit to a raceme. Ultimately, the abundance and visitation rate of the different bee species together with the number of flowers each pollinator trips per visit to a raceme determine their impact on pollination of alfalfa flowers [17]. A more abundant pollinator that trips fewer flowers per raceme per visit may have the same impact on pollination as a less abundant pollinator that trips a greater number of flowers per visit to a raceme. Similarly, a pollinator that is abundant in the area but has lower visitation rate to alfalfa flowers may have a similar impact on alfalfa pollination as a less abundant pollinator that has a high frequency of visits to alfalfa flowers. Finally, pollinator species may interfere with one another, as would be the case if a pollinator species with a low tripping rate of flowers depleted nectar from flowers and affected future visitations by pollinators with high tripping rates. Although this study was not designed to measure relative visitation rates or pollinator abundance, our sample sizes for visits to inflorescences and for number of flowers visited per patch indicate common visits by wild bee species to alfalfa flowers.

This study, together with previous work on alfalfa [1, 4], highlights the efficiency of wild pollinators for pollination of alfalfa flowers. In fact, wild bees were utilized as pollinators for leguminous crops in vast areas of the U.S. about 100 years ago [4]. However, increases in field sizes and use of insecticides and decreases in natural habitats around agricultural fields have reduced the use of wild pollinators in alfalfa and other leguminous crop pollination [4]. Current problems in maintaining sufficiently large populations of leaf cutting bees in the fields, combined with the fact that we rely on Canadian sources of bees with few alternative sources of leaf cutting bees in the event of catastrophes [20], strongly emphasize the need for the development of management practices that encourage and facilitate the establishment of wild bumble bees and solitary bees around alfalfa seed production fields. Although the goal should not be to rely entirely on wild pollinators for pollination of large alfalfa production areas, the presence of wild pollinators would decrease the risks associated with strictly relying on leaf cutting bees and could reduce the high price currently associated with alfalfa pollination.

4.2. *Effects of Bee Species and Plant Density on Potential for Gene Flow.* In alfalfa, plant density affected gene flow by insect pollinators but not all pollinators had the same response to a change in plant density. Although all bumble bee species visited more flowers per foraging bout in low relative to high density patches, honeybees were not affected by plant density and visited the same number of flowers in high and low density patches. Honeybees visited few flowers per patch irrespective of plant density. If the relative amount of transgenic pollen in the conventional field's flowers is inversely proportional to the total amount of pollen delivered by each bee during a bout of foraging in the conventional field as suggested by Cresswell [17], then the lower mean residence of the honeybees would suggest a higher relative transfer of transgenic pollen by honeybees relative to the other pollinators. The results obtained in this study imply that the higher transfer of transgenic pollen by honeybees would not be affected by plant density. These data suggest that honeybees may represent a greater risk of transgene escape relative to the bumble bee pollinators observed in this study. However, greater sample sizes for honeybees would be useful to confirm the trends reported here.

The lower mean residence observed at high relative to low density for all bumble bee species suggests a greater risk of transgene escape at high relative to low density for bumble bees. This pattern runs opposite to what had been observed in a previous study where increasing density decreased gene flow [11]. In this previous study, increasing plant density shortened flight distances between plants [12]. High density situations often do shorten intermate distances which tend to reduce dispersal distance [13, 27, 28] because it induces higher pollen turnover within shorter distances. However, in our system, all three species of bumble bees consistently visited more flowers per foraging bout in low density patches. In the high density treatment, plants were intertwined which would tend to limit the growth of each individual plant. Increasing plant density may lower gene flow up to a point but then increase it when plant growth becomes limited. This situation commonly occurs in agricultural field settings and therefore deserves further investigation. Future work is needed to clarify the impact of density on gene flow by insect pollinators. The fact remains, however, that except for honeybees, plant density influenced gene flow by insect pollinators in this study.

Our findings support previous studies by demonstrating differences in pollination efficiency of alfalfa flowers by distinct bee species. Our results also highlight the efficiency of many wild bee species as pollinators of alfalfa. Therefore, encouraging the establishment of wild bee species around commercial fields of alfalfa seed production fields could reduce the risks and the costs associated with our reliance on a single managed pollinator like the leaf cutting bee. However, with the potential increase in commercial production of transgenic alfalfa, it is also important to determine the potential impact of these distinct pollinators on gene flow. Here we demonstrate that distinct pollinators can have different potential impact on gene flow and risk of transgene escape and that not all pollinators respond similarly to changes in plant density, a feature of the landscape known to

influence gene flow. The impact of pollinator species on gene flow and risk of transgene escape will ultimately depend not only on the number of flowers visited per foraging bout in a patch but also on the absolute and relative abundance of the different pollinator species in the alfalfa field. We have a lot more to learn about the potential impact of wild pollinators on pollination, gene flow, and risk of transgene escape in outcross insect-pollinated crops but this study represents a first step in that direction.

Acknowledgments

The authors are grateful to Zachary Larson-Rabin for his help with setting up the alfalfa plots and raising the leaf cutting bees. A. Johnson helped with pollinator observations and S. Krauth with pollinator identification. Andrew Bonde provided the honeybee hive used in this experiment. Funding to the first author was provided by the United States Department of Agriculture, Agricultural Research Service.

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Review Article

Abundance and Diversity of Native Bumble Bees Associated with Agricultural Crops: The Willamette Valley Experience

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Received 20 August 2009; Accepted 2 December 2009

Academic Editor: James C. Nieh

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There are widespread concerns about declining populations of bumble bees due to conversion of native habitats to agroecosystems. Certain cropping systems, however, provide enormous foraging resources, and are beneficial for population build up of native bees, especially eusocial bees such as bumble bees. In this review, we present evidence of a flourishing bumble bee fauna in the Willamette Valley in western Oregon which we believe is sustained by cultivation of bee-pollinated crops which bloom in sequence, and in synchrony with foraging by queens and workers of a complex of bumble bee species. In support of our perspective, we describe the Oregon landscape and ascribe the large bumble bee populations to the presence of a pollen source in spring (cultivated blueberries) followed by one in summer (red clover seed crops). Based on our studies, we recommend integration into conservation approaches of multiple agroecosystems that bloom in sequence for sustaining and building bumble bee populations.

1. Introduction

There are widespread concerns about declines in the numbers and distribution of endemic bees [1–4]. In the Holarctic region, concerns relate, in particular, to eusocial bumble bees, *Bombus* spp. (Hymenoptera: Apidae), which are important pollinators of native plants and crops [5, 6]. In Europe, *Bombus* populations have been closely monitored for decades, and loss of bumble bees and their nesting sites have been attributed largely to anthropogenic activities such as habitat fragmentation due to agricultural intensification and urbanization [7]. To counteract the negative effects of modern agriculture on the environment, agri-environment schemes have been implemented through which financial incentives have been provided to farmers to manage their farms for the benefit of biodiversity, the environment, or the landscape [8]. These include strategies targeted at pollinators, such as appropriate management along field margins for providing food resources and nesting sites [9]. However, densities of bumble bees have been documented to be determined not by the proportion of seminatural habitats but by the presence of rewarding mass flowering crops in agricultural landscapes [10]. Bumble bee colonies live for several

months while bloom in a crop lasts for just a few weeks. Hence, one mass flowering crop alone is usually not adequate for sustaining a bumble bee colony through the year.

Here, we provide a contrary perspective to bumble bee declines while describing the abundant and diverse bumble bee fauna in the state of Oregon on the west coast of the United States. We believe that this rich fauna has been sustained by the practice of farming of bee-pollinated crops that bloom in sequence, and in synchrony with foraging by a complex of bumble bee species. In support of our opposing perspective to the pollination crisis, we describe the Oregon landscape and present results of our studies in which we estimated the diversity and abundance of native bumble bees in a spring crop and a summer crop. Based on our studies, we recommend integration into conservation approaches of multiple agroecosystems that bloom in sequence for sustaining and building bumble bee populations.

2. The Oregon Landscape

The state of Oregon lies north of California on the west coast of the United States. It has a land area of 25 million ha with a low population of only 3.79 million or

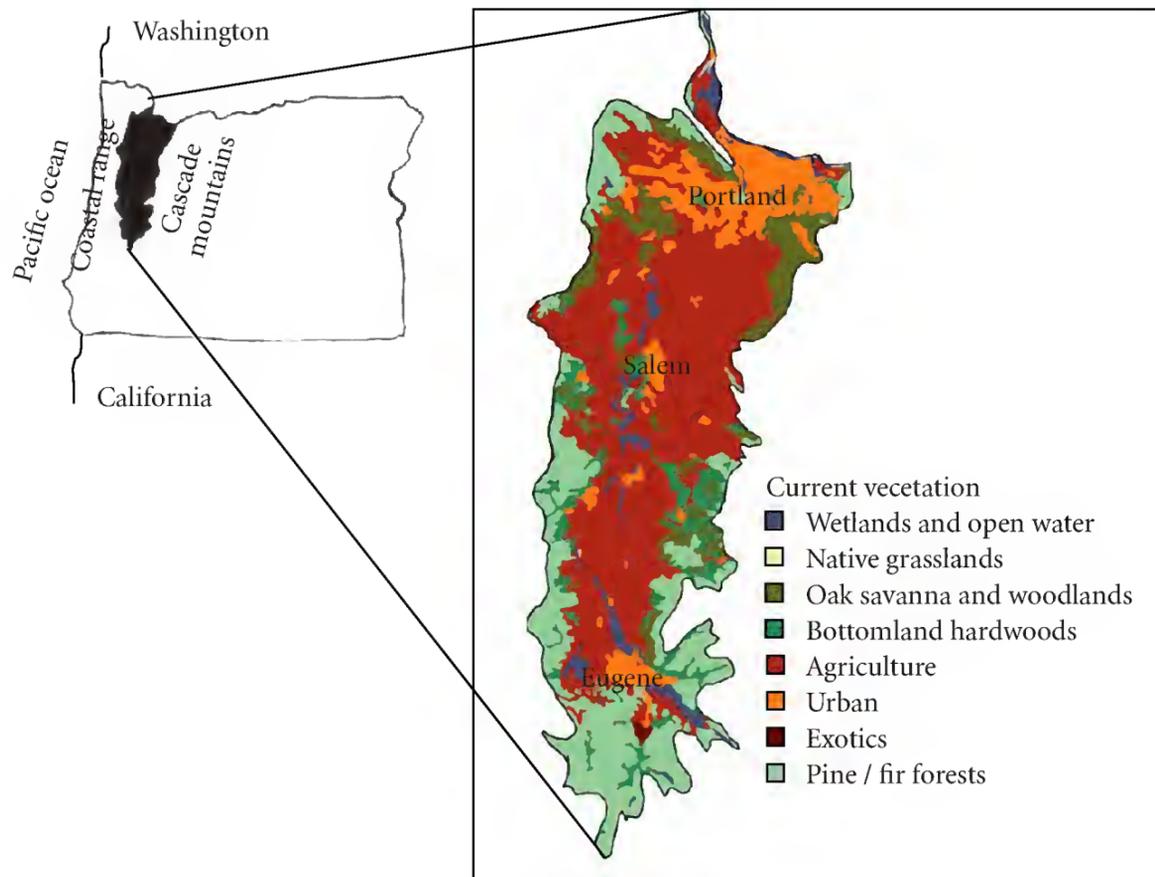


FIGURE 1: Map of the Willamette Valley in western Oregon showing agricultural, wooded, and urban landscapes (Modified from [11]).

0.15 people per ha, and 27% devoted to farming (<http://www.us-places.com/Oregon/Oregon.htm>). Urban development is concentrated in the three cities of Portland, Salem, and Eugene in western Oregon and, even within these cities, farm lands lie adjacent to housing developments. The vast landscapes of agricultural production are interspersed with remnant vegetation as the state was dominated by forests until recently.

The heart of the agricultural country in Oregon, and one of the most fertile agricultural areas in the U.S, is the Willamette Valley, the large valley of the Willamette River in the western part of the state (Figure 1) [11]. With the Cascade Mountains to the east and the Oregon Coast Range to the west, the valley stretches 177 km north to south and 97 km east to west. Due to its proximity to the Pacific Ocean, it receives close to 100–115 cm rainfall in the winter while the summer months of July and August are almost rain free. Crops grow vigorously as a result of winter rainfall, and harvest is facilitated by the dry conditions in summer. These climatic conditions have resulted in the production of over 200 crops including cereals, ornamentals, nursery crops, and bee-pollinated fruits, vegetables, and legumes raised for seed.

3. Bumble Bees Associated with Agricultural Crops in the Willamette Valley

Crop producers in other US states can use commercially reared *Bombus impatiens* Cresson for pollination. However, *B. impatiens* is not native to Oregon, and exotic bumble bees cannot be introduced into the state (http://www.oregon.gov/ODA/PLANT/IPPM/appr_insects.shtml). Hence, for crops serviced by bumble bees, producers in Oregon are dependent on native bumble bee populations.

Native bumble bees were first studied in depth in Oregon in the late 1950s [12]. Few follow up studies were conducted until we serendipitously discovered a highly bee-specific blue vane trap that facilitated evaluation and monitoring of native bee fauna [13]. Our recent bee census studies have documented that over 60 species belonging to 19 genera in five families are present in western Oregon [13–16]. These include a rich complex of spring and summer bumble bee species whose life cycles are synchronous with the bloom periods of many crops grown in the Willamette Valley. Here we present results of our studies on bumble bee composition and abundance during bloom in two Oregon cash crops in the Willamette Valley, namely highbush blueberries (*Vaccinium corymbosum* L., Ericaceae) and red clover (*Trifolium pratense* L., Fabaceae) raised for seed. In each crop, our objectives were to estimate the diversity and abundance of bumble bees: (1) in the landscape and (2) foraging on crop bloom.

4. Pollination and Blueberry Production in the Willamette Valley

Blueberry is native to North America but cultivated worldwide. In Oregon, highbush blueberries are raised in the Willamette Valley. With increasing consumer awareness about the health benefits of blueberries, the area under blueberry production in Oregon doubled in the last decade from 860 ha in 1997 to 1,782 ha in 2007 [15].

Under western Oregon conditions, bloom lasts for about 4 weeks in May. Pollination during this period is critical for larger fruit, better fruit quality, and earlier ripening of berries [17–20]. In Oregon, producers typically stock high numbers of hives of the European honey bee, *Apis mellifera*

TABLE 1: Bumble bee species observed foraging on bloom in commercial blueberry and red clover seed production fields in western Oregon.

Bumble bee species	% of all <i>Bombus</i> foragers ¹	
	Blueberry bloom ²	Red clover bloom ³
<i>Bombus appositus</i>	1.57	1.74
<i>Bombus californicus</i>	3.92	1.87
<i>Bombus griseocollis</i>	15.69	0.68
<i>Bombus melanopygus</i>	12.55	0.00
<i>Bombus mixtus</i>	8.23	0.31
<i>Bombus nevadensis</i>	5.49	3.17
<i>Bombus vosnesenskii</i> ⁴	52.55	92.23

¹Based on visual observations made while walking in the field.

²From [23].

³From [16].

⁴A small proportion (2-3%) of these were likely to have been *B. caliginosus* which is phenotypically very similar to *B. vosnesenskii* and cannot be accurately separated from it in the field.

L. (Hymenoptera: Apidae), ranging from 2.5 to 7 hives per ha to compensate for their low efficiency in pollination of blueberries (personal observation). Honey bees do not forage at temperatures below 12.7°C [21], which are common during blueberry bloom in the Willamette Valley. In addition, honey bees do not buzz pollinate, the mechanism by which certain bees visiting the flowers vibrate their body to remove the pollen grains from the anthers of blueberries [22]. In contrast, bumble bees are better foragers in western Oregon as they can forage under diverse conditions from cool to hot, and even in the rain (personal observation), and they are capable of buzz pollination. However, due to the dependence on honey bees for pollination, prior to the studies described below, there was little information about bumble bees associated with blueberry bloom in the Willamette Valley.

We quantified bumble bee composition during blueberry bloom by placement of blue vane traps in a blueberry orchard in the Willamette Valley in 2006 [23]. We captured 270 bumble bees belonging to seven species, including *B. appositus* Cresson (10 individuals), *B. californicus* Smith (132), *B. griseocollis* (DeGeer) (14), *B. melanopygus* Nylander (39), *B. mixtus* Cresson (19), *B. nevadensis* Cresson (17), and *B. vosnesenskii* Radoszkowski (39) [23]. The following year, the study was repeated at 5 other orchards which provided insights on distribution of each species [15]. *Bombus californicus*, *B. mixtus*, and *B. vosnesenskii* were collected at all five sites, while *B. melanopygus* was collected at four, and *B. appositus* and *B. nevadensis* were trapped at two sites. In 2007, *B. griseocollis* was not collected at any site probably because our sampling study ended before emergence of queens during that year.

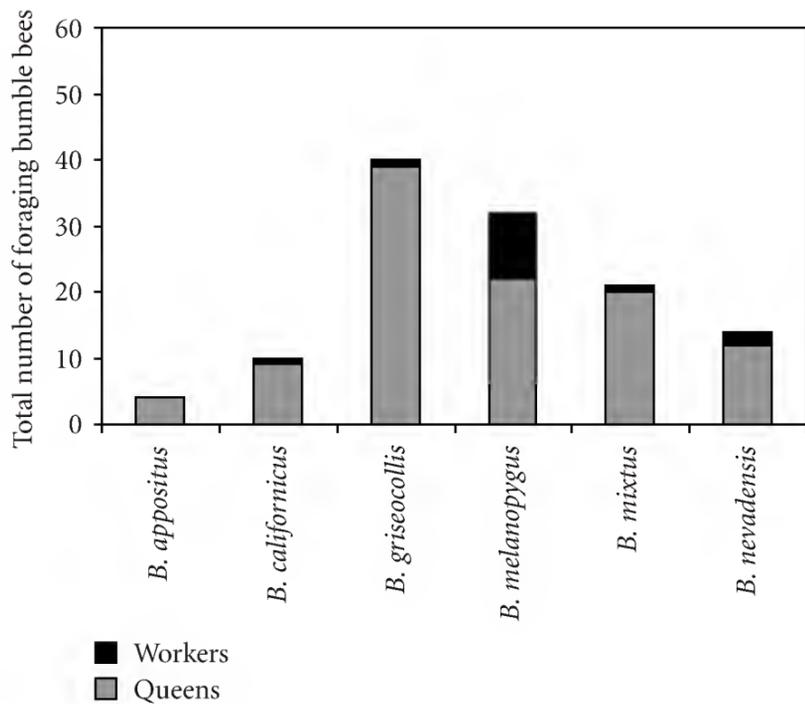
The presence and abundance of six to seven species of bumble bees during bloom in a spring crop are quite remarkable. However, presence of a species in the trap does not necessarily indicate that it forages on the surrounding crop. Hence, in 2006, to quantify the composition of bumble bees foraging on blueberry flowers, we made visual

observations during 2-minute periods while walking along rows of blueberry bushes [23]. We recorded 255 bumble bees from 127 sets of counts (=1 bumble bee/min) including *B. appositus* (4 individuals), *B. californicus* (10), *B. griseocollis* (40), *B. melanopygus* (32), *B. mixtus* (21), *B. nevadensis* (14), and *B. vosnesenskii* (134). Thus, the 7 bumble bee species captured in traps in the same year were also observed foraging on bloom, though in different proportions. While the seven species varied in the proportion of foragers on bloom (Table 1), all are likely to contribute to blueberry pollination as none was observed robbing nectar by chewing holes in blossoms at the base of the flowers, behavior exhibited by certain bumble bee species in other regions [24]. Of the 255 specimens observed, 208 (81.6%) were queens and 47 (18.4%) were workers indicating that the pollinating force was composed primarily of newly emergent queens (Figures 2 and 3). This is beneficial for blueberry pollination as the efficiency of bumble bee queens as blueberry pollinators is reported to be higher than that of bumble bee workers [25].

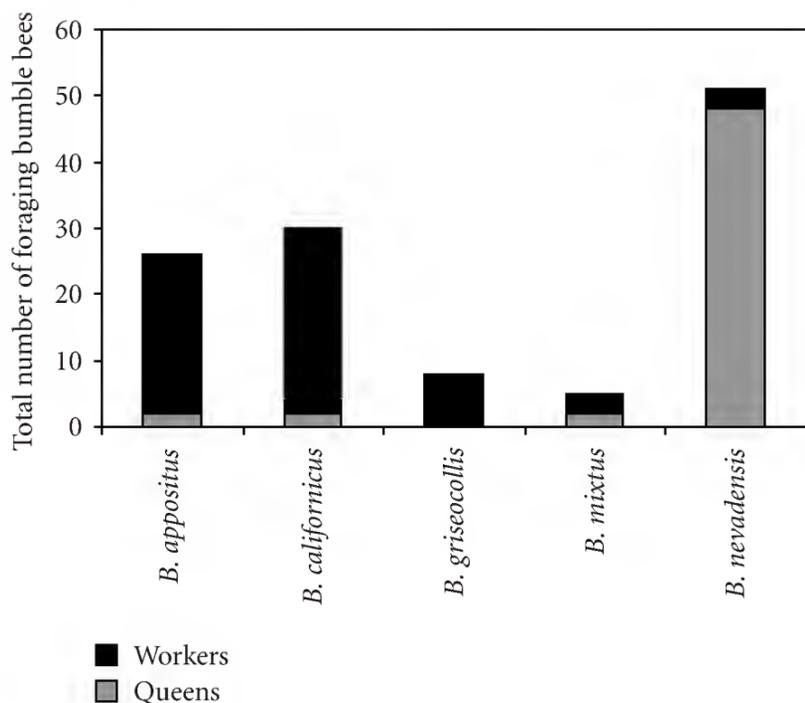
A comparison of the bumble bee fauna captured in the blue vane traps in our study with other trapping studies is a challenge due to differences in sampling protocols. However, bumble bee abundance in Willamette Valley blueberries appears to be greater compared to other regions based on estimates of foragers reported in other studies. As mentioned earlier, in our study we recorded an average of 1 bumble bee/min foraging on blueberry bloom [23]. In contrast, MacKenzie and Eickwort [26] estimated 0.04 bumble bees/min foraging on highbush blueberries in upstate New York, while in blueberry fields in the Fraser Valley in British Columbia, the mean number of native bees, including both bumble bees and solitary bees, recorded by MacKenzie and Winston [27] was 33/h (=0.55/min). Bumble bee diversity in foragers is also quite variable. We observed seven species in our study while in a study conducted in Michigan [28] only one bumble bee species, *Bombus bimaculatus* Cresson, was observed foraging on blueberry bloom.

We also assessed bumble bee activity in one blueberry field. Based on visual observations and the presence of 2,720 bushes/ha, we estimated an average of 0.055 bumble bees/bush/min (=150 bumble bees/ha/min). The average time spent by one bumble bee foraging on one flower was 4 seconds (=15 flowers/min) including the time spent moving from one flower to another. Based on these estimates, 135,000 flowers/ha/hour/bee were potentially visited, or over 11.3 million over a 14-day period assuming that the bumble bee population remained relatively constant for six hours (10:00 am and 4:00 pm) each day. The estimate is conservative as blueberry bloom extends beyond two weeks in the Willamette Valley, and bumble bees are active in blueberry fields up to 10 hours a day during that period (personal observations). With the abundance and long duration of their foraging activity observed in the current study, we believe that bumble bees could have contributed considerably to the high yield (>15 ton/ha) recorded by the producer (personal communication).

Honey bees are not considered to be effective pollinators of blueberries, but in the Willamette Valley, given the presence of 2.5 to 7 hives/ha in the field, they likely contributed



(a)



(b)

FIGURE 2: Comparison of the numbers of queens and workers of bumble bee species (excluding *B. vosnesenskii* which is included in Figure 3) observed foraging on bloom. (a) Blueberries ($n = 127$ counts). (b) Red Clover ($n = 187$ counts).

to some extent. Similarly, other native bees could also have played a role. In our studies conducted between 2005 and 2009, we have captured several hundred native solitary bees belonging to 23 species in ten genera in five families in the blue vane traps placed adjacent to blueberry fields in spring (Table 2). However, we rarely encountered any non-*Bombus* native bee foraging on bloom during our 2-minute counts (personal observation). In a study in British Columbia [27], while solitary bees were observed on blueberry bloom, there were ten times more bumble bees. Diversity of solitary bees was high in another study conducted in British Columbia [29], but blueberry mass was observed to be related not to abundance of solitary bees or of honey bees, but to that of bumble bees. Still, further research is needed for determining

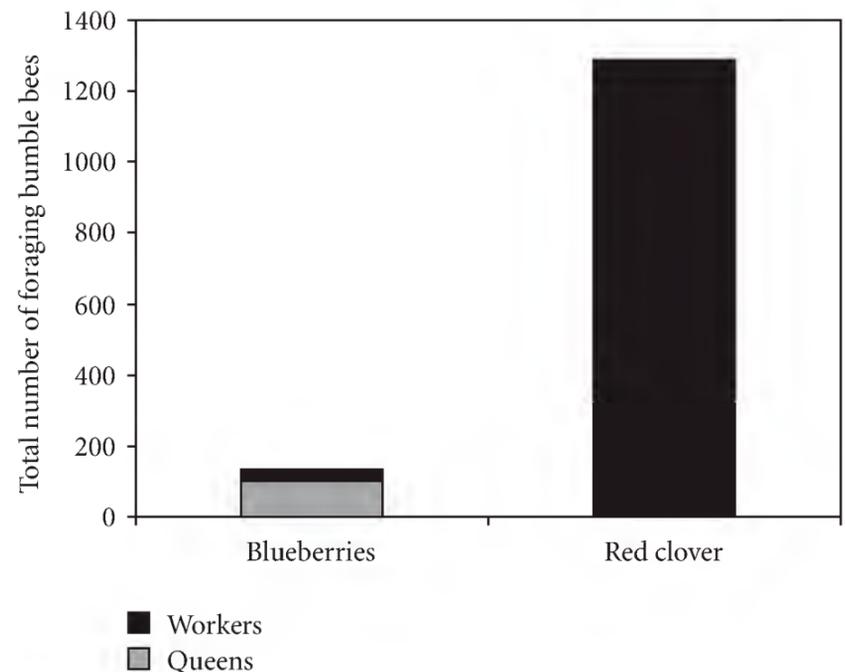


FIGURE 3: Comparison of the numbers of queens and workers of *B. vosnesenskii* observed foraging on bloom ($n = 127$ counts) and red clover ($n = 187$ counts).

the impact of solitary bees in blueberry pollination in the Willamette Valley.

5. Pollination and Red Clover Seed Production in the Willamette Valley

Red clover is grown worldwide in temperate regions as a forage legume, and as a rotation crop for soil improvement [30]. The Willamette Valley is a key region for red clover seed production in the US due to the favorable climatic conditions. The high rainfall during winter enables the production of red clover with minimal irrigation, while the relatively dry periods in summer facilitate harvest with little risk of rain damage (<http://www.oregonclover.org/seedproduction.html>). As a result, over 4,300 ha are under red clover seed production in this area [31].

The critical factor for seed production in red clover is pollination [32–34]. Red clover blooms over six weeks in the months of July and August in Oregon. The florets on each seed head open over six to eight days but due to rapid decrease in fertility, the florets must be pollinated within two to four days after opening [35]. Hollowell and Tysdal [36] indicated that 875 million florets are present in a hectare of red clover. This highlights the need for an abundance of pollinators for achieving high yield in red clover seed crops.

While bees are recognized as the primary pollinators of red clover, there has been considerable disagreement over the value of various species [37, 38]. Darwin's claim that bumble bees alone affected red clover yield [39] was disputed by Meehan [40] but dramatic evidence of their impact was provided by the introduction of bumble bees to New Zealand as this resulted in an enormous increase in seed production [37]. Since the honey bee was already present in the country, this appeared to confirm that honey bees were of limited value to red clover. Subsequent studies documented that while honey bees do pollinate this crop,

TABLE 2: Endemic bees captured and observed as foragers during bloom in blueberry and red clover seed crops in the Willamette Valley in studies conducted between 2005 and 2009.

Family	Species	Blueberry ^{1,2}	Red clover ^{1,3}	
Colletidae	<i>Hylaeus calvus</i> (Metz)	✓	✓	
	<i>Hylaeus rudbeckiae</i> Cockerell and Casad	✓	✓	
Halictidae	<i>Agapostemon texanus</i> Cresson	✓	✓	
	<i>Agapostemon virescens</i> (Fabricius)	✓	✓	
	<i>Halictus confusus</i> Smith	✓		
	<i>Halictus farinosus</i> Smith	✓	✓	
	<i>Halictus ligatus</i> Say	✓	✓	
	<i>Halictus rubicundus</i> (Christ)	✓	✓	
	<i>Halictus tripartitus</i> Cockerell	✓	✓	
	<i>Lasioglossum mellipes</i> (Crawford)	✓	✓	
	<i>Lasioglossum olympiae</i> (Cockerell)	✓	✓	
	<i>Lasioglossum pacificum</i> (Cockerell)	✓	✓	
	<i>Lasioglossum sisymbrii</i> (Cockerell)	✓		
	<i>Lasioglossum titusi</i> (Crawford)	✓		
	<i>Lasioglossum trizonatum</i> (Cresson)	✓	✓	
	<i>Sphecodes</i> sp.	✓	✓	
	Andrenidae	<i>Andrena</i> sp.	✓	✓
	Megachilidae	<i>Anthidium</i> sp.	✓	
<i>Heriades</i> sp.			✓	
<i>Megachile brevis</i> Say			✓	
<i>Megachile perihirta</i> Cockerell			✓	
<i>Osmia lignaria</i> Say		✓	✓	
<i>Osmia</i> sp.		✓ (2)	✓ (5)	
Apidae		<i>Anthophora bomboides stanfordiana</i> Cockerell		✓
	<i>Anthophora urbana</i> Cresson		✓	
	<i>Bombus appositus</i> Cresson	✓	✓	
	<i>Bombus bifarius nearcticus</i> Handlirsch	✓		
	<i>Bombus californicus</i> Smith	✓	✓	
	<i>Bombus caliginosus</i> (Frison)		✓	
	<i>Bombus griseocollis</i> (DeGeer)	✓	✓	
	<i>Bombus melanopygus</i> Nylander	✓	✓	
	<i>Bombus mixtus</i> Cresson	✓	✓	
	<i>Bombus nevadensis</i> Cresson	✓	✓	
	<i>Bombus occidentalis</i> Greene		✓	
	<i>Bombus sitkensis</i> Nylander		✓	
	<i>Bombus vosnesenskii</i> Radoszkowski	✓	✓	
	<i>Ceratina acantha</i> Provancher	✓	✓	
	<i>Ceratina micheneri</i> Daly		✓	
	<i>Ceratina nanula</i> Cockerell		✓	
	<i>Melissodes agilis</i> Cresson		✓	
	<i>Melissodes bimatrix</i> LaBerge		✓	
	<i>Melissodes robustior</i> Cockerell		✓	
	<i>Psythirus</i> sp.		✓	
	<i>Synhalonia</i> sp.	✓	✓	
	<i>Triepeolus</i> sp.		✓ (2)	

¹Numbers in parenthesis refer to number of species.²From [15, 23], and unpublished data.³From [16], and unpublished data.

their efficacy depends on the amount of competing foraging resources in the vicinity [32, 41]. In Oregon, red clover seed producers typically stock 2.5 to 5 hives per ha for pollination but the recent high costs of honey bee rentals have led producers to question the value of honey bee hive rentals (personal communication). However, they were unwilling to take the risk of depending on native bumble bees and other pollinators, as prior to the studies outlined below, there was little information about bumble bees present during red clover bloom.

We conducted a monitoring study with blue vane traps in three red clover seed production fields in 2007 to estimate the bumble bee composition in the area during bloom [16]. In all, 1,227 bumble bees including six of the seven species trapped in blueberries were also trapped in red clover. We collected *B. appositus* (15 individuals), *B. californicus* (19), *B. griseocollis* (26), *B. mixtus* (7), *B. nevadensis* (28), and *B. vosnesenskii* (1,132). Each species was collected at all three sites. While *B. melanopygus* was not collected in traps at any site in 2007, we recorded its presence in traps in red clover seed fields in other years [16]. It is an early spring emerging species, and colonies typically die out by the time red clover blooms.

As in the case of blueberries, we studied bumble bee foraging on red clover by recording the numbers of each species observed while walking through the field. The same six bumble bee species that were observed in the traps were also observed foraging on bloom (Figures 2 and 3) [16]. In 187 visual counts, 1-2 min each, we noted the presence of 1,609 bees (queens, workers, and males). There was a greater proportion of workers compared to queens in all species except *B. nevadensis* (Figures 2 and 3). The species observed included *B. appositus* (28 individuals), *B. californicus* (30), *B. griseocollis* (11), *B. mixtus* (5), *B. nevadensis* (51), and *B. vosnesenskii* (1,484). Proportions of bumble bee species differed from those recorded in blueberries but *B. vosnesenskii* was dominant in both cropping systems (Table 1). Interestingly, of these species, only *B. griseocollis* has been reported elsewhere as a pollinator of red clover [42–44].

Overall, we recorded an average of 6.2 bumble bees per minute across the six weeks of red clover bloom. Early bloom abundance of 0–4 bumble bees/min dramatically increased during peak bloom to 15–30 bees/min. In comparison, in a study by Morrison conducted in Quebec, 1,901 bumble bees were observed visiting red clover during 68 observations, 20 minutes each (=1.4 bumble bees/min) [43].

A cage study conducted by us demonstrated that *B. vosnesenskii* is an efficient pollinator of red clover [16]. Mean seed yield from the *B. vosnesenskii* cages was 661 kg/ha (range = 623 to 685 kg/ha), and variances in seed yield and seed set were low which is indicative of consistency in performance. The *B. vosnesenskii* cage yield was slightly higher than the average yield in Oregon (600 kg/ha) which in itself was almost 40% higher than the US average (430 kg/ha) in the same year [31]. Given the high proportion (>90%) of *B. vosnesenskii* observed foraging on red clover florets during visual observations and in the blue vane traps, we believe that it is a key contributor to Oregon becoming the second largest red clover seed producer in the US [31].

For a deeper understanding of its foraging behavior, we are currently characterizing pollen loads on *B. vosnesenskii* workers returning to colonies placed adjacent to red clover fields. We are also using genetic markers to determine nest composition and foraging range of this dominant bumble bee species in the Willamette Valley.

The role of other native bees in red clover pollination in the Willamette Valley is not known. We have caught several hundred solitary bees belonging to 35 species in 15 genera in five families during bloom (Table 2). However, as in the case of blueberries, we rarely encountered non-*Bombus* bees foraging on bloom during our 2-minute counts. According to Plath [37], while occasionally a solitary bee will forage on red clover, the crop would probably set little seed if its pollination depended on other insects besides bumble bees.

In Oregon, while honey bee hives are rented for red clover seed crop pollination, worker abundance is high only during early bloom in July [16]. Pollen traps placed in hives documented that midway through bloom the workers switch to foraging elsewhere (personal observation). Honey bee foraging away from red clover fields coincided with high numbers of bumble bee foragers in red clover. This suggests that the low number of bumble bees in early-mid July could be the result of competition with honey bees. Peterson et al. [41] reported that bumble bees tended to be more abundant in fields located >1.6 km away from apiaries (honey bee colonies). Also, earlier studies have documented negative impacts on factors such as reproductive success [45] and size of workers [46] of bumble bees in areas of high honey bee density. It is possible that foraging behavior could also be affected by the presence of honey bees, and hence bumble bee abundance may be even greater if producers do not stock honey bee hives. We are currently comparing bumble bee abundance and red clover pollination in the presence and absence of honey bee hive rentals.

6. Willamette Valley Model for Bumble Bee Abundance

It is believed that pollinator populations cannot be maintained by short-flowering agricultural crops alone because of the need of a continuous supply of nectar and pollen [47]. However, wild habitats do not necessarily satisfy these needs either. In contrast, cropping systems that flower in sequence can facilitate sustainability and build up of native bees especially eusocial bees. For maximum production of workers, initial vigor of spring queens is important [44] which can be achieved through provision of a spring-blooming bee-pollinated crop. Cultivated legumes are considered to be important in maintaining native bumble bee fauna [44], and if such crops bloom towards the end of summer, build up of bumble bees will be facilitated during the period when high numbers of reproductives are produced prior to hibernation of queens at the end of the year. We believe that the abundance of a complex of seven bumble bee species in the Willamette Valley is sustained due to the large areas under production of blueberries which provide large quantities of food resources in synchrony with queen emergence thereby

facilitating nest foundation, and of red clover seed crops which provide resources for drones and hibernating queens. The presence of six to seven bumble bee species has been reported in other regions in the US both in agricultural and native habitats [26, 29, 44, 48] but abundances are lower than what we observed. Of the species present in the Willamette Valley, while *Bombus vosnesenskii* was the most abundant bumble bee in both blueberries and red clover, queens and/or workers of all the remaining bumble bee species carried pollen loads in both blueberries and red clover, and thus they all contribute to some extent to both blueberry and red clover pollinations. In particular, queens of *B. nevadensis* >20 mm long and their large sized workers observed with large loads of pollen are likely to make a valuable contribution to pollination.

Besides the seven species that are flourishing, on occasion we have trapped the bumble bee species *B. bifarius nearcticus* Handlirsch and *B. sitkensis* Nylander in agricultural landscapes in the Willamette Valley (Table 1). In addition, a small proportion (2-3%) of the *B. vosnesenskii* observed in the studies were likely to have been *B. caliginosus* (Frison) which is phenotypically very similar to it. In the past, one more species, *B. occidentalis* Greene, was abundant in the Willamette Valley and in other parts of the Pacific Northwest [12]. However, since the late 1990s, it has all but disappeared from coastal and coastal valleys of its range presumably because of its vulnerability to introduced pathogens [49–51]. In the period leading up to its decline, queens of *B. occidentalis* collected from the west coast were sent to rearing facilities in the Midwest and Europe where they were raised commercially along with other bumble bee species. It is speculated that colonies returned to the west coast for pollination of greenhouse crops were infected with *Nosema bombi* and/or other pathogens to which *B. occidentalis* appeared to be highly susceptible [52]. The spillover effects from these commercial colonies to wild populations likely resulted in local extinction of *B. occidentalis* [53, 54]. We collected six individuals of *B. occidentalis* from clover fields in 2006 and 2007 [55], which suggests a possible recovery of the species in the area. The ban on introduction of exotic commercial bumble bees mentioned above should reduce further risk to the rich bumble bee fauna in Oregon.

The agricultural landscapes in the Willamette Valley also support a rich diversity of other native bees besides 11 bumble bee species. We trapped 39 species of solitary native bees belonging to 16 genera in five families in blueberry and red clover fields (Table 2). We rarely detected their presence on bloom but they could have escaped detection as we focused on bumble bees in our studies. The impact of Willamette Valley cropping systems on sustenance of diverse solitary bees, and the contribution of these bees to crop pollination, warrants investigation.

Besides the abundance of food resources provided by blueberry and red clover crops, other factors such as production practices also facilitate build up of bumble bees and other native bees in the Willamette Valley. While each crop is routinely subjected to pesticide sprays, there are few devastating pests perhaps due to the diversified nature of the agricultural landscapes. Except for one blueberry

orchard where organic practices were adopted, all other blueberry and all red clover seed fields in our studies were cultivated using herbicides, fungicides, and insecticides. However, pesticide applications were avoided during bloom or implemented at night to minimize negative impacts on honey bees, thus indirectly benefitting bumble bees too. Voles cause significant damage in agricultural crops in the Willamette Valley, and rodenticides are used for their management. This has been of benefit to bumble bees as empty rodent nests serve as nesting sites in close proximity to foraging resources in the crop. Remnant vegetation between agricultural fields may also be providing nesting habitats and overwintering sites for queens.

Urban developments adjacent to agricultural fields in the Willamette Valley also provide both foraging resources and nesting sites for bumble bees. Climatic conditions support growth of a great diversity of native and exotic annual and perennial flowering plants in gardens, and bumble bees have been observed to nest frequently in various urban locations. Due to the ban on commercial bumble bee colony introduction into Oregon, for our cage study, we sought colonies by placement of a request in the local newspaper in 2007 [16]. Since then we have received numerous calls from homeowners, and have noted the presence of nests of *B. griseocollis*, *B. melanopygus*, *B. mixtus*, and *B. vosnesenskii* in bird boxes, compost heaps, bags of potting soil, and insulation in sheds, homes, pump houses, and a diversity of other locations.

The current western Oregon landscape can serve as a model for bumble bee conservation as it provides both nesting sites and an abundance of foraging resources, the two critical needs of bumble bees. Based on our experiences, we recommend integration of multiple agroecosystems that bloom in sequence for conservation and build up of bumble bee populations. However, crops raised by farmers are dependent on markets, and hence even in the Willamette Valley, it may not always be possible to provide a sequence in forage resources in agricultural landscapes through cropping systems. In such situations, conservation efforts are essential. Irrespective of the approach adopted globally in agri-environment schemes or other pollinator initiatives, it is critical that attention is directed not just to providing foraging resources but to ensuring that there is a continuum in the presence of the food resources. Hence, in areas where a sequence in bloom in bee-pollinated cropping systems is not an option, rather than just recommending planting of hedgerows or providing lists of bee-friendly plants, researchers should develop and implement plans that include planting of a series of plants that bloom in sequence.

Acknowledgments

The authors thank blueberry and red clover seed producers for permitting them to conduct the studies in their fields. Support for the studies was provided by the Oregon Blueberry Commission, Oregon Clover Commission, and WSARE (Western Regional Agriculture, Research and Education).

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Review Article

Large Carpenter Bees as Agricultural Pollinators

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Received 12 September 2009; Accepted 9 January 2010

Academic Editor: Claus Rasmussen

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Large carpenter bees (genus *Xylocopa*) are wood-nesting generalist pollinators of broad geographical distribution that exhibit varying levels of sociality. Their foraging is characterized by a wide range of food plants, long season of activity, tolerance of high temperatures, and activity under low illumination levels. These traits make them attractive candidates for agricultural pollination in hot climates, particularly in greenhouses, and of night-blooming crops. Carpenter bees have demonstrated efficient pollination service in passionflower, blueberries, greenhouse tomatoes and greenhouse melons. Current challenges to the commercialization of these attempts lie in the difficulties of mass-rearing *Xylocopa*, and in the high levels of nectar robbing exhibited by the bees.

1. The Role of Non-*Apis* Bees in Agricultural Pollination

Insect pollination of agricultural crops is a critical ecosystem service. Fruit, vegetable or seed production from 87 of the 115 leading global food crops depends upon animal pollination [1]. The value of insect pollination for worldwide agricultural production is estimated at €153 billion, which represents 9.5% of the value of the world agricultural production used for human food in 2005 [2]. The area cultivated with pollinator-dependent crops has increased disproportionately over the last decades, suggesting that the need for pollination services will greatly increase in the near future [3]. This contributes to the concern to beekeepers, growers of insect-pollinated crops, and policy-makers over recent widespread declines in honey bee populations (Colony Collapse Disorder) [4–6].

Wild and domesticated non-*Apis* bees effectively complement honey bee pollination in many crops [7, 8]. Examples of management of non-*Apis* species for agricultural pollination include the use of bumble bees, primarily for the pollination of greenhouse tomatoes, the solitary bees *Nomia* and *Osmia* for the pollination of orchard crops, *Megachile* for alfalfa pollination, and social stingless bees to pollinate coffee and other crops [9–12].

This paper focuses on the large cosmopolitan genus *Xylocopa* as an additional provider of agricultural pollination

services. Aspects of these bees' life-history, social organization, and foraging ecology are discussed in the context of their potential role as crop pollination agents.

2. The Biology and Life History of Carpenter Bees

Large carpenter bees belong to the tribe Xylocopini within the subfamily Xylocopinae (Hymenoptera: Apidae). They are currently grouped into a single genus, *Xylocopa* [13]. The genus comprises at least three clades [14] and ca. 470 species [15]. Carpenter bees occur in tropical and subtropical habitats around the world, and occasionally in temperate areas [16]. Biogeographical analyses suggest that the genus probably has an Oriental-Palaearctic origin, and that its present world distribution results mainly from independent dispersal events [14].

As implied by their name, carpenter bees dig their nests in dead or decaying wood, except for the subgenus *Proxylocopa* that nests in the soil [17]. The wood-nesting carpenter bees construct two main types of nests: (i) unbranched (also called linear), with tunnels extending in either one or both directions from the nest entrance. Linear nests are usually constructed in hollow or soft-centered plant material, such as reeds; (ii) branched nests (>2 tunnels), usually constructed in tree trunks or timber [18]. The type of nest constructed usually varies with species, but some species show plasticity

in nest architecture, depending on the nesting substrate available to them [19]. The nesting female lays one or a few eggs along a tunnel during a brood cycle, provisions them, and constructs partitions of masticated wood to separate the offspring from one another. Maternal care in carpenter bees also involves guarding of the immature offspring and feeding of the newly matured ones by trophallaxis [20–22]. In some species, helper females participate in offspring care rather than nesting independently, thus nesting can be social (see below). Some species are univoltine, whereas others produce more than one brood per year [19]. The activity season of carpenter bees spans 8–12 months, depending on species (e.g., [21, 23–25]). Carpenter bees in temperate areas hibernate during the cold season [19, 26], but emerge to forage on warm winter days [21, 23].

The mating behavior of carpenter bees has been described for 38 species belonging to 16 subgenera [27]. Variation in mating strategies among subgenera has been recorded. In some subgenera, males search for females at nesting sites, flowers, or landmarks (non-territoriality). In others, they monopolize resources used by females, such as flowers or nesting sites (resource-based territoriality). Males may also monopolize areas lacking resources for females (non-resource-based territories, or leks) [18, 28]. A phylogenetic analysis suggests that resource defense is the ancestral state, and that this mating system is correlated with low color dimorphism between males and females and a small size of the mesosomal pheromonal gland [27].

Territorial males chase away intruding males [28, 29], which they identify by sight and by the odor emitted from the intruders' mandibular glands [30]. They also use a pheromone secreted from their mandibular gland to mark their territory [30]. When females enter the territories, males follow and try to mount them [28, 31]. Observations of copulations in carpenter bees are extremely rare [28] and were recorded only for a handful of species. In *X. varipuncta*, matings take place in the non-resource territories [32], while in *X. sulcatipes* and *X. flavorufa*, they occur at high elevation during flight [21, 31, 33].

3. Social Organization

Sociality, involving non egg-laying guard bees and a dominant egg-laying forager, has been described for ten species of *Xylocopa*. In nests of the African species *X. combusta*, first eclosing daughters remain in their natal nests and perform guarding duties while their mothers produce a second brood ([34] cf. [22]). Similarly, in nests of *X. pubescens* sociality generally occurs after the emergence of the young, where either the mother is the reproductive and a daughter guards or vice versa [20, 35]. Matrifilial nests of *X. virginica* (comprised of a mother and her daughters) also show reproductive skew, and guarding individuals become reproductive in the following year. In these nests, the mother performs all nest maintenance, foraging, cell preparation and oviposition, whereas the younger inactive females only perform guarding duties [36]. Nests of *X. sulcatipes* can be matrifilial, composed of sisters, or involve the joining

of unrelated females [21, 37]. Some *X. sulcatipes* nests are initially quasisocial (no reproductive division of labor), but after a brief period of reproductive competition involving oophagy, a division of labor is usually established. Eventually most nests contain one reproductive and a guard [38]. The helping role of female offspring has been suggested to promote greater maternal investment in daughters than in sons, leading to the female-biased sex ratio recorded in *X. sulcatipes* [37]. In both *X. pubescens* and *X. sulcatipes*, the reproductive females produce 100% of the offspring while the guards produce none [39].

Nests of *X. sonora* also exhibit high reproductive skew, where the forager (mother) reproduces and feeds nestmates via trophallaxis, and additional females (daughters and/or joiners) share guarding duties [40]. For *X. frontalis*, *X. grisescens*, and *X. suspecta* matrifilial, semisocial, and communal nests have been recorded [41]. Genetic analysis of *X. aeratus* and *X. bombylans*, which form multi-female nests during part of the breeding season, indicated the presence of multiple matrilineal lines in approximately 50% of nests. Socially nesting females were frequently sisters in one of the populations studied, and were often unrelated in a second population. The results also indicated that temporary high reproductive skew occurred in multi-female nests, that is, that different females were reproductive during different parts of the season [22].

Several ecological and life-history variables were suggested to promote social nesting in carpenter bees. Social living was found to correlate with late season [42] and older age [35] in *X. pubescens*, possibly because matrifilial nesting only occurs when mothers produce their second brood. Nest structure was proposed as an additional factor that affects social organization: in some species, females in branched nests build and provision separate tunnels at the same time, which can result in a communal social organization. In other species, females construct one tunnel for the first brood generation and only construct a new tunnel after the first brood has reached maturity. This can then result in eusocial nesting, where the daughters of the first generation assist their mother in building and provisioning subsequent tunnels [19]. Finally, a period of reproductive inactivity of mature offspring was proposed as a transition step toward social living. Such a period occurs in some solitary species (such as *X. frontalis* and *X. grisescens*), where newly emerged adult females remain in their natal nest for 20–30 days. During this time, they are provisioned by their mother or by their oldest sister, if the mother is absent. In some species, this association becomes permanent in a fraction of the nests (e.g., in *X. suspecta* [25]), which then become social.

Improved defense against parasites and predators has been suggested to favor the evolution of social nesting in bees (e.g., [43]). Carpenter bee nests are attacked by several types of natural enemies, including parasitoid wasps and flies, predatory wasps, ants, termites, and insectivorous birds [21, 44]. However, in *X. pubescens*, the frequency of parasitism did not differ between social and solitary nests [45]. Thus the role of guards in reducing nest parasitism is not supported so far.

The most extensive work on the consequences of sociality has been carried out for *X. pubescens*. In this species, the

frequency of social nesting increases as the reproductive season progresses. It has been suggested that this increase has evolutionarily been imposed on females by shortage in nesting sites [20]. Social nesters spend more time foraging outside their nests as compared with solitary individuals, perhaps because the presence of the guard in the nest reduces the risk of prolonged foraging [46]. Social nesters also suffer fewer nest takeovers by intruders than solitary nesters, providing a possible benefit for social nesting when competition for nests is high. The guards, in turn, may benefit from increased indirect fitness (if related to the reproductive), and increase their chances of eventually taking over the nest [46]. Thus, social organization can affect the fitness of *X. pubescens* females. Social and solitary nesters that foraged within a greenhouse differed in their food-plant preferences. Social females directed more of their foraging to a pollen source (*Portulaca oleracea*) than solitary nesters, possibly because of their higher brood production rates [47].

4. Foraging Ecology

4.1. Abiotic Requirements for Foraging. Carpenter bees tolerate high ambient temperatures during foraging, and most species are inactive at low temperatures. For example, the lower activity temperature thresholds are 23°C for *X. capitata* [48], 21°C for *X. sulcatipes*, and 18°C for *X. pubescens* [21]. Flower visit rates in *X. olivieri* are highest at a combination of high (25–35°C) temperatures and low (1–100 Lux) illumination levels [17]. *X. arizonensis* individuals that foraged on *Agave schottii* together with honey bees and bumble bees were active mainly during the late morning hours, while honey bees and bumble bees were more crepuscular. These patterns were suggested to reflect low competitive ability, together with high thermal tolerance, in the carpenter bees [49]. *X. varipuncta* maintains flight activity within an ambient temperature range of 12–40°C [50]. This heat tolerance suggests good heat regulation ability in carpenter bees, possibly controlled by a thermoregulatory center in the prothorax [51].

The activity period of some species, for example, *X. sulcatipes*, *X. cearensis*, and *X. ordinaria*, spans most of the daylight hours [21, 52, 53]. In other species (such as *X. pubescens*, *X. tabaniformis*, and *X. olivieri*), activity is crepuscular [17, 21, 54, 55]. A few species are nocturnal: *X. tenuiscapa* forages on its pollen host on moonless nights [56], and *X. tranquebarica* [57] has been observed foraging on moonlit nights.

4.2. Water Balance. Carpenter bees often ingest excess water during nectar foraging. Analysis of nectar consumed by *X. capitata* showed that it is very concentrated. Nevertheless, their hemolymph is only moderately concentrated, and their urine is very dilute. This suggests that ions, rather than water, may be limiting for carpenter bees [58]. This hypothesis is supported by the observation that bees often excrete water before and during flight, and that they often engage in water evaporation from ingested nectar [59]. A similar excess of water ingestion, which leads to copious excretion

and evaporation of water, was described for *X. pubescens* foraging on the nectar of *Callotropis*. On the other hand, physiological water requirements are finely balanced with the water contents of *Callotropis* nectar in the sympatric species *X. sulcatipes*, possibly due to extended coevolution with this plant [59].

4.3. Nectar Robbing. Nectar-foraging carpenter bees often perforate the corollas of long-tubed flowers, and thereby reach the nectaries without contact with the anthers. Such “illegitimate pollination” or “nectar theft” has been reported for *X. virginica* and *X. micans* foraging on blueberries. Nectar robbing in blueberries may reach 100% of the visits [60] and significantly reduces fruit set and seed number as compared with plants visited by honey bees ([61], but see [62]). Nectar robbing by carpenter bees has also been observed in the wild plants *Petrocoptis grandiflora* [63], *Fouquieria splendens* [64], *Glechoma longituba* [65], and *Duranta erecta* [66]. Corolla tube perforation contributed to the reproductive success of the plants in *P. grandiflora* and *F. splendens*, indicating that the nectar robbers were dusted with pollen during foraging, and functioned as pollinators. In *G. longituba* and *D. erecta*, on the other hand, nectar robbing by carpenter bees reduced seed set, as compared with plants visited by legitimate pollinators [63–66].

4.4. Food Sources. Carpenter bees in natural habitats are generalist nectar and pollen foragers. For example, foraging *X. cearensis* were recorded from 43 plant species in Bahia, Brazil [52], while *X. latipes* and *X. pubescens* foraged on 30 species in India [67]; In Israel, *X. pubescens* and *X. sulcatipes* used 61 species as forage plants [21]; *X. darwini* in the Pacific is known to visit the flowers of 79 plant species [29]; 28 plant species provide nectar and pollen for *X. ordinaria* in Brazil [53].

Carpenter bees can also be trained to collect sucrose solution from feeders in experimental settings. In laboratory experiments, *X. micans* were able to discriminate between sucrose solutions that differed in mean volume (1 versus 3 microliter) and concentration (10% versus 30%). They were indifferent to variability in both nectar volume and nectar sugar concentrations. This risk indifference was recorded if the bees were fed or starved [68].

5. Crop Plants That Are Pollinated by Carpenter Bees

Carpenter bees pollinate passionflower (*Passiflora* spp.) in their native habitats [69] and in commercial agricultural settings [70–73]. They provide better pollination service than honey bees for this crop [71]. *Xylocopa* subgenus *Lestis* has been successfully reared in greenhouses for tomato pollination in Australia. Their foraging activity led to an increase in tomato weight by 10% relative to a combination of wind and insect pollination. The efficiency of carpenter bees in pollinating tomatoes is increased by their ability to buzz the anthers [9]. In a pilot study in Israel, the fruit set of greenhouse-grown honeydew melons was three times

higher when pollinated by *X. pubescens* compared to honey bee pollination [74]. Social and solitary nesters had similar efficiency in pollinating this crop: they did not differ in the daily activity patterns and flower visitation rates. Pollination by both types of nesters led to similar fruit sets, fruit mass, and fruit seed number [47].

Carpenter bees are important pollinators of cotton in Pakistan, India, and Egypt [33]. *X. varipuncta* is compared favorably with honey bees (*Apis mellifera*) as pollinators of male-sterile cotton in field cages in the USA [75]. However, *X. pubescens* in Israel did not provide satisfactory pollination of cotton for hybrid seed production (D. Weil, personal communication). Finally, the night-flowering cactus *Cereus repandus* (syn. *C. peruvianus*) is pollinated by *X. pubescens* in Israel [76].

6. Domestication and Mass Rearing of Carpenter Bees for Agricultural Pollination

A major obstacle to the commercial use of native pollinators in agriculture is the need to mass-rear them, rather than collect them from nature. Devising efficient and cost-effective mass-rearing protocols for *X. pubescens* is a necessary step in this direction. Attempts to mass-rear carpenter bees have focused on the construction of nest boxes that are placed in natural habitats to enhance nesting success. Skaife [77] constructed observation nests of bamboo tubes and transferred hibernating *X. caffra* into them. Most of the females remained in these nests after they exited hibernation. Oliviera and Freitas [78] designed and tested nest boxes for *X. frontalis*, based on the general design of Langstroth honey bee hives. Each of nine wooden frames in these boxes was modified to serve as an independent *Xylocopa* nest. Colonization rates of these boxes ranged from 19% to 52%, and the proportion of males in the emerging brood was 0.38. Efforts to develop protocols for captive mating and rearing of carpenter bees have so far met with limited success (unpublished results). The endocrine and molecular pathways that underlie reproduction in carpenter bees are yet unknown. Elucidation of these pathways will help identify the bottlenecks in the bees' reproduction, which may include overwintering of adults, mating, sperm storage and choice, nest construction and/or brood care. Information on the potential reproductive pitfalls, and their physiological mechanisms, is expected to facilitate the development of effective captive breeding methods for *Xylocopa*.

7. Conclusions and Future Prospects

Carpenter bees possess several advantages as potential crop pollinators compared to other non-*Apis* bees. Many solitary bees have a short activity season and/or are specialist foragers, and therefore do not provide a broad alternative to honey bee pollination. Carpenter bees, on the other hand, have long activity seasons and feed on a wide range of plant species. In addition, they are capable of buzz-pollination. This makes them potentially more versatile as agricultural pollinators. Hibernation occurs in the adult

stage, and females start foraging whenever temperatures reach high enough values. This means that it is relatively easy to manipulate the onset of foraging in greenhouses. Another important advantage is that the genus has a worldwide distribution. This implies that local species of *Xylocopa* can potentially be used over wide areas, reducing the need to import exotic pollinators. The possibility to lure these bees into suitable artificial nesting material allows provisioning of nesting material that can be easily used in agricultural settings and moved to places where pollination services are needed [79].

In spite of higher per-capita pollination efficiency in some crops, carpenter bees are clearly inferior to honey bees in terms of pollinator work force, as they do not form large nests. Therefore they are expected to contribute most to crop pollination when honey bees are ineffective. For example, the high thermoregulatory ability of carpenter bees enables them to forage at higher ambient temperatures than honey bees. This makes them attractive candidates as pollinators in hot areas and in hot microclimates, such as in glass houses. The crepuscular and nocturnal activity of some species may also allow them to pollinate night-flowering crops, which are not visited by honey bees.

Several problems remain in the management of carpenter bees for crop pollination, which call for further research. Most important is the need to develop an efficient captive breeding program for carpenter bees, which would include controlled selection of genotypes, mating, and nest founding. Such protocols have already been developed for other non-*Apis* pollinators, such as *Osmia lignaria* [80] and *Osmia cornuta* [81]. They include guidelines for nest construction and placement, overwintering and transportation of the bees. A complementary challenge is to enhance reproduction of wild *Xylocopa* populations, through provisioning of nesting material to their natural habitat. The availability of nesting resources was shown to correlate with the community structure of wild bees [82]. Moreover, experimental enhancement of nest site availability has led to dramatic increases in wild populations of *Osmia rufa* [83]. These findings suggest that *Xylocopa* populations, and the pollination services they provide, may also benefit from nest site enhancement in agroecosystems. Additional information about the pathogens and parasites of the genus is needed as well [84]. A combination of ecological, physiological, and molecular genetic studies is likely to provide these essential data.

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Research Article

Flower Constancy in the Generalist Pollinator *Ceratina flavipes* (Hymenoptera: Apidae): An Evaluation by Pollen Analysis

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Received 25 July 2009; Accepted 11 December 2009

Academic Editor: Claus Rasmussen

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The food habits of the solitary bee *Ceratina flavipes* were studied by observation on foraging behavior and identifying the pollen grains that they collected. It appeared that *C. flavipes* tend to collect pollen from particular species; however, they visit multiple flowering species. We analyzed pollen sources from pollen loads of dried specimens from single foraging trips (SFT) and in pollen balls created from a single foraging day (SD). The pollen from all pollen balls in a nest represented the harvest from an entire breeding season (BP). This analysis showed that each bee on average collected pollen from 3.24 (SFTs), 2.02 (SD), and 3.12 (BP) flowering species. Bees collected pollen from a total of 14 flowering plant species. Furthermore, we calculated when pollen balls were created and found no significant interaction between seasonal pollen availability and bee preferences. Moreover, bees had consistent flower preferences, even if the preferred flower was not dominant at all times. These results indicate that *C. flavipes* exhibits flower constancy, and therefore, the generalist pollinator *C. flavipes* could function like a specialist pollinator.

1. Introduction

Flower constancy means that a bee restricts its foraging activity to one or a few flowering species, even when many other flowers are available. Since the last century, flower constancy has been studied in honey bees [1–6], bumble bees [7–10], and a few other bee species [11–13]. Flower constancy is an important behavior because it can enhance pollination efficiency for the plant and foraging efficiency for the pollinator. In eusocial bees, enhanced foraging efficiency by individual workers improves the colony survival rate. Thus, flower constancy has been studied extensively in eusocial bees [2, 10, 11].

The mechanisms of flower constancy in bees have been studied empirically [14, 15] and theoretically [16, 17], but are still unknown. Cognitive ability, vision [10], olfaction [6, 8], and memory [3, 5, 18] are thought to influence flower constancy. In solitary bees, foraging efficiency is also likely important; their olfactory sense is highly developed. For example, the solitary bee *Lasioglossum figueresi* uses odor

to recognize the nest [19]. Thus, solitary bees may also have flower constancy. Pollen balls provided for offspring by solitary bees have been examined in *Lasioglossum* [19], *Megachile* [12], *Heriades* [12], and *Osmia* [13]; most pollen balls of these species contain pollen from only two to three plant species, suggesting flower constancy in the preparation of the pollen ball. In these solitary bee species only the plant species used for pollen balls can be noted because no data on the available flowers were provided.

Previous studies of flower constancy in eusocial bee species did not examine temporal variation in flower constancy throughout the breeding period because many were laboratory-based studies. It is difficult to follow bees individually or to identify offspring age in the field, making laboratory studies advantageous. However, flower resources in the field might influence foraging behavior.

Therefore, we explored the relationship between the availability of flower resources and flower constancy in the solitary, generalist pollinator bee *Ceratina flavipes*. The life history of *C. flavipes* has been well studied in Japan [20–22].

We analyzed flower constancy based on pollen samples at three levels: a single foraging trip, a single day of foraging, and the entire breeding period. We defined flower constancy as when individual *C. flavipes* forages on fewer flowering species than the total number of plants used by all examined individuals of *C. flavipes* during the study.

2. Methods

2.1. Species and Study Site. *Ceratina flavipes* is the dominant species at the study site on the Ishikari Coast, Japan [23]. On the Ishikari Coast, a windbreak chaparral runs parallel to the shoreline, which is covered by a 200–300 m wide grassland vegetated by various wild flowering plants. Each female of *C. flavipes* digs a nest burrow in the stem of a dead grass shoot and oviposits several eggs during the breeding season. During the breeding season, females forage for pollen and nectar several times each day when weather conditions are suitable. Females make a pollen ball and lay an egg on it; the larva eats the pollen ball and grows within the cell. Generally, the female stores a pollen ball and an egg at each cell. The pollen balls and eggs are placed individually and in temporal order along the nest burrow. This behavior is advantageous because we can determine the order in which the eggs were laid. The breeding season of *C. flavipes* is from early June to late July in this study site.

The study site had about 22 flowering species, eight of which were observed in this study: *Calystegia soldanella* (Convolvulaceae), *Lathyrus japonicus* (Leguminosae), *Melilotus suaveolens* (Leguminosae), *Oenothera biennis* (Onagraceae), *Picris hieracioides v. glabrescens* (Compositae), *Rosa parvifolius* (Rosaceae), *Rosa rugosa* (Rosaceae), and *Vicia cracca* (Leguminosae). To study pollen resources used by *C. flavipes*, we placed 69 bee nests in the middle of a quadrat in the end of May 2000, before the bees started to oviposit. Set nests were collected from an area surrounding the study site.

2.2. Observation of Flower Visitation. A total of 13 nest-building female *C. flavipes* were followed and their foraging behavior observed from 8:00 to 14:00 on 18 and 20 June and 6, 7, and 11 July 2000. We observed marked bees as long as we could track them by eyes during observation periods (8:00–14:00). We then recorded (1) the flower species visited, (2) whether the individual moved between flowers within a plant, and (3) the behavior on the flower, which was classified into landing on the flower petals, staying on the central of flower without foraging pollen, and pollen foraging. For tracking observation, bees were caught and marked with paint marker at their abdomen. Each bee was marked with small dots of two colors and was identified by color combinations.

2.3. Pollen Analysis. We regarded the pollen load at the scopae of hind legs as the mean amount of pollen collected in a single trip. We regarded one pollen ball and all pollen balls in a nest as the mean amount of pollen collected in a single day and throughout a breeding season, respectively.

For the analysis of pollen collected in a single foraging trip, we used pollen attached to the scopae of 84 mounted specimens of bees sampled from several sites near the Ishikari Coast site in the past 10 years. These mounted specimens were caught at sites with more than two flowering plant species. Thus, we assumed that they had the opportunity to visit multiple plant species. To determine flower constancy within a single foraging trip, we used pollen loads from the pollen baskets of bee specimens that had been sampled at and near the study site within the last 10 years. We used dead specimens because collecting pollen loads from bees on each foraging trip would cause too much disturbance of the bee behavior.

For pollen collected in a single day or throughout the breeding period, we sampled 69 nests at the study site on 1 July 2000. We analyzed 253 pollen balls from these nests. When a pollen ball was already consumed by a larva, we collected the pollen ball particles and larval or pupal waste remaining in the cell.

We processed the pollen using the standard acetolysis method [24]. Pollen grains ($n = 200$) were randomly chosen from each sample and identified to species under a microscope, referring to technical pollen books [25, 26] and pollinic preparations. The pollinic preparations were samples of untreated pollen collected from flowers at the study site.

2.4. Estimation of Oviposition Date. *Ceratina flavipes* sequentially oviposits from the bottom upward in the nest. This behavior was used to estimate oviposition dates and the dates on which pollen balls were made. We divided the immature individuals into stages, and the developmental periods were allocated among the stages. Three larval stages were defined: “small larvae” whose legs were hard to identify (4 or 5 days after oviposition), “medium larvae” whose legs were easy to identify (11 to 13 days), and “large larvae” without a pollen ball (18 or 19 days). Eggs hatched within 1 or 2 days. Small larvae became medium larvae after 2 or 3 days. We used these developmental stages to back-calculate the oviposition dates of immature bees sampled from the set nests. We confirmed that the order of immatures in the nest and the pollen with each immature did not conflict with the phenological calendar. We recorded offspring stage (e.g., adult, pupa, larva, or egg) in order from the bottom of each nest. To determine the developmental period of each stage, we sampled 10 wild nests with immatures at the study site. After dissecting the 10 nests, each individual was placed in a vial with a pollen ball, kept at room temperature without air conditioning, and reared in the laboratory. For the hatching period, we selected the oldest and youngest eggs in the nest because we did not know when the eggs had been oviposited.

2.5. Available Pollen Resources and Flower Constancy. The availability of pollen resources in the field was compared with the pollen in the nests of individual bees from the pollen analysis. The availability was estimated by regularly counting the number of flowers and determining the average dry weight of pollen per flower in each focal species. We counted

Individual code	
1	- <u>B</u> - <u>A</u> = <u>A</u> -
2	- <u>A</u> = <u>A</u> - <u>A</u> = <u>A</u> = <u>A</u> - <u>A</u> -
3	- <u>B</u> - <u>B</u> = <u>B</u> = <u>B</u> = <u>B</u> = <u>B</u> -
4	- <u>B</u> - <u>B</u> - <u>A</u> - <u>B</u> = <u>B</u> - <u>B</u> -
5	- <u>B</u> - <u>A</u> - <u>B</u> - <u>A</u> - <u>B</u> -
6	- <u>A</u> - <u>D</u> = <u>D</u> - <u>A</u> -
7	- <u>A</u> - <u>D</u> = <u>D</u> - <u>A</u> -
8	- <u>A</u> - <u>A</u> - <u>A</u> - <u>A</u> -
9	- <u>C</u> - <u>C</u> = <u>C</u> -
10	- <u>C</u> - <u>E</u> - <u>C</u> -
11	- <u>B</u> - <u>B</u> - <u>B</u> -
12	- <u>A</u> - <u>A</u> - <u>A</u> -
13	- <u>A</u> - <u>A</u> - <u>A</u> -

FIGURE 1: Consecutive visits to flowers by marked *Ceratina flavipes* in late June 2000 at the study quadrat. Ishikari study site. Each alphabet indicate flowering species, A: *Rosa rugosa*; B: *Rosa parvifolius*; C: *Picris hieracioides v. glabrescens*; D: *Lathyrus japonicus*; E: *Calystegia soldanella*. And each marks indicated the behaviors, =: Moving within same stem; -: Moving to another stem. Bold face letter indicates collection of pollen, underline indicates staying at central of flower without collecting pollen, and standard face letter indicates landing on the flower petals.

the number of open flowers of the eight focal species, treating the spicate of *C. soldanella* as one flower, within a 50 × 100 m quadrat once per week during the observation period (12 June to 1 July 2000). We also recorded the date of first flowering in each species. To calculate the average amount of pollen provided by a single flower per day, we selected several intact flower buds from each focal species at the edge of the study site (10 to 35 buds per species) and covered each bud with a small bag (3 × 4 cm) of fine mesh cloth. We collected five covered flowers every day from the start of flowering until petals dropped. Sampled flower heads were dried at room temperature, and the pollen was separated from other parts (i.e., anthers and petals) using a 1 mm wire mesh filter. The pollen was then completely dried in an incubator at 40°C for more than 1 week and weighed on an electronic balance.

3. Results

3.1. Flower Visitation. We successfully followed 13 marked bees and observed their flower visits (Figure 1). Although six bees visited two species, all *C. flavipes* individuals foraged exclusively for pollen on a particular species, except bee number 5 that collected pollen from two plant species.

3.2. Pollen Analysis. The 14 plant species found in the pollen analysis included the eight focal species. The mean (maximum in parenthesis) number of plant species was 3.24 (7), 2.02 (5), and 3.12 (6) for pollen collected in a single foraging trip (SFT), a single day (SD), and the breeding period (BP), respectively (Table 1). We found that the mean number of plant species visited was relatively low, with 55 (SFT), 227 (SD), and 50 (BP) of pollen load composed by more than 80% of same species, furthermore, some of

them, 6 (SFT), 94 (SD), and 9 (BP), composed by 100% of same species within analyzed 200 pollen grants (Table 1). These results indicate that *C. flavipes* shows flower constancy, although it is a generalist pollinator. Flower constancy means that an individual visits some flowering species regularly, although, overall, different individuals of *C. flavipes* visit various flowering species to obtain resources.

3.3. Oviposition Date. Of the immature bees that we reared from 10 nests sampled in the field, 39 were female and 31 were male. Eggs and small, medium, and large larvae were oviposited on 30 June or 1 July; 26 or 27 June; 18, 19, or 20 June; and 12 or 13 June, respectively (Figure 2(b)). These results coincide with the pollen analysis and the phenology of the eightfocal plant species at the study site (Table 2, Figure 2(a)).

3.4. Available Pollen Resources and Flower Constancy. The pollen availability of each species was estimated as the product of the dry weight of pollen per flower head and the number of flowers (Table 3). The mean dry weight of pollen per flower decreased in the following order: *V. cracca*, *R. rugosa*, *R. parvifolius*, *M. suaveolens*, *P. hieracioides v. glabrescens*, *L. japonicus*, *O. biennis*, and *C. soldanella*. There was interspecific variation in the flowering period; the longest was that of *M. suaveolens* and the shortest was that of *C. soldanella* (Table 3). Pollen availability was not significantly related to bee flower preference at any developmental stage (Table 4; *G*-tests, egg: $X^2 = 9979.381$, $P < .01$; small larvae: $X^2 = 11782.85$, $P < .01$; medium larvae: $X^2 = 22632.59$, $P < .01$; large larvae: $X^2 = 24017.79$, $P < .01$).

In nine nests, all pollen balls in the nest were composed of a single plant species, that is, *R. rugosa* or *R. parvifolius*. Although *R. parvifolius* was not a dominant species at the beginning of the breeding season, three female bees constantly foraged on *R. parvifolius*.

4. Discussion

Flower gardens in temperate areas can be beautiful, because various species flower in a short period of time. In this study site, which was located in a cool-temperate area, 22 plant species flowered concurrently. There was interspecific variation in flower density with *R. parvifolius* being one of the rarest. Although a rare species might require a specialized pollinator, we did not observe specialist pollinators on 1 July. However, generalist pollinators can also function as specialized pollinators if they exhibit flower constancy. *C. flavipes* showed flower constancy in its pollen foraging (Table 1), and the intensity of its flower constancy seemed to vary intraspecifically.

We studied flower constancy of polylectic solitary bee, *C. flavipes* with observation of foraging behavior for SFT, pollen analysis from pollen attached specimens for SD, and that from pollen ball in the nest for BP. It is difficult to conclude with each result from SFT, SD, and BP, because there are some limitations due to the small number of foraging observations (SFT), uncertainty of foraging information of

TABLE 1: Composition of pollen grains ($n = 200$) randomly chosen from each of 84 pollen loads and 253 pollen balls.

Level of pollen load	N	The number of flowering species/pollen load		Percentage of pollen grains of the most dominated plant species within random 200 pollen grains			
		Range	Mean \pm S.D.	100%	100 $>\sim\geq$ 80%	80 $>\sim\geq$ 50%	50 $>\sim>$ 0%
Pollen loaf	84	1–7	3.24 \pm 1.27	6	49	26	3
Pollen ball	253	1–5	2.02 \pm 0.91	94	133	25	1
Nest	69	1–6	3.12 \pm 1.47	9	50	9	1

TABLE 2: Species of pollen grains contained in cells for each developmental stage collected on 1 July. +: present; -: absent; LL: large larva; ML: medium larva; SL: small larva; E: egg. Sum of set 69 nests and sampled 10 nests for determination of developmental period, was shown in this table.

Stage (no. of pollen ball)	Total no. of flowering sp.	Flowering sp.													
		Rr	Lj	Hr	Lm	Rp	Vc	Ob	Ph	Tp	Tr	Cs	Ms	Ea	Sa
E (17)	10	+	+	+	+	+	+	+	+	-	-	-	-	+	+
SL (12)	8	+	+	+	+	+	-	-	-	-	+	+	+	-	-
ML (52)	8	+	+	+	+	+	+	-	+	+	-	-	-	-	-
LL (93)	6	+	+	+	+	+	+	-	-	-	-	-	-	-	-

Rr: *Rosa rugosa*; Lj: *Lathyrus japonicas*; Hr: *Hypochoeris radicata*; Lm: *Lonicera morrowii*; Rp: *Rosa parvifolius*; Vc: *Vicia cracca*; Ob: *Oenothera biennis*; Ph: *Picris hieracioides*; Tp: *Trifolium pratense*; Tr: *Trifolium repens*; Cs: *Calystegia soldanella*; Ms: *Melilotus suaveolens*; Sa: *Silene armeria*; Ea: *Erigeron annuus*.

specimens (SD), and lack of uniformity in estimation of flower availability (BP). However, these limitation needs to be dealt with in a separate studies, considering all the results together in this study, it is possible to regard *C. flavipes* to have flower constancy.

Ceratina flavipes tended to prefer certain plant species (Figure 1), these data are insufficient because observations were made were not tested experimentally. Our results, however, indicate a preference of *C. flavipes* for *R. rugosa* and *R. parvifolius* pollen at this study site (Table 4). Other flowering species were uncommon in pollen balls, although the availability of some species was high (Table 4). The uncommon species in pollen balls may result from bee behavior, such as casual landing or nectar feeding. Although the individual bees exhibited flower constancy, many flowering species were used (Figure 1). Thus, the percentage of pollen grains represented by the most dominant plant species was low (Table 1), indicating that bees may choose to collect pollen from a particular flowering species.

The mechanisms and causes of flower constancy in pollinators still remain elusive. Many conceptual and empirical studies suggest that the cognitive and memorization abilities of pollinators are important determinants of flower constancy. In theoretical studies, based on a classical patch model [27], optimal strategies with an important parameter, that is, individual memory, have been constructed [16, 17]. Bees have the cognitive ability to recognize floral colors [10, 28]; furthermore, the cognitive ability to recognize odors has been explored, especially in bumble bees [7, 8] and honeybees [3–6]. The memory of an individual forager is the primary contributor to flower constancy [18]. Previous studies have suggested that generalist pollinators are effective pollinators for angiosperms [29–31]. Flower constancy increases the effectiveness of pollination by gen-

eralist pollinators [32]. The various determinants of flower constancy are connected via neural substrates [33]. These factors are regulated by the highly developed sensory systems in the bees [4, 34, 35].

Flower traits (i.e., odor, color, and shape) might motivate bees to select certain flowers when foraging. In particular, olfactory sensations might be important, particularly for bees, because olfaction is used to find particular plant species [5, 6, 8] and to recognize the nest [19]. However, bees' ability to remember flower traits is limited; it is unclear how many flower traits bees can memorize and/or discriminate among when foraging. To determine the mechanisms of flower constancy in bees, the relationship between memorization and learning of particular plant species and the foraging behavior of the bees must be determined.

Although the lifecycles of some bee species are known, the timing of memorization and learning remain unclear. In *C. flavipes*, prior studies describing the life cycle indicate that individuals have opportunities to memorize pollen species at different developmental stages: when growing on a pollen ball provided by the mother, when they eclose with frass in the cell, when they are provided with nectar and pollen by their mother or elder sisters after the breeding season, when they first forage by themselves during dispersal in the prehibernation season, or when they start foraging by themselves at the beginning of the nesting and/or breeding season after hibernation [23, 36–39]. Holometabolous insects have different nervous systems as adults than they do as juveniles [40]; thus, memories acquired as a juvenile may be lost during metamorphosis. Combined with the results of prior studies, our results suggest that the memorization required for flower constancy is more likely to occur in the prehibernation season, that is, the period from emergence to hibernation, than in other stages.

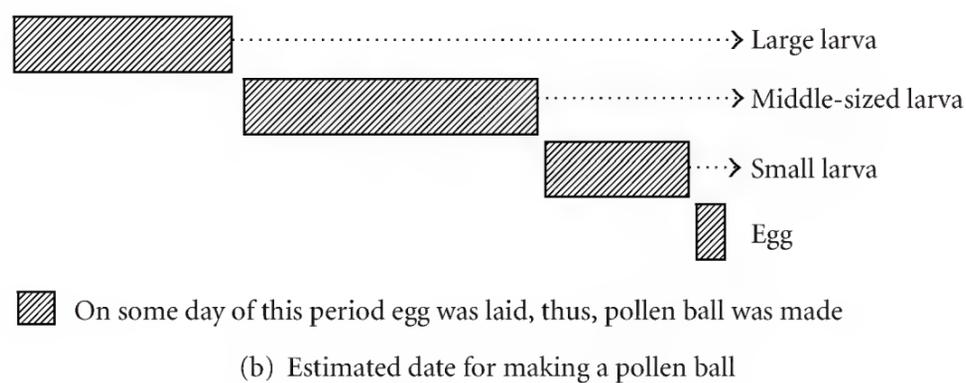
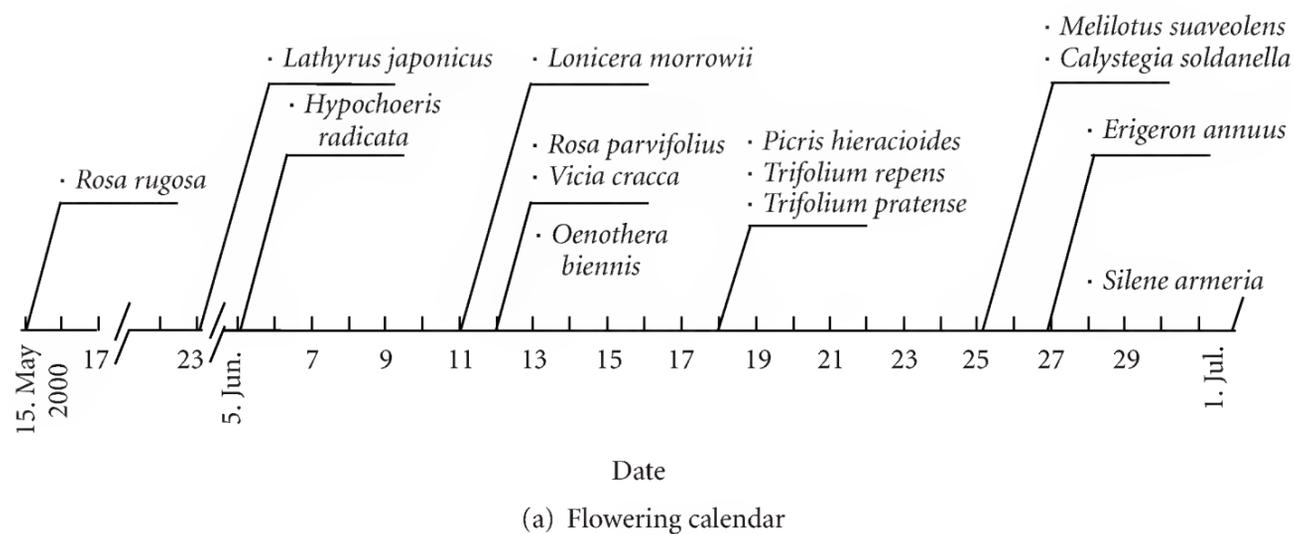


FIGURE 2: (a) Flowering phenology on the Isikakri Coast and (b) oviposition dates (i.e., dates when pollen balls were made) inferred from the rearing of immature individuals in the laboratory. Nests with pollen balls, eggs, larvae, pupae, and adults were sampled on 1 July. The thick line in (a) represents the starting dates of the flowering period of each flowering species at the field. The date axis in (a) is common with in (b).

TABLE 3: Weight (mg) of desiccated pollen per flower head on each day after the initiation of flowering (see Figure 2(a) for each species). Mean \pm standard deviation of five flower heads. Average pollen production (P) was used to calculate pollen availability within a 50×100 m quadrat (cf. Table 4). Names of flowering species are arranged in descending order average pollen production.

Flowering sp.	Days from the initiation of flowering							Average pollen productin (P)
	1	2	3	4	5	6	7	
<i>Vicia cracca</i>	33.26 \pm 41.9	34.30 \pm 18.61	22.83 \pm 9.11	33.50 \pm 47.46	19.34 \pm 30.77	13.55 \pm 3.64	—	26.13 \pm 8.80
<i>Rosa rugosa</i>	26.96 \pm 22.14	13.48 \pm 14.98	9.38 \pm 4.03	4.22 \pm 3.54	2.53 \pm 0.92	—	—	11.31 \pm 9.76
<i>Rosa parvifolius</i>	12.60 \pm 8.59	15.48 \pm 13.56	9.25 \pm 32.51	7.72 \pm 3.29	—	—	—	11.26 \pm 3.47
<i>Melilotus suaveolens</i>	1.99 \pm 1.42	3.05 \pm 1.24	10.00 \pm 1.65	13.04 \pm 4.13	18.22 \pm 8.33	9.05 \pm 1.92	3.42 \pm 16.58	6.76 \pm 4.54
<i>Picris hieracioides</i>	8.15 \pm 1.87	4.58 \pm 2.13	2.95 \pm 1.49	2.64 \pm 1.81	—	—	—	4.44 \pm 2.20
<i>Lathyrus japonicus</i>	0	11.84 \pm 30.67	0	0	—	—	—	2.96 \pm 0.00
<i>Oenothera biennis</i>	0.58 \pm 0.24	1.97 \pm 1.68	0.93 \pm 0.54	—	—	—	—	1.15 \pm 1.16
<i>Calystegia soldanella</i>	4.41 \pm 0.95	0.45 \pm 0.30	—	—	—	—	—	0.93 \pm 0.84

Our quadrat was near the maximum size in this study area, but there are some small vegetation patches around the study area, such as parking areas. The *V. cracca*, *R. parvifolius*, and *P. hieracioides* pollen were found from pollen balls; however, we did not observe these plant species at the study area during the putative period (6/12-13) (Table 4).

The results suggested that the bee might forage beyond our study quadrat to seek for the particular flowering species. Furthermore, a species might be memorized before hibernation, the first foraging period of *C. flavipes*, as the olfactory information acquired in the early days after emergence modifies bees' later behavior in honeybee [18].

TABLE 4: Comparison of pollen availability and pollen usage by *Ceratina flavipes* and Phenology of the total dry weight of pollen for each flowering species during the breeding season of *C. flavipes*. The number of flowers is shown in parentheses. Total pollen mass was calculated as $m \times n$, where m is the average dry pollen weight per flower (from Table 3) and n is the number of flowers. Availability and usage differed significantly among species.

Flowering sp.	Number of pollen grants (Number of flower head)							
	Availability 6/12-13	Using [83 balls] LL	Availability 6/18-20	Using [47 balls] ML	Availability 6/26-27	Using [8 balls] SL	Availability 6/30-7/1	Using [10 balls] E
<i>Vicia cracca</i>	0.00	106	2351.70 (90)	5	9145.50 (350)	0	12542.40 (480)	1
<i>Rosa rugosa</i>	7227.09 (639)	9372	51256.92 (4532)	5602	44991.18 (3978)	981	6853.86 (606)	979
<i>Rosa parvifolius</i>	0.00	4666	371.58 (33)	2546	675.60 (60)	518	35941.92 (3192)	794
<i>Melilotus suaveolens</i>	0.00	0	0.00	0	1453.40 (215)	0	17778.80 (2630)	0
<i>Picris hieracioides</i>	0.00	73	972.36 (219)	55	1602.84 (361)	0	5772.00 (1300)	6
<i>Lathyrus japonicus</i>	4091.49 (1521)	90	13619.47 (5063)	41	4040.40 (1365)	0	515.04 (60)	0
<i>Oenothera biennis</i>	0.00	0	0.00	0	1.15 (1)	0	623.3 (542)	0
<i>Calystegia soldanella</i>	0.00	0	16.86 (2)	0	0.00	0	1.41 (8)	0
others	0.00	2293	2205.64 (759)	1151	0.00	101	111.18 (631)	220
G-test	$X^2 = 24017.79$ $P < .01$		$X^2 = 22632.59$ $P < .01$		$X^2 = 11782.58$ $P < .01$		$X^2 = 9979.381$ $P < .01$	

These facts together suggest that the foraging behavior of adults is determined by adult experiences in the pre-hibernation season. However, this may not always be the case. *C. flavipes* is also found in temperate areas, where it is unlikely that bees use information memorized before hibernation because the flowering species are completely different at the beginning and ending of the breeding period. In addition, many solitary generalist bees eclose only after hibernation [12, 19, 41–43]. To determine the mechanisms of flower constancy in solitary, social, and generalist bees, the relationships between learning, memorization, and forging behavior should be examined using behavioral observations and neurobiological methods.

Acknowledgments

The authors thank M. Fukuda (Hokkaido University) for help with the study of *C. flavipes*, N. Hagihara for technical support with the pollen analysis, S. Sakai and Y. Kobayashi (CER Kyoto University) for helpful advice on the manuscript, and colleagues in the Higashi laboratory at the Hokkaido University, for useful discussion.

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Research Article

Male Remating Success and the Frequency of Copulatory Plugs in the Green Lynx Spider *Peucetia viridans* (Araneae, Oxyopidae)

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Received 15 August 2009; Accepted 4 January 2010

Academic Editor: Bethia King

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Peucetia viridans males were allowed to mate with three virgin females and most matings resulted in live spiderlings, even when males lacked palpal paracymbial processes. Among females, the presence of copulatory plugs was inconsistent, and when present, their condition was not uniform; broken-off male paracymbia were often found in epigynal orifices. There was no size effect in a male's ability to completely plug a female's genital orifices, as well as no significant change in the pattern of plug production over consecutive mating trials. Among mated, field-collected females, the presence of plugs and paracymbia was variable, with females from some sites possessing neither structure. Field-collected females with no plugs were in significantly better condition than those with two plugs and in nearly significantly better condition than those with two paracymbia. Females in the best condition may excel at resisting the emplacement of genital obstructions and/or voiding such structures, potentially enabling them to mate with multiple males. Enhanced prey access mediated by increased water availability may be why females at two sites were in relatively better condition. If plugs help prevent sperm desiccation in inseminated females, this may have contributed to the absence of plugs from females at these two moister sites.

1. Introduction

The green lynx spider *Peucetia viridans* (Hentz) (Araneae: Oxyopidae) is the largest member of its family, with a distribution throughout the southern United States, Mexico and Central America [1]. It is a diurnal, visual hunter that forages on plants, especially on flowers, where it lies in ambush for potential prey (pers. obs., [2]). Although little studied up to 1960, *P. viridans* has been the sole or partial focus of at least 25 papers since then [3], making it one of the best characterized hunting spiders in North America.

While much is now known about the reproductive biology of *P. viridans*, two details of its mating behavior are still unresolved, specifically the success of copulations with multiple females by individual males and the frequency of

copulatory plugs. *P. viridans* is one of only two oxyopids (the other is *P. longipalpis* F. O. P.-Cambridge) which are known to produce copulatory plugs [4, 5], structures which are commonly thought to delay and/or reduce the probability of female remating [6]. In addition, a portion of the male palp often breaks off within the female during mating in *P. viridans*, a mechanism which has been found to impede sperm transfer by subsequent males in other spiders [7–9].

Brady [1] was the first to note the presence of copulatory plugs in *P. viridans*, as he found that the two openings of a female's epigynum were usually plugged in preserved specimens with a hard, black material; often the distal portion of the paracymbium of a male palpus was also embedded in this material (see [10] for detailed illustrations). Brady stated that the black material must be deposited during

or immediately after insemination, a suggestion perhaps corroborated by Whitcomb and Eason's [11] observation of a large drop of shiny liquid on the epigynum of an individual female immediately following copulation, one which later disappeared. He reasoned that the plugging of the female epigynum and the loss of the male paracymbial process should prevent further mating by both female and male (although since males possess two palpi, an individual male could potentially mate twice). However, in a laboratory study of *P. viridans* mating behavior, Whitcomb and Eason [11] showed that each mating episode involves numerous copulations between female and male with both palps being inserted alternately into the epigynal openings. Males mated freely on successive days and one male mated with three different females over three days. In contrast, each female mated with only one male and actively rejected subsequent male suitors.

Since the paracymbial process acts as an orientation device to guide the embolus into an epigynal opening [10], its loss from both palpi would be expected to prevent repeated copulations by males. Hence, the results of Whitcomb and Eason [11] suggest that the presence of the paracymbial process may not be absolutely necessary for copulation. However, they did not report on the production of egg sacs and live young by their mated females; so it is not known whether the copulations they observed actually resulted in successful fertilization.

Whitcomb and Eason [11] also reported on the frequency of copulatory plugs, as did Exline and Whitcomb [10], who also provided data on the frequency of inserted paracymbia. Whitcomb and Eason [11] found copulatory plugs in all the mated females they examined, but not in any virgin females. In contrast, Exline and Whitcomb [10] found that not all mated females had plugs and noted that plugs can be easily removed and may sometimes be lost during egg-laying. They found that in approximately 20 mated females, 10 had at least one male paracymbium embedded in the plug. Among the remaining 10, the plug was either missing altogether or contained neither paracymbium. While decades old, these uncertainties concerning the success of copulations with multiple females by individual males and the frequency of copulatory plugs have remained unresolved.

Recently, Ramirez et al. [3] genetically documented cases of multiple paternity in field-collected *P. viridans* broods, indicating that females sometimes remate in the wild. There are a number of reasons why a species which is known to produce copulatory plugs may nonetheless exhibit multiple mating; these are often connected with a lack of uniformity in plug efficacy [12]. For example, with the funnel web spider *Agelena limbata* Thorell (Araneae: Agelenidae), the epigynum has a cavity (atrium) where both insemination ducts open [13]. In a laboratory study, Masumoto [14] found that the copulatory plugs of some males filled the atrium completely (complete plugs), while those produced by others (especially smaller males) did not (incomplete plugs). With incomplete plugs, second males were often able to pry off the plug and inseminate the female, resulting in both first and second males siring offspring, underscoring the potential significance of the size contrast between sexual

partners for plug presence. In contrast, with the orb weaver *Leucauge mariana* (Taczanowski) (Araneae: Tetragnathidae), the male's palp deposits small blobs of white paste on the female's epigynum near the openings of her insemination ducts; only if the female contributes, a clear liquid to these blobs will be retained as functional copulatory plugs [15]. Moreover, specific features of male copulatory courtship increase the likelihood that the female will contribute to the formation of a functional plug [16], highlighting the potential for female participation in determining the fate of copulatory plugs.

To improve our understanding of the success of copulations with multiple females by individual males and the frequency of copulatory plugs in *P. viridans*, this study reports on the outcome of a series of matings where individual *P. viridans* males were given the opportunity to copulate with three virgin females. Following each mating, the presence or absence of a copulatory plug (and its condition if present) and the subsequent production of fertile or infertile eggs was noted for each female, as was the presence or absence of the paracymbial process of each palp for males. The epigynal condition was also surveyed for females collected from various sites in southern California and the influence of female body condition on epigynal condition was assessed. Females across a range of taxa often attempt to impede or remove obstructions to their genital tract (e.g., [17–20]) and females in better condition may be more successful at doing so, hence our analysis of body and epigynal condition in *P. viridans*.

2. Materials and Methods

2.1. Mating Trials. We collected subadult *P. viridans* ($n = 21$ females, 7 males) from six sites in Los Angeles, Riverside, and San Diego Counties, California, in July–August 2004 and reared them to adulthood individually to ensure virginity. Collection sites and identification numbers of all participants are as follows: *Los Angeles Co.*—Ernest Debs Regional Park (σ^1); Loyola Marymount University (♀ 1, 3, 4, 7, 8, 9, 10, 11, 12, 14, 19, 20); *Riverside Co.*—Along Cajalco Rd., east of Eagle Canyon Rd. intersection (♀ 21; σ^7); Davis Rd., east edge of Lake Perris State Recreation Area (♀ 13, 17; $\sigma^3, 4, 5$); *San Diego Co.*—Crest Canyon Preserve, Del Mar (♀ 15); Carmel Valley Road, Del Mar (♀ 2, 5, 6, 18; $\sigma^2, 6$). The collection site for ♀ 16 is uncertain due to the accidental loss of locality data from its rearing jar, although it was derived from one of these six populations. Spiders were maintained in glass apothecary jars (8.5 cm diam \times 21.5 cm tall) and were fed bees and other flying insects every few days. Water was added periodically with an eye dropper.

Once the spiders reached adulthood as indicated by fully developed female epigyna and male palps, each male was allowed to mate with three randomly chosen females. For each mating trial, a virgin female was removed from her rearing jar and was placed on the flower head of a potted buckwheat (*Eriogonum* Michaux spp.) branch sitting in the center of a large bell jar (22 cm diam \times 30 cm tall). After she had settled quietly on or under the flower head, the male was

placed in the bell jar near the base of the buckwheat branch. A lid was then placed on the bell jar and the spiders were left together until the next morning, at which time they were separated and their genitalia were examined microscopically. Specifically, the female epigynum was inspected for copulatory plug material and male paracymbial processes, while the presence or absence of the paracymbial process was noted for each male palp. Plug condition was scored as “complete” if it completely filled the funnel-shaped epigynal orifice, or “partial” if it was not large enough to occlude the opening at the base of the “funnel” or was positioned in such a way that it did not physically block this orifice. Thereafter, the male and female were returned to their rearing chambers. The female was fed on subsequent days in anticipation of egg sac production. To ensure the absence of potential chemical residues, the bell jar was washed out between mating trials and fresh buckwheat branches were used for each trial.

Since *P. viridans* males require 12–16 hours to recharge their palpi [11], males in our study were offered females every other day until they had been given access to three females. Insemination of a female was considered successful only if living, active spiderlings emerged from her egg sac(s) (e.g., [21]). Following the conclusion of the study, the adult males and females were preserved in 80% ethanol and their sizes (carapace width (mm)) were determined using a dissecting microscope with a calibrated ocular micrometer. We were unable to obtain the weights of these specimens due to unexpected abdominal deterioration following their preservation.

2.2. Field-Collected Females. We collected adult female *P. viridans* ($n = 54$) found guarding egg sacs from five sites in Los Angeles and San Diego Counties, California, in October–November 2004 (population abbreviation and sample size are indicated in parenthesis): *Los Angeles Co.*—Kenneth Hahn State Recreation Area (HSR, 34); Ernest Debs Regional Park (DEB, 7); Loyola Marymount University (LMU, 9); *San Diego Co.*—Crest Canyon Preserve, Del Mar (CCN, 2); Carmel Valley Road, Del Mar (CVR, 2). Each female was microscopically examined and the presence and condition of copulatory plugs and retained male paracymbial processes were recorded. We also measured female size (carapace width, mm) and weight (mg). Size and weight values were ln transformed and the computational procedures of Jakob et al. [22] were used to generate the residual index (*RI*, the residuals of body mass on body size), a nondestructive measure of body condition, for each spider. Individuals with higher residual index scores are heavier for their size [23] and scores can be reliably compared among conspecifics regardless of sex, age, reproductive state, geographical population, or date of capture [24]. While the assessment of body condition (overall energy balance [25]) using the residual index has been a topic of debate (e.g., [26] versus [27]), Ardia [28] and Schulte-Hostedde et al. [29] empirically revalidated its use as a measure of body condition and found that it was superior to major alternatives which have been proposed.

2.3. Statistical Analyses. Unless otherwise specified, all analyses were carried out using the StatView 5.0.1 [30] statistical analysis program.

2.3.1. Mating Trials. To assess the cause of variation of copulatory plug presence among the mating trial females which mated ($n = 18$), we first assigned them to two groups based on their respective postmating epigynal condition (e.g., [14]): “both” if the left and right epigynal orifices were completely blocked by plug material, or “incomplete” if this was not the case. For females assigned to each group and their respective male partners, we then compared male carapace width, female carapace width, and relative male to female size (male carapace width/female carapace width) using Mann-Whitney *U* tests. While pseudoreplication may be a concern given the reuse of individual males in multiple trials, each female was used only once and thus each male-female pairing was unique. In addition, to assess whether the pattern of plug production varied among trials, we used a contingency table analysis in which the columns were the different plug combinations and the rows were the three trials. However, since a standard asymptotic chi square test of this table would have been questionable given the small overall sample size [31], we instead used an exact permutation inference approach developed for small-sample categorical data sets by Rugg [32] and implemented in his TableSim program. For each of these analyses, we report a G^2 value and its associated exact *p*.

2.3.2. Field-Collected Females. To assess whether the pattern of plug and paracymbia production varied among sites, we used contingency table analyses in which the columns were the different plug or paracymbial combinations and the rows were the five sites. However, since some cells of these tables had values of less than 5, we again used TableSim [32] to obtain exact permutation inference results. In addition, while we attempted to test for variability among site-specific plug \times paracymbia contingency tables, comparing these tables using TableSim was not successful, as the program would abort prematurely, probably due to an excessive number of cells with values of 0. For this reason, the plug and paracymbia data were treated separately in the analyses of intersite differences.

We used one-way analysis of variance (ANOVA) to test for differences in means of the residual index for females grouped by epigynal condition. There were three groups based on plug combination (two: complete plugs present in both left and right orifices; one: a single complete plug present in the left or right orifice; none: complete plugs absent from both orifices) and three based on paracymbium combination (two: paracymbia present in both left and right orifices; one: a single paracymbium present in the left or right orifice; none: paracymbia absent from both orifices). We also used ANOVA to test for differences in means of the residual index among the five sample sites (HSR, DEB, LMU, CCN, CVR). Since only a single female was found with a partial plug (HSR-49) and another was found with two (HSR-52), these spiders were excluded from the ANOVA analyses to

TABLE 1: Mating trial results for male *Peucetia viridans* with consecutive female partners (A, B, C). Postmating condition of pairs is as follows: *For Females*: plug in epigynum (left: L, right: R, orifices): C, complete; Pr: partial; N: absent (male paracymbial processes (p) are noted when present); female fertile (= live young from resulting egg sac(s)), yes or no; *For Males*: palp (paracymbial) process missing, left (L), right (R) palp. Source and identification numbers of spiders are given in the text.

Male #1	Post-Mating Outcomes	Female A #1	Female B #5	Female C #9
	Plug in epigynum (L/R)	N/N(p)	N(p)/N	Pr/N
	Palp process missing	R	L/R	L/R
	Female fertile	yes	yes	yes
#2		#2	#13	#17
	Plug in epigynum (L/R)	N/N	C(p)/C(p)	C/C
	Palp process missing	none	L/R	L/R
	Female fertile	no ¹	yes	yes
#3		#3	#7	#11
	Plug in epigynum (L/R)	C(p)/C(p)	C/C	C/Pr
	Palp process missing	L/R	L/R	L/R
	Female fertile	yes	yes	yes
#4		#4	#8	#12
	Plug in epigynum (L/R)	N/N	N/N	N/N
	Palp process missing	L/R	L/R	L/R
	Female fertile	yes	no ²	no ³
#5		#6	#10	#15
	Plug in epigynum (L/R)	C(p)/C(p)	N/N	Pr/C
	Palp process missing	L/R	L/R	L/R
	Female fertile	yes	yes	yes
#6		#14	#16	#18
	Plug in epigynum (L/R)	N(p)/N(p)	C/C	C/C
	Palp process missing	L/R	L/R	L/R
	Female fertile	yes	yes	yes
#7		#19	#20	#21
	Plug in epigynum (L/R)	N/C(p)	Pr/C	C/C
	Palp process missing	R	L/R	L/R
	Female fertile	no ⁴	yes	yes

¹Two egg sacs produced, both with undeveloped eggs.

²No egg sac produced.

³Two egg sacs produced, first with undeveloped eggs, second with no eggs.

⁴One egg sac produced, filled with undeveloped eggs.

avoid having groups made up of single individuals. The remaining females ($n = 52$) were therefore used in all the ANOVA tests. In cases where a significant difference was indicated by ANOVA, Fisher's protected least significant difference (PLSD) test [33] was used to conduct post hoc pairwise comparisons among the means.

3. Results

3.1. Mating Trials. The males were generally successful in inseminating their female partners: of the 21 females involved in mating episodes, 17 later spun egg sacs from which live spiderlings emerged, and of these, 12 were second and third partners in the mating sequence (Table 1). Of these 12 females, 9 were inseminated by males which had lost both their paracymbial processes, demonstrating that the

presence of this palpal structure is not essential for successful insemination and mating with multiple partners.

When one or both paracymbial processes were lost, they were generally found in the epigynal orifices of the female partner (7 of 9 cases) (Table 1), though there were two exceptions. The presence of a paracymbial process in a female orifice was sometimes accompanied by an epigynal plug and sometimes not. In all three cases in which a single process was transferred to a female during a mating episode, it was found in the epigynal orifice on the corresponding side (left or right) of the female.

Among the 17 females that produced fertile eggs, the epigynal plug was quite variable: 8 (47%) females had a complete plug in both epigynal orifices; 5 (29.4%) females had no plug in either orifice; 3 (17.6%) females had a complete plug in one orifice and a partial one in the other orifice; 1 (5.9%) female had a partial plug in one orifice

TABLE 2: Means (\pm SE) of physical characteristics for laboratory pairs of *Peucetia viridans* who mated ($n = 18$) by postmating epigynal condition of females. Epigynal condition was classified as both if the left and right epigynal orifices were completely blocked by plug material, or incomplete if this was not the case. The results of Mann-Whitney U tests for differences between the groups are also shown.

Physical characteristics	Epigynal condition		Mann-Whitney	
	Both	Incomplete	z	P
$n =$	8	10		
Male carapace width (mm)	3.493 (0.128)	3.393 (0.089)	-0.622	0.534
Female carapace width (mm)	3.992 (0.142)	3.998 (0.103)	-0.178	0.859
Body size ratio (male/female)	0.876 (0.015)	0.852 (0.028)	-1.200	0.230

and no plug in the other (Table 1). In addition, one female (#19) produced a single egg sac with infertile eggs, even though she possessed a complete plug in one orifice (as well as a paracymbial process). Hence, of the 18 females that had certainly engaged in copulations with their partners, 13 (72%) had at least a partial plug in one epigynal orifice.

Finally, for mating pairs which included the 18 females that had engaged in copulations and which were grouped by plug condition as defined in Section 2.3.1, male size, female size, and the body size ratio (male/female) did not significantly differ for the Both and Incomplete plug groups (Table 2). Moreover, the pattern of copulatory plugs organized by mating trial for these females (Table 3) showed no significant difference among trials ($G^2 = 11.686$, exact $P = .306$).

3.2. Field-Collected Females. The epigynal plug was also quite variable among the field-collected females (see Table 1 in supplementary Material available online at doi:10.1155/2010/602897). Of the entire sample ($n = 54$), 50% had no plug in either orifice and 20.4% had a complete plug in both epigynal orifices; the remaining females had combinations of partial and complete plugs (Supplementary Table 1(a)). As for the presence of paracymbial processes, 72.2% of the entire sample had no process in either orifice, while approximately equal numbers of the other 15 females had either one or two processes present in their epigynal orifices.

Comparison of the field-collected female data for the individual sites (Kenneth Hahn State Recreation Area (Supplementary Table 1(b)); Ernest Debs Regional Park (Supplementary Table 1(c)); Loyola Marymount University (Supplementary Table 1(d)); Crest Canyon Preserve (Supplementary Table 1(e)); Carmel Valley Road (Supplementary Table 1(f))) reveals considerable interpopulation variation. For example, while the percentage of females with no plug in either orifice was somewhat less than that for the entire sample (50%) at both the Hahn (35.3% (12/34)) and Debs (42.9% (3/7)) sites, no plugs of any sort were found in the females from Loyola Marymount University and Carmel Valley Road, while a single plug was found in a female

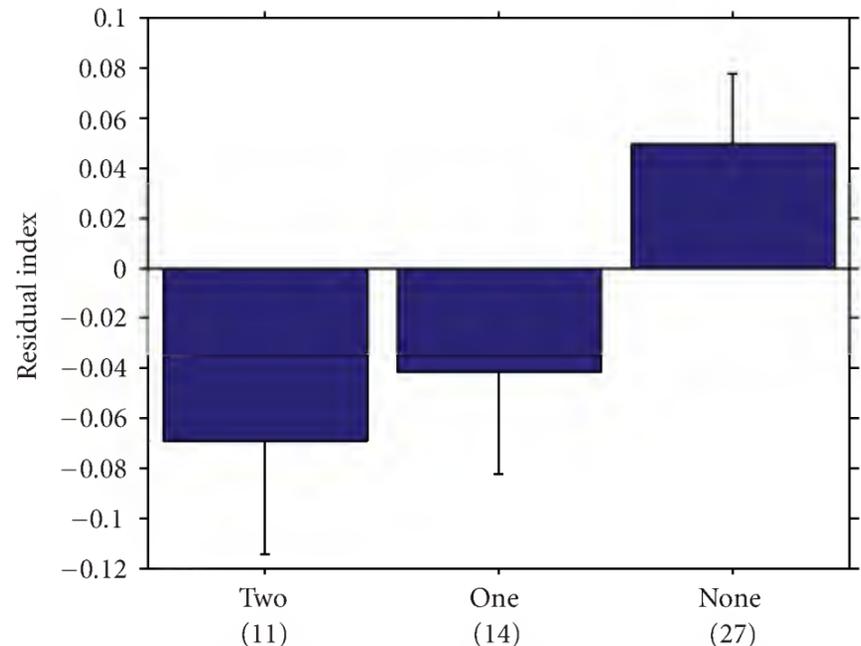


FIGURE 1: Means (\pm SE) of the residual index for field-collected *Peucetia viridans* females grouped by combinations of copulatory plugs in their epigyna. Combinations are defined in the text and the number of spiders in each group is indicated in parentheses.

from Crest Canyon Preserve (Table 4(a)). Similarly, while the representation of females with no paracymbial processes in either orifice was less than that for the entire sample (72.2%) at both Hahn (67.7% (23/34)) and Debs (42.9% (3/7)), no Loyola Marymount University, Crest Canyon Preserve or Carmel Valley Road females were collected with processes in her orifices (Table 4(b)). Surprisingly, this interpopulation heterogeneity was only marginally nonsignificant for plugs ($G^2 = 22.110$, exact $P = .051$) and was not significant for paracymbia ($G^2 = 13.253$, exact $P = .102$). However, since these results may have been affected by the minimal sample sizes ($n = 2$) for the adjacent Del Mar, San Diego Co. sites (Crest Canyon Preserve, Carmel Valley Road), we combined them into a single Del Mar sample ($n = 4$) and reran these analyses with TableSim. This time, inter-population heterogeneity was significant for both plugs ($G^2 = 20.384$, exact $P = .033$) and paracymbia ($G^2 = 13.253$, exact $P = .040$).

The field-collected females grouped by copulatory plug combination differed significantly for the residual index (Supplementary Table 2, Figure 1). Both the Two and One plug females had negative mean RI values while the None (no plug) females exhibited a positive mean, with posthoc tests (Supplementary Table 2) showing that only the Two and None females differed significantly ($P < .05$), though the difference between the One and None females approached significance ($P = .067$). When these females were grouped based on combinations of inserted paracymbia, heterogeneity for the residual index approached significance ($P = .061$), with the Two females exhibiting a negative mean RI and the One and None females positive means (Figure 2), echoing the relative difference between the Two and None groups based on plug combination (Figure 1). Thus, females with two copulatory plugs were in significantly poorer body condition than those possessing no plugs; females with

TABLE 3: Copulatory plug combinations for laboratory *Peucea viridans* females who mated by order of mating trials.

Mating Trials	Copulatory Plugs					<i>n</i> =
	Two Complete	One Complete	Complete + Partial	One Partial	Both Absent	
First	2	1	0	0	3	6
Second	3	0	1	0	2	6
Third	3	0	2	1	0	6
<i>n</i> =	8	1	3	1	5	18

TABLE 4: Combinations of copulatory plugs and paracymbial processes in the epigyna of field-collected *Peucea viridans* females by sample site. Site abbreviations are defined in the text.

(a) Plugs						
Site	Copulatory Plugs					<i>n</i> =
	Two Complete	One Complete	Two Partial	One Partial	Both Absent	
HSR	8	12	1	1	12	34
DEB	3	1	0	0	3	7
LMU	0	0	0	0	9	9
CCN	0	1	0	0	1	2
CVR	0	0	0	0	2	2
<i>n</i> =	11	14	1	1	27	54

(b) Paracymbia						
Site	Paracymbial Processes			<i>n</i> =		
	Two	One	None			
HSR	4	7	23	34		
DEB	3	1	3	7		
LMU	0	0	9	9		
CCN	0	0	2	2		
CVR	0	0	2	2		
<i>n</i> =	7	8	39	54		

two paracymbia appeared to be in similarly poor condition relative to their peers.

Finally, field-collected females grouped by sample site differed significantly for the residual index (Supplementary Table 3, Figure 3). Loyola Marymount exhibited a large positive mean, one which was significantly greater than the negative means for Hahn, Debs, and Crest Canyon (posthoc tests, Supplementary Table 3). Conversely, Crest Canyon displayed a large negative mean, one which differed significantly from that for Hahn. In addition, Crest Canyon was also nearly significantly different ($P = .068$) from Carmel Valley Road, the other site with a positive mean. In sum, females from Loyola Marymount were in better body condition than those from Hahn, Debs, and Crest Canyon, as was also nearly true for Carmel Valley Road females compared with those from Crest Canyon. Crest Canyon females were also in poorer condition than those from Hahn.

4. Discussion

4.1. Mating Trials. The mating trial results conclusively demonstrate that *P. viridans* males have the potential to successfully inseminate multiple female partners, even if they have lost both their paracymbial processes. These trials also

showed that when these male structures do break off, they are not always transferred to the female; yet when they are lodged in an epigynal orifice, they can do so even if a plug is absent. Furthermore, the mating trial results validate Exline and Whitcomb's [10] observation that the probable manner of palpal insertions into the two openings of a female's epigynum during copulation is ipsilateral (right in right, left in left; also known as equilateral [34]).

Since the paracymbial process is not essential for effective sperm transfer, its role during reproduction is unclear. Exline and Whitcomb [10] stated that the breakage of the paracymbium occurs when the male withdraws the embolus from the epigynal orifice and Brady [1] observed that this event "insures fertilization of the female," although he did not elaborate. If the paracymbial process is an orientation device for the palpal embolus during copulation as stated by Exline and Whitcomb [10], males with and without paracymbial processes may differ in the amount of time it takes for them to insert their emboli into the epigynal openings of their female partners, potentially allowing males that position themselves more quickly (those with processes?) to transfer greater amounts of sperm during a copulation [35]. If the paracymbial process does serve to facilitate sperm transfer, it may be that its loss is partly the result of sexual

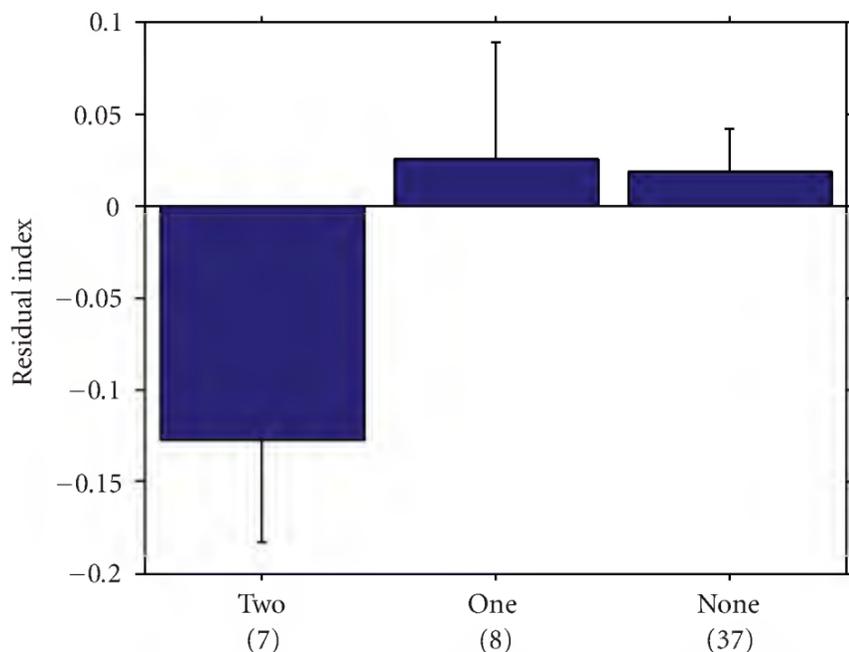


FIGURE 2: Means (\pm SE) of the residual index for field-collected *Peucetia viridans* females grouped by combinations of paracymbial processes in their epigyna. Combinations are defined in the text and the number of spiders in each group is indicated in parentheses.

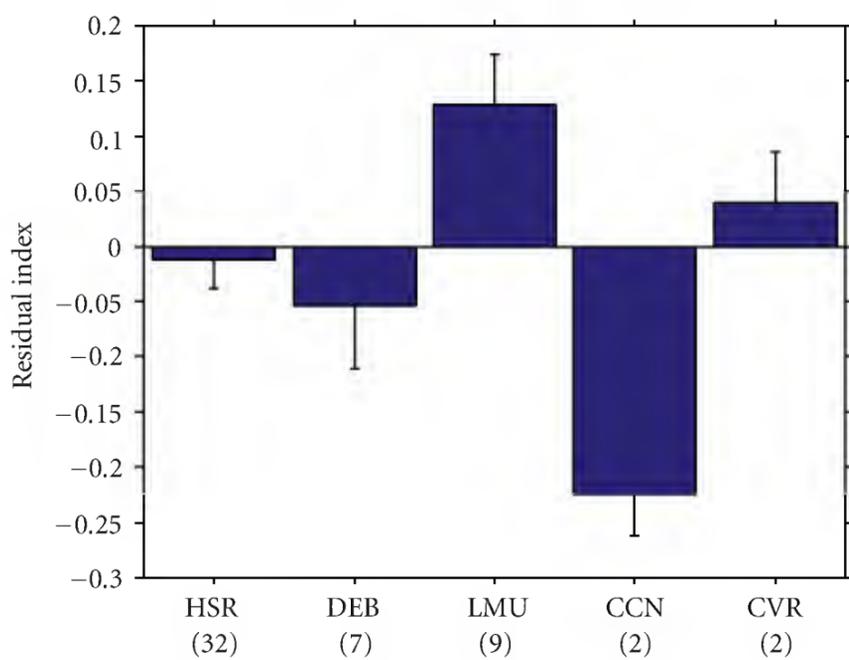


FIGURE 3: Means (\pm SE) of the residual index for field-collected *Peucetia viridans* females by sample site. Site abbreviations are defined in the text and sample sizes are indicated in parentheses.

conflict over when to terminate copulation, as has been suggested for the curved tip of the male palpal conductor in the orb weaver *Nephila plumipes* (Latreille) (Araneae: Tetragnathidae), which physically attaches a copulating male to a female until her attempts to dislodge him frequently result in its breaking off [36]. Finally, as noted earlier, paracymbial processes may function as plugs when lodged in a female. Experiments have shown that palpal structures in the female genital tract do act as effective plugs in some spider species but not in others [37, 38]. As for *P. viridans*, Ramirez et al.'s [3] study of multiple paternity in field-collected broods led them to conclude that inserted paracymbial processes (and copulatory plugs) were not associated with a reduction in female remating, though their sample size was limited. Since paracymbial processes

were so often absent from freshly mated females in the mating trials (Table 1), we agree that this structure may not represent an effective plug in *P. viridans*. Nevertheless, even structures which only occasionally delay or prevent female remating will be favored as male adaptations for sperm competition (W. Eberhard, pers. comm. [39]). Clearly, a more detailed investigation will be needed to clarify the reproductive nuances of copulations when the paracymbial process is present or absent from one or both male palpi as well as the reproductive significance of its presence or absence in the female epigynal orifice.

As with the paracymbial process, the presence of copulatory plugs was inconsistent among the freshly mated *Peucetia viridans* females (Table 1), and when present, their condition was not uniform. In addition, the presence of plug material was not an absolute indicator that successful insemination had occurred. Since the origin of the copulatory plug in *P. viridans* is unknown [10], it is difficult to assess the significance of the variation in plug presence documented for the mating trials. Masses of material (copulatory plugs) that form within or near the entrance of the female reproductive tract as a result of copulation occur in a diversity of taxa, including spiders [4, 40], and they have long been considered devices which interfere with female access to further sexual partners [12, 41, 42]. As reviewed by Eberhard [13], the material that forms the mating plug in spiders may be deposited by the male, by the female, or by both partners, and for males, may be generated by glands in the abdomen, palps, and/or mouthparts. The drop of liquid on the epigynum of a freshly mated female observed by Whitcomb and Eason [11] could be this material in *P. viridans*, though they were uncertain as to whether it was an actual plug precursor and they did not know its source. In the future, the analysis of video recordings of *P. viridans* copulatory activities, coupled with the fixation of spiders in copula and the examination of serial sections of the copulatory organs in physical contact, may help identify male and/or female contributions to plug formation (e.g., [15, 16, 34, 43]).

In whatever manner plugs are formed in *P. viridans*, many factors could potentially influence their presence and condition in mated females (reviewed in [5, 12, 13]). While a thorough assessment of these factors was beyond the scope of this study, we did evaluate whether relatively smaller males may have greater difficulty in completely filling a female's genital orifices (e.g., *Agelena limbata* [14]) and whether multiple mating by males may produce of a greater proportion of partial or absent plugs in their later mates, due to a decrease in male plugging ability and/or contributions (e.g., *Oedothorax retusus* (Westring) [6]). However, our analysis of epigynal condition and the male/female size ratio of sexual partners found no evidence for a size effect in the ability of male *P. viridans* to completely plug a female's paired genital orifices. Moreover, we also saw no significant change in the pattern of plug production over the course of the three mating trials, as might be expected if the males were declining in their ability to promote plug formation. Of course, given the limited number of males and females involved in the trials, these findings should be considered preliminary. Nonetheless, these results may indicate that

plug presence and persistence in *P. viridans* is largely due to differences among individual females, a possibility which seems consistent with findings for the field-collected females.

4.2. Field-Collected Females. The presence of plugs and paracymbia in the epigyna of mated *P. viridans* females varied widely among individuals and among sites. Particularly notable was the contrast between the varied mix of both structures in females from Kenneth Hahn State Recreation Area and Ernest Debs Regional Park, and the absence of plugs from females at Loyola Marymount University and Carmel Valley Road, as well as the absence of paracymbia from these same two sites, along with Crest Canyon Preserve (Supplementary Table 1). Obviously, the minimal representation of these structures at Loyola Marymount University, Crest Canyon Preserve and Carmel Valley Road could be largely the result of limited sampling at these sites. However, the fact that the small Debs Regional Park sample ($n = 7$) contained females with various combinations of both plugs and paracymbial processes (Supplementary Table 1(c)) suggests that the absence of these structures from the Loyola Marymount University sample ($n = 9$) may not be simply due to limited sampling but may in fact have a biological basis, as we discuss later.

Our analysis of the influence of body condition on epigynal condition showed that mated females with no copulatory plugs were in significantly better condition than females with two copulatory plugs, and that females with two paracymbia appeared to be in similarly poor condition. These results suggest that females in the best condition may be the most able to avoid the emplacement of obstructions to their genital openings during copulation and/or to void such structures thereafter, potentially enabling them to mate with another male(s). There are many potential benefits and costs for females which mate multiply (reviewed in [17, 44]); though since female remating has been widely described in general [45] and among spiders ([5], including *P. viridans* [3]), it appears that females typically gain from remating in terms of increased reproductive fitness [46]. Correspondingly, female efforts to prevent (e.g., [15, 47]), dissolve (e.g., [48, 49]), and/or physically expel (e.g., [50, 51]) genital obstructions are also well known, though to the best of our knowledge, the energetic costs of these activities have never been quantified. However, among anopheline mosquitoes whose males produce copulatory plugs which act as physical barriers to remating [52, 53], Giglioli [54] found that well fed *Anopheles melas* Theobald females were able to dissolve mating plugs more quickly than poorly fed females. Similarly, since a spider with a positive residual index score is fatter than one that has a negative score [22], *P. viridans* females in the best condition presumably had the most acquired resources available for allocation to important tasks, perhaps including keeping their epigyna obstruction-free. While accurately measuring the energetic costs of behaviors can be empirically challenging, techniques such as flow-through respirometry and the measurement of organismal glycogen and lipid levels have facilitated the energetic assessment of mating activities in other invertebrates

[55–57] and their use with *P. viridans* might help clarify the relationship between female body condition and epigynal condition detected in this study.

Finally, the fact that body condition also varied significantly by site might be explained by *P. viridans* females experiencing different levels of foraging success depending on their location. Since there are a multitude of factors which can influence insect diversity and abundance in the seasonally dry Mediterranean climate of southern California [58], the availability of insect prey likely differed among the geographically disparate sites sampled for this study. Among these factors, moisture is thought to play a key role in driving insect abundance in this arid region, via its impact on plant primary productivity [59–61]. In this regard, while the Loyola Marymount site is a bluff face with natural coastal sage scrub vegetation [62], it receives surface water runoff from an irrigation system located along the blufftop, given its location on the edge of a university campus. Since *P. viridans* of all instars were concentrated along the blufftop in fall 2004, they were situated in well-watered vegetation and so may have had access to significant numbers of insect prey. This situation may prevail to a lesser degree at the Carmel Valley Road site, which is a larger patch of similar vegetation surrounded by the backyards of urban homes and road edges, with some irrigation systems in place along the road. Thus, enhanced access to insect prey mediated by increased water availability beyond normal rainfall may be one reason why females at Loyola Marymount were in such better condition than their counterparts at the three natural preserves, Hahn, Debs, and Crest Canyon. This may also be why body condition differed between the adjacent Carmel Valley Road and Crest Canyon Preserve sites.

The increased water availability at Loyola Marymount and Carmel Valley Road may have potentially played a more direct role in facilitating the absence of plugs from females at these sites. Specifically, since the genital orifices of female spiders are otherwise always open to the surrounding air unless sealed by copulatory plugs, Huber [63] suggested that their presence may help resist sperm desiccation. Plug frequency may therefore partly reflect the dryness of a population's setting, particularly if they are produced by females. While we are not aware of empirical support for Huber's hypothesis and while we lack the environmental data to thoroughly assess its applicability to *P. viridans*, it is possible that better body condition and an absence of copulatory plugs at the Loyola Marymount and Carmel Valley Road sites may have both been facilitated by anthropogenically enhanced moisture levels. Clearly, a detailed study of abiotic factors, plant and arthropod phenologies, and spider dietary intake (e.g., [64–66]) at nearby mesic and xeric sites over the course of a year will be needed to explicitly evaluate the influence of site-specific environmental parameters on the body and epigynal condition of resident spiders. Indeed, since copulatory plugs are often externally visible when they are present in the epigynum of *P. viridans*, if adult females were inspected for the presence of plugs over time at these sites (e.g., [67, 68]) prior to their eventual collection for measurement, it might also improve our understanding of the frequency and persistence of this structure and its

relationship to female body condition and environmental parameters.

Acknowledgments

The authors are grateful to C. Chagouri and S. Khajavi for laboratory assistance. For facilitating access to Ernest Debs Regional Park, they thank R. Delgado, M. Renaker, P. Sun, and R. Valdivia (Audubon Center at Debs Park). Financial support was provided by Loyola Marymount University (William F. McLaughlin Chair in Biology).

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Research Article

The Bumble Bees of Ukraine: Species Distribution and Floral Preferences

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Received 4 August 2009; Accepted 11 December 2009

Academic Editor: Claus Rasmussen

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The bumble bees were studied in Ukraine between 2002 and 2009 under field conditions, by examining historical and recent collections and by the literature data. Forty one species are reported from the whole territory, 32 of them being recorded from the forest-steppe zone. Ten species are rare in all their habitats: *B. confusus*, *B. distinguendus*, *B. fragrans*, *B. gerstaeckeri*, *B. ruderatus*, *B. armeniacus*, *B. mesomelas*, *B. laesus*, *B. veteranus*, and *B. cullumanus*. The present persistence of the steppe species *B. armeniacus*, *B. laesus*, and *B. cullumanus* is restricted to the eastern part of the country, and *B. fragrans*—to the Crimean Peninsula. The information on distribution, abundance, habitat, and floral preferences of bumble bees is also provided.

1. Introduction

It is rather strange that at present so little is known in the World about the Ukrainian bumble bees, since the territory of this country lies in the middle of the European continent, bordering Poland, Slovakia, Hungary, and Romania in the west, the Republic of Belarus in the north, Russia in the east, and washed by the Black Sea and Sea of Azov in the south. In this paper we are going to enrich the knowledge on the bumble bees, which inhabit the vast range of the country landscapes.

The history of studies in the Ukrainian bumble bees embraces about 140 years. The historical records of different bumble bee species from the Ukrainian territory can be traced by the collections and the literature [1–12]. Since the late sixties to the end of the 20th century studies of bumble bees were nonexistent. Recently we have continued the research and a number of papers have been published [13–19].

For understanding the structure of bumble bee communities and for predicting persistence of any one species in certain habitat types, the distribution of different landscapes in the country should be taken into consideration.

2. Materials and Methods

2.1. Geographical Regions of Area Studied. The Ukrainian territory occupies the south-western part of East-European Plain, the eastern part of the Carpathians (named the Ukrainian Carpathians), and the Crimean Peninsula. The area of the country stretches almost 1300 km from the west to the east, and 900 km from the north to the south. The landscape of the flat country is quite diverse and form clear latitudinal zones: the mixed coniferous-broad-leaved forest zone in the north, the forest-steppe zone in the centre, and the steppe zone in the south, which is adjacent to the costal line of the Black Sea and occupies the most part of the Crimean Peninsula (Figure 1). The landscapes of both highlands (the Ukrainian Carpathians and the Crimean Mountains) are characterized by different altitudinal zones (or belts). There is a considerable difference in natural conditions between and within zones, resulting in great diversity of floral and animal communities. The zone of the mixed coniferous-broad-leaved forests (the so-called Ukrainian Polissia) has a lowland relief with broad flooded river valleys and is characterized by high levels of ground waters and precipitation (550–650 mm per year), and by a

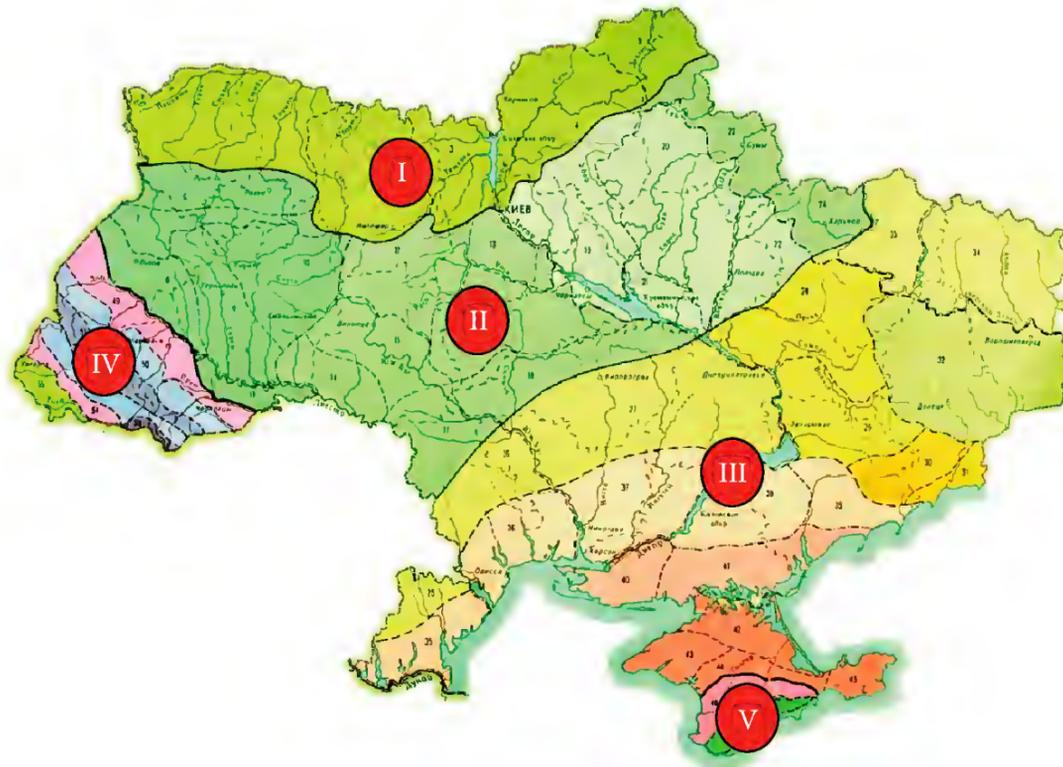


FIGURE 1: Geographical regions of Ukraine (zone limits are marked in continuous line). I: the zone of mixed coniferous-broad-leaved forests; II: forest-steppe zone; III: steppe zone; IV: the Ukrainian Carpathians; V: the Crimean Mountains.

great mosaic of natural habitats. Large areas are occupied by pine forests with an admixture of broad-leaved trees. The climate is temperate continental and mild. Owing to the large areas of marshlands and boggy woodlands, unsuitable for intensive human activities, the nature of Ukrainian Polissia is the least transformed in comparison with other regions of Ukraine. The forest-steppe zone stretches from SW (the foothills of the Ukrainian Carpathians) to NE (western spurs of the Middle-Russian Hills). Eastwards the climate grows more continental. The vegetation cover has been essentially transformed; the natural forests are not available, and the steppe vegetation of natural type has been preserved in low sections of Volyn and Podolia Hills (in the west) and in the Dnieper River lowland (in the east). The steppe zone is a dry warm-temperate zone, covered mostly by grasses that decrease in forbs' diversity as one moves south. Arable land covers above 75% of the zone and little of the virgin steppe has been preserved. The Ukrainian Carpathians stretch 280 km long and 100 km wide, the highest point being Goverla Mt. (2061 m a. s. l.). The altitudinal climatic belts are strongly pronounced, and differ in vegetation. The foothills are occupied by the broad-leaved forests (up to 600 m altitude), giving place to the abies-beech forests (up to 900–1200 m altitude), which change into the spruce forests (up to 1400–1600 m altitude). The highest elevations are occupied by the subalpine and Alpine meadows. The inversion of altitudinal belts is often present, depending on elevation, the slope exposition, and climatic conditions. To the southwest of the Ukrainian Carpathians the Transcarpathians' Lowland lies (Figure 1), with an absolute altitude of 102–120 m. The plain landscapes are covered with the oak and black-alder forests (15% of the whole area) and with small remnants of the meadow-steppe vegetation. The climate is warm and moist (precipitation about 700 mm per year). The lowland is densely populated and arable land covers 50%.

The Crimean Mountains occupy the southern part of the Crimean Peninsula and stretch for 150–160 km, being 50–60 km wide. They are covered with dry forests of the different type and with the steppe meadows.

2.2. Sampling Localities. Bumble bees were collected regularly in all habitat types of the western Ukraine between 2002 and 2009. The bumble bee communities of the Crimea and of the eastern part of the forest zone were studied in 2006. The material from the steppe zone was collected by our colleagues during 2005–2008 and kindly placed at our disposal. The permanent monitoring of the bumble bee communities was accomplished in marshlands and marshy woodlands of Western Polissia, in protected areas within the forest-steppe zone in the west, in mixed forests and meadows of the Nature Reserve “Roztochia,” in the different habitats of all altitudinal belts of the Ukrainian Carpathians and in the Transcarpathians' Lowland. The agricultural landscapes were regularly investigated as well. The bumble bees were observed on flowers and identified in field conditions, with part of them being captured for precise identification. Forage plant species were identified as well. The community structure, species relationships, foraging activities, abundance, and phenology of every species were studied throughout the season, from emerging the queens from hibernation to the last available males in autumn.

2.3. Studied Collections. We have thoroughly examined the historical and recent collections in Ukraine and housed at State Museum of Natural History and National University in Lviv, national universities in Nizyn, Uzhgorod, Simferopol, Kharkiv, Donetsk, Institute of Zoology (Kyiv), and at Kharkiv Entomological Society. As well as the bumble bee collections once made from the Ukrainian territory and

now deposited in Russia (Zoological Museum of Moscow State University, Zoological Institute in Saint-Petersburg, and Belgorod State University) and Poland (Institute of Systematics and Evolution of Animals in Krakow). In total 10 000 bumble bee specimens were examined.

3. Results and Discussion

3.1. Distribution and Habitat Preferences. The complete checklist of the Ukrainian bumble bees includes 41 species (Table 1). The subgenus *Thoracobombus* is the most representative in Ukraine and embraces 12 species. Four of them, *B. pascuorum*, *B. humilis*, *B. muscorum*, *B. sylvarum*, are widely distributed almost all over the country. *B. pascuorum* is a eurytopic species, which is capable of persistence in different conditions, including the high pressure of urban habitats [19]. In steppe zone it inhabits mainly urban and rural areas, where it can find suitable nesting sites and sufficient feeding resources. *B. humilis* is distributed mainly in the eastern part of the forest zone, all over the forest-steppe zone, and sparsely in the steppe zone, preferring dry meadows with steppe flowering vegetation. In general, its abundance everywhere is low, with the exception of the Crimean foothills and mountains. This species also occurs from foothills to about 1000 m altitude in the Ukrainian Carpathians, where it is rare. *B. muscorum* is the common inhabitant of the marshlands in Western Polissia (the forest zone), where it dominates. However, eastwards and southwards its abundance decreases rapidly, and it can be found locally in small numbers. The species avoids large swamps and flooded woodlands, as well as dry meadows, where it apparently cannot find appropriate nesting sites and feeding plants. Its foraging range is too small for searching feeding resources elsewhere than the nesting site [20]. The persistence of *B. muscorum* in the steppe zone (including the Crimea) is restricted to small natural or artificial moist “oases,” and in the Ukrainian Carpathians to foothills. On the contrary, *B. sylvarum* prefers more dry habitats and its abundance in the forest-steppe zone is higher than in the forest zone. It also occurs in steppes, excluding arid regions.

Out of 26 bumble bee species inhabiting the forest zone (Table 1), four can be regarded as “strictly” forest species: *B. hypnorum*, *B. jonellus*, *B. pratorum* and *B. schrencki*. The boreal species *B. jonellus*, and *B. schrencki* occur all over the mixed-forest zone. Their largest populations are restricted to the western part of the zone (Rivne Region), where flooded woodlands and swamps with specific floral resources are optimal for their persistence. A small population of *B. jonellus* occurs in the west of the forest-steppe zone near Lviv City in marshy area with numerous fish-breeding ponds. We failed to find this species in the Ukrainian Carpathians, although it has been recorded from adjacent areas in Poland [21].

The greatest number of bumble bee species was recorded in the landscapes of the forest-steppe zone till the half of the 20th century (Table 1). No wonder that both forest and steppe species could find there a diverse number of habitats appropriate for their existence. Thus, “strictly” steppe species

B. fragrans, *B. cullumanus* (subspecies *serrisquama* Morawitz, 1888), *B. armeniacus*, and *B. laesus* previously were recorded in a series of localities all over the forest-steppe zone (Figures 2 and 3). At present all of them apparently extinct from the zone. Although, there are several nature reserves within the zone, protected steppe plots are too small for persistence of steppe species populations.

As for *B. distinguendus* and *B. subterraneus*, which have been included in the Species’ Red Lists of many European countries as very threatened or extinct [22, 23], their distribution in Ukraine and in the forest-steppe zone in particular, is almost within the same ranges, as was recorded in the past century. Their populations are small and sparsely distributed, especially of *B. distinguendus*. It is noteworthy, that these species were considered as rare ones more than 100 years ago, at least in the west of Ukraine [1–3, 5]. Both species prefer broad meadows, with *B. distinguendus* being restricted to the northern half of Ukraine and *B. subterraneus* being more abundant southwards. The largest population of the latter species exists in the Crimean Mountains, including foothills [18].

The occurrence of *B. semenoviellus* in Ukraine has been reported only recently [14, 24]. It has been considered that this species extended its range from East towards Western Europe at the end of the 20th century [25]. At least, any specimen has not been available in the historical collections of Ukraine. At present it is distributed in the forest and the forest-steppe zones with low numbers being abundant in few localities. Its persistence is connected with wetlands.

The steppe zone of Ukraine is inhabited by 22 bumble bee species (Table 1), among which are “strictly” steppe species and those with wide ecological valence, tolerant to dry and warm conditions. As well, some species of woodlands have adapted themselves to urban and rural habitats, with proper microclimate. The only steppe species, which is considered as common in the steppe zone, including the Crimean steppe, is *B. argillaceus*. In the forest-steppe zone it has been extinct from many localities, especially in the west. It is noteworthy that recently we have recorded *B. argillaceus* from the Transcarpathians’ Lowland [17].

Other steppe species, *B. armeniacus*, *B. laesus*, *B. zonatus*, *B. cullumanus*, *B. mesomelas*, are rare in all their habitats, and nowadays persist mainly in pristine steppe areas, preserved in the east of the country. As far back as the beginning of the 20th century, *B. cullumanus* was considered an extremely rare species in the west of Ukraine, where it inhabited the steppe meadows in the Dnister River valley [2, 7]. We failed to confirm its persistence in the same area at present. Recently, a few specimens of the species were recorded only from the eastern part of the steppe zone (Figure 3). The recent populations of *B. armeniacus* persist in the Crimean steppe [18] and in the Ukrainian Steppe Reserve in the east (Figure 2).

At present, the availability of the rare species *B. fragrans* in Ukraine remains in question. Till the first half of the 20th century this species was widely distributed all over the forest-steppe and steppe zones (Figure 2). The only area, from which the species records were made in the second half of the 20th century, was the Crimean steppe [18] (Figure 2).

TABLE 1: Bumble bees of Ukraine and their distribution among geographical zones.

Species	Distribution				Restricted to
	Mixed-forest zone	Forest-steppe zone	Steppe zone		
<i>B. (Bi.) confusus</i> Schenck, 1859	•	•			
<i>B. (Kl.) soroeensis</i> (Fabricius, 1777)	•	•			
<i>B. (St.) distinguendus</i> Morawitz, 1869	•	•			
<i>B. (St.) fragrans</i> (Pallas, 1771)		•	•		
<i>B. (St.) subterraneus</i> (Linnaeus, 1758)	•	•			
<i>B. (Mg.) argillaceus</i> (Scopoli, 1763)		•	•		
<i>B. (Mg.) gerstaeckeri</i> Morawitz, 1881					The Ukrainian Carpathians
<i>B. (Mg.) hortorum</i> (Linnaeus, 1761)	•	•	•		
<i>B. (Mg.) ruderatus</i> (Fabricius, 1775)		•	•		
<i>B. (Th.) armeniacus</i> Radoszkowski, 1877		•	•		
<i>B. (Th.) mesomelas</i> Gerstaecker, 1869		•	•		
<i>B. (Th.) pomorum</i> (Panzer, 1805)		•			
<i>B. (Th.) laesus</i> Morawitz, 1875		•	•		
<i>B. (Th.) humilis</i> Illiger, 1806	•	•	•		
<i>B. (Th.) muscorum</i> (Linnaeus, 1758)	•	•	•		
<i>B. (Th.) pascuorum</i> (Scopoli, 1763)	•	•	•		
<i>B. (Th.) ruderarius</i> (Müller, 1776)	•	•			
<i>B. (Th.) schrencki</i> Morawitz, 1881	•				
<i>B. (Th.) sylvarum</i> (Linnaeus, 1761)	•	•	•		
<i>B. (Th.) veteranus</i> (Fabricius, 1793)	•	•			
<i>B. (Th.) zonatus</i> Smith, 1854			•		
<i>B. (Ps.) barbutellus</i> (Kirby, 1802)	•	•	•		
<i>B. (Ps.) bohemicus</i> Seidl, 1837	•	•	?		
<i>B. (Ps.) campestris</i> (Panzer, 1801)	•	•	•		
<i>B. (Ps.) maxillosus</i> Klug in Germar, 1917			•		
<i>B. (Ps.) norvegicus</i> (Sparre-Schneider, 1918)	•	•			
<i>B. (Ps.) quadricolor</i> (Lepeletier, 1832)	?				The Ukrainian Carpathians
<i>B. (Ps.) rupestris</i> (Fabricius, 1793)	•	•	•		
<i>B. (Ps.) sylvestris</i> (Lepeletier, 1832)	•	•			
<i>B. (Ps.) vestalis</i> (Geoffroy in Fourcroy, 1785)	•	•	•		
<i>B. (Pr.) haematurus</i> Kriechbaumer, 1870					The Crimean Peninsula
<i>B. (Pr.) hypnorum</i> (Linnaeus, 1758)	•	•	•		
<i>B. (Pr.) jonellus</i> (Kirby, 1802)	•				
<i>B. (Pr.) pratorum</i> (Linnaeus, 1761)	•	•			
<i>B. (Pr.) pyrenaeus</i> Pérez, 1880					The Ukrainian Carpathians
<i>B. (Bo.) lucorum</i> (Linnaeus, 1761)	•	•	•		
<i>B. (Bo.) terrestris</i> (Linnaeus, 1758)	•	•	•		
<i>B. (Ag.) wurflenii</i> Radoszkowski, 1859					The Ukrainian Carpathians
<i>B. (Ml.) lapidarius</i> (Linnaeus, 1758)	•	•	•		
<i>B. (Cu.) cullumanus</i> (Kirby, 1802)		•	•		
<i>B. (Cu.) semenoviellus</i> Skorikov, 1910	•	•			
In total: 41	26	32	22		

•: availability of species;

?: probability of occurrence.

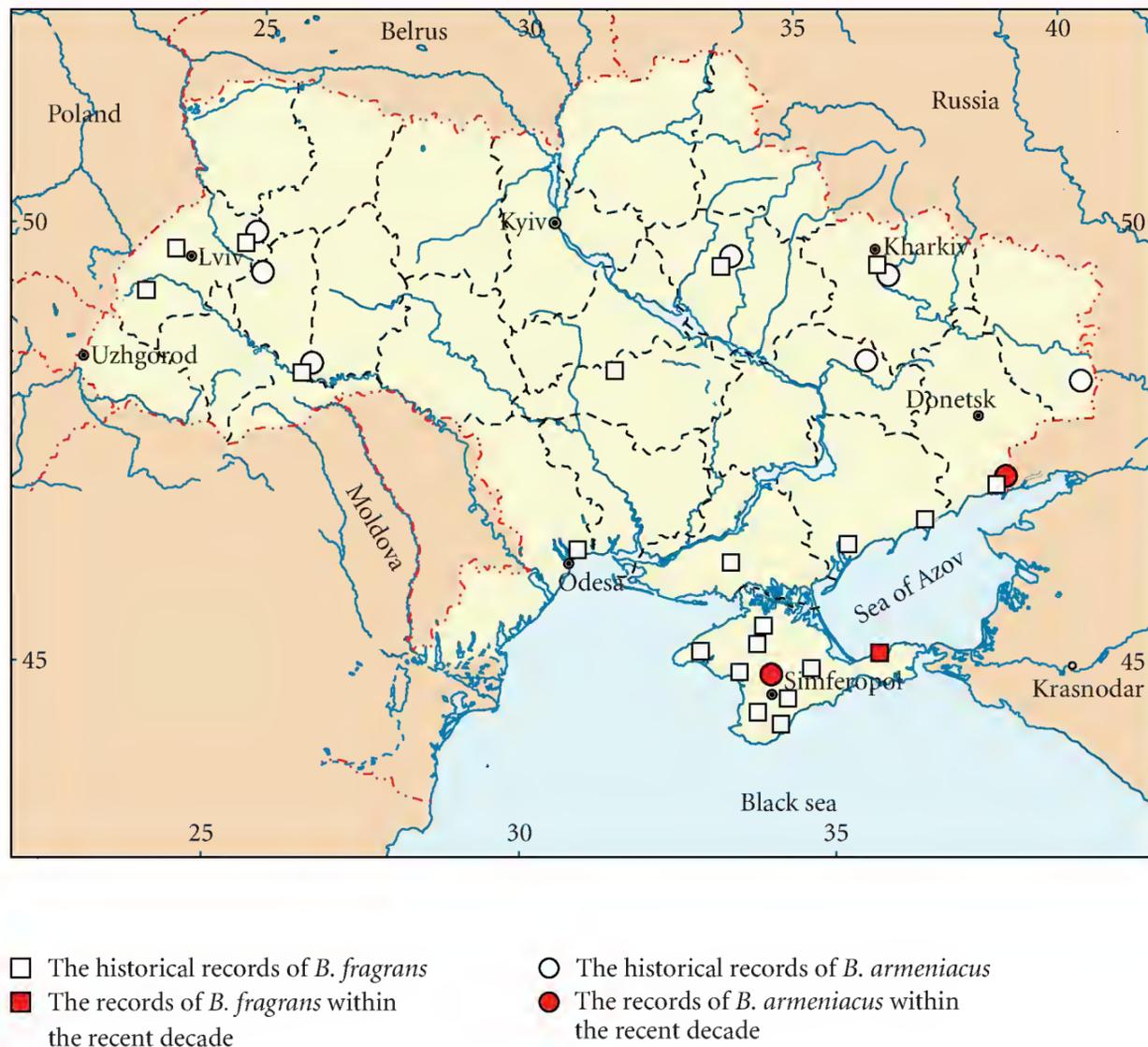


FIGURE 2: The distribution of *B. fragrans* and *B. armeniacus* in Ukraine (the records made since 1868).

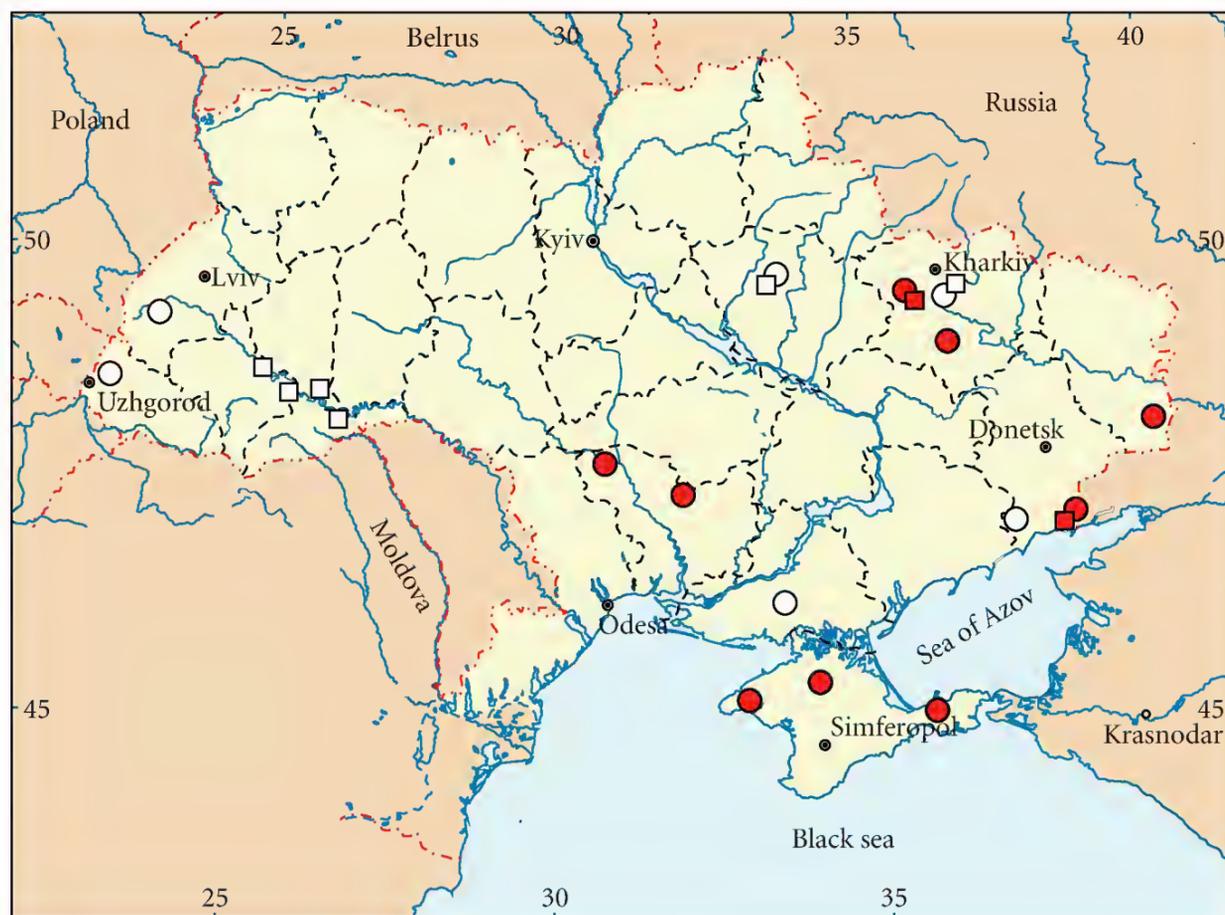
Twenty three species of bumble bees occur in the Ukrainian Carpathians [14], occupying different habitats in accordance with their ecological requirements. In general, the altitudinal distribution of bumble bees concurs with that reported from the Polish Carpathians [26]; however, some differences exist. With growing altitude the species composition of bumble bee communities changes, most of species being restricted to the upper forest limit (1400–1500 m alt.). *B. terrestris*, *B. subterraneus*, *B. lapidarius* occur up to 600–700 m altitude, the latter rarely rising beyond 1000 m in SW mountain macroslope. Only two mountain species, *B. wurflenii* and *B. pyrenaicus*, and also eurytopic *B. lucorum* founded colonies in subalpine meadows, the latter two rising to the Alpine. Although, two other species, *B. hortorum* and *B. gerstaeckeri*, occasionally forage in the subalpine, they never move far away from the upper forest limit. The distribution of the rare cuckoo-bee *B. quadricolor* remains in question, as only a single record is known from the Ukrainian Carpathians made in 1939 [8]. Recently, this species has been collected from woodlands in the Republic of Belarus, adjacent to Ukrainian Polissia (two specimens are available in the collection of Nizyn University). Hence, its existence in the forest zone of Ukraine is very likely.

About 20 bumble bee species occur in the Crimean Peninsula, which are distributed over the habitats according to their ecological preferences. The only representative of the subgenus *Pyrobombus* in the Crimea is *B. haematurus*, which

occupies the niche similar to that of *B. pratorum* in the forest and the forest-steppe zones.

The abundances of the same species, which inhabit different landscapes or zones, are different. We try to generalize the data on a large scale of the entire territory of Ukraine (Table 2). The group “locally abundant” was created for those species, which in general can be regarded as vulnerable in Ukraine for many reasons: the occurrence near the limits of their geographical ranges, the specific habitats they need, lack of habitats due to anthropogenic influence, and so forth [27, 28]. Only in a small number of localities all over the country these species are abundant enough.

3.2. Floral Preferences. It is known that bumble bees select the flowers fitting to the length of their tongues [29, 30]. Besides, there exists a preference for particular plant species in each bumble bee species [30–32] and in individual foragers as well [33]. These preferences change during the season, depending on plant phenology, stage of the colonial development in bumble bees, the diversity of forage plant, and on the competition in insect communities for feeding resources [30, 34]. It is also known that “majoring” and “minoring” in foraging behavior is typical of all bumble bee species [30, 35]. In Ukraine, every species of bumble bees, which occurs in different types of zonal landscapes, shows seasonal preferences to the same flowering plant species, if they are available in a habitat. Most Ukrainian bumble bees



- The historical records of *B. cullmanus*
- The historical records of *B. laesus*
- The records of *B. cullmanus* within the recent decade
- The records of *B. laesus* within the recent decade

FIGURE 3: The distribution of *B. cullmanus* and *B. laesus* in Ukraine (the records made since 1868).



FIGURE 4: Newly emerged young queen of *B. pratorum* feeding on *Cirsium oleraceum*.



FIGURE 5: *B. hortorum* worker taking reward from *Galeopsis speciosa* flower, its favorite pant species.

are polylectic species, with the exception of *B. gerstaeckeri*, which almost completely forages from *Aconitum* spp. [15], there is a difference in diet between the subgenera. The bumble bees of the subgenus *Pyrobombus* can be regarded as the main pollinators of Ericaceae plant species, *Vaccinium myrtillus*, *V. uliginosum*, and *Rhodococcum vitis-idaea* in particular. The persistence of *B. pratorum* and *B. jonellus* in flooded woodlands of flat country and of *B. pyrenaeus*

in the mountains highly depends on these plants, mass-blooming in the crucial period of colonies' foundation. Besides, their diet always includes *Geum rivale*, *Pulmonaria* spp., and *Galeobdolon luteum*, *Rubus* spp., *Frangula alnus*, *Symphytum* spp., *Geranium phaeum*, *Arctium* spp., *Cirsium* spp. (Figure 4).

The long-tongued species *B. hortorum* (subgenus *Megabombus*) and the medium-tongued *B. pascuorum*, *B. humilis*,

TABLE 2: Abundance of bumble bee species within their distributional ranges.

Species	Rare in all their habitats	Locally abundant	Common	Ubiquitous
<i>B. confusus</i>	+			
<i>B. soroeensis</i>			+	
<i>B. distinguendus</i>	+			
<i>B. fragrans</i>	+			
<i>B. subterraneus</i>		+		
<i>B. argillaceus</i>			+	
<i>B. gerstaeckeri</i>	+			
<i>B. hortorum</i>			+	
<i>B. ruderatus</i>	+			
<i>B. armeniacus</i>	+			
<i>B. mesomelas</i>	+			
<i>B. pomorum</i>		+		
<i>B. laesus</i>	+			
<i>B. humilis</i>		+		
<i>B. muscorum</i>		+		
<i>B. pascuorum</i>				+
<i>B. ruderarius</i>			+	
<i>B. schrencki</i>		+		
<i>B. sylvarum</i>			+	
<i>B. veteranus</i>	+			
<i>B. zonatus</i>	+			
<i>B. barbutellus</i>			+	
<i>B. bohemicus</i>				+
<i>B. campestris</i>				+
<i>B. maxillosus</i>			+	
<i>B. norvegicus</i>			+	
<i>B. rupestris</i>				+
<i>B. sylvestris</i>			+	
<i>B. vestalis</i>				+
<i>B. haematurus</i>			+	
<i>B. hypnorum</i>			+	
<i>B. jonellus</i>		+		
<i>B. pratorum</i>				+
<i>B. pyrenaicus</i>				+
<i>B. lucorum</i>				+
<i>B. terrestris</i>				+
<i>B. wurflenii</i>			+	
<i>B. lapidarius</i>				+
<i>B. cullumanus</i>	+			
<i>B. semenoviellus</i>		+		
In total	11	7	12	10

B. muscorum, *B. sylvarum* (*Thoracobombus*) everywhere prefer deep-corolla flowers of *Lamium* spp., *Melampyrum* spp., *Salvia* spp., *Stachys* spp., *Vicia* spp., *Trifolium* spp., *Galeopsis* spp. (Figure 5). We have observed young queens

of *B. muscorum* feeding on *Betonica officinalis* inflorescences year after year in the same place at the end on July (Figure 6).

There is a number of foraging plant species, which grow abundantly everywhere, have long periods of blooming, and



FIGURE 6: Newly emerged *B. muscorum* queen feeding on *Betonica officinalis* inflorescence.



FIGURE 8: *B. terrestris* foudress queen foraging from *Echium vulgare* flower.



FIGURE 7: *B. (Ps.) vestalis* female feeding on *Ajuga reptans*.



FIGURE 9: *B. pomorum* worker foraging from *Betonica officinalis*.

provide much reward. In all types of habitats these plants are always preferred by bumble bees, offering great rewards to many species in crucial periods of the season. These are early spring *Glechoma hederacea*, *Ajuga reptans*, *Geum rivale*; late-spring *Lamium* spp. and *Symphytum officinale*; summer species *Rubus idaeus*, *Echium vulgare*, *Tilia* spp., *Salvia verticillata*, *Trifolium* spp., *Galeopsis* spp., *Centaurea jacea*, *Cirsium* spp., *Carduus* spp., *Ballota ruderalis*, *Impatiens balsamina*, and others (Figures 7 and 8).

In the west of the forest-steppe zone the major forage plants of bumble bees in summer are *Trifolium* spp., *Salvia pratensis*, *S. verticillata*, *Betonica officinalis*, and *Veronica longifolia* (Figures 9 and 10). In the Ukrainian Carpathians the most abundant and most preferable to bumble bees in summer are *Rubus idaeus*, *Rubus hirtus* agg., *Knautia arvensis*, *Succisa pratensis*, *Carduus bicolorifolius*, *Cirsium waldsteinii*, *Chamaenerion angustifolium*, *Trifolium pratense*, *T. medium*, *Vicia cracca*, *Telekia speciosa*, *Centaurea jacea*, and *C. carpatica* (Figure 11). Out of 190 forage-plant species observed in the mountains, those of the families Asteraceae (30), Lamiaceae (29), and Fabaceae (20) were particularly favored by bumble bees, making up almost 42% of the whole

diet. This coincides with the results obtained in the adjacent mountain areas in Poland [36].

Studying the diet of bumble bees, inhabiting the western regions of Ukraine (Western Polissia, western part of the forest-steppe zone, and the Ukrainian Carpathians) for several years, we have recorded only those plant species, which were directly visited by foraging bumble bees. In total 352 plant species belonging to 50 families were visited, of which perennials made up 80% (282 species, including trees and shrubs). Apparently, the forage resources of bumble bees, inhabiting the whole Ukrainian area, embrace more than 500 plant species. The necessity of further research is evident.

It is beyond doubt that bumble bees are the most effective pollinators of natural and cultural flowering plants, and their declines world-wide, which have taken place during the last 50 years [28, 37–41] may have a detrimental impact on pollination networks [42, 43]. As a result of land-use changes in the steppe zone, the steppe bumble bees have suffered a great decline both in diversity and numbers (Figures 2 and 3). As all steppe species in Ukraine occur near the northern limit of their distributional ranges, the loss of habitats or their fragmentation, and reduction in the abundance of food



FIGURE 10: Newly emerged *B. lucorum* males feeding on *Veronica longifolia* inflorescences.



FIGURE 11: Young queen of *B. sylvarum* feeding on *Centaurea carpatica*.

plants have proved to be the crucial factors which caused their extinction from most localities in the forest-steppe and steppe zones of Ukraine [27, 28, 44].

Although at present there is a lack of conservation strategies in Ukraine aimed at pollinating insects, the level of agricultural industry in general is much lower than in most European countries. There are regions, especially in the forest and forest-steppe zones, and in the Ukrainian Carpathians, where the organic farming is a common practice. A lot of fallow lands and seminatural habitats enable persistence of wild bee populations. Nevertheless, the precautions should be made to prevent extinction of diverse habitats in particular cases, when large areas are set to fire in spring and autumn, what is a common practice of local people all over Ukraine. It would be useful to take advantage of the West-European countries in working out the schemes aimed at raising public awareness of the role the pollinators play both in natural and transformed ecosystems [44].

Acknowledgments

The author is much obliged to Vladimir V. Martynov for providing material from steppe reserves; to Mihail Filatov, Sergei P. Ivanov, Alexander Bokotei, Alexander N. Drogvalenko, Alexander V. Prisnyi, Waldemar Celary, Yuri

A. Pesenko, Alexander Antropov, and others for placing the bumble bee collections at her disposal; to Alexander Kuziarin for help in plant species identification; to Volodymyr B. Rizun for help in preparing illustrations.

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Research Article

Floral Resources and Nesting Requirements of the Ground-Nesting Social Bee, *Lasioglossum malachurum* (Hymenoptera: Halictidae), in a Mediterranean Semiagricultural Landscape

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Received 24 July 2009; Accepted 11 December 2009

Academic Editor: Claus Rasmussen

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In order to adopt correct conservation strike plans to maintain bee pollination activity it is necessary to know the species' resource utilisation and requirements. We investigated the floral resources and the nesting requirements of the eusocial bee *Lasioglossum malachurum* Kirby at various sites in a Mediterranean landscape. Analysis of bees' pollen loads showed that Compositae was the more exploited family, although interpopulations differences appeared in the pollen types used. From 5 to 7 pollen types were used by bees, but only as few as 1–1.9 per load. Variations of the pollen spectrum through the annual nesting cycle were conspicuous. At all sites, bees nested in horizontal ground areas with high soil hardness, low acidity, and rare superficial stones. On the other side, the exploited soil was variable in soil granulometry (although always high in % of silt or sand) and it was moderately variable in content of organic matter and highly variable in vegetation cover. Creation of ground patches with these characteristics in proximity of both cultivated and natural flowering fields may successfully promote colonization of new areas by this bee.

1. Introduction

Bees (Apoidea) provide pollination of many wild and cultivated plant species [1, 2] and important services to agriculture [3, 4]; however, their biodiversity in crop areas is threatened by increasing agricultural intensification, which includes the loss of natural and seminatural habitats and extensive monoculture plantings [5–7]. Despite the importance of wild bees in crop pollination, farmers, in particular in the Mediterranean area, still underestimate the importance of managing wild bee populations. Wild bees may be very successful in the pollination of crops, in particular some plants rarely exploited by honey bees or in areas affected by strong decline of honey bee populations [8–10]. It is thus necessary to adopt correct conservation management plans in order to maintain bee pollination

activity. A first step to build conservation plans for bees, and eventually to evaluate if a given wild bee species may be useful for crop pollination, is to quantify its resource utilisation and requirements [11]. Excluding the honey bee, important data on these aspects are available in particular for bumblebees (*Bombus* spp.) (e.g., [12–14]), while very little is yet known on other wild bees, both solitary and social (e.g., [15, 16]). Likewise, we still have little information on which species pollinate crops [17]. Because, at least in part, natural history traits such as sociality and nesting requirements can determine how bees respond to land use [8, 18], a good knowledge of resource use (both food and nesting habitat) of wild bee species is necessary to eventually manage successfully populations in agricultural areas.

The aim of this study was to evaluate the pollen resources and the nesting requirements of a common European

wild bee, the ground-nesting and eusocial *Lasioglossum malachurum* Kirby (Hymenoptera: Halictidae), in a Mediterranean area in Central Italy. This area is included in a protected reserve (Maremma Regional Park) and contains both extensive crop fields and natural and seminatural areas. Mediterranean ecosystems are still poorly investigated with regard to bee ecology [19–22], and floral preferences are much better known in the bee species of Central Europe and North America (e.g., [23, 24]). On the other hand, data on physical-chemical features of nesting sites, such as soil granulometry, acidity, and organic matter, are even more poorly known in ground-nesting bees of Mediterranean, despite it is known that nest founding occurs only if substrate possesses determined characteristics, which may change among bee genera or species [25–27].

Lasioglossum malachurum is a typical primitively eusocial sweat bee [28]. Queens establish their colonies in subterranean nests in spring and then produce one (in Northern Europe) to three (in Southern Europe) worker phases and a last phase composed of males and gynes; these phases are separated by several days during which no foraging activity takes place [29–31]. Mated gynes (queens) overwinter and found new colonies in the following spring. Workers from different European areas were often observed to visit many different flowers, mainly yellow composites [30]. Despite its abundance in most part of Europe, almost nothing is known on the relative importance of each foraged plant in the diet and on nesting requirements of this species, except anecdotal observations [30, 32].

To evaluate floral resources and nesting requirements of *L. malachurum*, we studied different populations located in diverse environments, from agricultural areas to urban areas to seminatural woody areas of the Park.

2. Materials and Methods

2.1. Study Area and Studied Species. The study area, of about 8 km², was located near Alberese, a small town inside the Maremma Regional Park (Tuscany, Italy: 42° 40' 5" N, 11° 6' 23" E). This area is typically Mediterranean, with the average annual temperature around 14–15°C (7.1°C in January, 23.1°C in August); the average yearly rainfall is about 690 mm, with a maximum in November–December and a minimum in July–August. The main part of the park is characterised by the Uccellina mountains, a chain of hills parallel to the coast and covered by the thick Mediterranean maquis. In this area, *L. malachurum* is commonly found nesting in small (<50) to very large (>2000) nest aggregations in a variety of locations, such as along tracks in the woods, along cultivated fields, or even in small bare soil patches inside the town. Two bee nesting sites were chosen for the study of pollen resources: site A was located in a *Quercus* wood, while site B was located along an alfalfa field about 100 m from the Ombrone river. The two sites were separated by about 3 km, and in both *L. malachurum* nested copiously (>1000 nests at both sites). Other bee or wasp species nested in the same areas, although with a minor abundance [33]. For the study of nesting substrate, we used both sites A and

B and, additionally, 3 other nest aggregations: site C within Alberese town where nests were found in a small area of bare soil (about 20 nests), site D along a tomato field (about 40 nests), and site E on a pathway bounding a farm (about 100 nests). No less than 1 km separated these sites.

2.2. Flowering Plants Richness, Bee Pollen Collection, and Identification. Pollen loads were collected from bees returning to their nest after a foraging trip, from 15 to 20 April (only at site B), from 15 to 30 May (at sites A and B), from 15 to 30 June (at sites A and B), and from 23 July to 8 August (only at site B) in 2005 (pollen were not sampled daily). Except in April, when queens were still foraging alone, we collected pollen from workers. Considering that this bee species is characterized by 7–25 days-periods of foraging separated by similar length periods of null foraging (activity breaks) during the annual nesting cycle (e.g., [32]), we believe that sampling through 15 days during each foraging phase can give a realistic picture of pollen use. The collections took place between 9.00 and 15.00 in April and May, between 9.00 and 18.00 in June, and between 9.00 and 12.00 in July/August, according to the different daily periods of foraging by the bees in the three periods. Then we associated each pollen sample with one of the three daily periods (9.00–11.59, 12.00–14.59 and 15.00–17.59). Site A was not sampled in April and July/August because only very few females were active in those periods (<5). On the first and the last day of each period we also sampled all the species of flowering pollen-producing plants in a 1 km radius from the nest aggregation in order to obtain plant taxa richness. This radius was chosen because females of Apoidea generally forage at relatively short distances from the nesting site, rarely more than 1 km [34, 35]. Greenleaf et al. [34] recorded a maximum foraging distance for *L. malachurum* of 600 m.

Once a pollen-carrying bee was collected, it was placed in a 1.5 mL eppendorf tube and placed in a box for 10–15 minutes, where, in the dark, they readily downloaded the pollen grains as normally occurs in the brood cells. The bee was then released and the pollen load preserved until the laboratory analysis. From 8 to 20 (average = 14, standard deviation = 5.4) bee pollen loads were collected per period at each site.

Pollen loads were then prepared in the laboratory for identification. After acetolysis [36], from 1400 to 1800 pollen grains of the pollen load of each forager were observed with a light microscope and classified by morphology into taxonomically distinct pollen types. Identification was based on pollen keys available in the literature on the European flora [37–47], on the pollen collection preserved at the laboratory of palynology CNR-IDPA (Milan), and by comparison with the pollen obtained by the plants sampled by us in the area. Identification reached at least the family level, sometimes the genus-level or type-level (which may include species of a single genus or of different related genera of a single family).

On the whole, roughly 95000 pollen grains were classified, and relative abundances of pollen types estimated as %. Those types accounting for less than 1% were excluded for

further analysis because they may be contaminations [48]. By preserving individual pollen loads in separate tubes in the field, however, we excluded contamination as much as possible.

2.3. Soil Sampling and Nesting Substrate Characterization.

From the five nesting aggregations chosen for the study of nesting requirements we recorded directly environmental variables of the nesting site and collected and analysed in laboratory soil samples. This part of the study was done in July 2008. The substrate variables of the field were recorded in 3 plots of 1 m × 1 m at each nesting site. They were the following: (i) % of vegetation cover (estimation by eye), (ii) number of stones with diameter >5 cm, (iii) % of stone cover (estimation by eye), (iv) slope of soil surface (with an Abney level, to the nearest 0.1 degree), and (v) soil hardness (measured with a penetrometer to the nearest 0.1 Kg/cm²; 3 measures were taken per plot).

Soil samples comprised 500 g of soil in the upper 10–15 cm; one sample per nesting site was obtained. The samples were then transported to the laboratory in closed plastic bags, weighed to the nearest 0.0001 g, and then opened to obtain the dry sample used for the further analysis.

Once dry, we reweighed the sample and passed it through a 2000 μm-sieve, suspended in H₂O₂ to remove the organic matter, and passed again in sieves of 1400, 1000, 710, 500, 355, 250, 180, 125, 90, and 63 μm [49]; a further passage through aerometry was performed on the soil sample which passed the 63 μm-sieve (clay) [49]. Weighting all the samples from each of the sieves we obtained the data to build granulometric curves, which are based on the cumulative percentage of particles falling in the granulometric classes (expressed as φ-intervals, where φ = -log₂ (particle diameter in mm)). We thus evaluated the relative proportions of gravel, sand, silt, and clay in the soil samples [49].

Organic carbon was quantified following the method described in a technical guide of the Italian Ministry of agriculture, food and forest resources [50] and then converted to organic matter with the equation: organic matter = 1.724*organic carbon.

To quantify pH of the soil we used only the subsample with granulometry <2000 μm. Ten g were added 25 mL of bi-distilled water, and the following day the pH was recorded with a pH sensor to the nearest 0.1 unit.

Thus, from the analysis of soil, we obtained 6 variables: (i) quantity of organic matter (mg), (ii) pH, (iii) % of gravel, (iv) % of sand, (v) % of silt, and (vi) % of clay.

2.4. Statistical Analysis. Nonparametric statistics were used to analyse the data. The χ²-test and the G-test was used to compare richness and relative abundance (resp.) of pollen families/types in samples across periods; the Yates' continuity correction was applied to these tests in case of df = 1. The Spearman correlation test was used to look for linear correlations between sets of data. The Mann-Whitney test and the Kruskal-Wallis test were used to compare medians between two or more samples, respectively. A series of Kolmogorov-Smirnov tests was used to test for differences

in distributions of cumulative % of the φ-classes in the granulometric curves between sites.

In the text, average numbers are given ± standard deviation.

3. Results

3.1. Pollen Resources. A total of 27 blooming plant families were collected around the nest aggregation at site A and 28 at site B (Table 1). The floral community was slightly different between the two sites, with some families collected exclusively at the wood site (site A) (e.g., Apocynaceae, Boraginaceae) and some other only at the crop site (site B) (e.g., Chenopodiaceae, Verbanaceae) (Table 1). On the whole, 23 families were collected at both sites, although sometimes represented by different genera (e.g., about half of the genera of Compositae were collected at both sites) and much more often by different species (on the whole, 67 species were sampled at the wood site and 56 at the crop site, but only 17 species were present at both sites).

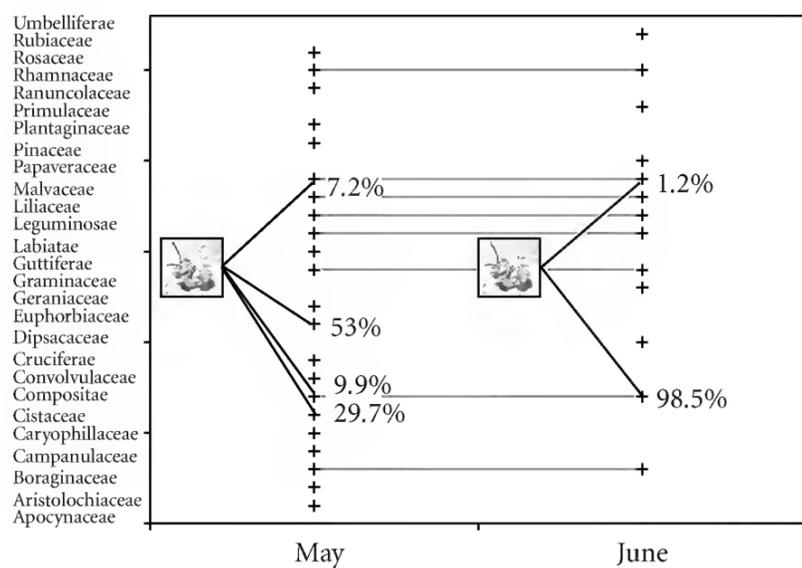
L. malachurum workers foraged for pollen on plants belonging to 4 families at site A and 6 at site B; however, the resource exploitation, in terms of the number of families used out of the number of available families, was not less at the wood site (14.3%) than at the wood site (20.7%) (G-test, $G = 0.082$, $df = 1$, $P > .05$). The number of families used relative to their number in the environment changed slightly through the period of collection at both sites (Figures 1(a) and 1(b)). At the wood site, richness in plant families did not change significantly from May to June (χ²-test, $\chi^2 = 2.31$, $df = 1$, $P > .05$) (Figure 1(a)). Richness in plant families in the environment at the crop site also did not change significantly from April to July/August (χ²-test, $df = 3$, $\chi^2 = 3.10$, $P > .05$). The number of plant families used by bees seems to follow the same slight trends across the season: at the wood site, it decreased from May to June (Figure 1(a)), while at the crop site it reached the maximum in June and the minimum in April, decreasing in July/August (Figure 1(b)). As a result, there was a marginally significant correlation between plant richness in the environment and that used by bees (using all the periods/sites) (Spearman correlation test, $r = 0.74$, $n = 6$, $P = .046$).

With the exception of Rosaceae (which were exploited at site B only in June despite they were still flowering in July/August) and Cruciferae (which were used at site B in May but not in April despite already flowering in that month), bees at both sites did not seem to change plant families until their pollen source is no longer available. However, the relative use of these plant families conspicuously changed across periods.

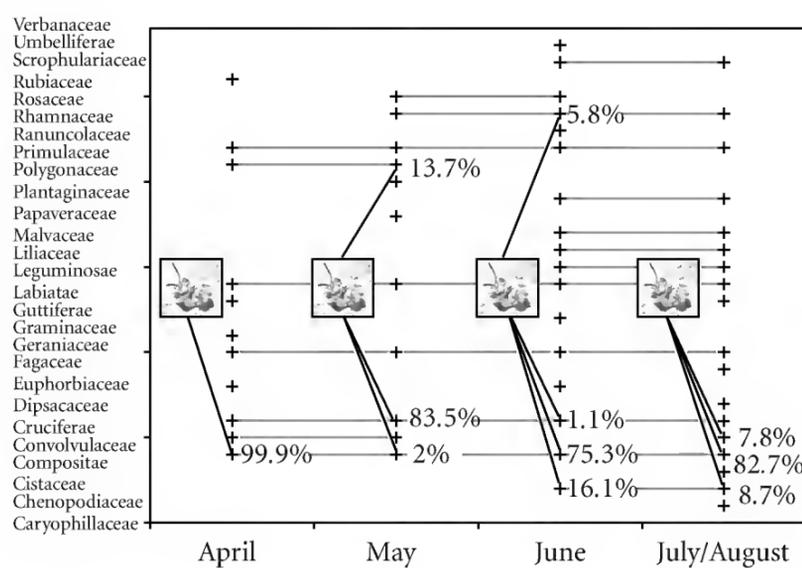
In fact, Compositae (34.4%) and Euphorbiaceae (38.3%) were, on the whole, the most abundant families represented in pollen loads at the wood site (followed by Cistaceae (21.4%) and Papaveraceae (5.5%)), but not in all the periods: Compositae was almost the only family used in June (G-test, $G = 3433$, $df = 1$, $P < .0001$), while Euphorbiaceae was the most used family in May (G-test, $G = 1672.4$, $df = 3$, $P < .0001$) (Figure 1(a)). By contrast, Compositae was by far the most used family at the crop site (80.3%), followed

TABLE 1: List of families and genera collected in the environment around *L. malachurum* nesting sites, and relative use of plant genera/pollen types (richness of used taxon/richness in the environment* 100) in the families where genera/type-level identification of pollen from bees was possible. Genera/types in bold were found in bee pollen loads. Only taxa represented in pollen loads by more than 1% were considered.

Site A	Site B	Plant family	Collected by bees at site A	Collected by bees at site B	Plant genera in the environment at site A	Plant genera in the environment at site B	Use by bees at site A	Use by bees at site B
X	—	Apocynaceae	no	no	<i>Vinca</i>	—	—	—
X	—	Aristolochiaceae	no	no	<i>Aristolochia</i>	—	—	—
X	—	Boraginaceae	no	no	<i>Echium, Myosotis</i>	—	—	—
X	—	Campanulaceae	no	no	<i>Campanula</i>	—	—	—
X	X	Caryophyllaceae	no	yes	<i>Petrorrhagia, Silene, Stellaria</i>	—	—	—
—	X	Chenopodiaceae	no	yes	—	not determined	—	—
X	X	Cistaceae	yes	yes	Cistus, Helianthemum	<i>Cistus</i>	50%	—
X	X	Compositae	yes	yes	Anthemis, Bellis, Chicorium, Cirsum, Coleostephus, Crepis, Evax, Filago, Hieracium, Inula, Pallenis	Anthemis, Bellis, Centaurea, Hieracium, Pallenis, Picris, Doronico, Aster, Senecio, Sonchus	18.2%	50%
X	X	Convolvulaceae	no	yes	<i>Convolvulus</i>	Convolvulus, Calystegia	—	50%
X	X	Cruciferae	yes	yes	<i>Arabis, Capsella, Rapistrum, Sysimbrium</i>	<i>Arabis, Brassica, Capsella</i>	—	—
X	X	Dipsacaceae	no	yes	<i>Knantia</i>	<i>Scabiosa</i>	—	—
X	X	Euphorbiaceae	yes	yes	Euphorbia	<i>Euphorbia</i>	100%	—
—	X	Fagaceae	no	yes	—	<i>Quercus</i>	—	—
X	X	Geraniaceae	no	no	<i>Geranium</i>	<i>Geranium</i>	—	—
X	X	Graminaceae	yes	yes	<i>Alopecurus</i>	not determined	—	—
X	X	Guttiferae	no	yes	<i>Hypericum</i>	<i>Hypericum</i>	—	—
X	X	Labiatae	no	no	<i>Lamium, Marrubium</i>	<i>Mentha, Stachys</i>	—	—
X	X	Leguminosae	no	yes	<i>Lathyrus, Spartium, Trifolium, Vicia</i>	<i>Medicago, Ononis, Scorpiurus, Trifolium, Vicia</i>	—	—
X	X	Liliaceae	no	yes	<i>Allium</i>	<i>Ornithogalum</i>	—	—
X	X	Malvaceae	no	no	<i>Malva</i>	<i>Malva</i>	—	—
X	X	Papaveraceae	yes	yes	Papaver	<i>Papaver, Fumaria</i>	100%	—
X	X	Pinaceae	yes	yes	<i>Pinus</i>	<i>Pinus</i>	—	—
X	X	Plantaginaceae	no	yes	<i>Plantago</i>	<i>Plantago</i>	—	—
—	X	Polygonaceae	no	yes	—	Polygonum	—	100%
X	X	Primulaceae	no	no	<i>Anagallis</i>	<i>Anagallis</i>	—	—
X	X	Ranunculaceae	no	yes	<i>Anemone, Clematis</i>	<i>Anemone, Clematis, Migella, Ranunculus</i>	—	—
X	X	Rhamnaceae	no	yes	<i>Paliurus</i>	<i>Rhamnus</i>	—	—
X	X	Rosaceae	yes	yes	<i>Prunus, Rosa, Sanguisorbia</i>	<i>Rosa</i>	—	—
X	X	Rubiaceae	no	no	<i>Galium</i>	<i>Galium</i>	—	—
—	X	Scrophulariaceae	no	no	—	<i>Veronica</i>	—	—
X	X	Umbelliferae	no	yes	<i>Daucus, Pimpinella, Ridolfia</i>	<i>Daucus, Peucedanum</i>	—	—
—	X	Verbanaceae	no	yes	—	<i>Verbena</i>	—	—



(a)



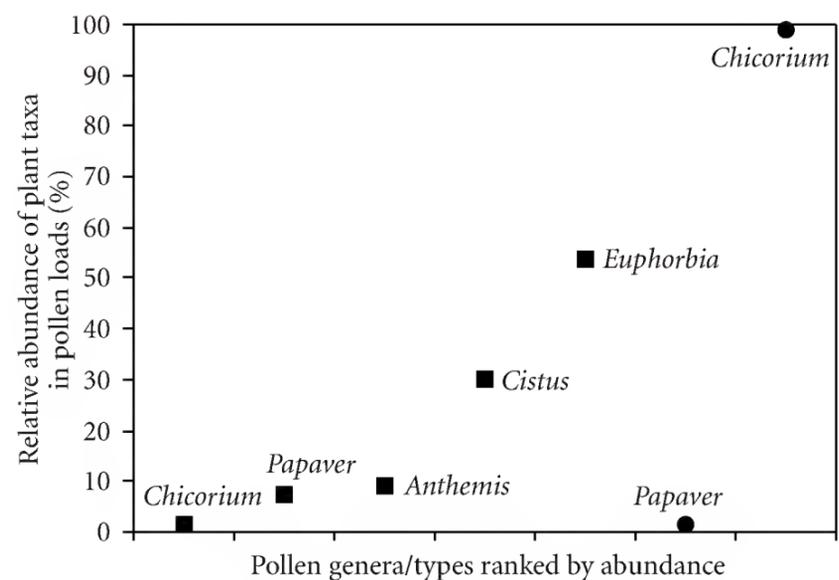
(b)

FIGURE 1: Presence/absence of plant families sampled in the environment and in bee pollen loads (with associated the relative abundance) (indicated by a line to a bee picture) through the period of collection. (a) Site A (wood site). (b) Site B (crop site). For bees, only families with abundance >1% were considered.

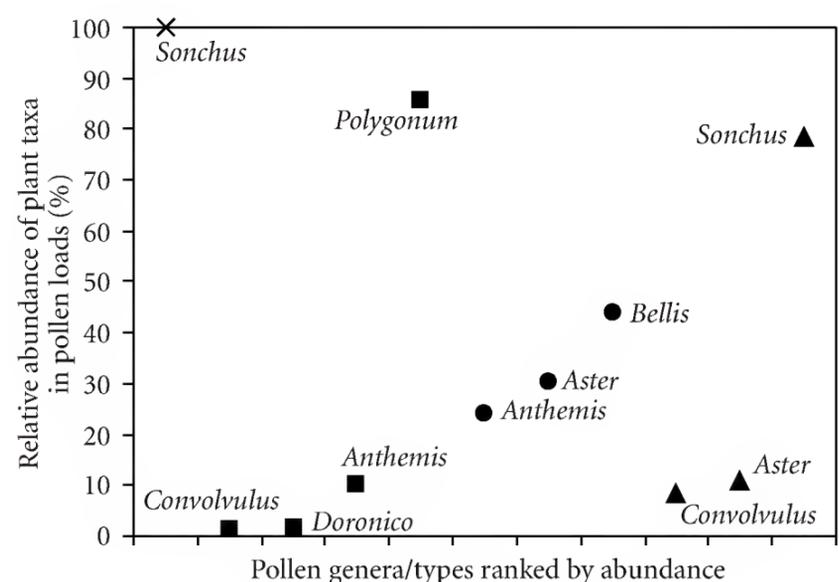
by Chenopodiaceae (8.1%), Convolvulaceae (6.8%), and Cruciferae (3.3%). Compositae was the only family used in April, and the most used family in June (G -test, $G = 6780.4$, $df = 3$, $P < .0001$) and July/August (G -test, $G = 69417.6$, $df = 2$, $P < .0001$), while Cruciferae was the most used family in May (G -test, $G = 6104.6$, $df = 2$, $P < .0001$) (Figure 1(b)).

At a daily level, differences resulted in the relative abundance of pollen plant families used by bees in the morning (9.00–11.59), midday (12.00–14.59), and afternoon (15.00–17.59). In particular, half of the families were mostly collected in the morning and half at midday at site A, while 4 families were mostly collected in the morning and 2 at midday at site B (Table 2).

Considering the pollen collected at genus or type-level, we noted that *L. malachurum* workers foraged for pollen on plants belonging to 5 genera/types at the wood site and 7 pollen genera/types at the crop site. Resource exploitation, in terms of number of genera used out of the number of available genera (in the exploited families), varied from 18.1% in the Compositae to 50% in Cistaceae at wood site



(a)



(b)

FIGURE 2: Relative abundance (%) of different pollen genera/types sampled in bee pollen loads through the period of collection. Marking symbols identify periods: × = April, ■ = May, ● = June, ▲ = July/August. (a) Site A (wood site). (b) Site B (crop site). Only taxa with abundance >1% were considered.

and it was 50% in Compositae and Convolvulaceae at crop site (Table 1). In case of families represented by one single genus in the environment and used by the bees the overlap was obviously complete (Table 1).

Pollen from *Chicorium* (Compositae) was almost the only sort used in June at wood site (G -test, $G = 3424.2$, $df = 1$, $P < .0001$), while *Euphorbia* (Euphorbiaceae) predominated in May at this site (G -test, $G = 4233.3$, $df = 4$, $P < .0001$) (Figure 2(a)); on the other hand, bees foraged only on *Sonchus* (Compositae) in April and mostly on this genus in July/August at the crop site (G -test, $G = 43368.9$, $df = 2$, $P < .0001$), while *Polygonum* (Polygonaceae) predominated in May (G -test, $G = 490.7$, $df = 3$, $P < .0001$) and a mixture of *Bellis*, *Aster* and *Anthemis* (Compositae), with significant differences in their proportions, predominated in June at this site (G -test, $G = 195.8$, $df = 2$, $P < .0001$) (Figure 2(b)). Note that pollens from Cruciferae were not determined at the genus/type level, so that although *Polygonum* was an abundant genus, it was less abundant than Cruciferae.

TABLE 2: Relative abundance of pollen plant families collected by bees in three periods of the day. Only families with abundance >1% were considered.

Site	Family	9.00–11.59	12.00–14.59	15.00–17.59	G-test (df = 2)
A	Cistaceae	75.1	16.0	8.9	$G = 1292.2, P < .0001$
A	Compositae	70.2	20.5	9.3	$G = 1650.6, P < .0001$
A	Euphorbiaceae	38.7	47.3	13.9	$G = 555.2, P < .0001$
A	Papaveraceae	5.8	68.8	25.4	$G = 272.9, P < .0001$
B	Chenopodiaceae	92.2	2.4	5.4	$G = 19356.9, P < .0001$
B	Compositae	91.0	8.2	0.8	$G = 184939, P < .0001$
B	Convolvulaceae	100.0	0.0	0.0	—
B	Cruciferae	55.6	44.4	0.0	$G = 3222.9, P < .0001$
B	Polygonaceae	39.8	60.2	0.0	$G = 35.4, P < .0001$
B	Rosaceae	7.1	90.1	2.8	$G = 502.1, P < .0001$

TABLE 3: Superficial characteristics of the nesting sites of *L. malachurum* sampled in 2008. Measures were taken at 3 plots of 1 m × 1 m at each site. Except for soil hardness, which was taken 3 times at each plot (9 measures per site), all the other variables have $n = 3$, so that statistical comparisons were not possible.

Sample code	Soil hardness (Kg/cm ²)	Vegetation cover (%)	Soil surface slope (degrees)	Number of stones with diameter >5 cm	Stone cover (%)
A	7.9 ± 2.1	2.7 ± 2.5	13.3 ± 20	3.3 ± 0.6	13.3 ± 7.6
B	8.6 ± 1.2	0	0	0	0
C	10.7 ± 0.3	11.7 ± 16.1	0	2.7 ± 3.8	10.0 ± 0.0
D	10.9 ± 0.2	63.3 ± 5.8	0	0	1.7 ± 2.9
E	6.5 ± 1.8	1.7 ± 2.9	0	0	2
Statistics	Kruskall-Wallis test: $\chi^2 = 29.12, P < .001$	—	—	—	—

The average number of pollen genera/types per load did not vary through periods at wood site: it was 1.5 ± 0.7 in May (median: 1), and 1.2 ± 0.4 in June (median: 1) (Mann-Whitney test: $U = 246, n_1 = 20, n_2 = 16, P = .12$); on the contrary, it changed across periods at the crop site, being 1 ± 0 in April (median: 1), 1.9 ± 0.8 in May (median: 2), 1.3 ± 0.4 in June (median: 1) and 1.2 ± 0.4 in July/August (median: 1) (Kruskall-Wallis test: $\chi^2 = 23.42, df = 3, P < .001$). However, one must consider that such difference is due only to the median value for May, which is the double of those of all the other periods.

3.2. Nesting Requirements. The typical nesting site of *L. malachurum* in Maremma Regional Park consisted almost invariably of a horizontal ground area with moderately high to very high soil hardness, which differed between sites, and little stone coverage (Table 3); by contrast, nest aggregations may be covered or not by vegetation (Table 3). Soil characteristics varied among sites: some presented high percentages of sand and other high percentages of silt; gravel and clay percentages were in general low, except at one site each where they were moderate (Table 4). As a consequence, in some cases granulometric curves (Figure 3) did not differ between sites (site A versus site D, site B versus site E, site C versus site D, and site C versus site A: Kolmogorov-Smirnov test, $0.17 < D < 0.21, n_1 = n_2 = 28, P > .05$), while in all the remaining cases the differences in the results of comparisons

of the granulometric curves between sites were significant (Kolmogorov-Smirnov test: $0.35 < D < 0.49, n_1 = n_2 = 28, P < .05$).

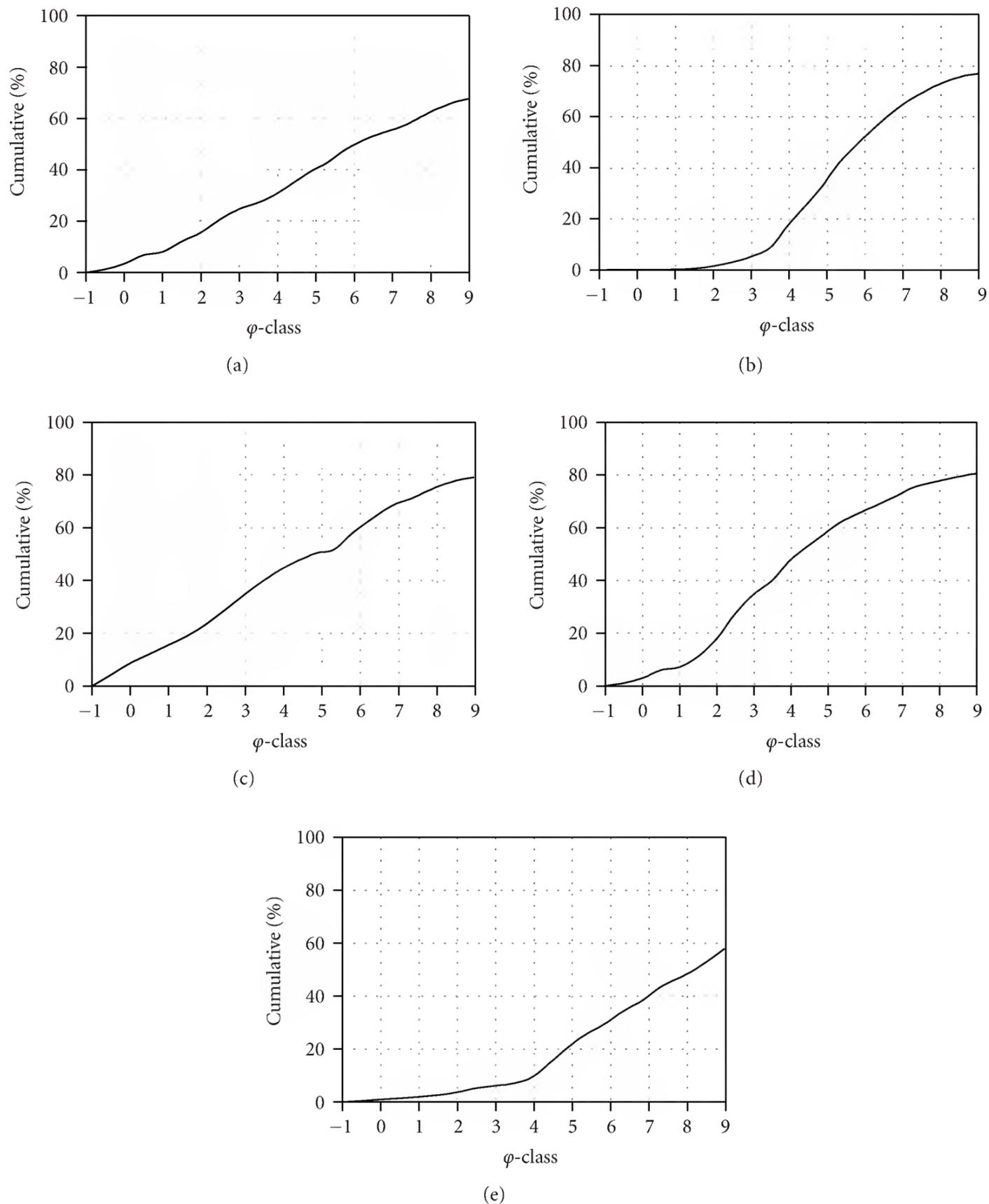
Amount of organic matter also varied from low to moderate (Table 4). The value of pH was, on the contrary, quite constant (between 7.4 and 7.8) (weakly alkaline soils) (Table 4).

4. Discussion

Lasioglossum malachurum was defined in the past as a polylectic species, being recorded on a variety of flowers, mainly yellow composites [29]. In our study, the conspicuous variation of pollen types sampled from the bee loads across the nesting cycle supports the opportunism of this species. However, it is interesting that our studied populations showed, at least during short periods, a quite narrow pollen spectrum. In fact, in any given period of collection, only a small portion of the available flowering plants present in the environment were actually found in the bee pollen loads. Observing bee foraging at flowers rather than analysing pollen quantitatively, Knerer [29] observed similar situations for this species. In fact, he found that *Bellis* supplied exclusively the food source of queens in southern England, while *Andryala* and *Plantago* were the almost exclusive plants foraged by workers in Spain and in Camargue, respectively.

TABLE 4: Physical-chemical and granulometry characteristics of the soils sampled in 2008 at nesting sites of *L. malachurum*.

Sample code	Organic matter (mg)	pH	% gravel	% sand	% silt	% clay
A	12.5	7.7	14.6	26.4	31.9	27.1
B	11.7	7.4	3.0	12.5	62.7	21.8
C	6.5	7.8	25.7	33.3	25.8	15.2
D	17.5	7.5	13.9	40.6	28.9	16.5
E	48.2	7.5	4.0	10.0	45.6	40.4

FIGURE 3: Cumulative frequency curves of soil particles sizes from 5 nesting sites of *L. malachurum*. On the x -axes there are represented the values expressed as φ -units ($-\log_2$ of the diameter of the particles, in mm) and on the y -axes the cumulative percentages of the particles representing the different granulometric classes. (a)–(e): sites A-E.

Other halictid bees seem to be much more broadly polylectic than *L. malachurum*. For example, pollen of 42 plant species from 17 families has been found in the food stores of the halictid bee *Halictus subauratus* (Rossi), with 61%–86% of the pollen being accounted for by the Compositae [51]. However, also in that case the foragers are characterized by strict flower preferences: more than 90% of the pollen in each cell belongs to two or three, sometimes four, plant species [51]. Other case studies support the fact that, even in polylectic halictids, the females often collect most food from a small number of plant species. *Seladonia confusa* (Smith) foragers regularly visit only a few plant species, although they have been observed on the flowers of 165 species [52], and *Lasioglossum imitatus* (Smith) collects pollen from only one plant species during one flight, although this species was recorded on different plant species [53]. Floral constancy, that is, the tendency to return several times to the same plant species when the pollen is abundantly available, may account for this difference, in these and in other bee species [54, 55].

Another factor which probably accounts for differences found among the number of visited plant species and the number of actual pollen types recorded in bee loads or in bee nests is the differential use of flowering plants for nectar and pollen exploitation, with most of plant species visited often only for nectar [56]. We found in bee pollen loads a total of 8 pollen types at wood site and 21 types at crop site, but only 5 and 7 had abundances greater than 1%, suggesting that maybe some pollen grains may attach to the bee body during nectar feeding on nonpollen exploited plants.

Compositae remains probably the most abundant family exploited by polylectic *Lasioglossum* spp. (e.g., [16, 57]). Data available in literature on pollen load composition of species of the genus showed that the number of pollen types varies broadly: *L. villosum* Kirby collects pollen of one single type and *L. pauxillum* Schenck of only 2 kinds, *L. morio* (Fabricius), *L. fulvicorne* (Kirby), and *L. albipes* (Fabricius) use between 7 and 13 pollen types, while both *L. leucozonium* and *L. calceatum* use 17 and 23 pollen types, respectively [16]. *L. malachurum* in our study collected 11 pollen types (populations combined), which results in this species to be moderately polylectic. According to Michener [57], the long season of activity of social bees compared to solitary ones must usually be associated with dependence on a diversity of lowers. *L. calceatum* and *L. malachurum* are both eusocial species and have a broad pollen spectrum, but *L. pauxillum* (eusocial and oligolectic) and *L. leucozonium* (solitary and polylectic) are evident exceptions to this rule.

On the other side, a lower variance appears in the average number of pollen types per load in *Lasioglossum* species: in the literature values reported range from 1 to 2 [16], exactly as in our study. This suggests once more the possibility that these bees exploit repeatedly a narrow spectrum of profitable flowering species and that maybe they change their preference only when plant community structure changes. Previous qualitative observations on the pollen load of *L. malachurum* already suggested this possibility: Knerer [29] reported different bees returning to the nests with pollen

of specific colours. Our quantitative data even more suggest floral constancy in *L. malachurum*.

The recorded variation in our study of the pollen preference through the nesting period of the bee suggests the possibility of using *L. malachurum* to increase pollination of those target plants normally present in the diet of the bee and most preferred in certain periods of the year. For example, *L. malachurum* foraged quite exclusively for chicory (*Chicorium*) at the wood site during June and about half of the pollen collected in June at the crop site was of daisy (*Bellis*). The only two species of these genera sampled in the environment were *C. intybus* L. and *B. perennis* L., which are commonly cultivated and used by humans as food (the former) or for pharmaceutical infusions (the latter). Moreover, in May at the crop site, bees foraged predominantly on Cruciferae; although it was not possible to determine the pollen type, our environmental survey showed *Brassica oleracea* L. cultivars (cabbage, broccoli, seed rape) to be cultivated in the farms around the nesting site, and it could be pollinated by *L. malachurum*. In fact, *Brassica* crops were importantly visited by other *Lasioglossum* spp. (e.g., [58]). However, since two other Cruciferae were sampled in the area, *Arabis glabra* Bernch. and *Capsella bursa-pastoris* (L.) Medicus, this hypothesis should be explicitly tested with new data.

Other abundant pollen types recorded in bee loads do not seem to be of economical importance but of course may preserve the pollination system and thus the environmental service [4, 7].

The quantitative data presented on the nesting site and soil used for nesting of *L. malachurum*, here reported for the first time for this species, revealed that probably founding queens need horizontal ground surfaces with low stone coverage, hard-packed, and alkaline soils to initiate nest construction, while other factors, such as vegetation cover, seem to be less important. Compact soil and variable presence of spots of vegetation were recorded qualitatively in other nest aggregations of this species [29, 32]. Other halictid bees seem to respond differently from *L. malachurum* concerning the recorded variables. Across sites, *Halictus rubicundus* (Christ) prefers softer soils (but not within dense aggregations), sloped ground surfaces, and soils with low to moderate % of gravel [27]. Potts and Willmer [27] suggested that in dense aggregations females are forced to use hard soils to maintain structural integrity of the nests; however, despite we could not statistically test it, this hypothesis does not seem to work for *L. malachurum*: in fact, no differences seem to exist between the soil hardness of the smallest (site C) and the largest (site A and B) nesting aggregations, and in any case at least the site C would be the one with a slightly harder soil. A solitary species, *Dieunomia triangulifera* (Vachal), prefers areas that avoid vegetation, maybe because vegetation perches could increase the presence of parasitic flies (Conopidae) [26]. In our studied population, no parasitic flies attack *L. malachurum* with the exception of *Megaselia leucozona* Schmitz (Diptera: Phoridae), which does not wait for the host on perching sites [59].

Soils of other species of *Lasioglossum* were analysed by Cane [25], and all showed major levels of silt (*L. sisymbrii* Cockerell, *L. laevissimus* Smith: from 44% to 53.8%) or sand (*L. cinctipes* (Provancher): 54.8%) but no gravel and low % of clay thus on the whole similar proportions found in our study on *L. malachurum*, possibly suggesting a recognizable pattern in nesting habits for the whole genus. It is likely that only some parts of a particular area meet all the apparent criteria necessary for nest initiation, and this may account for aggregation patterns at a landscape level.

Ground-nesting bee species may be more threatened than those that utilise the cavities of wood or plant stems, particularly where intensive agriculture has resulted in loss of nesting habitat as well as floral diversity [60]. However, it was suggested that arable farmland can be enhanced as a habitat for these insects by growing annual nectar- and pollen-producing herbaceous plants for them in noncropped areas such as set-aside and field margins, and by providing additional suitable nesting substrate zones [60, 61]. This may work better for polylectic species in particular. For example, Cane et al. [62] found that habitat fragmentation reduced the abundance and richness of oligolectic but not polylectic bees, suggesting higher extirpation rates in the former.

For what concerns *L. malachurum* in Central Italy, the creation of patches of ground (following, e.g., the method described in [61]), characterized by the needed features mentioned above, in proximity of target plant species populations such as chicory and daisy, may increase the fitness of such economically valuable species; presence of other mixed species of plants, mainly composites, may be readily used by the bees during periods of low blooming of the target species. *L. malachurum* is very widespread in the Park (Polidori et al., unpublished data) and thus probably possesses good dispersion and colonization capacities.

Acknowledgments

Thanks are due to the Maremma Regional Park offices for support and permit to perform the field work. C. Ravazzi kindly has permitted the use of his laboratory for the palynological analysis, and F. Andrietti gave useful suggestions on an early draft of the manuscript. The authors are indebted to R. Henry L. Disney, which kindly revised the English. This work was supported by a 3-year grant FIRB from the Italian Ministry of University (RBAU019H94-001-2001).

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Research Article

Analysis of Pollen Collected by *Andrena flavipes* Panzer (Hymenoptera: Andrenidae) in Sweet Cherry Orchards, Afyonkarahisar Province of Turkey

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Received 29 July 2009; Revised 4 December 2009; Accepted 7 January 2010

Academic Editor: James C. Nieh

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Andrena, which is the largest genus in the Andrenidae, is a very important genus for the pollination of fruit trees. *Andrena flavipes* Panzer is one of the most common species observed in the study area and can continue the flight activity even under low temperature. In this study, the pollen collected by *A. flavipes* was determined. In addition, the potential to carry sweet cherry pollen of the aforementioned species was also researched. For the pollen preparates, the scopae of 34 females were used. As a result of the diagnosis studies, it was determined that *A. flavipes* species collected the pollens of 13 families and that the dominant pollen group belonged to the Brassicaceae. It was ascertained that *A. flavipes* collected sweet cherry pollen and that the sweet cherry flowers do not represent a primary pollen source, however.

1. Introduction

Sweet cherry (*Prunus avium* (L.) Moench) is the earliest ripening species among temperate climate fruit species. In about the current 1500 sweet cherry varieties throughout the world, except a few (such as the Stella) that are generated by artificial mutation, all are self-incompatible [1]. High fruit-set can only be accomplished through cross-pollination among the compatible varieties [2, 3]. Henceforth, sweet cherry orchards require a huge population of pollinator bees that would carry out the adequate amount of pollen transfer between the different varieties [4]. In practice, the honeybee is the main pollinator used in sweet cherry orchards, due to reasons like its colony's purchase at relatively lower prices, breeding in easier conditions, and having many members that collect food in a colony [2] compared to the commercially managed other pollinators. However, sweet cherry's budding in very early periods, the negative effect of rain during the blooming period, and low temperatures limit the pollinator activities of honeybees [5, 6]. In addition, pests

and diseases of honeybees, natural competition between the bees that are natural inhabitants and brought later, loss of the habitat, invasive plant species spreading and pressuring the nectar and pollen producing vegetation, the reduction in honeybee genetic variety, and colony losses seen due to the chemicals used in cultivated areas form the main problems of beekeeping [7]. In this case, it is clear that a more diverse pollination strategy would be beneficial to long-term sustainability of crops that require insect pollinators. For this reason, the first step is to determine the presence of the wild bees in agroecosystems. Then, their floral resources and nesting habitat should be identified in order to protect them and increase their quantity [8].

In Turkey, there are two important activities related to bee-keeping. One of these is honeybee breeding that is especially made for bee products, and the other is the *Bombus* bee that is used to perform pollination operations (cross-fertilization) in the greenhouses. However, Özbek [9] indicates that there are about 2000 species of bee operating as pollinators in Turkey, which is one of the richest regions

of the world in terms of bee fauna. Diagnosis of the pollen samples gathered by wild pollinators in agricultural ecosystems, particularly in extreme climatic conditions contributes to understand clearly whether or not there is a relationship between pollinators and products. On the other hand, understanding the roles of pollinator species in the ecosystem will facilitate to adopt pollinator friendly practices (foraging habitat, nesting areas, monitoring, etc.) that make it possible for them to survive in agricultural ecosystems. Therefore, it is mandatory to undertake researches on the species of bees to evaluate their current potential.

Andrena Fabricius is the most common genus of Andrenidae family within the Holarctic [10]. Although different levels of social organisation from solitary to pre-social in Andrenidae have been detected [11], *Andrena* species are solitary bees [12]. These species are also called “mining bees” as they nest in the soil [13]. Klug and Bünemann [14] state that *Andrena* species are very effective for the pollination of fruit trees. *A. flavipes* which is one of the most common species of *Andrena* genus is bivoltine. Spring generation displays activity between March and May, whereas summer generation between July and September. It is known that the species is polylectic species as it feeds on the pollens of Apiaceae, Asteraceae, Brassicaceae, Lamiaceae, Fabaceae, and other families [15]. Furthermore, it is found that it is dominant species in some of the agricultural ecosystems such as apple orchards [16], alfalfa [17, 18], and onion fields [19].

In this study, it was aimed to identify the pollen collected of *A. flavipes* Panzer, which is considered as potential pollinators of fruit trees, in the sweet cherry orchards. The reason for selecting *A. flavipes* for the current study is that it is one of the most abundant species during the blooming period of the sweet cherry orchards and that it can fly even though the temperature is under 12°C. Vicens and Bosch [20] also state that some early-flying bees including the species of *Andrena* are known to forage on *Prunus* flowers when weather conditions are unfavorable for honeybees. Thus, whether or not it would be an important pollinator for sweet cherry flowers was tried to be determined. The study was undertaken in the sweet cherry orchards in Sultandağı town (Afyonkarahisar). This town is in fourth place in Turkey’s sweet cherry production with 18,434 tons [21] per year [22].

2. Materials and Methods

2.1. Study Area. This study was undertaken in the sweet cherry orchards in Sultandağı town located in the east of Afyonkarahisar, Turkey. Bee samples were collected by Malaise trap from two sweet cherry orchards in the period between 15 March–15 May in 2007 and 2008. There were 300 sweet cherry trees in the first and 700 in the second orchard, of 0900 Ziraat sweet cherry variety which were produced to be exported. Bing and Stella cultivars were used as pollenizers. The traps were set in the bud swell period and lifted in the green fruit period. Samples collected in the killing bottle were killed by ethyl acetate.

The diagnosis of *Andrena flavipes* Panzer species was carried out by Dr. Tomozei (Museum of Natural Sciences “Ion Borcea” Bacău, Romania).

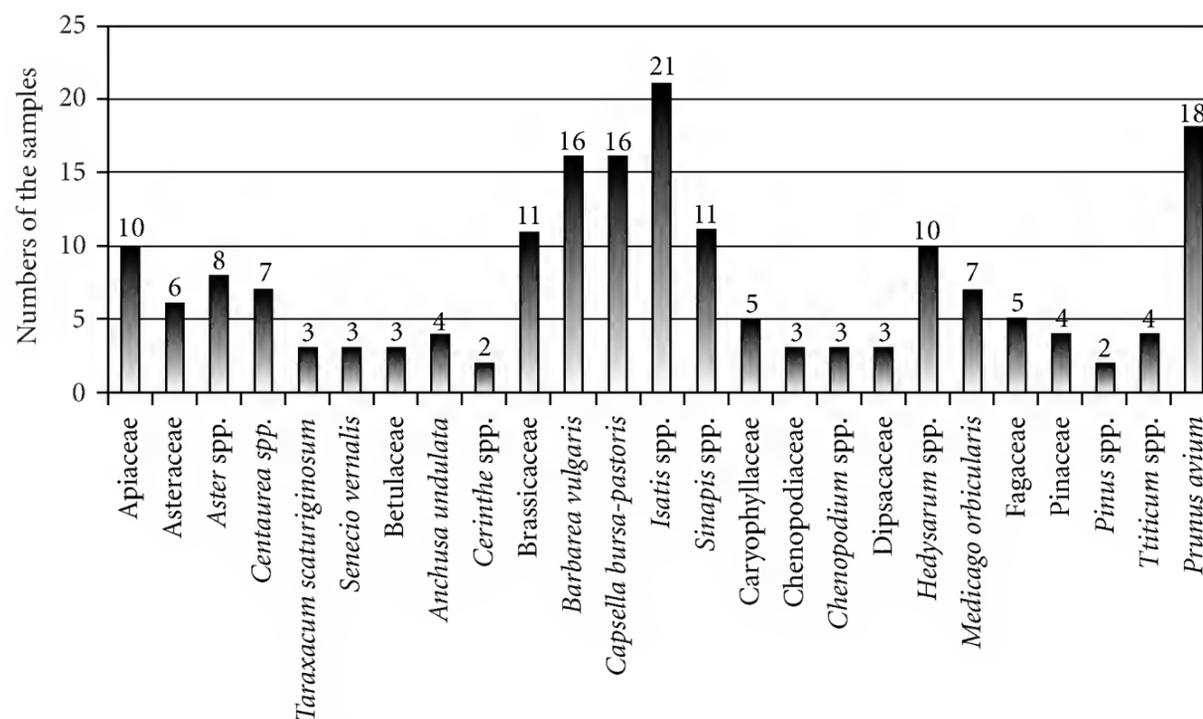
2.2. Pollen Analysis. For the pollen preparates; among the collected female *A. flavipes*, 34 samples that had pollens in the scopa were used. The pollen preparation was made following Güler and Sorkun [23]. In order to separate pollen from the scopa, third pair of legs was placed inside 25 mL glass tubes. Five ml of 70% alcohol were added to the tubes and mixed by glass baget for 15 minutes. The contents were filtered into clean tubes using wire filter of 250 µm pore size. The sample tubes were centrifuged at 3500 rpm for 30 minutes. The supernatant was decanted and 5 mL distilled water was added to the pollen pellet. The tubes were centrifuged at 3500 rpm for 15 minutes. The supernatant was decanted and the tube caps were left open on the benchtop for 10 minutes for the pellet to dry.

Basic fucsin-glycerin-gelatine mixture taken with the edge of a sterile needle was added to the pollen pellet. The stained sample was transferred to a microscope slide and put on a hotplate set at 40°C. When the gelatine was melted, 18 × 18 mm cover slips were placed on the samples. The analysis was carried out through the use of a Nikon Eclipse E400 microscope. Diagnosis was carried out following the related literature [24–29]. In order to verify the diagnosis, reference microscope slides prepared from pollens of formerly diagnosed plant species were used. Two hundred of pollen grains were counted in each slide and percentage of pollen was calculated according to taxa. Those percentages were used to determine abundant of taxa. The following terms were used for frequency classes: dominant pollen (more than 20% of pollen grains counted), secondary pollen (11–20%), minor pollen (6–10%) and rare pollen (less than 5%) [30–33].

In addition, throughout the study in the orchards, the plants that were in the blooming period were collected and pressed and transformed into herbarium material. The diagnosis of this material was carried out by Dr. Mutlu (Inönü University, Malatya).

3. Results and Discussion

Pollen diagnosis were able to be made on a genus level, all of the pollen samples which could not be diagnosed down to this level were collected and evaluated under their associated family’s names as well. While pollens belonging to 21 families were diagnosed as a result of the study, pollens belonging to families of the Campanulaceae, Geraniaceae, Lamiaceae, Oleaceae, Papaveraceae, Plantaginaceae, Salicaceae and Zygophyllaceae were only seen in singly preparates, and their amounts in these preparates again did not exceed one or two. Thus, pollens belonging to these families were accepted as accidental infection and were left out of evaluation. As a result of the diagnosis of the plant samples that were collected in orchards and transformed into herbarium material; the species of *Capsella bursa-pastoris* (L.) Medik., *Barbarea vulgaris* R. Br., *Senecio*

FIGURE 1: The plants that *A. flavipes* collected pollen.

vernalis Waldst. & Kit., *Taraxacum scaturiginosum* G. Hagl., *Anchusa undulata* L. and *Medicago orbicularis* (L.) Bart. were identified. In addition; as the samples associated with the *Prunus* genus were the only species that were blossomed at the time of the study, *Prunus* spp. was taken into evaluation as *P. avium* (L.) Moench (sweet cherry). Other genera identified in the pollen preparates were not diagnosed on a species level as they were not present in the plant species collected in the orchards. It is thought that these pollen samples were collected by the bees in the areas surrounding the sweet cherry orchards. Because it is known that *A. flavipes* generally went 120–150 m away from their nests in order to nourish, and flew maximum 415 m away [34].

Other identified families and genera and species associated with these families are shown in Table 1

In the samples taken into evaluation, those pollens belonging to the Brassicaceae family were the commonest ones (40.11%). This finding supports the assumption of Tadauchi [35] that regards *A. flavipes* as the most significant pollinator for *Brassica* in spring in Kazakhstan and Kyrgyzstan. In addition, the dominant pollen in every preparate again belonged to this family as well. Within the Brassicaceae; among the *Isatis*, *Barbarea*, *Capsella* and the *Sinapis* spp., the pollen of *Isatis* spp. was the most preferred by *A. flavipes* (Figure 1). This genus's pollen production is secondary, and nectar production is at minor level. While *Capsella bursa-pastoris* that is one of its second preferred species does not have too much importance in terms of bee-keeping, both pollen and nectar production of the other species (*Barbarea vulgaris*) are at secondary level. On the other hand, the pollen production of *Sinapis* spp. is minor and nectar production is secondary [36].

The families identified outside the Brassicaceae, especially the Asteraceae and Rosaceae, were found in many bee samples. Asteraceae is the family with the highest number of species within Turkey [37, 38]. D'Albore [29] has formed a grading system in which he rated the species of plants between 1 (being the rare) and 4 (being the

TABLE 1

(1) Apiaceae
(2) Asteraceae
<i>Aster</i> spp.
<i>Centaurea</i> spp.
<i>Senecio vernalis</i> Waldst. & Kit.
<i>Taraxacum scaturiginosum</i> G. Hagl.
(3) Betulaceae
(4) Boraginaceae
<i>Anchusa undulata</i> L.
<i>Cerinthe</i> spp.
(5) Brassicaceae
<i>Barbarea vulgaris</i> R. Br.
<i>Capsella bursa-pastoris</i> (L.) Medik.
<i>Isatis</i> spp.
<i>Sinapis</i> spp.
(6) Caryophyllaceae
(7) Chenepodiaceae
<i>Chenopodium</i> spp.
(8) Dipsacaceae
(9) Fabaceae
<i>Hedysarum</i> spp.
<i>Medicago orbicularis</i> (L.) Bart.
(10) Fagaceae
(11) Pinaceae
<i>Pinus</i> spp.
(12) Poaceae
<i>Triticum</i> spp.
(13) Rosaceae
<i>Prunus avium</i> (L.) Moench

dominant) according to their pollen and nectar production for honeybees. In this system, the species of *Centaurea* scored 3 and 4 for pollen and nectar productivity, whereas the scores

for *Aster* were 2 and 1, respectively. The species of *Taraxacum* are the earliest blooming species in the spring. In addition, it is dominant in terms of both pollen and nectar production [36].

Weeds in the orchards function as alternative food resources for the bees in the environment, prior to the blooming of sweet cherry. As a matter of fact, Bosch et al. [4] determined that populations of *Osmia lignaria* (Hymenoptera: Megachilidae), which they released to the sweet cherry orchard where blooming delayed due to bad climatic conditions, survived feeding on *Taraxacum* sp. that was present in the orchard. While the Rosaceae family was represented by a single species (*P. avium*) in the orchards at the time the study was undertaken, other families had more species in the blooming period. Although pollen and nectar production of *P. avium* are on minor levels, it was found *P. avium* pollens in 18 of the 34 bee samples (Figure 1). The results of the pollen analysis indicate that the sweet cherry flowers do not represent a primary pollen source. However, *A. flavipes* seems to play an important role in cherry orchards particularly when the populations of honey bees are insufficient or when there is low temperature conditions.

Acknowledgments

The authors would like to thank Dr. Bogdan Tomozei (Museum of Natural Sciences “Ion Borcea” Bacău, Romania) for the diagnosis of *Andrena flavipes*, and Dr. Birol Mutlu (İnönü University, Malatya) for the diagnosis of herbarium materials in the manuscript. The research was financially supported by the General Directorate of Agricultural Research, Ministry of Agriculture and Rural Affairs.

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Research Article

Several New Aspects of the Foraging Behavior of *Osmia cornifrons* in an Apple Orchard

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Received 3 August 2009; Revised 13 November 2009; Accepted 15 December 2009

Academic Editor: Claus Rasmussen

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We investigated the foraging behavior of *Osmia cornifrons* Radoszkowski, which is a useful pollinator in apple orchards consisting of only one kind of commercial cultivars such as “Fuji”, and of different types of pollinizers, such as the red petal type, “Maypole” or “Makamik”. It was confirmed that, in terms of the number of foraging flowers per day, visiting flowers during low temperatures, strong wind, and reduced sunshine in an apple orchard, *O. cornifrons* were superior to honeybees. We indicated that *O. cornifrons* seemed to use both petals and anthers as foraging indicator, and that not only female, but also males contributed to apple pollination and fertilization by the pollen grains attached to them from visiting flowers, including those at the balloon stage. It was confirmed that *O. cornifrons* acts as a useful pollinator in an apple orchard consisting of one kind of cultivar with pollinizers planted not more than 10 m from commercial cultivars.

1. Introduction

Osmia cornifrons Radoszkowski is one of the more useful pollinators of Rosaceae fruit production including apples. Although honeybees (*Apis mellifera* Linnaeus) are the most important natural carriers of pollen in an apple orchard, the use of *O. cornifrons* is on the increase in Japan due to its superior characteristics over honeybees, such as its higher pollination rates produced by not moving along the rows, its superior safety from being stingless, and the fact that it flies and pollinates apples in cooler and damper weather [1, 2]. Moreover, since the fertilization area it covers while collecting pollen in an apple orchard is smaller than that of honeybees, *O. cornifrons* are especially useful in relatively small and densely planted apple orchards in Japan consisting of one kind of cultivar with Crab-apples as pollinizers that are planted not more than 10 m from the cultivar [3].

Previously, we investigated the usefulness of the foraging behavior of *O. cornifrons* in an apple orchard consisting of a pollinizer and a commercial cultivar, “Fuji” [4]. We demonstrated that *O. cornifrons* showed strong flower constancy during one pollen-nectar foraging trip of 4–8 minutes,

though the bees seemed to forage different types of flowers, for example, from pollinizers with red petals to commercial cultivars with white petals during their 16–22 pollen-nectar foraging trips [4]. From the results showing that the pollen from pollinizers not brushed off from the pollinator’s body could be used for the fertilization of commercial cultivars visited on their next foraging trip, *O. cornifrons* seemed to be a useful pollinator in apple orchards consisting of a single cultivar, such as “Fuji” and of pollinizers of different types, such as the red petal types, “Maypole” or “Makamik” [4]. Moreover, *O. cornifrons* seemed to be a useful pollinizer for “Delicious,” which is difficult for honeybees to access as a pollinator due to its sideways approach [4, 5].

In this paper, we investigated the foraging behavior of *O. cornifrons* and elucidated their daily foraging time, foraging indicators, and the likelihood of male contributions to fertilization.

2. Materials and Methods

2.1. Experimental Area. Our research was conducted from 2006 to 2009 at a 9.0-ha apple orchard at the Nagano Fruit

Tree Experiment Station, in Nagano, Japan, as well as in sectors of experimental farms at both Gifu University and Nagoya University [3, 4].

At Gifu University, the area occupied by one nesting shelter of *O. cornifrons* consisted of ca. 400 females together with four “Seirin Spur” (a “Fuji” sport) and three “Maypole” trees. We planted four-year-old apple trees in March, 2006, and established 1 to 8 rows composed of either “Maypole” or “Seirin Spur” and “Maypole”, with the distance from the nesting shelter to rows 1 to 8 being 1.8 m, 3.7 m, 7.6 m, 13.4 m, 20.3 m, 21.2 m, 30.8 m, and 33 m, respectively [3]. In March, 2009, we cut down 14 “Maypole” trees located in rows 2 to 7.

2.2. Foraging Time of *O. cornifrons*. The apple orchard at Nagano was partitioned into 49 blocks (most were 2000 m²) with 9 nesting shelters of *O. cornifrons* comprised of at least 1000 females, which means that a sufficient number of *O. cornifrons* were present everywhere in the orchard [3]. *Osmia cornifrons* uses a reed tube as its nest, and the *O. cornifrons* we used were originally captured by Mr. Takazawa in 1966 on the thatched roof of his house located close to the Nagano Fruit Tree Experimental Station. The number of bees has increased two to three times in one year without any troubles, such as an attack of natural enemies. To observe the daily foraging period of *O. cornifrons*, we counted the average number of flying individuals in front of the nesting site in block no. 14 at 5-minute intervals on an hourly basis from 8:00 to 18:00. We recorded 20 when more than 20 individuals were counted since counting their exact number was difficult. We measured the temperature, solar radiation, and wind speed using the FreeSlot-68KD system (M. C. S Co., Sapporo, Hokkaido, Japan) settled at block no. 19 at the Nagano Fruit tree experimental station [3]. All of the data are recorded automatically every minute from morning till night.

2.3. Indicators for Foraging and Pollination by Males. In Nagano, we randomly selected 80 or 83 “Fuji” flowers in one tree (2 total of 160 or 163 flowers in two trees) in block no. 17, then removed the petals (20 or 21 flowers), anthers (20 or 21 flowers), and both petals and anthers (20 flowers) to determine what part of the flower was most attractive to visiting *O. cornifrons*. From 2006 through 2008, the percentage of “Fuji” fruit set was determined to be 18 to 23 days after full bloom. We confirmed that the flowers with standing sepals and a tendency toward swollen ovaries and surrounding receptacle tissues had succeeded in fertilizing and setting fruit. We also manipulated some flowers in “Maypole” and “Dolgo” and observed that *O. cornifrons* were visiting them.

At Gifu (2008) and Nagoya (2009), we used nets to cover three “Alps-Otome” planted at one-meter intervals with three “Maypole” planted along the same line at one meter intervals. Again using nets, we covered one “Alps-Otome” and one “Maypole” planted at one-meter intervals at Gifu (2008) and Nagoya (2009) for the control experiment. All of the four-year-old “Alps-Otome” and “Maypole” plants were grown in individual pots until February of 2009, then

planted in soil at the experimental farm of Nagoya University. Between the 13th to 22nd of April, a total of 40 and 30 males of *O. cornifrons* were introduced to the planting area at Gifu (2008) and Nagoya (2009), respectively. The percentage of “Alps-Otome” fruit set was determined as mentioned. We investigated the fruit set of 100 flowers located at the top of each tree (2008) as well as that of all the flowers (2009). The data of each year from 100 to 575 flowers were averaged out (mean \pm SD).

3. Results and Discussion

3.1. Foraging Time and Distance of *Osmia cornifrons*. We observed the activity of *O. cornifrons* at the nesting side for 16 days (5 days in 2006, 4 days in 2007, and 7 days in 2008) and discovered several characteristics of their foraging behavior. First, they started foraging from 9:00 AM (29th or 30th of April 2007 and 2008, Figures 1(a) and 1(b)) or 8:00 AM (2nd of May 2007, Figure 1(c)) or 7:00 AM (1st of May 2008, Figure 1(d)), and the temperature seemed to be the critical point from which they began foraging. As we found that some individuals started foraging at 10.7°C and 10.9°C on April 28th and May 4th 2006, respectively, the temperature at which they began daily activity appeared similar to that of *O. cornuta* (10°C to 12°C), but lower than that of *A. mellifera* (12°C to 14°C) [6]. In addition, *O. cornifrons* showed longer periods of activity in fine weather (from 7:00 A.M. to 6:00 PM, Figure 1(d)) than might be expected by Mr. Kitamura (from 8:00 A.M. to 5:00 PM, unpublished results). We found that *O. cornifrons* were already in flight at an apple orchard at 6:10 A.M. on May 2nd 2008 and confirmed that they were collecting pollen from “Fuji” flowers at 6:30 A.M. Since the pollen of flowers under bright sunlight had thoroughly dried by 6:15 A.M., they seemed to have started foraging for pollen at 6:30 A.M. As *O. cornifrons* visit ca. 15 flowers per minute, which is a rate higher than that of honeybees (6 flowers per minute) (Kitamura, personal communication), the rate of apple flowers pollinated by *O. cornifrons* must be higher than that by honeybees. Although they could not forage under very strong winds (3.9–5.4 m/s) and reduced sunshine (0.61–1.66 MJ/m²) (Figure 1(c), Kitamura, unpublished results) [6], in 2008, they were able in fact to fly under relatively severe conditions (strong winds of 2.5–4.2 m/s and reduced sunshine of 0.48–0.79 MJ/m²) (3:00 to 4:00 PM in Figure 1(d)). Weather conditions in 2008 were hurriedly for *O. cornifrons* since fine weather with no trace of rain continued from April 27th to May 2nd, so that the flowering period of “Fuji” (10 days) was the shortest in the last 3 years. This might explain why they flew in spite of such severe conditions.

Previously, we showed that the pollination of apple trees by *O. cornifrons* maintained high levels at a 33 m location from the nesting side [4]. For that reason, both the pollinizers (Maypole) and commercial cultivars (Seirin Spur) were planted at 3.7 m, 7.6 m, 13.4 m, and 33 m from the nesting side. We cut down all “Maypole” except for three plants at the closest point (1.8 m) to the nesting side, thus reducing the number of pollinizers. In that rearranged area, fruit set was

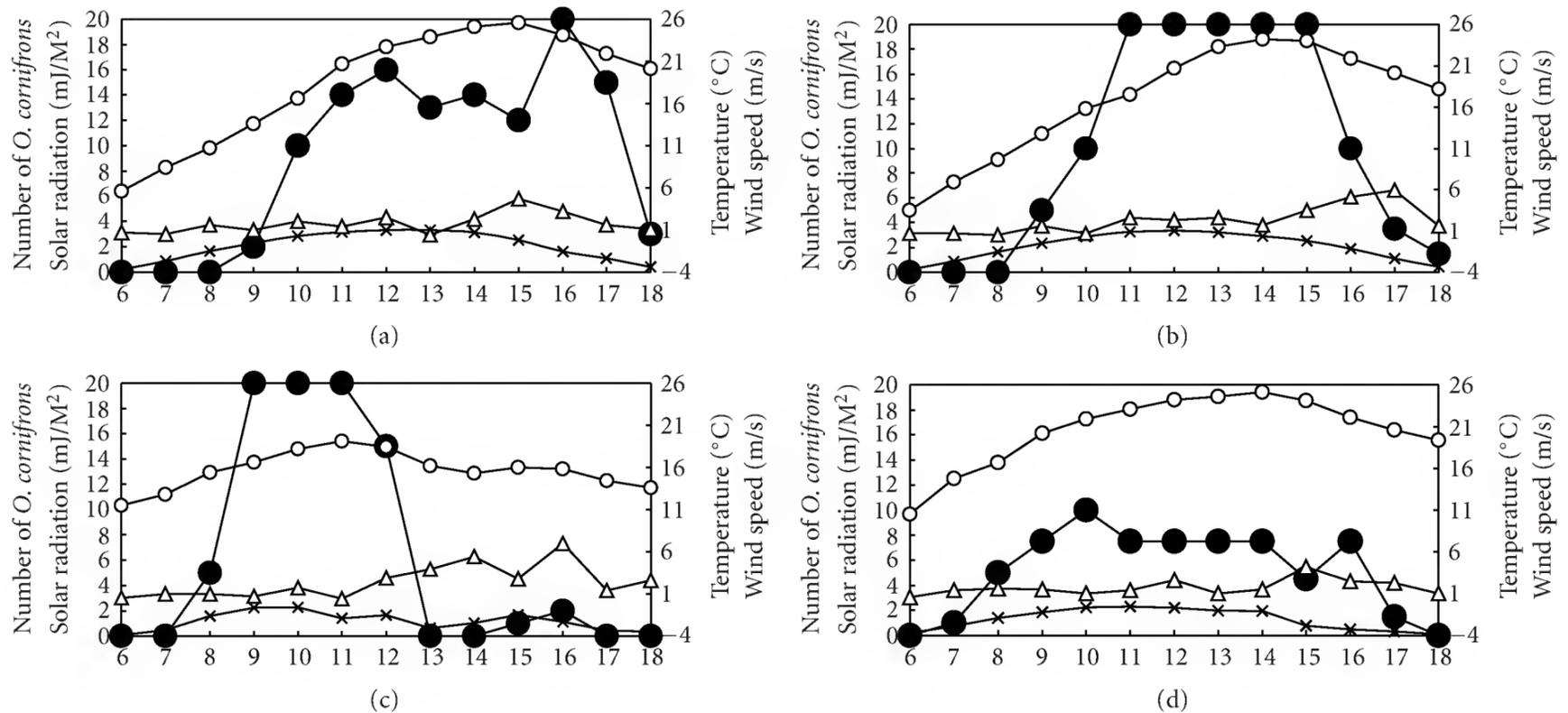


FIGURE 1: Activity of *O. cornifrons* (black circles) during 4 days at a nesting site in the Nagano orchard. Ambient temperature (°C, white circles), solar radiation (MJ/m², cross), and wind speed (m/s, triangles) are also shown. (a) 30th of April, 2007, (b) 29th of April, 2008, (c) 2nd of May, 2007, and (d) 1st of May, 2008.



FIGURE 2: Stamens and a pistil morphology of apple flowers. (a) "Starking Delicious", (b) "Fuji", (c) "Shinano Gold", (d) "Tsugaru", (e) "Dolgo", and (f) "Maypole".

still maintained at high levels up to 13.4 m from the nesting side (rate of fruit set (%) of 3.7 m, 7.6 m, and 13.4 m were 87.0%, 84.3%, and 72.6%, resp. (No. of fruits/No. of flowers of 3.7 m, 7.6 m, and 13.4 m were 849/976, 848/1006, and 863/1188, resp.)) but was significantly reduced at the 33 m point (rate of fruit set (%) was 43.9% (No. of fruits/No. of

flowers was 220/501)). Although we did not count the exact number of *O. cornifrons* visiting the 33 m point, they seemed to be scanty compared to those from 2006 to 2008. Moreover, since we cut down 82% of the "Maypole" pollinizers (14/17), pollen amounts available to the pollinizers might have been insufficient. These findings suggested that our previous

TABLE 1: Fruit set of “Fuji” fruit formed by open-pollination.

Flower morphology	Year	No. of flowers	No. of fruits	Rate of fruit set (mean \pm S.E.%)	No. of seeds/fruit (mean \pm S.E.%)
Anthers removed	2007	21	18	85.7	8.2
	2007	20	15	75.0	9.7
	2008	20	15	75.0	8.5
	2008	20	15	75.0	8.9
	2007-2008			77.7 \pm 2.7	8.8 \pm 0.7
Petals removed	2007	21	14	66.7	7.7
	2007	20	9	45.0	8.7
	2008	20	10	50.0	6.3
	2008	20	13	65.0	5.5
	2007-2008			56.7 \pm 5.4	7.1 \pm 0.7
Anthers and petals removed	2007	20	13	65.0	7.1
	2007	20	5	25.0	5.8
	2008	20	4	20.0	5.0
	2008	20	4	20.0	3.8
	2007-2008			32.5 \pm 10.9	5.4 \pm 0.7
Untreated	2007	21	21	100.0	8.2
	2007	20	18	90.0	8.9
	2008	20	17	85.0	10.2
	2008	20	20	100.0	9.0
	2007-2008			93.8 \pm 3.8	9.1 \pm 0.4

TABLE 2: Rate of dehiscere anthers within a balloon-stage “Maypole”.

No. of flowers	Year	No. of flowers having at least 1 dehiscere anther	Rate of flowers having at least 1 dehiscere anther (mean \pm S.E.%)	No. of anthers/flower	No. of dehiscere anthers/flower	Rate of dehiscere anthers/flower (mean \pm S.E.%)
10	2006	7	70.0	17.5	2.0	11.4
10	2007	4	40.0	19.4	1.1	5.7
10	2007	5	50.0	19.6	2.9	14.8
10	2007	4	40.0	19.1	1.6	8.4
10	2007	5	50.0	19.2	1.7	8.9
10	2007	3	30.0	18.1	0.3	0.02
			46.7 \pm 5.6			8.2 \pm 2.1

TABLE 3: Fruit set of “Alps-Otome” fruit formed by “Maypole” pollen carried by *Osmia cornifrons* male.

Distance from “Maypole”	Year	No. of flowers	No. of fruits	Rate of fruit set (mean \pm S.E.%)
1	2008	100	18	18.0
1	2009	365	129	35.3
	2008-2009			26.7 \pm 8.7
2	2008	100	19	19.0
2	2008	575	128	22.3
	2008-2009			20.7 \pm 1.7
3	2008	100	26	26.0
3	2009	480	140	29.2
	2008-2009			27.6 \pm 1.6
1*	2008	100	1	1.0
1*	2009	121	1	0.83
	2008-2009			0.9 \pm 0.1

* Control, no male.

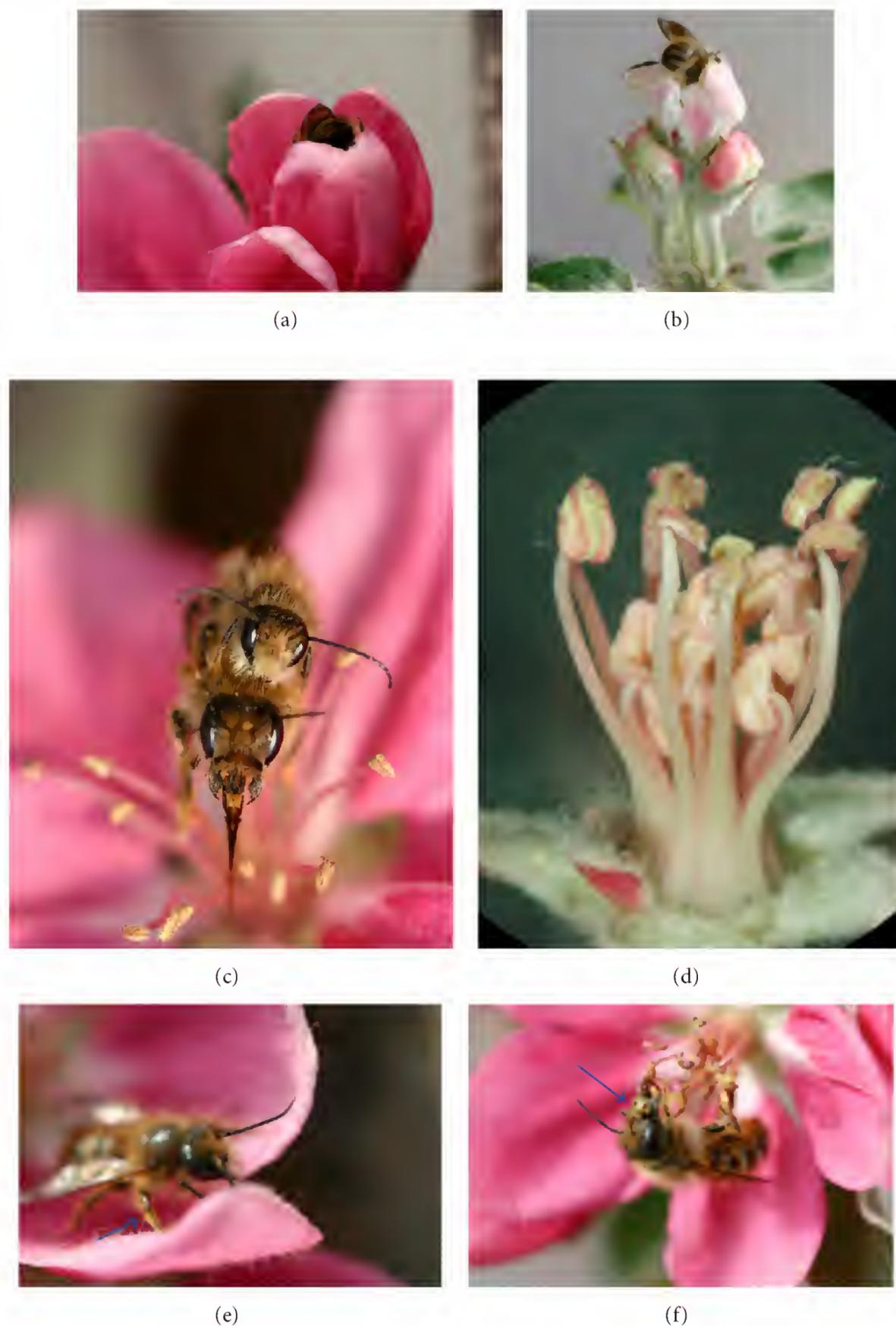


FIGURE 3: *Osmia cornifrons* visiting balloon stage flowers. (a) *O. cornifrons* visiting “Maypole”, (b) *O. cornifrons* visiting “Fuji”, (c) *O. cornifrons* male mounted on female drinking “Maypole” nectar, (d) Dehiscere anthers within a balloon-stage “Maypole” (Petals removed.), and (e) and (f) Pollen grains adhered to male’s leg (e) and female’s mouth (f).

pollinizers should have been planted not more than 10 m from commercial cultivars in an apple orchard.

3.2. Foraging Indicators of *Osmia cornifrons*. Previously, we found that *O. cornifrons* preferred pollen from “Delicious” flowers in spite of their considerable distance from the nesting side. As shown in Figure 2, the stamens of “Starking Delicious” (Sport of “Delicious”) flowers were arranged in an upright position compared to those of other pollinizers and cultivars [7]. The upright stamens of “Delicious” flowers

might be suitable for pollen collecting bees, such as *O. cornifrons*, since that made it easier to attach an abundance of pollen to their abdomen at one time. Since collecting pollen is the most important task for *O. cornifrons* females seeking to make pollen loaves for their larvae, it is also recognized that anthers, in addition to petals, also must be seen as important indicators for their visiting flowers. As shown in Table 1, fruit set levels (%) of “Fuji” (93.8%) were reduced by the removal of flower petals or anthers (56.7% and 77.7%, resp., in Table 1) and extremely reduced by the removal of

both anthers and petals (32.5% in Table 1). We observed *O. cornifrons* visiting flowers lacking either petals or anthers, but rarely approaching flowers lacking both petals and anthers. Bees could be navigated by U.V. light, and in the case of apple flowers, their anthers, pollen, and petals showed high levels of U.V. absorption (data not shown). As a result, *O. cornifrons* seemed to visit petals or stamens of flowers such as visible guidance.

3.3. Visiting Balloon Stage Flowers. We kept *O. cornifrons* at 4°C for a few weeks to adapt their foraging behavior to apple flowers. *Osmia cornifrons* males and females began to emerge at the apple orchard of the Nagano Fruit Tree Experiment Station ca. two weeks and one week earlier, respectively, than the flowering time of “Fuji”. They visited *Veronica persica* Poir., *Vicia angustifolia* L., *Taraxacum officinale* Weber, *Prunus avium* L., and *Pyrus commusis* L. in an effort to survive until apple flowering and then turned to visiting apple flowers once they bloomed.

We found that males and females visited “Maypole” and “Fuji” flowers at the balloon stage (Figures 3(a) and 3(b)). Some individuals visited “Maypole” flowers for 6 to 8 seconds. Females were mainly concerned with collecting pollen and drinking nectar, while the main purpose for males was randomly to search for females and drink nectar (Figure 3(c)). We investigated the conditions of anthers at the balloon stage of flowers. As shown in Table 2 and Figure 3(d), 46.7% of the stamens of balloon stage “Maypole” flowers had at least one dehiscent anther, and 8.2% dehiscent anthers at the balloon stage, suggesting that *O. cornifrons* would accumulate pollen on their bodies while visiting balloon stage flowers for 6 to 8 seconds. We found that both males and females picked up pollen grains around their mouth and legs (Figures 3(e) and 3(f)).

3.4. Male Contributions to Pollination. As already mentioned, we found that some males picked up pollen around their mouth and/or legs (Figure 3(e), unpublished results). Although as has been suggested, such accumulations made no contribution to pollination, since their foraging trips were mainly concerned with finding nectar and/or females, we considered that the pollen attached to a male’s body could incidentally result in the pollination and fertilization of apples. We investigated whether or not the pollination and fertilization of “Alps-Otome” only occurred by the intervention of *O. cornifrons* males. The experiment was carried out using three “Maypole” and three “Alps-Otome” covered by nets. As shown in Table 3, all “Alps-Otome” trees were fertilized by “Maypole” pollen carried by *O. cornifrons* males (20.7%–27.6% fruit set in Table 3), suggesting that males could serve as apple pollinators.

Acknowledgment

This research was supported by the Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries, Japan.

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Research Article

Two New Species of the Genus *Thoracophorus* Motschulsky, 1837 (Coleoptera: Staphylinidae, Osoriinae) with Remarks on Ecology of the Genus in the Neotropical Region

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Received 5 November 2009; Revised 8 January 2010; Accepted 1 February 2010

Academic Editor: Christopher Carlton

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The new species *Thoracophorus venezuelanus* and *T. silvaticus* are described. The ecological information from labels and that produced by more detailed studies in Central Amazonia indicate that tree canopies and tree trunks are main habitats of *Thoracophorus* species, and that many species might be associated with ants or termites.

1. Introduction

New species of the genus *Thoracophorus* have been found in recent collections of Volker Assing and colleagues of the Natural History Museum, Lawrence, Kansas.

A review on the species richness of the genus in the Neotropical region is given based on the numbers and species in my database, which includes 1186 specimens determined to species from different museums. Additionally, material collected by myself in 1971 and 1972 was analysed. This material was collected in three inundation forests near Manaus [1], but could not be identified to the species level at that time. Two years ago I received the rove beetle material from J. Adis (†) for identification; it was collected by different methods in a Varzea forest near Manaus, it also included two *Thoracophorus* species. Thus, this study has the following aims: (1) descriptions of new species and (2) analysis of the overall ecology of the genus and the specific ecology of single species.

2. Material and Methods

The material includes the new species derived from the collection of Volker Assing (VAC), Hannover, Germany, and from the Snow Entomological Museum Collections of the University of Kansas Natural History Museum, Lawrence, USA (SEMC). I thank V. Assing and J. S. Ashe (†), formerly

University of Kansas, for lending the material and for the deposition of some paratypes for my collection. Types are deposited in these collections and in my own collection (UIC). The types deposited now in private collections will be deposited in public collections later. Length was measured along the middle of the tagmata and width across widest part of tagmata. Total length measures cover the fore-body and length of the abdominal tergites without intersegmental spaces.

Ecological information was derived from the 1186 *Thoracophorus* specimens included in my database. Specimens were identified over the past 25 years and originated from different museums and private collections [2–4]. In addition, the rove beetle material of J. Adis was studied. This material was collected between April 1981 and March 1982 using different methods on Ilha de Marchantheria (58°58'W, 3°15'S), an island in the Rio Solimões approximately 10 km upstream from the inflow of the Rio Negro. The material included 16 samples collected at 4 sites by canopy fogging, 5 replicate samples at two sites each by trapping at tree trunks, 21 replicate samples of a Kempson heat extraction of litter, 3 ground emergence traps containing a pitfall trap, and 7 pitfall traps [5].

My own samples that were collected during the dry season between September 1971 and April 1972 in three inundation forests near Manaus were reinvestigated. These forests are directly influenced by the white waters of the

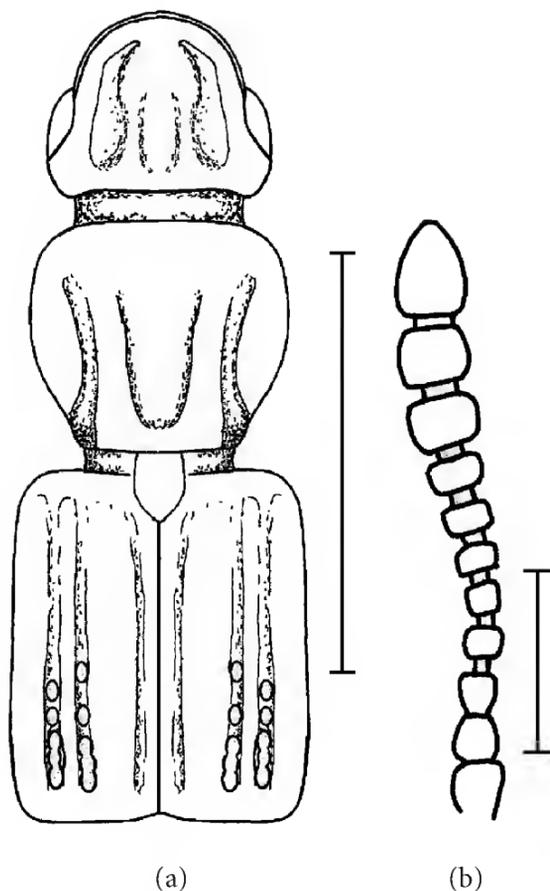


FIGURE 1: Fore body (a), antenna (b) of *Thoracophorus simplex* (scale bar (a) 0.5 mm, (b) 0.1 mm).

Rio Solimões (Ilha do Curarí), by the mixed waters (Lago Janauarí), and by the black waters of the Rio Negro (Rio Tarumã Mirím). Further information about sampling dates and methods is given in Irmeler [1]. Abundance of *Thoracophorus guadalupensis* Cameron, 1913 was measured by hand-sorting from 0.1 m² leaf litter collected at sites located in a flooding gradient in the three forests in this investigation.

3. Description of the New Species

3.1. *Thoracophorus venezuelanus* n.sp. (see Figures: 2(a)–2(c), 4(c)–4(d)).

Material. Holotype: Venezuela: Carabobo, Mun. Bejuma, El Maquero (10°15.90'N, 68°17.60'W), 1200 m elevation, male, 21.01.2007, leg. L. Brachat (VAC). Paratypes: 1 male, 4 females with the same data as for the holotype (VAC, UIC).

Diagnosis. *T. venezuelanus* is closely related to *T. simplex* (Figures 1(a) and 1(b), 4(a) and 4(b)) due to its small size and the weakly developed elytral carinae, pronotal depressions, and head elevations. *T. venezuelanus* is slightly larger than *T. simplex* Wendeler, 1930 that is only 1.5 mm in size. Distinct differences are found in the antennae. The penultimate antennomeres in *T. simplex* are only twice as wide as long, whereas they are three times as wide as long in *T. venezuelanus*. Moreover, the elytra are totally polished in *T. simplex*, but matte in *T. venezuelanus*. The aedeagus provides no valuable differentiating characters.

Description. Length: 1.8–2.0 mm. Colour: light red, head and pronotum slightly darker. Head: 0.25 mm long, 0.33 mm wide; eyes distinctly prominent; temples short and abruptly narrowed to neck; disc with a moderately deep depression

on each side of the middle; finely punctate, but with deeper and larger punctures in paired depressions; without usually developed pair of longitudinal elevations at centre of disc; surface shiny and with extremely weak longitudinal microsculpture. Antennae short; as long as head and half of pronotum combined; first two antennomeres thick; following four antennomeres distinctly smaller; 3rd antennomere conical, 4th to 6th antennomere quadrate; antennomeres 8 to 10 at least 3 times as wide as long. Pronotum: 0.27 mm long, 0.33 mm wide; sides nearly parallel in anterior half; front angles obtusely rounded; in dorsal aspect, lateral margin visible in posterior half only; lateral furrows weakly depressed and nearly obsolete in the anterior half; central depression also weakly developed; with extremely fine punctation and longitudinal microsculpture; surface shiny except in the lateral and central depressions with a slightly more distinct microsculpture and thus matter surface. Elytra: 0.48 mm long, 0.37 mm wide; with fine longitudinal carinae; intercarinate space with dense and deep microsculpture; surface matte.

Etymology. The specific name is derived from the country in which the species was found.

3.2. *Thoracophorus silvaticus* n.sp. (see Figures: 3(a)–3(c), 4(e) and 4(f)).

Material. Guyana: Region B, Iwokrama forest, Turtle Mt., base camp (4°43.5'N, 58°43.5'W), 50 m elevation, male, 1 Jun. 2001, leg. R. Brooks, Z. Falin, #GUY1BF01 100, collected under bark (holotype, SEMC). Paratypes: Peru: Madre de Dios, Pentiacolla Lodge, 8 km NE Mirador, Alto Madre do Dios River (12°38.23'S, 71°16.23'W), 950 m elevation, female, 24. Oct. 2000, leg. R. Brooks, #PERU1B00 083, collected under bark (SEMC); Suriname: Marowijne, Palumeu (3°20.56'N, 55°26.18'W), ca 169 m elevation, male, 8. Jul. 1999, leg. Z.H. Falin, #SUR1F99 182, collected in splintered log (pyrethrum fogging) (UIC); Saramacca West Suriname Road, 178 km, WSW Zanderij Airport (4°59.6'N, 56°18.48'W), 25 m elevation, female, 13. Jun. 1999, leg. Z. Falin, #SUR1F99 068, collected under bark (UIC).

Diagnosis. *T. silvaticus* can be distinguished by its characteristic colour and the triangular shape of the head. It resembles *T. bruchi* Bernhauer, 1933, *T. sahlbergi* Irmeler, 1985, and *T. susannae* Irmeler, 2001 by the shape of the pronotum with the strong emargination in front of the posterior angles. The overall shape is similar to *T. columbinus* Irmeler, 2001, but the pronotal emargination in *T. columbinus* is less developed. Moreover, differences to *T. susannae* are also found in the more or less even lateral pronotal margin, although an indistinct denticulation can be developed in *T. silvaticus*. However, it is never as distinct as in *T. susannae*.

Description. Length: 2.4 mm. Colour: light red; head at temples darker, on small parts nearly black; elytra darkened to posterior edge; legs and antennae yellow. Head: 0.25 mm long, 0.40 mm wide; shaped like a triangle with slightly rounded sides; temples behind eyes short, tooth-like prominent; directly narrowed to distinct neck; eyes prominent; central elevations indistinct and short; lateral elevations absent; without punctation, but with distinct and dense

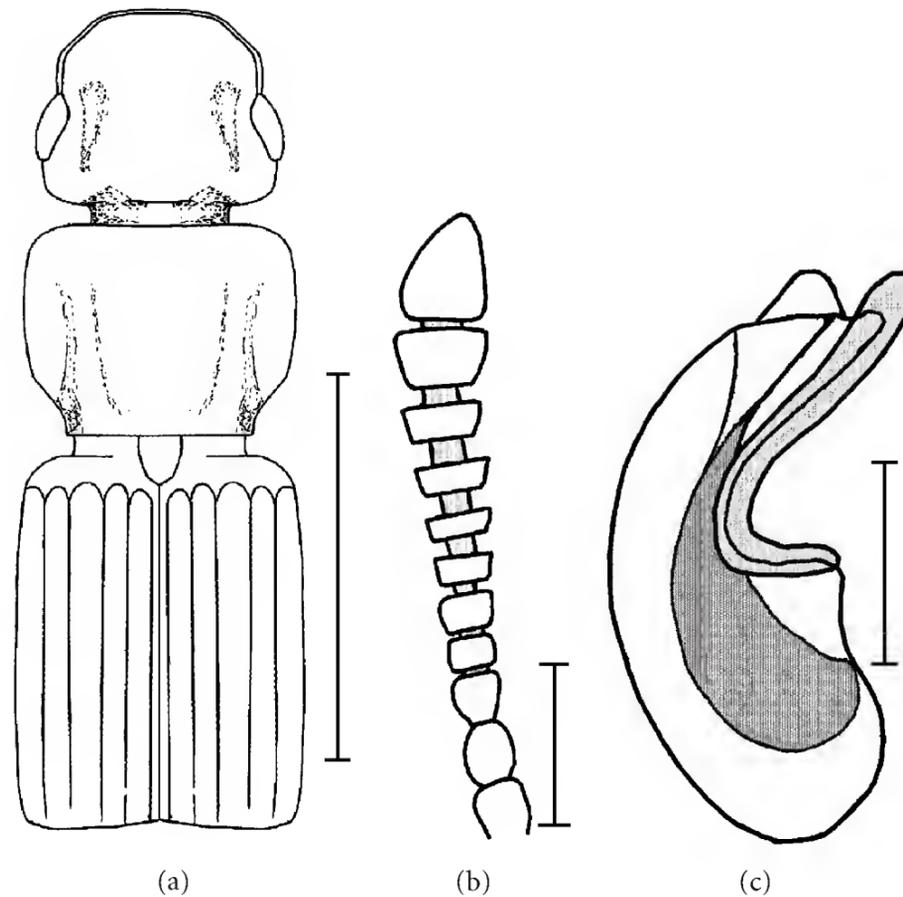


FIGURE 2: Fore body (a), antenna (b), and aedeagus (c) of *T. venezuelanus* (scale bar (a) 0.5 mm, ((b and c) 0.1 mm).

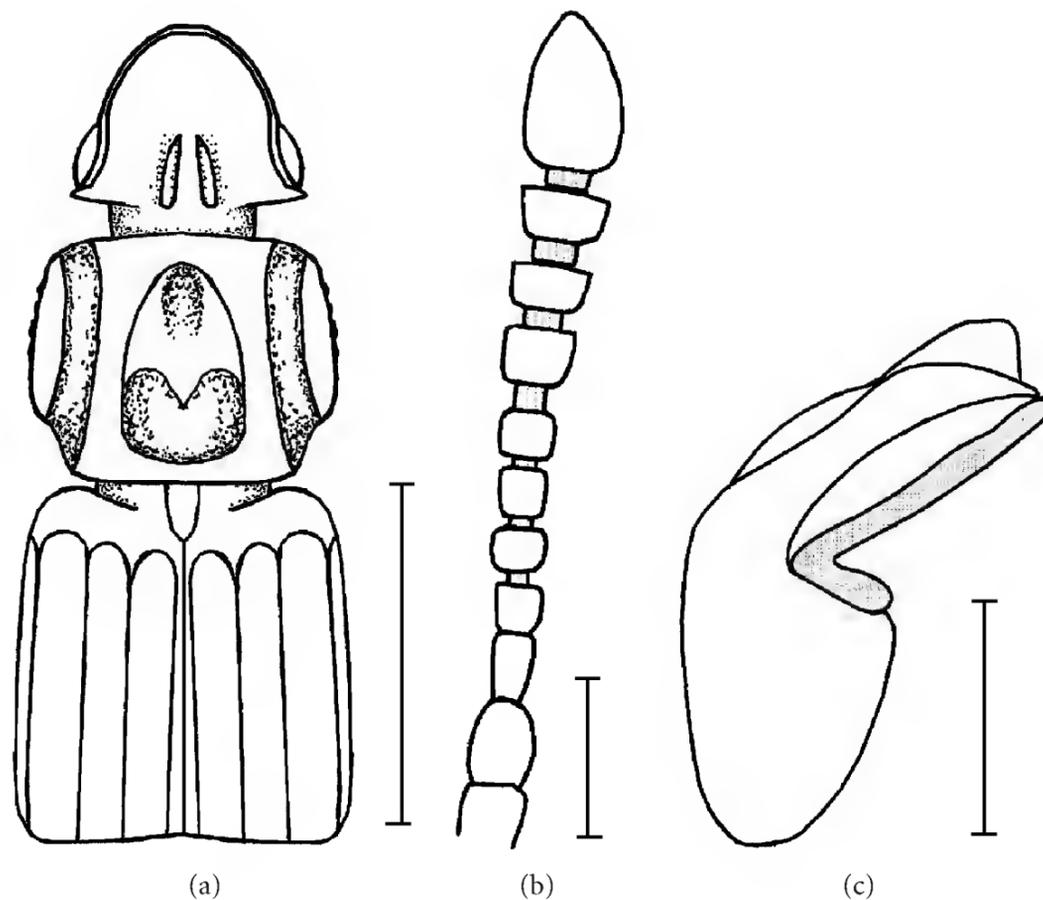


FIGURE 3: Fore body (a), antenna (b), and aedeagus (c) of *T. silvaticus* (scale bar (a) 0.5 mm, ((b and c) 0.1 mm).

microsculpture; surface matte. *Antennae* as long as head and half of pronotum combined; with antennomeres 1 and 2 thicker than following antennomeres; 2nd antennomere oblong, 3rd conical; antennomeres 4 to 6 quadrate, following antennomeres increasing in width and wider than long; penultimate antennomere twice as wide as long. *Pronotum*: 0.38 mm long, 0.50 mm wide; widest in posterior third;

slightly narrowed to anterior angles, anterior angles obtusely rounded; a deep emargination in front of posterior angles; with deep lateral furrow and distinct central depression; within central depression an indistinct elevation; without punctation, but with distinct and deep microsculpture; surface matte. *Elytra*: 0.50 mm long, 0.55 mm wide; with three carinae on disc; without punctation, but with distinct

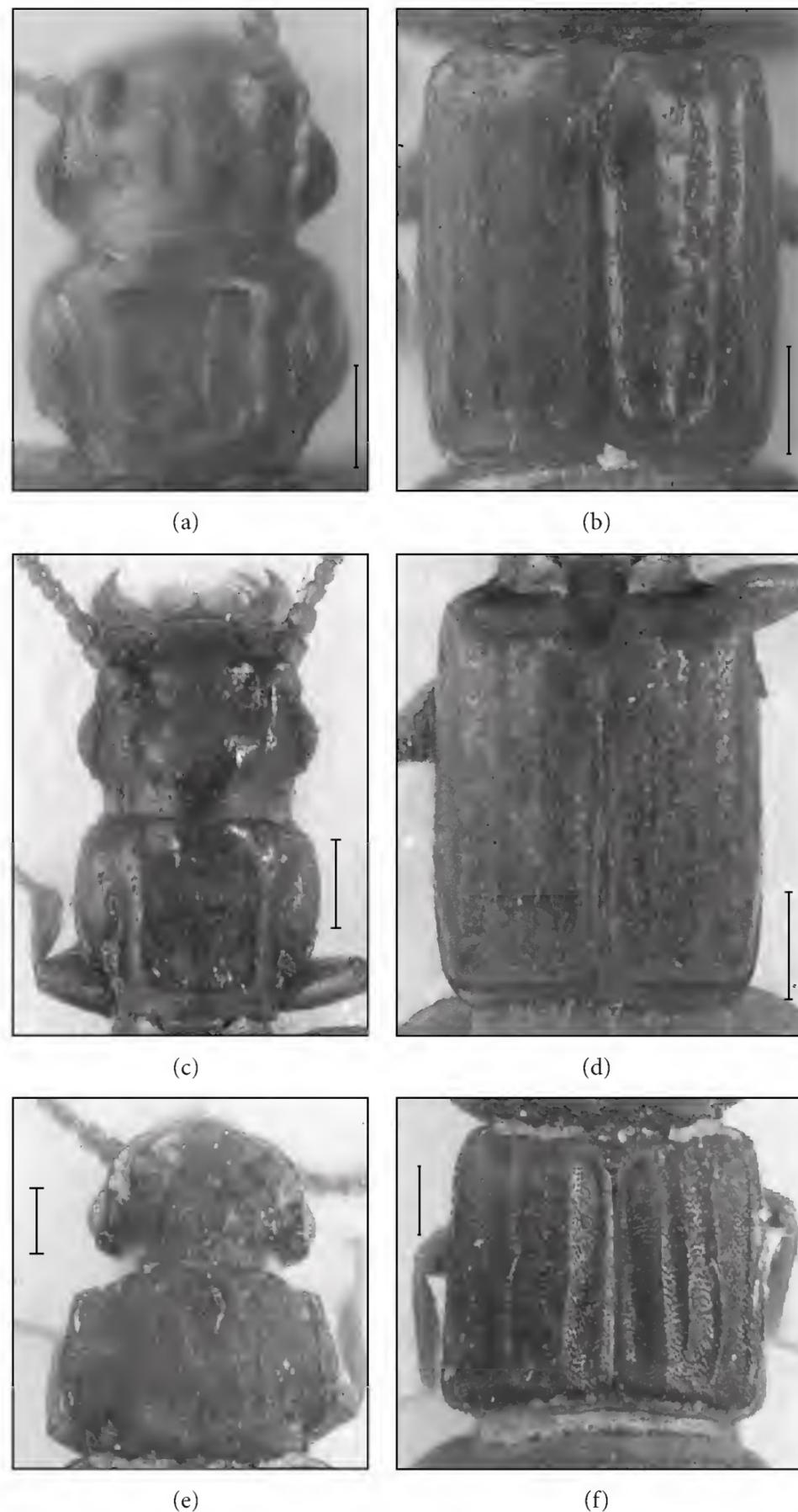


FIGURE 4: Surface of head, pronotum (a) and elytra (b) of *Thoracophorus simplex*; surface of head, pronotum (c) and elytra (d) of *T. venezuelanus*; and surface of head, pronotum (e) and elytra (f) of *T. silvaticus* (scale bar: 0.1 mm).

and dense microsculpture, surface matte; microsculpture slightly less dense than on pronotum and head and, thus, surface slightly shiny. *Abdomen* without punctation, but with dense microsculpture, surface matte as on pronotum and head; tergites without striate structure, even at base of tergites; only with short and sparse setae laterally. *Aedeagus* form nearly rectangular angles between basic part and apical part; paramera as long as central lobe.

Etymology. The specific name is derived from the same Latin word meaning “living in the woods.”

4. Remarks on Ecology

Thirty-six species of the genus *Thoracophorus* from the Neotropical region are known at present, including the two newly described ones here. According to Herman [6], 45 species of the genus are known worldwide. Thus, the Neotropical region is by far the most species-rich region in the world which, however, might be due to a better investigation status of the continent than of the African or Indo-Malaysian regions. Nevertheless, we are far from a more

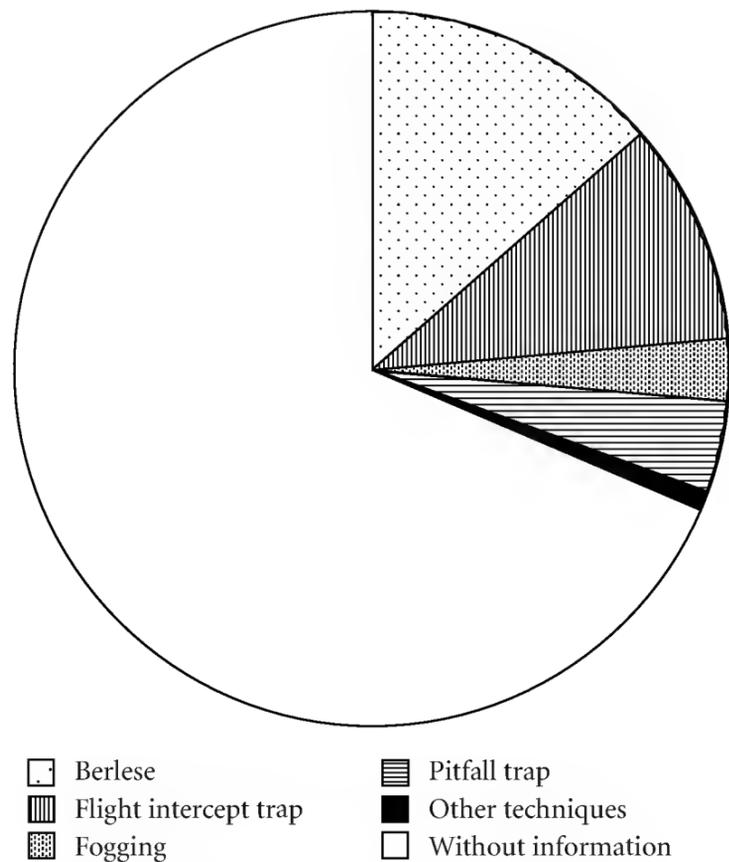


FIGURE 5: Fraction of specimens caught by different methods.

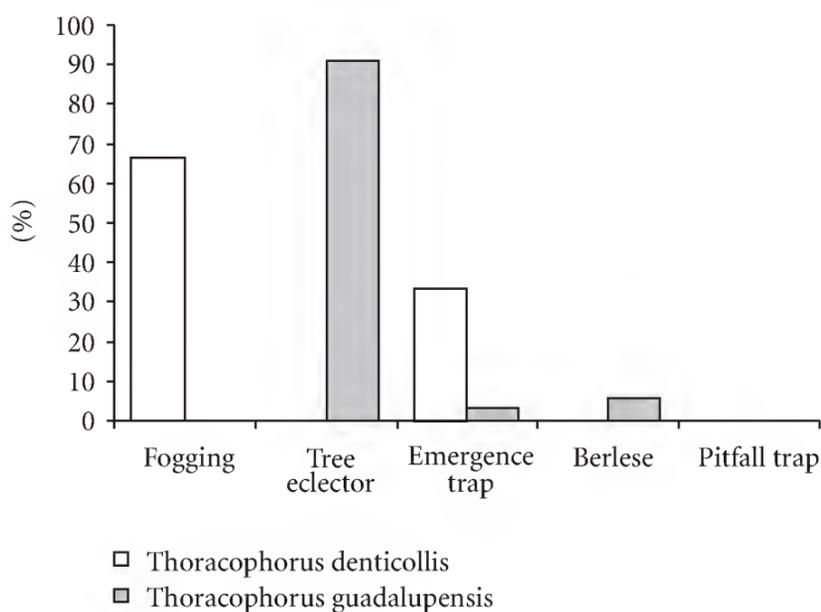


FIGURE 6: Fractions of specimens found by different methods in the Central Amazonian Varzea forest of Ilha de Marchantheria of the two *Thoracophorus* species.

or less complete knowledge of the species richness in the Neotropics.

The ecological requirements are known from only a few species. Regarding the information given by collectors on the pinned labels, nothing is known about 19 species except the location and sampling date. Two species, *T. brevicristatus* Horn, 1871 and *T. heyeri* Wasmann, 1902, live in nests of termites. *T. brevicristatus* can be regarded as an inquiline of *Neotermes* termites [7] and *T. heyeri* was found in nests of the termite *Eutherms fulviceps* [8]. Extraction of leaf litter and flight intercept trapping were the overall most efficient methods to capture *Thoracophorus* species (Figure 5). However, this differs between species. If

specimens with no information on their collecting methods are omitted, *T. aequalis* Sharp, 1887 (86%), *T. exilis* (Erichson, 1840) (78%), *T. filum* Sharp, 1887 (67%), and *T. sculptilis* (Erichson, 1840) (74%) were mainly collected by flight intercept traps, whereas *T. distinguendus* Irmeler, 2005 (79%), *T. guadalupensis* (51%), and *T. susannae* (63%) were more efficiently caught by Berlese extraction from leaf litter.

The main habitat of the species can be derived from the specific methods that produced the highest numbers of species (Figure 6). According to the investigations performed by J. Adis in the Central Amazonian Varzea with different methods, *T. denticollis* (Erichson, 1840) ($n = 3$) was found by canopy fogging ($n = 2$) and trapping at tree trunks ($n = 1$), whereas *T. guadalupensis* ($n = 784$) was mainly caught by trapping at tree trunks, but was also taken in low abundances by litter extraction or by litter emergence traps, and even very rarely in pitfall traps ($n = 1$). It can be supposed that both species mainly inhabit the canopy or tree trunks and only rarely the soil floor. This is supported by investigations made by J. C. Hurtado in the terra firme forest of Reserva Ducke, located about 40 km north-east of Manaus, where several specimens of *T. guadalupensis* were found from March to May 1996 on *Eschweilera wachenheimii* (Benoist) Sandwith, 1932 and *Corythophora alta* Knuth, 1939 trees (material in Natural History Museum, London).

The most common species, *T. guadalupensis*, showed an abundance ratio of 27 : 8 : 0 in the investigations in 1971 and 1972 in three inundation forests of white water, mixed water, and black water, respectively. *Thoracophorus guadalupensis* obviously preferred the nutrient and sediment rich site at Ilha de Curarí in the Varzea of the Rio Solimões. Regarding the seasonal occurrence of the species at Ilha do Curarí, most specimens occurred in the late half of the dry season in the upper zones of the Varzea forest up to 3 m inundation during the immersion period (Figure 7). This corresponds to an emersion period from mid-September to mid-March. An equally seasonal and zonal occurrence was found in the mixed water inundation forest at Lago Januari. In sites with longer and deeper immersion and shorter emersion periods, respectively, no specimens were found. According to the hand-sorting results, the abundance on the forest floor ranged from 3 ind·m⁻² to 14 ind·m⁻² at Ilha de Curarí and from 0.9 ind·m⁻² to 7.7 ind·m⁻² at Lago Januari during the late half of the emersion period.

5. Discussion

The biology of Neotropical *Thoracophorus* species is largely unknown, with the exception of two species that are certainly inquilines of termites [7, 8]. Moreover, the sampling methods used to study these species provide no additional information about the living habitat of most species as records are mainly based on flight intercept traps or Malaise traps. The high number of records by Berlese extraction shows that the forest floor is used by many species, which is in concordance with the predatory Osoriinae genus *Tannea* that lives in the forest floor litter [9]. In contrast, the fungi-feeding genus

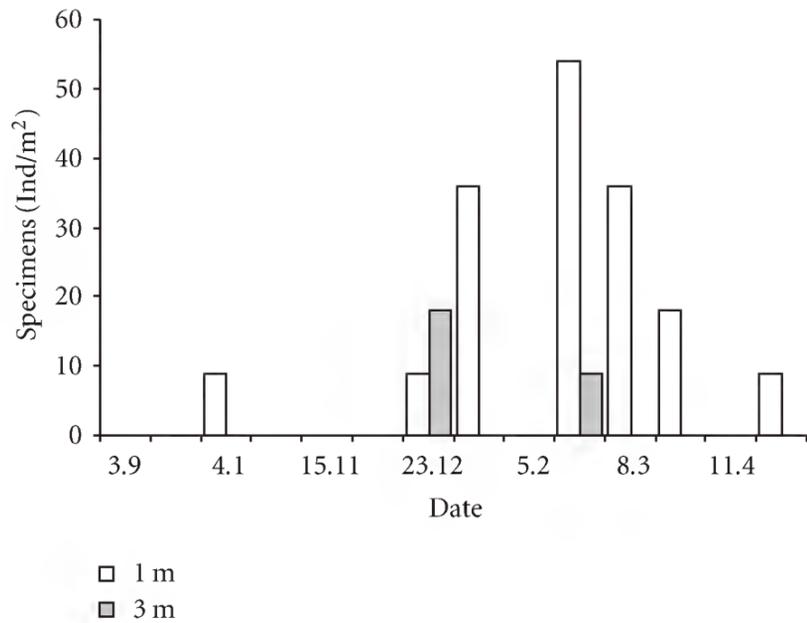


FIGURE 7: Abundance of *Thoracophorus guadalupensis* on forest floor during the dry period in the Varzea forest of Ilha de Curari at sites inundated 1 or 3 m during immersion period. No specimens were found at 4 m and 5 m immersed sites.

Lispinus nearly exclusively inhabits the under-bark habitat [10].

The example of *T. guadalupensis* indicates that species found in the litter layer mainly live on tree trunks, but apparently come to the forest floor in lower numbers. The lack of records from the tree trunk habitat in many species probably indicates the low investigation status of this habitat. Thus, tree trunks may be regarded to be a main habitat for species found on the ground as well. A different species from the Central Amazon, *T. denticollis* (Erichson, 1840), supports this hypothesis: the species was previously known from the type specimen from Puerto Rico, but was mainly found in the tree canopy of the Central Amazonian forest. The limited records of this species and the far distance between the two locations can be attributed to its canopy habitat which has hardly been investigated anywhere. Indeed, the fact that many Neotropical species are known from only a few specimens from flight intercept traps indicates that the upper tree zones in the rain forest are important habitats for many *Thoracophorus* species. Insects of upper zones in forests are generally organisms that fly well, as they have to move from tree to tree. During their flights, the species are generally caught by flight intercept traps or similar trapping methods. It is possible that more species of *Thoracophorus* are associated with ants or termites living in the canopy, on tree trunks, or on the ground of rain forests. In this case, records of *Thoracophorus* species on the soil floor can be referred to the larger habitat of their host species. Unfortunately, nothing is known about the host specificity of the species. The European *Thoracophorus corticinus* Motschulsky, 1837, is associated with the ant species *Lasius brunneus* [11]. However, the authors underlined the diversity of habitats and the opportunistic use of resources in the genus. If the canopy and tree trunk habitats are two of the main habitats of *Thoracophorus* species in the Neotropics, we may assume that the number of species will dramatically increase if these

rarely-studied habitats will be investigated with more effort in the future.

Acknowledgment

This study is dedicated to the author's colleague and friend Professor Joachim Adis, who put his material on Central Amazonian rove beetles to the author's disposal and who passed away too early to publish the results together with him.

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Research Article

Patch Departure Behavior of Bumble Bees: Rules and Mechanisms

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Received 1 August 2009; Accepted 6 February 2010

Academic Editor: Koos (J. C.) Biesmeijer

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I present an increment-decay model for the mechanism of bumble bees' decision to depart from inflorescences. The probability of departure is the consequence of a dynamic threshold level of stimuli necessary to elicit a stereotyped landing reaction. Reception of floral nectar lowers this threshold, making the bee less likely to depart. Concurrently the threshold increases, making departure from the inflorescence more probable. Increments to the probability of landing are an increasing, decelerating function of nectar volume, and are worth less, in sequence, for the same amount of nectar. The model is contrasted to threshold departure rules, which predict that bees will depart from inflorescences if the amount of nectar in the last one or two flowers visited is below a given level. Field tests comparing the two models were performed with monkshood (*Aconitum columbianum*). Treated flowers contained a descending series of nectar volumes (6 to 0 μL of 30 % sucrose solution). The more nectar that bees encountered in the treated flowers, the more likely they were to remain within the inflorescence after subsequently visiting one to three empty flowers. I discuss the differences between rules and mechanisms in regard to cognitive models of foraging behavior.

1. Introduction

For the majority of flowering plants, successful reproduction depends on a mutualism with insect pollinators. As with all such coevolved interactions, the two parties are motivated by self-interest: the plant is provided with an efficient means of pollen transfer, while the pollinator receives energy and nutrients in the form of floral nectar and pollen. The fine details of this interaction include factors such as the number of flowers that the pollinator should visit within an inflorescence before departing and moving to another plant of the same species. Plants should maximize the amount of pollen exported to the stigmas of conspecifics, while simultaneously minimizing the level of geitonogamous self-pollination. The pollinator, meanwhile, should behave so as to maximize its net rate of energy gain, and should stay within an inflorescence until it is more profitable to depart and move to another. Pollinator departure from inflorescences thus falls within the scope of patch departure in foraging theory, a central sub-discipline of behavioral ecology [1].

Evolutionary study of patch departure began with the marginal value theorem (MVT) [2], which specifies how

foragers should exploit patches in order to maximize the long-term net rate of energy gain. However, the MVT itself does not specify a realistic departure rule or policy [3, 4]. This is due to the MVT's assumption of complete information: the forager is in effect omniscient, knowing all the relevant data about a patch before entering it. Proximal or "cognitive" models of patch departure should produce roughly the same decisions as would the MVT, while making realistic use of available environmental cues.

Theoretical work has shown how information gained while foraging within patches can be used to construct an optimal departure policy [3, 5, 6]. A general, flexible formalism makes use of Bayes' Theorem to derive optimal departure rules given various distributions of the number of prey within patches [3, 7–11]. Graphical representations of several such models are shown in Figure 1. Following Stephens and Krebs, I refer to these models as increment-decay processes, due to the continuous dynamics of the expected remaining patch time [12]. These models are interesting for reasons other than just the possibility of stochastic optimization. Notably, the incrementing and decrementing dynamics have neurobiological analogues, in processes such as habituation

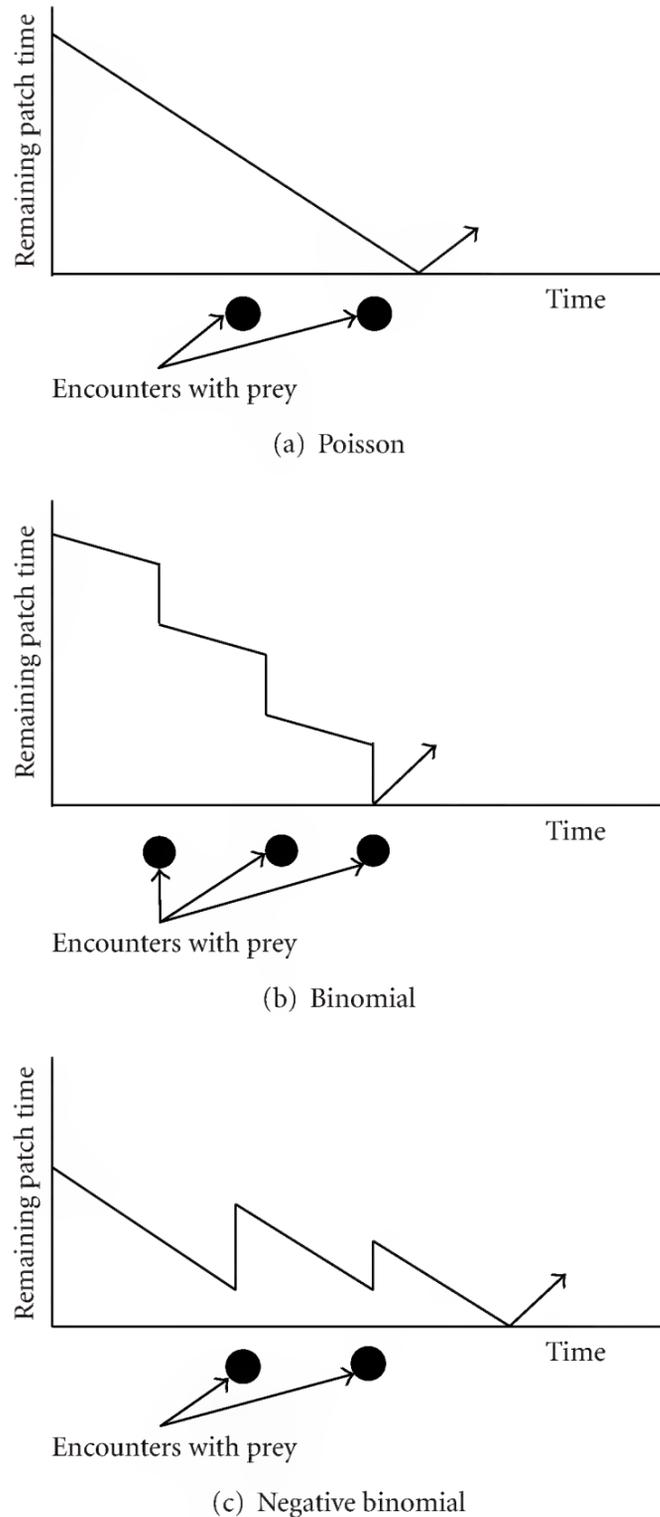


FIGURE 1: Graphical Representations of several Bayesian patch departure rules [7, 12], for three distributions of the number of prey within patches. The ordinate represents expected remaining time in the patch. Solid circles represent encounters with prey. (a) For the Poisson distribution, prey captures yield no information and departure occurs after a fixed time independent of the number of prey encountered. (b) For the binomial distribution, each prey capture decrements expected remaining patch time. (c) For the negative binomial, each prey capture increments expected remaining patch time, but each increment in sequence is smaller.

and sensitization to stimuli. Waage [13] first suggested that the parasitic wasp *Venturia* (= *Nemeritis*) *canescens* uses an increment-decay process for searching and departing from patches of its host.

One can see that, in outline, an increment-decay model could describe the behavior of bumble bees or other nectarivores visiting multiple-flowered plants. An inflorescence can be considered as a patch, and each encounter with a prey

(in this case, a flower that contained nectar) would affect the expected remaining number of flowers to be visited on the plant before departing. However, such models have only very rarely been explored in regard to bee foraging behavior [14–16]. The reason is that departure from inflorescences can often be analyzed via a discrete version of the MVT that applies to the situation of overlapping encounters, meaning that the forager meets more than one prey at a time [17, 18]. Many plants have an inflorescence in which the flowers are arranged vertically on a central stem, blooming sequentially from the bottom upward. As a result, flowers near the bottom often contain more nectar than others higher on the stem [19]. To maximize the long-term net rate of energy gain, bees should begin at the bottom of such an inflorescence and work upward. At some point the diminishing amounts of nectar in higher flowers would make it more profitable to depart the inflorescence and move to another plant. We should then observe that bees often depart from such vertical inflorescences before visiting all available flowers, and this has been observed in the field [19–22] and in the laboratory [23]. Thus a number of authors have proposed the following “threshold departure rule”: the bee should depart the inflorescence when

$$E(S) < E(L), \quad (1)$$

where $E(S)$ (stay) is the expected profitability from the next flower visited within the current inflorescence, and $E(L)$ (leave) is the expected profitability from the first flower visited after departure [24–28].

The original threshold departure rules are now generally thought to be too simplistic a description of patch departure in bumble bees [1, 14, 15]. In this paper I present an increment-decay model, similar to those that have been proposed for parasitoids, but specifically tailored to bumble bees. I present the results of field experiments designed to contrast the model’s predictions with those of the threshold departure rules.

2. The Model

The increment-decay model for patch departure presented here was originally applied to bumble bee foraging at a higher level, the situation in which there is patchiness in nectar within large meadows of the relevant plant species, but no discrete patches as such [14]; it was soon realized that a similar model in outline could apply to departure behavior within inflorescences.

The first assumption is that bumble bees land on inflorescences and begin to probe the flowers for nectar if the set of stimuli presented by the inflorescence are sufficient to release a stereotyped behavioral pattern, hereafter called the landing reaction [29]. In vector notation, let \mathbf{s} be a vector of stimulus intensities from various modalities, including visual, olfactory, and tactile, and let \mathbf{w} represent a vector of weightings for each $s_i \in \mathbf{s}$; the scalar product $z = \mathbf{s}^T \mathbf{w}$ defines a real number z that is mapped onto a decision function $f(z)$ that returns the conditional probability of landing given z ,

$P(\text{Land} | z)$. The simplest form of $f(z)$ would be the step function

$$f(z) = \begin{cases} 1, & z \geq z^*, \\ 0, & z < z^*, \end{cases} \quad (2)$$

where z^* is a threshold level necessary to evoke the landing behavior. In (2) the response is all or none for fixed z ; modification to make the response probabilistic may easily be done by making $f(z)$ a sigmoid function:

$$f(z) = \frac{1}{1 + \lambda \exp(-\beta(z - z^*))}. \quad (3)$$

Equation (3) approaches a step function in the limit as $\beta \rightarrow \infty$. Since (2) and (3) return a conditional probability, we may use for $f(z)$ any function that is also a distribution function of a random variable; thus the requirements are that $f(z)$ is right-continuous, is nondecreasing, approaches 0 in the limit as $z \rightarrow -\infty$, and approaches 1 in the limit as $z \rightarrow \infty$.

The above statements are an extremely simplified description of a cognitive system that integrates sensory information and initiates behavioral output. Note that the weightings to various stimuli presented by the flowers may include highly negative weightings, making the bee less likely to land; such stimuli include scent marks left by other bees, holes in nectar-robbled flowers, or marks on flower petals made by bees' tarsal claws. In addition, the measures of and the weightings to these stimuli may be both dynamic and varied among individuals. In other words, the inputs $s_i \in \mathbf{s}$ and the weights $w_i \in \mathbf{w}$ are not assumed to be fixed, but may change due to learning, or may vary due to individual differences among bees: for example, visual accuracy can vary with bee head size [30].

The core assumption of the model is that the threshold parameter z^* (or equivalently, the function $f(z)$ itself) is a dynamic variable, continually changing with time and in the light of recent experience. The probability that a bee will probe the next flower or will depart the inflorescence thus depends on how $f(z)$ changes with time and experience. Finding nectar causes z^* to decrease, making the bee more likely to probe the next flower, and thus incrementing the expected remaining time within the inflorescence. Concurrently, I assume that z^* spontaneously increases, so that the expected remaining time within the inflorescence decays between nectar encounters.

The second assumption is that there are maximum and minimum values of z^* , and these are reflecting boundaries. The minimum is automatically reflecting since z^* increases continually. Upon reaching its maximum, I assume that the threshold then lowers until the next nectar encounter; if the bee has departed the inflorescence at that point, the probability of flower stimuli evoking the landing reaction increases monotonically during interplant travel. Thus the longer the distance to the next plant, the lower will be the threshold z^* upon encountering the next inflorescence. This means that bees will tend to stay longer within inflorescences the greater the average distance between plants. This is

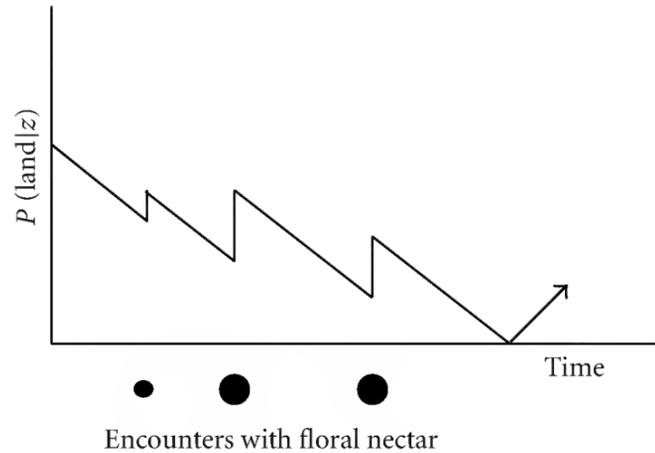


FIGURE 2: A graphical interpretation of the increment-decay model for bumble bee patch departure. The conditional probability of landing on the next flower given the weighted stimuli sum z , $P(\text{Land} | z)$, is plotted as a function of time. The probability declines monotonically with time. If the bee finds nectar within a flower, the probability increases in proportion to the nectar volume. Solid circles represent reception of nectar, with the radius proportional to the nectar volume. Note that for the same amount of nectar the amount of incrementing is less in sequence, following (4) in the text. When the threshold z^* reaches its maximum value, it then spontaneously decreases, increasing the landing probability during interpatch travel (arrow).

a fundamental prediction of the MVT, here produced via a mechanism similar to that proposed by Ollason [31].

The above assumptions can be interpreted graphically as an increment-decay process (Figure 2). Each reception of nectar (solid circles) decreases z^* , thus increasing the probability of landing on the next flower, increasing the expected remaining patch time by an amount proportional to the volume of nectar. Concurrently, z^* increases between nectar encounters, meaning that the expected remaining time within the inflorescence decays.

The dynamics of expected remaining patch time resemble the potential function of McNamara [6], with several important differences. Here the processes of decay and increment affect a threshold response to stimuli, with the result that the effects of nectar reception may be spread across several discrete patches, for example, flower groupings within the same plant or on adjacent plants [32, 33]. In the model the decision to depart is influenced by possibly many attributes of inflorescences besides the standing crops of nectar. This explains, for example, the observation by Pyke that bees stay longer within larger inflorescences of *Aconitum columbianum* even when these contain no more nectar than smaller inflorescences [19]. Larger inflorescences will have a greater value of z , all other things being equal.

Several points should be clarified concerning the decay process. In Figure 2 the rate of decay is constant for illustrative purposes, but in general it may be nonlinear, following an exponential or hyperbolic trajectory, for example. The increments are shown happening instantaneously, as in other such models (Figure 1); in reality each flower visit includes a handling time. We may keep the convenient form shown by assuming that the increments represent the net effect of increase due to nectar minus decay incurred during the time of the flower visit. This allows for the possibility that a flower

visit may actually result in a net decrement in expected remaining patch time, if the handling time for that flower is long enough. In addition, the model to this point does not explicitly specify the probability of departure from the whole inflorescence, which may be less than 1 given that the bee does not land (i.e., bees may reject the current flower, but stay within the same inflorescence). The conditional probability of departure given z may be specified by any nonnegative function of z bounded above by $1 - P(\text{Land} | z)$.

Reception of energy in the form of floral nectar lowers the threshold z^* , thus incrementing the expected remaining patch time. In the formal Bayesian foraging models cited above, the increments from prey capture have been constant or limited to a small number of values [3, 8, 10]. In the current model the “prey” is a continuous variable, an amount of energy depending on a volume of nectar and the nectar sugar concentration. The change in z^* will be given by a function ϕ that has as arguments the volume of nectar (given a constant sugar concentration) and other information, such as the order of encounter of flowers within the inflorescence. I assume that $\phi = \phi(V, k)$ is a function of at least two arguments, the volume of nectar V in the flowers, and the order of their encounter within the inflorescence, k . I make the following assumptions for the form of $\phi(V, k)$:

$$\frac{\partial \phi}{\partial V} > 0, \quad \frac{\partial^2 \phi}{\partial V^2} < 0, \quad \frac{\partial \phi}{\partial k} < 0, \quad (4)$$

where V is the volume of nectar and $k = 1, 2, \dots, n$ is the order of encounter, with $k = 1$ being the first flower encountered after the threshold has attained its maximum and thus begun to decrease. The first two partial derivatives mean that increments are an increasing and decelerating function of the amount of nectar. This is simply the familiar Weber-Fechner Law for the subjective scaling of stimuli (see [34–37] for further application of the Weber Law to amount in foraging models). The final assumption in (4) states that the increments are a declining function of order; each increment in sequence is worth less for the same amount of nectar, until the threshold z^* has reached its maximum.

The evolutionary reasons for these assumptions about the form of ϕ follow from the typical distribution of nectar standing crop within and among plants. Often there is a highly clumped distribution, caused by differences in secretion rates between plants or due to local search by the bees themselves [38, 39]. Responding to occasional bonanzas of nectar within flowers in a linear manner might cause bees to stay much longer than would be optimal within an inflorescence. The decline with order follows from the optimal response of a Bayesian forager when there is a clumped distribution of prey within patches. Iwasa et al. derived optimal Bayesian policies for foragers encountering Poisson, binomial, and negative binomial distributions of prey within patches [7]. An overlooked feature of their equations was that for the negative binomial distribution, which indicates clumping of prey, each increment in sequence is less than the previous increment until the forager has departed the patch (Figure 1(c)). In neurobiology, this decline in the response to stimuli with order of presentation is known as adaptation [40].

The mathematical details in the model are important in distinguishing it from other psychological or constrained optimality models for patch departure. The major difference concerns a horizon effect: the “time window” over which past experience affects departure decisions may appear to change. For example, decisions in one patch may be influenced by experience in previously visited patches if the bee departed from those earlier patches before the threshold z^* had reached its maximum. If the latter occurs, however, the bee’s behavior in the current patch may appear to be a function of the last travel distance only. This difference in the apparent time window provides direct contrast with threshold departure and other “run of bad luck” (ROBL) models. In a one-flower threshold departure rule the decision to depart at each flower is independent of experience at previous flowers, while in the general ROBL model there is always a specified time or number window beyond which past experience has little or no bearing on present decisions. Similar arguments apply to general memory window rules of the Linear Operator Model (LINOP) or exponentially weighted moving average (EWMA) forms [4, 41, 42]. All such models employ a weighting of events within the time window to predict current decisions. A heavy weighting to more recent experience can explain the often observed strong effect of last travel distance on patch departure [42]. However, this weighting then predicts that foragers will rapidly adapt to changes in conditions such as average interpatch travel time, which is usually not the case.

The proposed model can explain the results of diverse experiments with bumble bees. Thomson et al. observed that bumble bees departed from empty umbels of *Aralia hispida* after visiting two flowers [32]. After leaving an umbel that had been enriched by placing $0.5 \mu\text{L}$ sucrose solution into all 12 open flowers, the bees probed an average of five flowers on a subsequent empty umbel before departing. On the enriched umbels, bees visited an average 14 flowers before departing, meaning that they revisited two flowers. These revisited flowers presumably contained the strongly negative stimulus of a marking pheromone, meaning that z for recently visited flowers will be lower than that for a simply empty flower. To be consistent with the model, other bees arriving at such recently visited flowers would probe fewer than two on average before departing; they would either visit one-flower or not land on the umbel at all; this behavior has been noted in other experiments [26, 43–45]. After departing from the enriched umbel, if the threshold z^* remained lowered due to the reception of nectar, there would then be a carryover effect on the ensuing empty umbel.

In a set of laboratory experiments with artificial umbels, Taneyhill and Thomson observed that bumble bees probed approximately the same number of flowers in umbels containing either 2 or $4 \mu\text{L}$ of nectar in each of eight artificial flowers, but fewer in umbels containing $1 \mu\text{L}$ in each flower [33]. For the increment-decay model, this would suggest that there was little difference in the value of ϕ for 2 and $4 \mu\text{L}$ due to its decelerating form, but that the effect of $1 \mu\text{L}$ was significantly less. When bees visited sets of three artificial

umbels, one empty and the other two containing two of the above three volumes of nectar, the numbers of flowers probed within the empty umbels depended on both the nectar volumes in previously visited umbels and their order of encounter. The bees probed almost identical numbers of flowers within empty umbels after first visiting either an umbel filled with 2 or 4 μL in each flower, and also nearly identical numbers after visiting umbels with both amounts in either order. However, they probed more after a 2 than a 1 μL umbel, and more after first a 2 and then a 1 μL umbel than vice versa. These results were consistent with the assumption that each increment is worth less, in sequence, for the same value of V : the asymmetry occurs because $(\phi(2, k) + \phi(1, k + 1)) > (\phi(1, k) + \phi(2, k + 1))$. If 4 and 2 μL are interchangeable, however, then $(\phi(4, k) + \phi(2, k + 1)) = (\phi(2, k) + \phi(4, k + 1))$, and thus the order of presentation makes no difference.

Cresswell also studied bumble bees foraging from ring-like inflorescences, in wild bergamot *Monarda fistulosa* [26]. He obtained estimates of departure probability as a function of nectar volume by finding the amount of nectar that was just sufficient to make bees always stay, recording the probability of departure from empty flowers, and drawing a line between the two in a plot of departure probability as a function of nectar volume. When values from this function were used in computer simulations, the predicted numbers of flowers visited consistently overestimated the actual numbers visited in study plots. This can easily be explained by the increment decay model due to its assumptions that departure probability increases continually and that each increment with nectar is worth less in sequence. In the stochastic threshold departure rule, departure probabilities remain the same at each flower for the same nectar volume.

Formal parameterizations of the model will be unique to each experimental system. In the field, the important flower stimuli and their relative weightings in the landing decision can be determined by statistical techniques such as principal components analysis, as Cresswell and Robertson have done for *Campanula rotundifolia* [46]. Recently much progress has been made in the analysis of how stimuli such as marking pheromones affect bees' decisions to exploit patches, and how such reactions are blended with experience and variation within individuals [47, 48].

The increment decay model makes several robust predictions that can be tested in comparison to the threshold departure rules. A deterministic, one-flower threshold departure rule predicts that bumble bees will always depart an inflorescence after finding an empty flower, since the threshold volume must be equal to or greater than zero. The analogous stochastic rule predicts that bees will depart after finding an empty flower with a fixed probability independent of experience at other flowers. The increment decay model, however, predicts that departure probability at empty flowers will decrease with increasing nectar in previously encountered flowers. The same arguments may be applied to two-flower and similar departure rules. I thus tested the two alternative models in the field, using one of the field systems that spawned the original threshold departure rules.

3. Methods

Field work was done in the vicinity of the Rocky Mountain Biological Laboratory, Gothic, Colorado. The protocol for the field experiments was to remove nectar from flowers within plants with vertical inflorescences and to place known amounts of sucrose solution (in all experiments I used reagent grade sucrose, 30 g solute per 100 mL distilled water) into flowers that bees would be likely to visit prior to the empty flowers. Since bumble bees often forage from plants with vertical inflorescences by starting near the bottom and moving upward [20, 21], I placed the sucrose solution into the bottom flowers in all experiments, and drained the second or second and third flowers from the bottom of all floral nectar.

The experiments reported here were done using monkshood, *Aconitum columbianum*. The monkshood inflorescence has dark purple, zygomorphic flowers borne on a central spike, with two cup-shaped nectaries hidden inside a hood-shaped cap formed by the fusion of two petals (see [49] for illustrations of how bees forage from monkshood). This species is well suited to tests of the threshold departure rules, because the structure of the flower makes it difficult for bees to sense the presence of nectar before landing and probing the flower, and the nectar can easily be removed from the nectaries within the flowers.

The first experiment tested one-flower threshold departure rules. I used 4 nectar treatments for the bottom flowers: 4, 2, 1, and 0 (=control) μL of 30% sucrose solution. The largest amount was chosen because this was approximately the highest standing crop volume found in surveys of the study populations (sugar concentration of samples was usually higher, generally in the range of 40% to 50% sucrose equivalents, so the treatments actually contained less sugar). I placed these amounts of nectar into the bottom flowers of study plants and drained the flower above the bottom of all the nectar. Although it would be easy to ensure that empty flowers contained no nectar at all by removing the nectaries, I did not do this for fear of altering the bees' behavior. Instead I checked the flowers for nectar every 20 minutes using 1 μL microcapillary tubes. Hamilton dispensing microsyringes were used to place the sucrose solution into the flowers. Observations were made with 6 plants on each observation day, and I used plants with at least 6 open flowers on the inflorescence. Plants were not selected at random; rather they were chosen from those that had nectaries in very good condition. While the bees visiting the plants were not marked, due to identification of caste and species it was determined that a minimum of 6 individual bees visited the plants.

I recorded the pattern of visitation, time of visit, caste, and species for all bees visiting the test plants. Because I found it difficult to accurately record data for all five nectar amounts at the same time, each day of observation was devoted to one nectar volume only; data for each replication of each treatment are thus analyzed separately.

A second experiment with *A. columbianum* tested both one- and two-flower threshold departure rules. I used as nectar treatments 6, 4, 2, 1 and 0 μL 30% sucrose solution.

TABLE 1: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. Four microliters of 30% sucrose solution were placed in bottom flowers of the inflorescence, with zero microliters (empty) in second-from-bottom flowers. The notation in this and all subsequent tables is X, 2 standing for bees that began by visiting the empty second flower first, without visiting the bottom flower. 1, 2 stands for bees visiting the treated bottom flower and then the empty second flower. Goodness of fit test for the null hypothesis of equal probability to depart: $\chi^2 = 4.67$, $P < .01$.

	Stay	Depart	Probability (Depart)
X, 2	13	6	0.32
1, 2	19	1	0.05

TABLE 2: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. Second replication of the experiment with four microliters of 30% sucrose solution placed in bottom flowers of the inflorescence, zero in second-from-bottom flowers. Goodness of fit test for the null hypothesis of equal probability to depart: $\chi^2 = 5.24$, $P < .01$.

	Stay	Depart	Probability (Depart)
X, 2	11	5	0.31
1, 2	22	1	0.04

TABLE 3: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. Two microliters of 30% sucrose solution placed in bottom flowers of the inflorescence, zero in second-from-bottom flowers. $\chi^2 = 1.73$, $P > .05$.

	Stay	Depart	Probability (Depart)
X, 2	22	10	0.31
1, 2	19	3	0.15

I drained the second and third flowers from the bottom of all nectar, and again checked these every 20 minutes using microcapillary tubes. I used a Rainin digital dispensing pipet to place sucrose solution into the bottom flowers. During this experiment, I was able to gather data for all five amounts of sucrose on each day of observation. I changed the amount of sucrose in each study plant every two hours during each day. Bee density in this experiment was greater than for the first experiment; at least 10 individuals were visiting the experimental plants.

Data from the first experiment were cast into 2×2 contingency tables and analyzed via two-way tests of independence, with the null hypothesis that the probability of departure from an empty flower was independent of previous experience (i.e., whether the bee had first visited the treated flower). Data from the second experiment were analyzed via an $R \times C$ contingency table test [50].

4. Results

In tests of one-flower threshold departure rules using *Aconitum columbianum*, bumble bees' probability of departure from empty flowers decreased with increasing volume of nectar in previously encountered bottom flowers (Tables 1, 2, 3, 4, 5, 6, and 7). With $4 \mu\text{L}$ of sucrose solution

TABLE 4: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. Second replication of two microliters of 30% sucrose solution placed in bottom flowers of the inflorescence, zero in second-from-bottom flowers. $\chi^2 = 3.95$, $P < .05$.

	Stay	Depart	Probability (Depart)
X, 2	31	17	0.35
1, 2	24	4	0.14

TABLE 5: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. One microliter of 30% sucrose solution placed in bottom flowers of the inflorescence, zero in second-from-bottom flowers. $\chi^2 = 0.269$, $P > .5$.

	Stay	Depart	Probability (Depart)
X, 2	21	5	0.19
1, 2	19	3	0.14

TABLE 6: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. Second replication of one microliter of 30% sucrose solution placed in bottom flowers of the inflorescence, zero in second-from-bottom flowers. $\chi^2 = 3.72$, $P < .06$.

	Stay	Depart	Probability (Depart)
X, 2	33	7	0.18
1, 2	31	1	0.03

TABLE 7: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. Control treatment with zero microliters of 30% sucrose solution in bottom flowers of the inflorescence, and zero in second-from-bottom flowers. $\chi^2 = 1.63$, $P > .1$.

	Stay	Depart	Probability (Depart)
X, 2	25	14	0.36
1, 2	10	8	0.44

in bottom flowers, the bees almost never departed after subsequently finding the empty flower if they had first visited the treated flower (probabilities of departure = 0.04 and 0.05 for two replicate experiments). In the control treatment with both flowers empty, the probability of departure from the empty second flower was greater if the bee had first visited the treated (empty) bottom flower, as is predicted by the increment-decay model, although the two probabilities were not statistically different. The probability of departure from the empty second flower, given that it was visited first, was about 0.3 (overall $P = .29$, $N = 220$), and in each experiment was fairly close to this value except for the two experiments using $1 \mu\text{L}$. Observations collected on different days showed nearly identical patterns, except for the second $1 \mu\text{L}$ treatment.

Results of the two-flower tests (Tables 8 and 9) were in accord with the one-flower experiments. The probabilities of departure depended on both the amount of nectar in the treated bottom flower and the pattern of visitation (3-way test of independence using a log-linear model; $G = 28.085$

TABLE 8: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. Control treatment with zero microliters of 30% sucrose solution in bottom flowers of the inflorescence, and zero in second-from-bottom flowers (2), and zero in third-from-bottom flowers (3). The notation in this and Table 9 is X, 2 or 3 stands for bees that began by visiting the empty second or third flower first, without visiting the bottom flower. X, 2 and 3 stands for bees visiting both empty flowers first. 1, 2, or 3 stands for visiting the treated bottom flower and then the empty second or empty third flower. 1, 2 and 3 stands for visiting the treated bottom flower and then both the empty second and empty third flowers.

	Stay	Depart	Probability (Depart)
X, 2 or 3	65	27	0.29
X, 2 and 3	14	6	0.30
1, 2 or 3	20	1	0.048
1, 2 and 3	8	1	0.11

TABLE 9: Departure patterns of bumble bees on treated inflorescences of *Aconitum columbianum*. The experiment used a descending series of volumes of 30% sucrose solution, from 6 to 0 microliters, in bottom flowers of the inflorescence, with second-from-bottom and third-from-bottom flowers empty. The notation for visit patterns is as detailed in Table 8. Three-way log-likelihood test of independence, $G = 28.085$, $P < .01$ for the 3-way interaction.

	Stay	Depart	Probability (Depart)
<i>6 μL</i>			
X, 2 or 3	38	12	0.24
X, 2 and 3	2	4	0.67
1, 2 or 3	30	0	0
1, 2 and 3	11	0	0
<i>4 μL</i>			
X, 2 or 3	33	17	0.34
X, 2 and 3	7	2	0.22
1, 2 or 3	55	1	0.018
1, 2 and 3	33	4	0.11
<i>2 μL</i>			
X, 2 or 3	30	17	0.36
X, 2 and 3	3	4	0.57
1, 2 or 3	38	2	0.5
1, 2 and 3	20	3	0.13
<i>1 μL</i>			
X, 2 or 3	26	11	0.3
X, 2 and 3	7	2	0.22
1, 2 or 3	49	4	0.08
1, 2 and 3	21	3	0.125
<i>0 μL</i>			
X, 2 or 3	97	43	0.31
X, 2 and 3	25	9	0.265
1, 2 or 3	52	13	0.20
1, 2 and 3	22	7	0.24

$P < .01$ for the 3-way interaction). As in the one-flower study, the probabilities of departure from the empty second flowers decreased with increasing amounts of sucrose placed

in the bottom flower. With $6 \mu\text{L}$ sucrose solution placed in the bottom flower, the bees never departed after next probing either one or two empty flowers. The probabilities of departure from empty second or third flowers visited first were again about 0.3, as in the one-flower experiments (overall, $P = .31$, $N = 324$), with relatively little variation across treatments (range, 0.24 to 0.36).

5. Discussion

The results from field experiments with *Aconitum columbianum* strongly suggest that the bumble bees did not use a one- or two- flower threshold departure rule. In many cases the bees did not depart after visiting from one to three flowers that were, to the limit of the experimental techniques, empty of nectar. The probabilities of departure from empty flowers were instead influenced by the amounts of nectar in previously encountered flowers, meaning that the bees were also not using a stochastic threshold departure rule. When testing threshold departure rules one could continue to advocate them with an increasing number window; in the present case the results do not falsify a three-flower stochastic threshold departure rule. However, this line of reasoning could be extended indefinitely. To interpret the results of Thomson et al. [32] in that manner, bees visiting umbels of *Aralia hispida* would have to have been switching between a two-flower and a five-flower stochastic threshold departure rule. It seems much more parsimonious to think of expected departure time or number as being flexible, able to vary with time and experience.

In this paper I have presented the increment-decay process as a general framework for understanding patch departure in bumble bees. Such a model, in which patch departure is considered as an observable aspect of a dynamical system, addresses the important question of how cognitive mechanisms of foraging behavior should be considered in theory above the neurobiological level. The terms “rules” and “mechanisms” are perhaps considered interchangeable by some, yet I argue that there can be subtle but important differences between the two. The word “rule” often denotes the type of if-then procedure found in expert computer systems or other such human constructs; many such rules have been proposed in foraging theory, including give-up time rules, fixed number rules, failure rules, and the threshold departure rules [3, 8, 24, 51]. However, other proposed mechanisms, such as those described in the present paper, cannot easily be expressed in this form. An ever-present danger in building cognitive models of behavior is that they run the risk of assigning to the animal processes that may account for the observations, but which may not in fact exist. Representing mechanisms as dynamical systems may bring the models closer to the underlying neurobiology while sacrificing ease of understanding them in commonsense terms.

Constructing cognitive models for patch departure behavior in this manner (adding details of mechanism peculiar to each organism) means that the results become further removed from the MVT [4]. What, then, is the purpose of the optimality models? The standard answer is

that they help build intuition about the mechanisms that one expects to find [52, 53]. The model presented in this paper was suggested by observations of bees and by the results of experiments. However, the approach was guided by principles from optimality theory, in both the overall structure of the model and in its assumptions concerning how experiences affect the model's dynamics. Increment-decay processes appear to be a fortunate compromise between the proximal and adaptationist descriptions, having their roots in Bayesian inference while at the same time bearing resemblance to neurobiological processes. Since they have the potential to explain so many aspects of patch departure [11], they should be investigated in depth for bumble bees and other foragers.

Acknowledgments

The author thanks James Thomson and Lars Chittka for discussion of the ideas presented herein, and the staff of the Rocky Mountain Biological Laboratory, Gothic Co. for field support.

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Research Article

Nest Relocation and Colony Founding in the Australian Desert Ant, *Melophorus bagoti* Lubbock (Hymenoptera: Formicidae)

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Received 14 January 2010; Accepted 27 February 2010

Academic Editor: Lars Chittka

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Even after years of research on navigation in the Red Honey Ant, *Melophorus bagoti*, much of its life history remains elusive. Here, we present observations on nest relocation and the reproductive and founding stages of colonies. Nest relocation is possibly aided by trail laying behaviour, which is highly unusual for solitary foraging desert ants. Reproduction occurs in synchronised mating flights, which are probably triggered by rain. Queens may engage in multiple matings, and there is circumstantial evidence that males are chemically attracted to queens. After the mating flight, the queens found new colonies independently and singly. Excavation of these founding colonies reveals first insights into their structure.

1. Introduction

The Australian desert ant, *Melophorus bagoti* Lubbock, is a widespread species of arid Central Australia. It inhabits low-shrub and grassland deserts, where it builds fairly large underground nests [1]. The outdoor activity is mainly restricted to the hotter summer months, when the ants are active during the heat of the day. Foragers usually begin their activity at soil surface temperatures of about 50°C and continue to forage at temperatures above 70°C [2]. They forage solitarily for food such as dead insects, seeds, and sugary plant exudates ([3], personal observations) and are well known for their ability to store liquids in the abdomens of specialised workers, the so-called repletes or “honey pots” (hence their common name “Red Honey Ant” and indeed the genus name *Melophorus*, meaning “honey carrier”). This method of food storage is also adopted by several other seasonally active ants, for example, *Cataglyphis* [4] of North Africa, *Camponotus* [5] of Australia, and *Myrmecocystus* [6] and *Prenolepis* [7] of North America (the latter store fat, not sugar).

In the recent years, *M. bagoti* has attracted increasing attention for its navigational abilities (e.g., [8–13]; for a review see [14]), thus making a broader understanding of its behaviour and life history desirable.

2. Materials and Methods

The study site is located 10 km south of Alice Springs, NT, Australia, on the grounds of CSIRO Alice Springs. The area is characterised by an arid climate, with an average annual rainfall of 279.4 mm [15]. The soil consists of sandy flood plain alluvium [16], and the vegetation is a mosaic of *Acacia* low open woodland and *Triodia* low open hummock grassland [17], although much of the latter has been replaced by the invasive Buffel Grass *Cenchrus ciliaris*. *M. bagoti* is common in the area, and their nests occur at a density of ~3/ha, which is much lower than previously reported by Muser et al. [3] from a different location.

The observation of a nest move was made in December 2008, and colony founding was observed between December 2008 and March 2009. As these incidents were unpredictable, observations could not be made systematically. Due to unusually high rainfall in November 2008 (wettest November on record with 156 mm rain), much of the area was covered by fresh vegetation for most of the summer.

3. Results and Discussion

3.1. Nest Move. After a full week of rainy weather, some nests of *M. bagoti* reopened their entrance holes on 21 November

2008. In the following three weeks, 12 of 16 observed nests relocated the position of their entrances several times by 5–191 cm (average: 73 cm). This behaviour is usually displayed much rarer. Occasionally several entrances were in use at the same time. In preparation for other experiments, the area around one of these nests was cleared of vegetation on 25 November whereby a nest chamber very close to the surface was accidentally opened. In the following days, the nest relocated its entrance to this new opening (distance: 47 cm, bearing: 190°), closing the old entrance. On 3 December (partly cloudy, max. temp. 40.9°C) at 17.00 hour we noticed that this nest was in the middle of relocating to a new nest site (distance: 17.75 m, 205°). A continuous but sparse moving column of ants, including repletes, was observed between the two nest sites. The column was directed to the new nest in almost a straight line. Although most workers went from the old to the new nest, some were observed going the other way. The width of the column varied from a few cm to about 1 m but always seemed to consist of distinct trails. Most, but not all of the repletes, were pulled or pushed out of the old nest opening by workers and proceeded to move to the new nest on their own (see Supplementary Material), where some were dragged into the entrance by workers. Because foragers are usually the only ants that leave a nest, repletes are necessarily unfamiliar with the environment around the nest. They must therefore rely on other cues to find the direction and location of the new nest. There are three possible explanations. Other workers within the nest could convey the information, they might simply follow other ants on the trail, or they might use a system of chemical (olfactory) marking. Indeed, on several occasions workers were seen dragging the tip of their abdomens across the sandy soil (see Figure 1 and the Supplementary Material), a behaviour which has not been observed in *M. bagoti* or any other solitary foraging desert ant so far. These ants may be laying intermittent odour trails. If this conclusion holds true, it will have important implications for future studies on the navigational strategies of this ant species.

We could distinguish two types of repletes, as previously described by Conway [1]: ones with clear, amber-coloured abdomens and ones with milky white abdomens. The sizes of their inflated abdomens were variable. One dealate queen was also observed, and one winged male, but no eggs, larvae or pupae. The queen was dragged all the way from the old to the new nest (see the Supplementary Material). All activity ceased at 17.30 hour. Over the next few days we checked for activity sporadically. The old nest was now presumably abandoned. On one occasion some workers and one replete from another nearby nest (distance: 19.98 m) entered the old abandoned nest. However, no further activity was observed at the old nest after this incident. At the new nest excavating activity was at first very high, but during the following days the activity slowed down considerably and eventually came to a stop. The nest reopened on 8 January and remained active until the end of the season.

Although nest emigration behaviour seems to be common in forest-dwelling ant species [18], this does not seem to be the case for *M. bagoti*. Once a nest is established, its location usually does not change over many years (personal



FIGURE 1: A worker of *M. bagoti* dragging her abdomen across the sandy surface during a nest relocation. Arrows indicate the track left behind in the sand. Still photo taken from a film sequence, credit A. Wystrach.

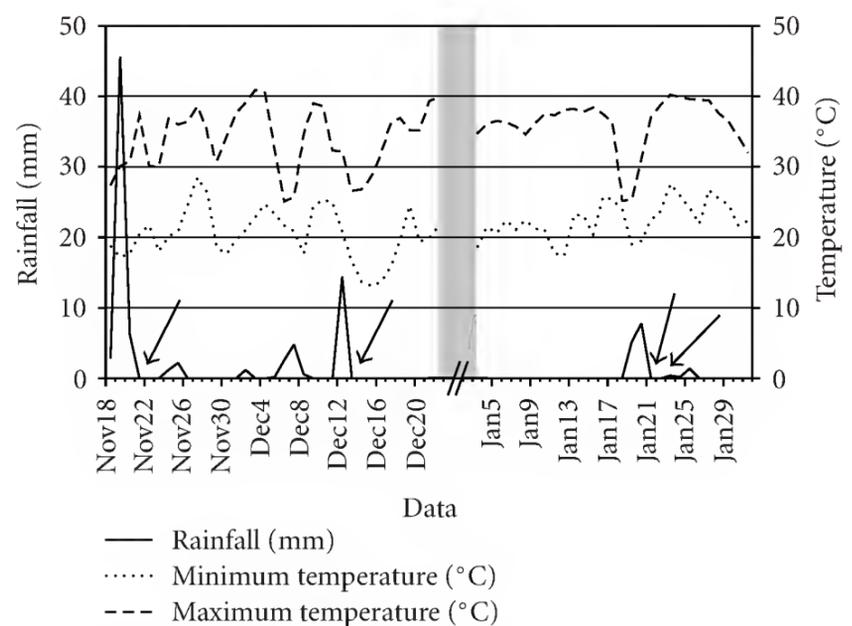


FIGURE 2: Timing of mating flights in *M. bagoti* during the summer 2008/09. Daily rainfall and temperature (min./max.) are shown for the time period from 18.11.08 to 31.01.09, excluding the period from 23.12.08 to 02.01.09 when no observations were made (indicated by grey bar). Arrows indicate observed mating flights. Climate data from [15].

observation). In the described case the move was probably triggered by our disturbance.

3.2. Colony Founding. The founding stage of an ant colony is usually characterised by the same sequence of events. The virgin queen leaves the nest in a mating flight and is inseminated by one or several males. She then looks for a new nest site and starts excavating a small nest, where she lays eggs and rears a small brood [19].

Several nuptial flights were observed during the summer of 2008/09, always after rainy days (see Figure 2) and always in the mornings. Heavy rain is a common trigger for the timing of mating flights in desert ants [19]. Sometimes queens and males left the nest together to fly off, at other

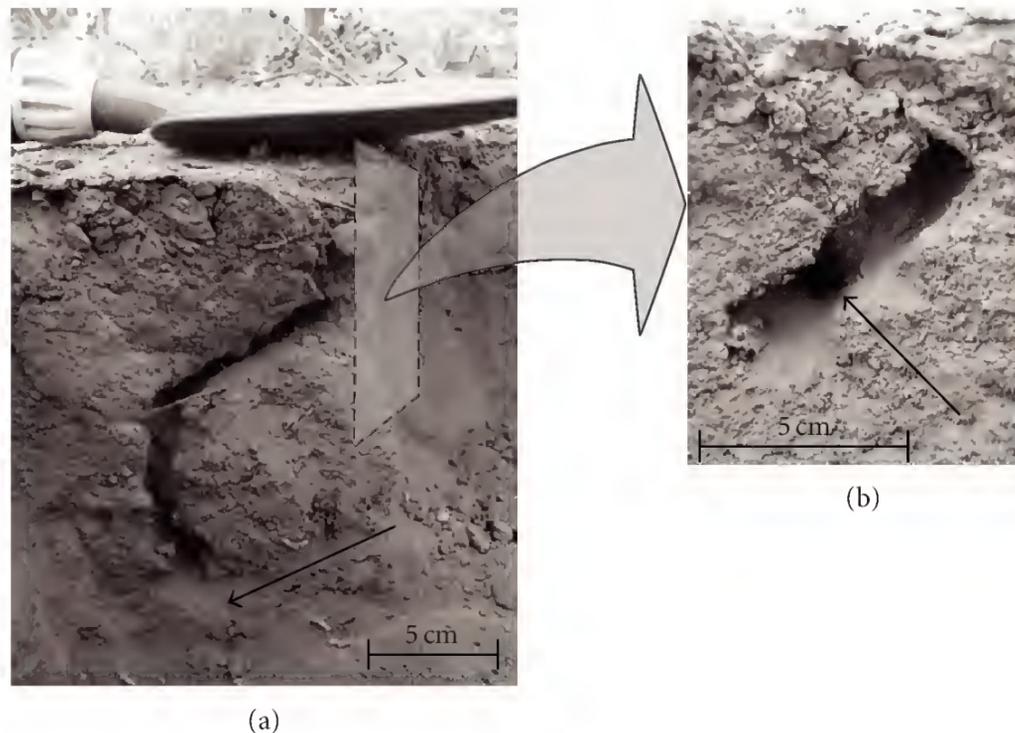


FIGURE 3: (a) Overview of an excavated founding colony of a *Melophorus bagoti* queen. Arrow indicates the location where the dead queen was found. (b) Close-up of the chamber encountered during excavation, the part of the channel leading to the chamber has been removed. Arrow indicates the channel leaving the chamber on the other side; see text for details. Photo credit P. Schultheiss.

times only queens did so. At about 10.30 hour on 21 January 2009, mating flights occurred at four nests simultaneously. As it had rained for the two previous days, it was humid, overcast, and warm (61% RH, 29°C at 9.00 hour). From this synchronised behaviour, we can surmise that mating occurs in swarms, although no such mating site could be located. One mating was actually observed: an already dealate queen was found on the ground, surrounded by several males, of which one copulated with the queen once for a few seconds.

The following day, a dealate queen was observed leaving a nest at 10.15 hour and was followed as she wandered around the area up to a maximum distance of 50 m from the nest entrance, regularly seeking thermal refuge on small plants and twigs. During this time, she copulated once with one male and three times with another male. On both occasions the queen had climbed onto a small plant and remained motionless while the male flew around her. This behaviour is somewhat reminiscent of the sexual calling behaviour of some ponerine ants [20]. The copulations lasted from a few seconds to about half a minute. As all the observed copulations involved dealate queens, they were obviously not regular matings; it seems though that queens readily mate even after they have broken off their wings and possibly even attract males chemically. After 1 hour 50 minutes we stopped following the queen; it is not known if she returned to the nest.

Another dealate queen was seen being followed by a flying insect (probably Diptera, Syrphidae, of which the subfamily Microdontinae has larvae that prey on ants in their nests; the adults are usually found in the vicinity of ant nests [21]). It followed the exact path the ant took at a constant distance of about 10 cm (see the Supplementary Material) until it eventually lost the ant and flew away after searching for a little while.

Queens founded new colonies independently and without the help of other queens or workers (haplometrosis, see [22]); this mode of colony founding is common in formicine ants [19, 23]. However, nothing is known about the number of queens in later colony stages or other populations of *M. bagoti*. For example, in North American ants of the genus *Myrmecocystus*, which can be regarded as the ecological equivalent to *Melophorus* [24], founding queens are often joined by other queens after they have excavated the first nest chamber alone [25]. Also, some desert ants in North America, including *Myrmecocystus*, display considerable geographic variation in their mode of colony founding [26]. We observed a total of 21 dealate queens at their attempts to establish new colonies (all on 21 January). Of these, only five were in a completely open place, while the remaining queens chose a spot in the shade of a little plant or twig. Here the queens started to dig at a shallow angle, using their mandibles (see the Supplementary Material). They continued digging for sometimes several hours. In one case, the queen had chosen a site that was close to an already existing nest (distance: 7.70 m), and workers from this colony apparently attacked and killed the queen. While several workers dragged the dead queen away, one worker closed the hole of the queen rapidly. After two days, 12 of the 21 holes were closed, rising to 15 after another four days; by 10 March, only one remained open (although obstructed by a branch). All colonies can thus be regarded as failed, for reasons unknown. Four of the closed founding colonies were then excavated. Three of these continued as a narrow channel underground for 2–10 cm, ending in a dead end with no remains of the queen, being wholly or partially filled with debris. The fourth hole started as a narrow channel, slowly sloping downward before opening into a small chamber (length: 7.5 cm). This was oriented at a right

angle to the channel but diagonally to the surface, at a depth of 4–9 cm below ground (see Figure 3(b)). The channel then continued downwards at roughly 45° for another 8 cm, turned abruptly downward, and ended without a chamber at a total depth of 16 cm below ground (see Figure 3(a)). Remains of a dead queen were found at the end of the channel, and parts of the channel were filled with debris.

The fact that there was no nest chamber at the end of the channel indicates that the queen died before she had fully excavated the founding nest. Although the observations presented here are necessarily incomplete and many important questions remain unanswered, they do offer a fascinating insight into the early stages of an ant colony.

Acknowledgments

The authors would like to thank CSIRO Alice Springs for permission to use their property, and Ken Cheng for contributing observations. The funding was provided by Macquarie University, Sydney, and the Australian Research Council.

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Research Article

Foraging Activity in *Plebeia remota*, a Stingless Bees Species, Is Influenced by the Reproductive State of a Colony

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Received 30 July 2009; Accepted 12 March 2010

Academic Editor: Koos (J.C.) Biesmeijer

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Colonies of the Brazilian stingless bee *Plebeia remota* show a reproductive diapause in autumn and winter. Therefore, they present two distinct reproductive states, during which colony needs are putatively different. Consequently, foraging should be adapted to the different needs. We recorded the foraging activity of two colonies for 30 days in both phases. Indeed, it presented different patterns during the two phases. In the reproductive diapause, the resource predominantly collected by the foragers was nectar. The majority of the bees were nectar foragers, and the peak of collecting activity occurred around noon. Instead, in the reproductive phase, the predominantly collected resource was pollen, and the peak of activity occurred around 10:00 am. Although the majority of the foragers were not specialized in this phase, there were a larger number of pollen foragers compared to the phase of reproductive diapause. The temperature and relative humidity also influenced the foraging activity.

1. Introduction

Stingless bees collect several types of material on their foraging flights. Most of these materials have vegetal origin, as pollen, nectar, resin, latex, leaves, trichomes, fragrances, oils, seeds, and so forth. In addition, stingless bees also collect materials of other origins, as animal feces, clay, water, and fungi spores, for example [1–3].

Among all these resources, pollen, and nectar are the ones used as food [4]. In some bee species oil is also used to provision brood cells, as in *Centris (Hemisiella) tarsata* [5] and *C. (H.) trigonoides* [6]. The other materials can be used for several purposes, especially construction and protection [2, 4]. The flight activity includes waste removal, namely, to remove garbage (detritus) from the colony, besides foraging. The detritus comprise feces, old combs, dead bees, larval and pupal exuvia, among others [1, 2, 7].

The foraging behavior varies seasonally throughout the year, especially in relation to the amount of pollen collected by the colonies. Climatic factors such as temperature, light

intensity, wind, rain, and relative humidity, as well as plant resource availability, influence foraging. Colony internal factors, such as population size and amount of stored food, also influence the foraging behavior of the individual bees and of the colony [2, 4, 8–12].

Several aspects of the flight and foraging activity of some stingless bee species have already been studied: (i) the influence of external and internal factors, (ii) the size and the physiology of the bees, and (iii) the effect of daily and seasonal patterns of availability of floral resources on foraging. However, among the species that present reproductive diapause, the foraging pattern and the flight activity in relation to the phase of diapause and the phase of oviposition by the queen were studied comparatively only in *Plebeia saiqui* [13, 14].

Reproductive diapause is characterized by an interruption in cell provisioning and oviposition process (POP) in autumn and winter [15–17]. In stingless bees the provisioning and oviposition process (POP) comprises (i) the construction of brood cells one by one, (ii) provisioning

of them by the workers, (iii) the oviposition by the queen, and (iv) sealing of the cells by the workers, then new cells are started [1]. Diapause also occurs in other stingless bee species, especially in the genus *Plebeia*: *P. remota* [15, 17], *P. droryana* [18], *P. julianii* [19], and *P. wittmanni* [20] and has also been observed in some colonies of *Melipona marginata obscurior* [21]. In this phase many changes in the architecture (i.e., construction of storage pots on the top of the pile of old combs) of the nest and in the behavior of the queens and workers occur, at least in *P. remota* [17]. In this species even the defensive behavior of the bees is modified during the reproductive diapause [22].

Hilário et al. [23–25] studied the influence of climatic factors on the flight activity of *P. remota*, but he did not present observations on the influence of diapause in the foraging and waste removal behavior for this species. The main aim of this study was to test whether the foraging behavior of this species varies according to the reproductive state of the colony. More specifically we examined whether there are differences on the type and amount of resource collected by the bees in the different phases of reproduction and in the removal of detritus. The influence of the temperature and the relative humidity on this activity and the daily rhythm of the foraging of the colonies and individual foragers were also investigated, as the individual activities of the foragers.

2. Material and Methods

The study was carried out in the Bee Laboratory (Bioscience Institute, University of São Paulo; 23°33'S, 46°43'W) in two periods: from May 8th to July 7th 2006 (reproductive diapause of colonies) and from November 13th 2006 to January 24th 2007 (reproductive phase).

We used two colonies of *P. remota* from Cunha (23°05'S, 44°55'O, São Paulo State). These colonies were hived in wooden boxes covered with glass lids and connected to the exterior of the laboratory by a plastic tube. Outside the building the tube was 15 cm long, so as to allow better observation of the activity of individual bees. Four hundred newly emerged bees were individually marked in each colony using a color code made with paint. This color code is based on five colors, each color representing a number, and on the position of the dot on the thorax (Figure 1). Dots in the center of the thorax mean 100 (white) to 500 (green). This system allows the researcher to mark 599 bees individually by combining the dots. For example, bee number 456 is marked with a blue dot on the center of the thorax, a green dot on the inner left side of the thorax, and a white dot on the upper right side. This is a marking system modified from Sakagami's system [26].

The observations were made between 8:00 and 18:00 (local time), for 20 minutes per hour in each colony, for 30 days distributed throughout each phase (a minimum of 3 times a week and maximum of 5 times a week). In these observations we counted the number of bees entering the colony and the number of bees taking out garbage, and registered the type of material carried by them (nectar, pollen, or resin). We also recorded the time and what

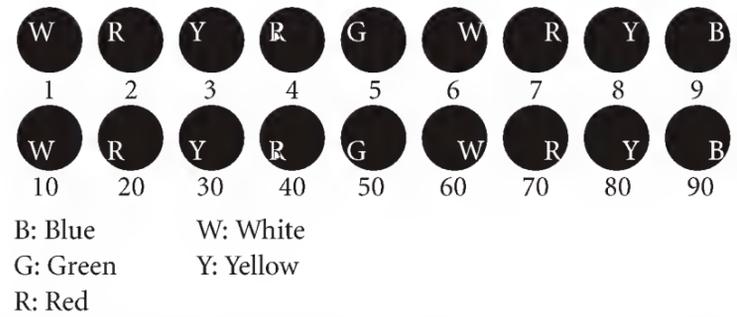


FIGURE 1: Marking system based on the position of the dots made with paint on the thorax of the bee. The black balls represent the thorax of the bee and the letters where the dots are made and the color of the paint.

resource individually marked bees foraged for. It was not possible to distinguish among bees bringing nectar, water, or nothing. Bees entering the hive without pollen or resin on the corbiculae were considered to bring nectar. To avoid an over estimation of bees collecting nectar, the number of bees removing garbage was subtracted from the number of bees collecting nectar, since these bees do not collect resources and come back rapidly without resources on the corbiculae, and they had been previously counted as bees collecting nectar.

We calculated the mean and standard deviations of the numbers of bees collecting nectar, pollen, and resin and removing garbage per hour, as well as the minimum and maximum numbers registered per colony. Since the datasets did not follow a normal distribution (Shapiro-Wilk test, $P < .05$), we used Mann-Whitney test to compare two groups of data and Kruskal-Wallis test or Wilcoxon signed-rank test to compare more than two groups [27].

We calculated the partial correlation indexes between air temperature ($^{\circ}\text{C}$) and relative humidity (%) and between these two environmental factors and nectar, pollen, and resin collection, total number of incoming trips in the colony and garbage removal. The controlled factors were the relative humidity and the air temperature. We also calculated Spearman correlation indexes between air temperature and relative humidity, and nectar, pollen, and resin collection and the total number of incoming trips in the colony. Weather data were provided by the Climatology and Biogeography Laboratory (Geography Department, Faculty of Philosophy, Letters, Science and History, University of São Paulo) from their experimental meteorological station at the University of São Paulo campus. The data were provided as means of five minutes of the temperature recordings. We used the mean of the four mean temperatures that corresponded to the 20 minutes of observation. To compare the air temperature and relative humidity between the two phases studied we used the Mann-Whitney test.

The analysis of the individual behavior of foragers was based on the activity performed (type of material collected or garbage removal) and on the frequency they performed it. We considered that bees that collected only one type of resource (nectar, pollen, and resin) in 80% or more of their flights were specialists in the collection of that resource, as Biesmeijer and Tóth [28] did. We also observed for how many days the marked bees foraged and their age.

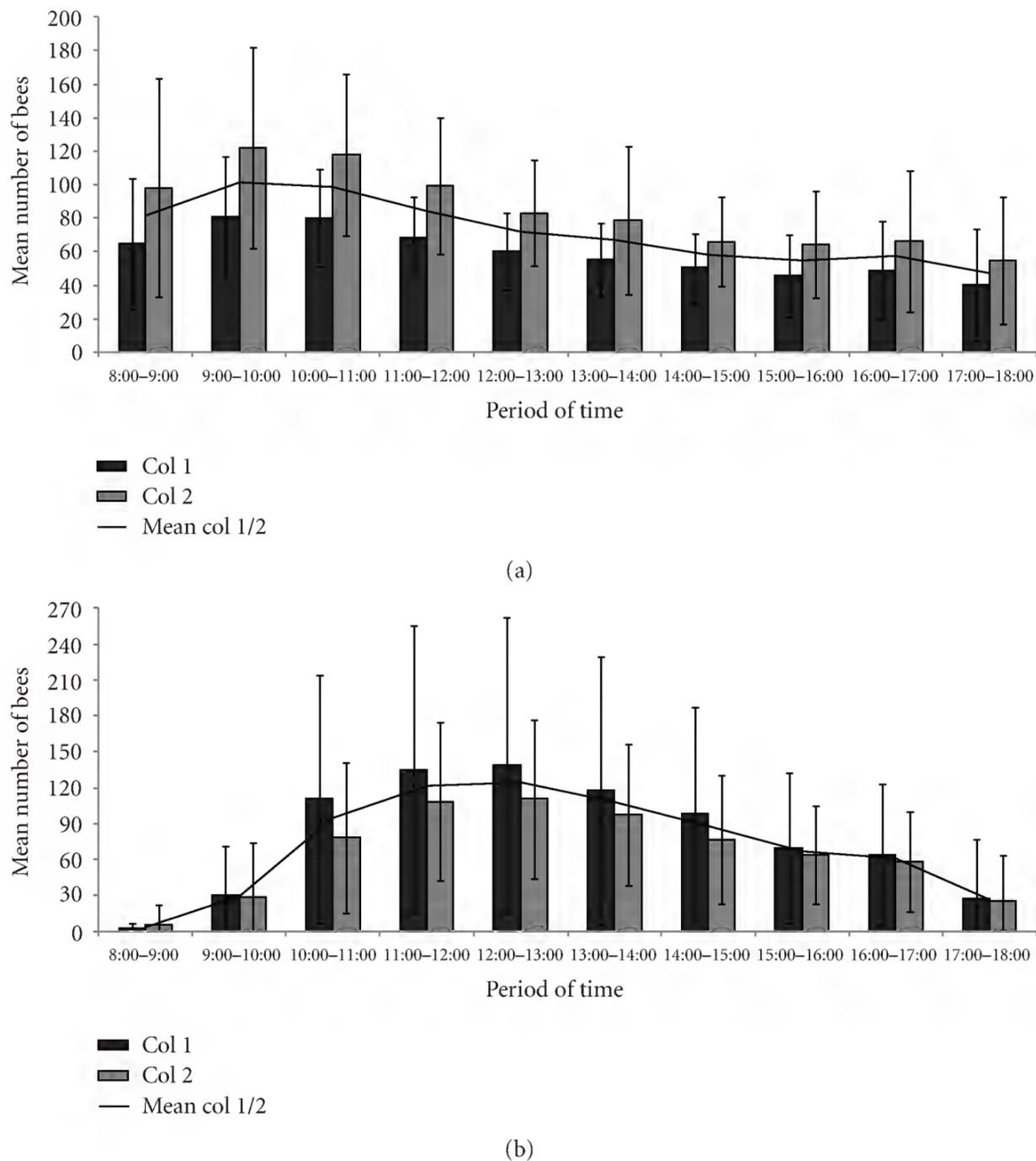


FIGURE 2: Total of entrances in two colonies of *Plebeia remota* in the reproductive phase (a) and in the diapause (b).

The foraging behavior of the colony and the individual foragers was also analyzed using rhythm tests. We tested whether the foraging of individual bees and of the colonies showed an acrophase (hr:min; local time) with the Rayleigh test ($P = .05$) [27]. We calculated the value of the acrophase of the colony and of individual marked bees (when the bee made six or more activities), the angular deviation of the acrophases and the mean vector r , which indicates the dispersion of the data around the acrophase; the greater the value of r , the less dispersion of the data around the acrophase [27].

3. Results

3.1. Foraging Patterns of Nectar, Pollen, Resin Collection, and Garbage Removal. There were differences in the foraging patterns between the reproductive and diapause phases. There was a statistically significant difference between the total number of bees collecting resources in the reproductive phase and during diapause (colonies 1 and 2, Mann-Whitney test, $P < .05$).

In both colonies the total number of bees collecting resources in the reproductive phase increased until 9:00. A peak of activity was found between 9:00 and 11:00. After 11:00, the income of resources decreased until 13:00, remaining constant for the rest of the day (Figure 2(a)).

In the diapause, the total number of foragers increased from 8:00 to 11:00. The resource income remained constant from 11:00 to 13:00, when it started decreasing until 18:00 (Figure 2(b)). The nectar collection pattern was similar to the pattern of the total number of bees bringing resources to the colony. During the reproductive phase it was nearly constant along the day in both colonies (Figure 3(a)). From 8:00 to 11:00 it increased and remained constant until 16:00, when it decreased slightly.

In the diapause (Figure 3(b)), the collection of this resource increased from 8:00 to 11:00 and showed a peak between 11:00 and 12:00 in colony 1, but in colony 2 this peak lasted until 13:00. In general, the collection of nectar started to decrease after this peak until cessation.

Although distinct patterns in nectar collection in the two phases were found along the day, there was no difference

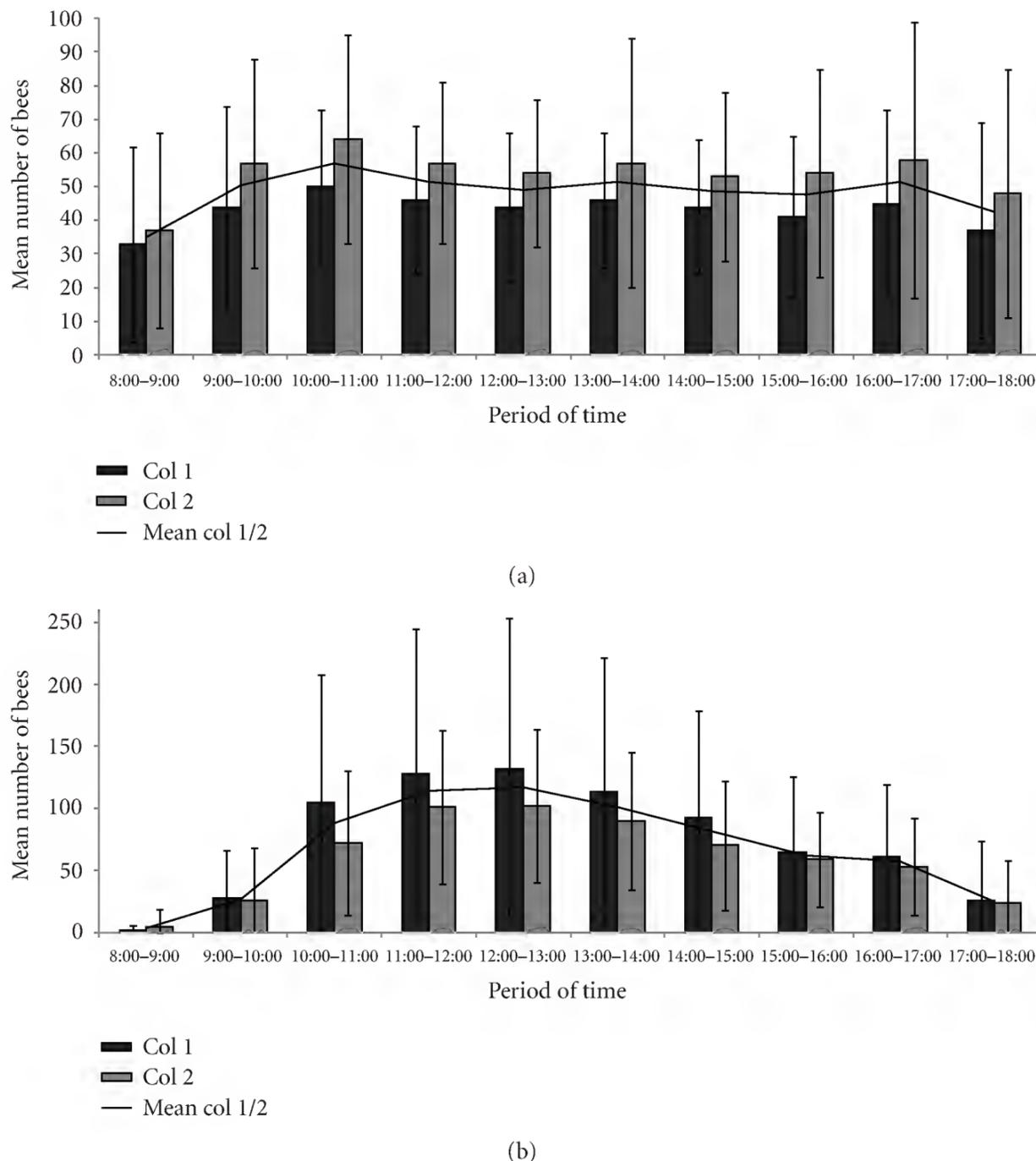


FIGURE 3: Nectar collection patterns of two colonies of *Plebeia remota* in the reproductive phase (a) and in the diapause (b).

between the total number of bees collecting nectar in the reproductive phase and in the diapause (Wilcoxon sign-rank test, colony 1: $P > .05$; colony 2: $P > .05$).

The pollen collection pattern also showed differences between the two phases (Figure 4). In the reproductive phase the pollen collection showed a peak at the beginning of the morning, between 8:00 and 10:00. After that, pollen collection decreased along the day. The diapause was characterized by a low number of bees bringing pollen to the nest (Figure 4(b)). The number of bees collecting pollen increased between 8:00 and 11:00, remaining nearly constant for the rest of the day. There was a statistically significant difference between the total number of bees collecting pollen in the reproductive phase and in the diapause (Wilcoxon sign-rank test, colony 1: $P < .05$; colony 2: $P < .05$). The total number of bees bringing pollen to the nest in the reproductive phase was higher than in the diapause.

Resin foraging in the reproductive phase was constant along the day (Figure 5(a)). In the diapause, this activity was nearly constant along the day, with exception of few periods of time (Figure 5(b)).

There was a significant difference between the total number of bees collecting resin in the reproductive phase and in the diapause (Mann-Whitney test, colony 1: $P < .05$; colony 2: $P < .05$). However, different situations occurred in each colony. In colony 1, the total number of bees bringing resin to the nest in the reproductive phase was higher than in the diapause. In colony 2, the opposite was found (Figure 5).

The garbage removal in the reproductive phase and in the diapause was concentrated at the end of the day, after 15:00 (Figure 6). In general, it increased along the day. The total number of foragers removing garbage during the reproductive phase was smaller than during diapause in colony 1 (Mann-Whitney test, $P < .05$), but in colony 2 the opposite was found (Mann-Whitney test, $P < .05$; Figure 6).

There was a difference in the number of foraging trips for the different resources in the two phases in the two colonies (Kruskal-Wallis test, $P < .05$). Nectar was always the most collected resource (Figure 7). In the reproductive phase, pollen was the second most collected resource (Figures 7(a) and 7(b)). In the diapause, different situations were found regarding pollen and resin collection. In colony 1, pollen

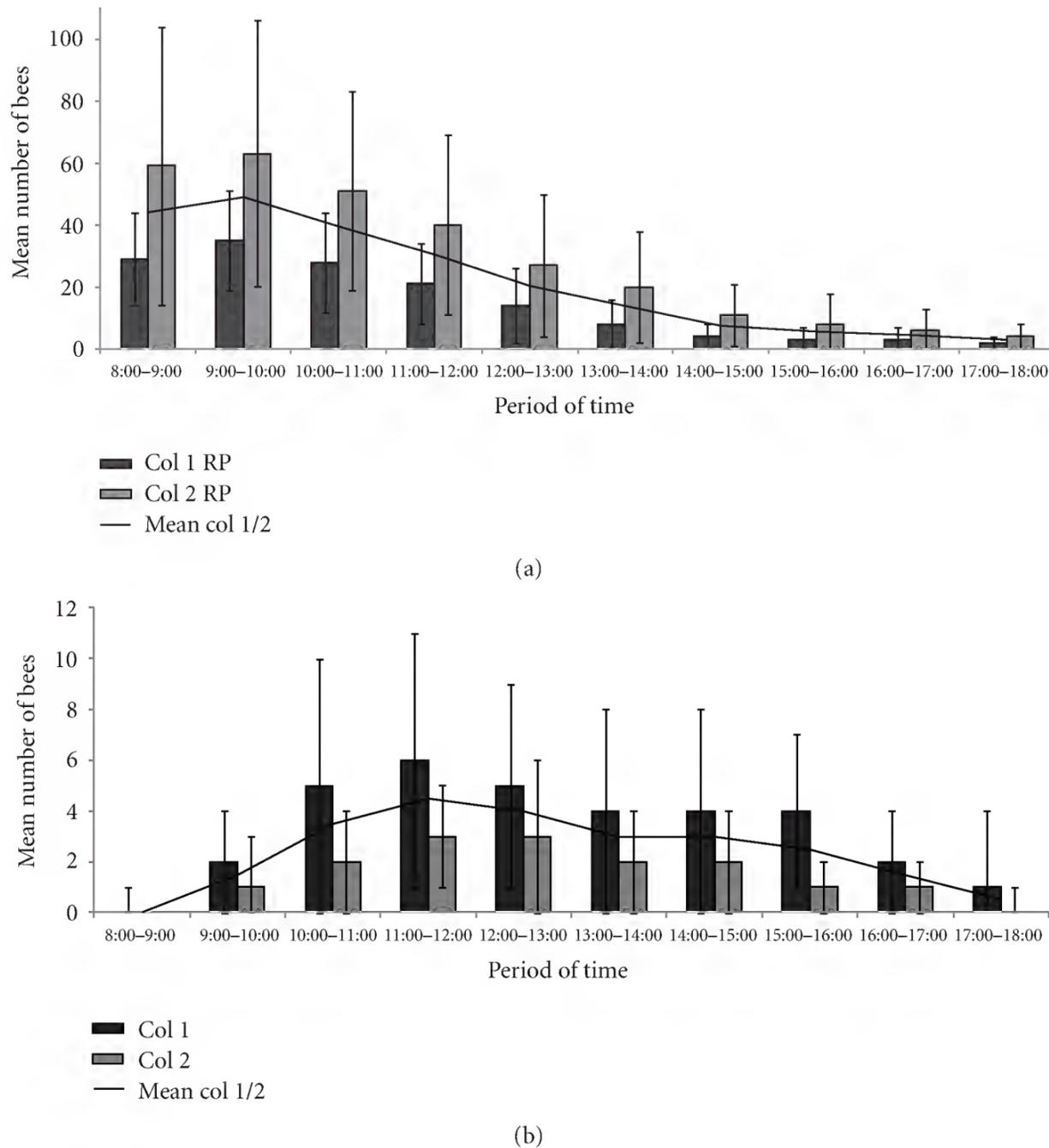


FIGURE 4: Pollen collection patterns of two colonies of *Plebeia remota* in the reproductive phase (a) and in the diapause (b).

collection was greater than resin collection (Mann-Whitney test, $Z = 6.7$ and $P < .05$; Figures 7(a) and 7(c)), however, colony 2 had the opposite behavior (Mann-Whitney test, $Z = 6.1$ and $P < .05$; Figures 7(b) and 7(d)). Garbage removal was more frequent when the resource (nectar, pollen, and resin) collection decreased at the end of the day (between 16:00 and 17:00) both in the reproductive phase and in the diapause (Figure 7).

3.2. Flight Activity and Climatic Factors. The air temperature and the relative humidity in the reproductive phase were different from the diapause (Mann-Whitney test, $P < .00$; Table 1).

In the diapause, for colony 1 the minimum temperature for foraging was 14.7°C (only one entry) and for colony 2 it was 14.3°C . No bees were observed foraging below these temperatures. In the reproductive phase, none of the temperatures restricted the foraging behavior.

As expected, air temperature and relative humidity were always correlated (Table 1). In the reproductive phase and in the diapause, the total number of incoming workers

depended on the air temperature and relative humidity (Table 2). The flight activity depended more on the temperature in the diapause than in the reproductive phase (higher r values). The partial correlation between the total number of incoming trips and the relative humidity was not statistically significant for colony 1 in the reproductive phase and for colony 2 in the diapause (Table 2).

Nectar collection also depended on the air temperature. However, only the partial correlation between nectar collection and relative humidity in colony 1 in the diapause were statistically significant. Nectar collection was highly correlated with the total incoming trips in the colony (Table 2).

Pollen collection depended on the air temperature in the diapause, but not in the reproductive phase. In contrast, it depended on the air relative humidity in both phases. This activity was correlated with the total number of entrances in the colony only in the diapause (Table 2).

In colony 1, resin collection showed a relationship with the temperature only in the diapause. In colony 2, on the other hand, this activity depended to a minor extent on the temperature in both phases. The partial correlation between

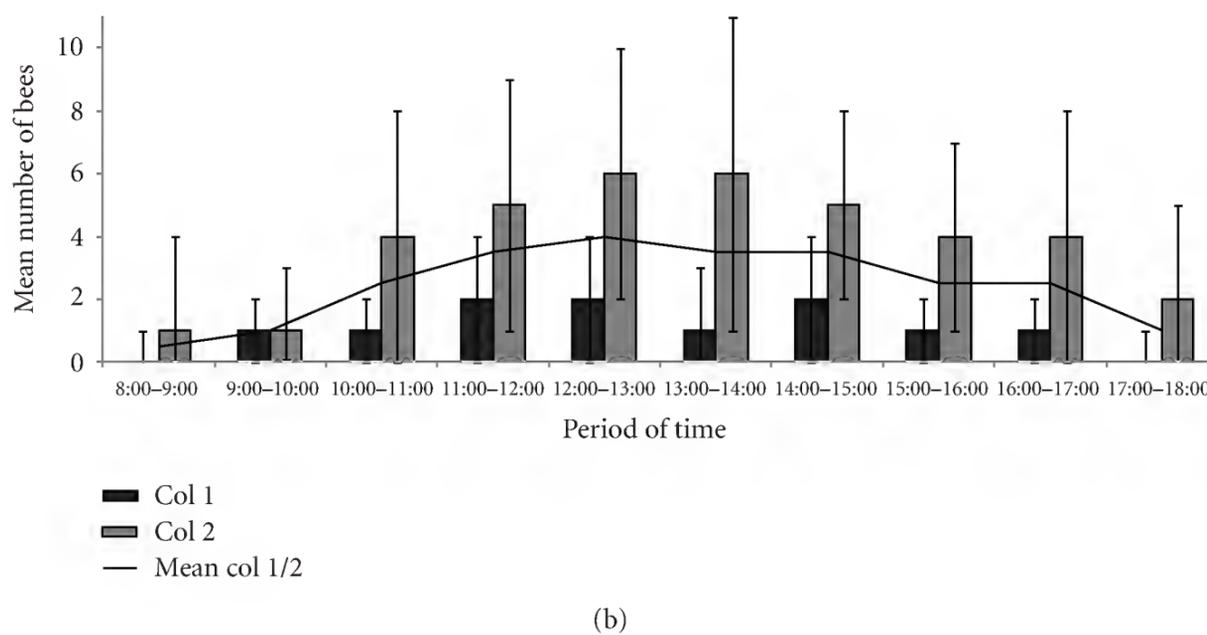
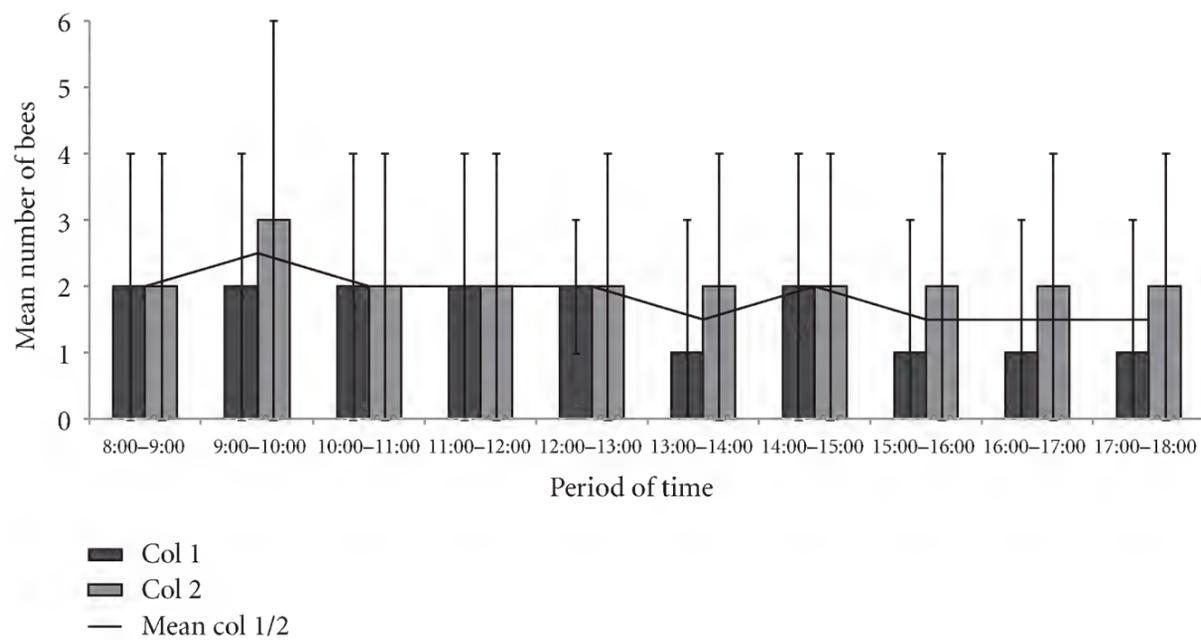


FIGURE 5: Resin collection patterns of two colonies of *Plebeia remota* in the reproductive phase (a) and in the diapause (b).

TABLE 1: Mean, minimum, and maximum temperatures ($^{\circ}\text{C}$) and relative humidity (%) during the reproductive phase and reproductive diapause.

		Colony 1	Colony 2
Reproductive phase	Temperature	Mean	$24.5^{\circ}\text{C} \pm 3.1^{\circ}\text{C}$
		Minimum	17.5°C
		Maximum	32.4°C
	Relative humidity	Mean	$64.6\% \pm 15.2\%$
		Minimum	26.3%
		Maximum	93.3%
Reproductive diapause	Temperature	Mean	$20.0^{\circ}\text{C} \pm 3.2^{\circ}\text{C}$
		Minimum	11.3°C
		Maximum	27.0°C
	Relative humidity	Mean	$58.0\% \pm 15.4\%$
		Minimum	28.3%
		Maximum	95.2%

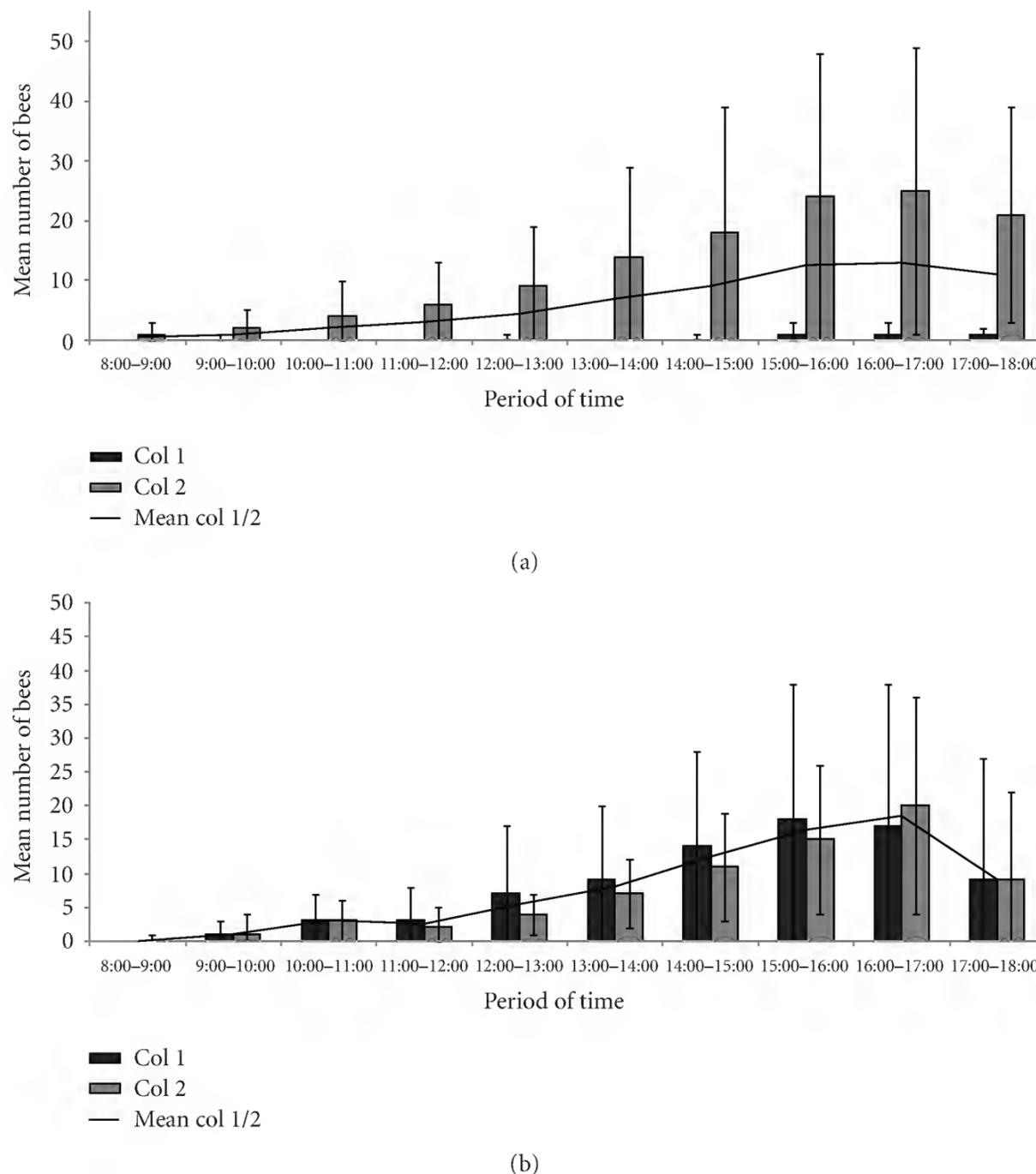


FIGURE 6: Garbage removal patterns of two colonies of *Plebeia remota* in the reproductive phase (a) and in the diapause (b).

resin collection and relative humidity was opposite in the two colonies. In colony 1, this activity was not correlated with relative humidity in the reproductive phase, but it depended to a minor extent on this climatic factor in the diapause. In colony 2, resin collection depended on the relative humidity in the reproductive phase, but not in the diapause. Generally, this activity was not significantly correlated with the total number of incoming trips in the hive, with the exception in colony 1 during the diapause (Table 2).

The partial correlation between garbage removal and temperature was not significant only in colony 2 in the reproductive phase. In colony 1, these two parameters showed a negative partial correlation in the reproductive phase. The partial correlation between garbage removal and relative humidity was not significant only in colony 1 in the reproductive phase. None of the partial correlations between this activity and the total number of incoming trips in the colony was statistically significant (Table 2).

3.3. Individual Activity of Foragers and Colony Rhythm. In the reproductive phase only eight (2%) marked workers

(from the ones we observed) in colony 1 and six (1.5%) in colony 2 were observed while foraging (entering and exiting the colony). In the reproductive diapause, 131 (32.75%) and 32 (8%) marked bees were observed in colonies 1 and 2, respectively. Additional marked bees were observed exiting the colony; however they were not considered in the analyses because we do not know what foraging activity they performed.

Marked bees contributed little to nectar, pollen, and resin collecting during the reproductive phase and reproductive diapause (Table 3).

We observed no marked bees removing garbage from colonies 1 and 2 in the reproductive phase. In the reproductive diapause, we observed only one marked bee in each colony performing this activity. In colony 1, this bee was responsible for only one removal flight (0.04%), but in colony 2, the observed bee was responsible for 7.21% of the removal flights.

We also verified if the marked bees were specialized (nectar, pollen, or resin foragers) or not. In the reproductive phase in colony 1, 50% of the marked bees were nectar foragers (37.5% collected only nectar and 12.5% collected

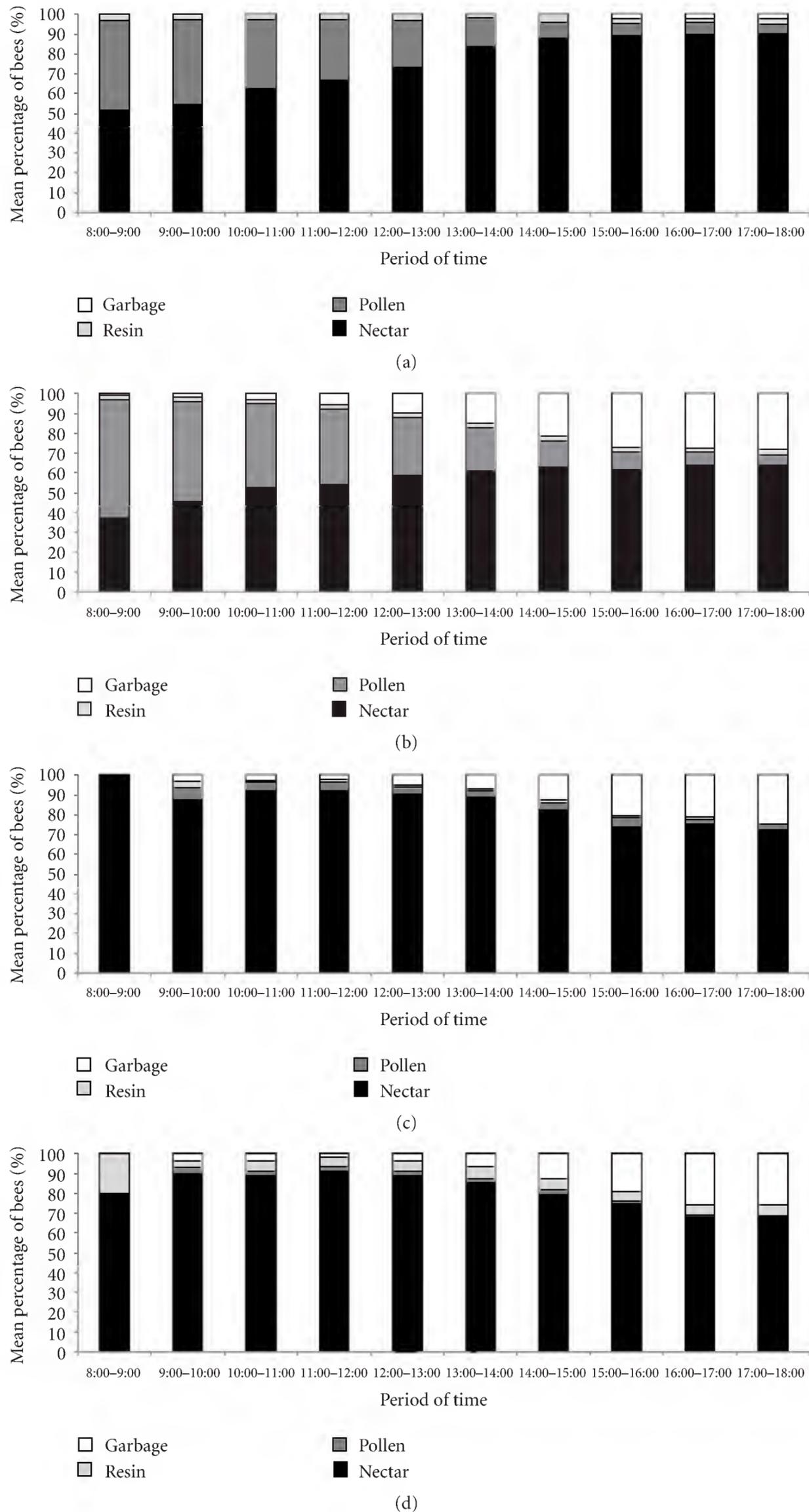


FIGURE 7: Mean percentage of *Plebeia remota* bees collecting nectar, pollen, and resin and removing garbage. Reproductive phase: (a) colony 1; (b) colony 2. Diapause: (c) colony 1; (d) colony 2.

TABLE 2: Partial correlations ($r_{ab,c}$, where a and b are the correlated variables and c the controlled variable) between nectar (N), pollen (P), resin (R), garbage (G), and total number of incoming trips (TE) and temperature (T) and relative humidity (RH); and Spearman correlations (r_{ab} , where a and b are the correlated variables) between temperature (T) and relative humidity (RH), and between nectar (N), pollen (P), resin (R), garbage (G), and total number of incoming trips (TE) in the reproductive phase and in the diapause, in colonies 1 and 2 of *Plebeia remota*.

	Colony 1		Colony 2	
	$r_{\text{reproductive phase}}$	r_{diapause}	$r_{\text{reproductive phase}}$	r_{diapause}
r_{TRH}	-0.7287*	-0.8993*	-0.7236*	-0.8969*
$r_{NT,RH}$	0.2824*	0.4135*	0.2823*	0.2188*
$r_{NRH,T}$	-0.0505 ^{ns}	0.1553*	0.0294 ^{ns}	0.0846 ^{ns}
r_{NTE}	0.8346*	0.9895*	0.7543*	0.9923*
$r_{PT,RH}$	-0.0511 ^{ns}	0.3584*	-0.0106 ^{ns}	0.3319*
$r_{PRH,T}$	0.1368*	0.2957*	0.1243*	0.2470*
r_{PTE}	0.5005 ^{ns}	0.6942*	0.6429 ^{ns}	0.7468*
$r_{RT,RH}$	0.0996 ^{ns}	0.4580*	0.1697*	0.1862*
$r_{RRH,T}$	-0.0261 ^{ns}	0.1565*	0.2750*	0.0742 ^{ns}
r_{RTE}	0.3524 ^{ns}	0.7751*	-0.0116 ^{ns}	0.5223 ^{ns}
$r_{GT,RH}$	-0.1854*	0.2460*	0.0478 ^{ns}	0.2256*
$r_{GRH,T}$	-0.0979 ^{ns}	0.0546*	0.1513*	0.1749*
r_{GTE}	-0.0183 ^{ns}	0.6354 ^{ns}	0.2903 ^{ns}	0.5973 ^{ns}
$r_{TE T,RH}$	0.1914*	0.4539*	0.1816*	0.2512*
$r_{TE RH,T}$	0.0279 ^{ns}	0.1734*	0.1588*	0.1152 ^{ns}

* statistically significant ($P < .05$); ^{ns} not statistically significant ($P > .05$).

TABLE 3: Total number of incoming trips of nectar, pollen, and resin and the percentage of marked bees that performed these activities during the reproductive phase and reproductive diapause in colonies 1 and 2 of *Plebeia remota*.

		Colony 1		Colony 2	
		Total number of incoming trips	% of marked bees performing the activity	Total number of incoming trips	% of marked bees performing the activity
Reproductive phase	Nectar	12332	0.30	15364	0.06
	Pollen	4406	0.36	8483	0.32
	Resin	466	0	627	0
Reproductive diapause	Nectar	22316	8.0	17291	2.76
	Pollen	974	5.85	472	0.21
	Resin	349	1.72	1114	0.09

also pollen), 25% pollen foragers, and 25% were non-specialized (collected nectar and pollen). In colony 2, 66.7% of the marked bees were pollen foragers and 33.3% were non-specialized (collected nectar and pollen).

In the reproductive phase, 94.6% of the marked bees were nectar foragers in colony 1 (86.2% collected only nectar and 8.4% collected also pollen or resin) and 96.9% in colony 2 (87.5% collected only nectar and 9.4% collected also pollen), respectively. In colony 1, 1.5% of the marked bees were pollen foragers in this phase and the rest was not specialized. In colony 2, 3.1% of the marked bees were specialized in garbage removal.

The period of time that a marked bee foraged for was variable. In the reproductive phase foragers of colony 1 collected nectar for an average of 2.2 days (standard deviation: 1.5 days; $n = 6$; maximum: four days) and pollen for 1.4 days (standard deviation: 0.9 days; $n = 5$;

maximum: three days). The mean number of days that the bees foraged (collected any resource or removed garbage) was 2.3 (standard deviation: 2.1 days; $n = 8$; maximum: six days). In colony 2 we observed only two nectar foragers; one foraged for two days and the other for three. The bees foraged for pollen for an average of 2.7 days (standard deviation: 1.6 days; $n = 6$; maximum: five days). The mean number of days that the bees foraged (collected any resource or removed garbage) was 3.3 (standard deviation: 2.3 days; $n = 6$; maximum: six days).

In the reproductive diapause, foragers of colony 1 collected nectar for an average of 3.3 days (standard deviation: 3.5 days; $n = 126$; maximum: 16 days), pollen for 1.9 days (standard deviation: 1.1 days; $n = 16$; maximum: 4 days) and resin for 1.3 days (standard deviation: 0.5 days; $n = 4$; maximum: 2 days). The mean number of days that the bees foraged (collected any resource or removed garbage) was 3.4

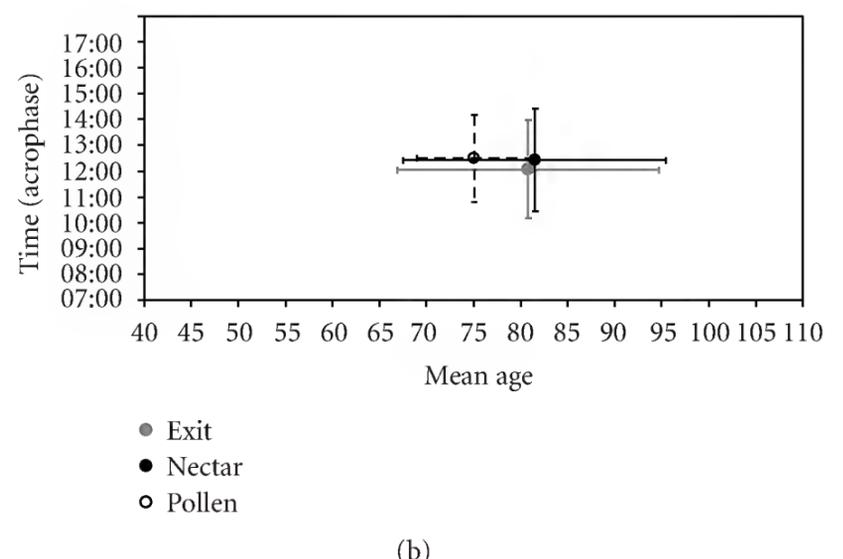
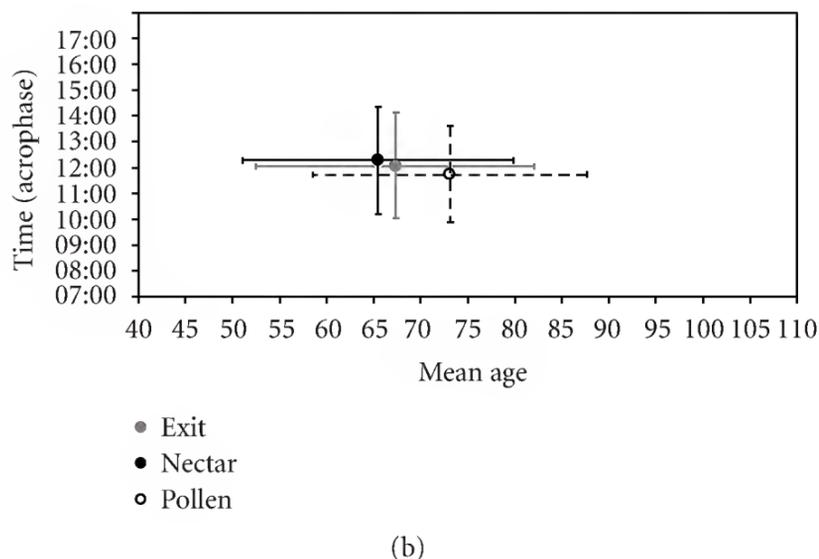
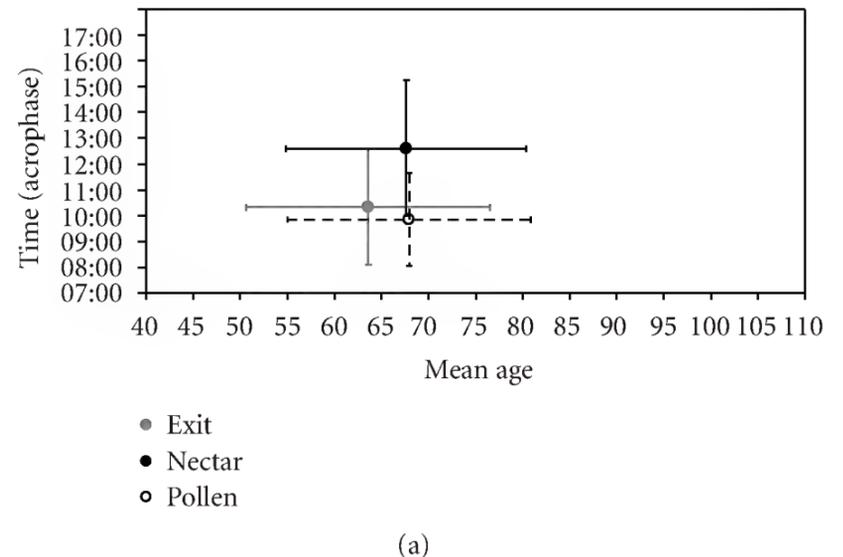
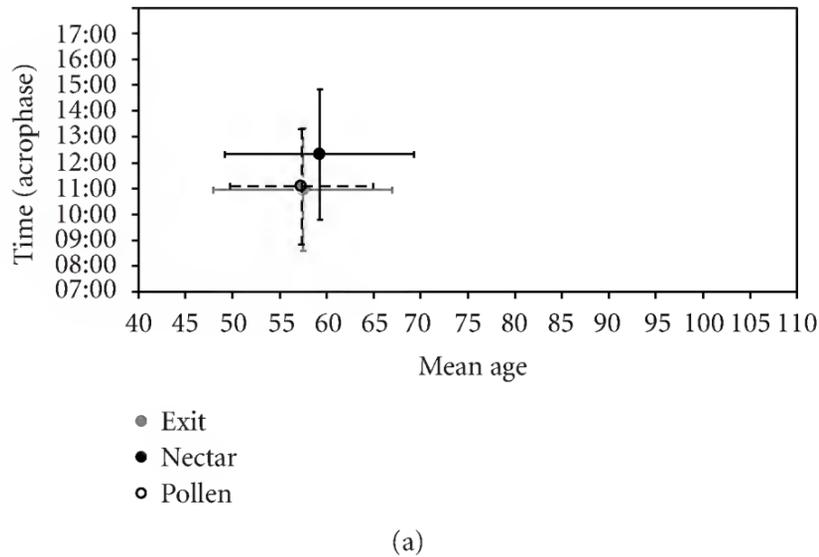


FIGURE 8: Acrophases with their respective angular deviations (y axis) and mean ages with their respective standard deviations (x axis) of nectar and pollen foraging and exit of marked bees of colony 1 of *Plebeia remota*, in the reproductive phase (a) and reproductive diapause (b).

FIGURE 9: Acrophases with their respective angular deviations (y axis) and mean ages with their respective standard deviations (x axis) of nectar and pollen foraging and exit of marked bees of colony 2 of *Plebeia remota*, in the reproductive phase (a) and reproductive diapause (b).

(standard deviation: 3.6 days; $n = 131$; maximum: 16 days). In colony 2, the foragers collected nectar for an average of 4.4 days (standard deviation: 3.6 days; $n = 31$; maximum: 13 days). We observed only two pollen foragers; one foraged for one day and the other for two. Only one bee collected resin and did it for one day. Another marked bee removed garbage from the colony for five days. The mean number of days that the bees foraged (collected any resource or removed garbage) was 4.5 (standard deviation: 3.5 days; $n = 32$; maximum: 13 days).

Although a great variability was observed as to age of marked bees, the foragers of the reproductive diapause were older than the foragers from the reproductive phase (Figures 8 and 9, Table 4) in both colonies.

Acrophases were detected in nectar, pollen, and exit of marked bees (Table 4). These acrophases occurred in different times in the reproductive phase and diapause, with the exception of nectar collection (Figures 8 and 9, Table 4). The acrophase of pollen collection occurred earlier in the reproductive phase (Table 4), but the variation around it (angular deviation) was similar between the two phases (Table 4).

We compared the foraging of marked bees with the colony foraging (marked and nonmarked bees observed). The acrophase of the marked foragers was within the interval of the acrophases of the colony foraging activity (Table 5). This indicated that the foraging activity of the marked bees is representative of the foraging activity of the colony.

3.4. Other Behavioral Observations. We observed the occurrence of nectar transfer between a forager and other bee that was in the tube. This behavior was not quantified, because it was not the aim of the observations, but tropholaxis was seen many times in the entrance tube. Sometimes the forager did tropholaxis with other bee in the tube as soon as it arrived and left the tube immediately. Hence, there is task partitioning in nectar collection in *P. remota*.

The garbage removal is also a task that is partitioned among workers of *P. remota*. We observed that one or two workers remained in the tube carrying a pellet of garbage with their mandibles. These workers passed the pellet to other workers that were in the entrance tube. Those caught the pellet with their first pair of legs, hold it with their mandibles and then flew out of the colony. Many times an

TABLE 4: Acrophases (Ac; hr:min) and angular deviations (AD; hr:min), mean ages (MA; days), standard deviations (SD; days), minimum (MinA) and maximum ages (MaxA), mean vectors (r), number of activities (NA), and number of marked bees observed (Nobs) in the nectar (N) and pollen (P) foraging (F) and in the exit (E) of the marked bees of colonies 1 and 2 of *Plebeia remota*, in the reproductive diapause (RD) and reproductive phase (RP).

	F	Ac \pm AD	MA \pm SD	MinA	MaxA	r	NA	Nobs	
Colony 1	E	12:04 \pm 02:02	67,3 \pm 14,8	42	105	0,856	1469	129	
	RD	N	12:17 \pm 02:05	65,4 \pm 12,4	42	95	0,851	1791	128
		P	11:45 \pm 1:51	73,1 \pm 14,6	50	101	0,882	58	16
	RP	E	10:57 \pm 02:23	57,5 \pm 9,5	38	73	0,804	42	9
		N	12:19 \pm 02:32	59,2 \pm 10,0	44	78	0,779	37	6
		P	11:05 \pm 02:14	57,3 \pm 7,7	48	70	0,827	16	5
Colony 2	E	12:04 \pm 01:55	80,8 \pm 13,9	55	107	0,874	455	32	
	RD	N	12:26 \pm 01:59	81,5 \pm 13,9	55	107	0,864	481	32
		P	12:30 \pm 01:41	75,0 \pm 6,1	68	79	0,903	6	2
	RP	E	10:21 \pm 02:15	63,6 \pm 12,9	43	79	0,825	27	5
		N	12:37 \pm 02:37	67,6 \pm 12,8	45	76	0,763	10	2
		P	09:52 \pm 01:49	67,9 \pm 12,9	43	90	0,885	27	6

TABLE 5: Acrophases (Ac) and angular deviations (AD) of the total number of bees collecting nectar and pollen, and of the marked bees of colonies 1 and 2 of *Plebeia remota*, in the reproductive phase and diapause.

	Phase	Resource	Bees	Ac \pm AD
Colony 1	Reproductive phase	Nectar	Colony	12:28 \pm 02:39
			Marked	12:19 \pm 02:32
		Pollen	Colony	10:11 \pm 02:00
	Reproductive diapause	Nectar	Colony	11:05 \pm 02:14
			Marked	12:32 \pm 02:01
		Pollen	Colony	12:17 \pm 02:05
Colony 2	Reproductive phase	Nectar	Colony	12:25 \pm 02:04
			Marked	11:45 \pm 01:51
		Pollen	Colony	12:32 \pm 02:39
	Reproductive diapause	Nectar	Colony	12:37 \pm 02:37
			Marked	10:17 \pm 02:04
		Pollen	Colony	09:52 \pm 01:49
Reproductive diapause	Nectar	Colony	12:38 \pm 2:05	
		Marked	12:26 \pm 01:59	
	Pollen	Colony	12:10 \pm 01:51	
			Marked	12:30 \pm 01:41

incoming forager caught the pellet from a worker and flew out of the colony immediately. Sometimes the worker that holds the pellet could resist and not give the pellet to the other bee. In this case, they pulled the pellet like in “tug of war” (rope pulling) or the worker ran away from the other bee that tried to catch the pellet while the other chased her.

4. Discussion

The foraging pattern of the reproductive phase was different from the one found in the diapause. Although we found some differences between times of the day, the foraging activity of the bees, during the reproductive phase, was nearly constant. Nevertheless, this activity in the diapause was more concentrated in the middle of the day, as already

found by Imperatriz-Fonseca et al. [29] and Hilário [23]. The foraging pattern of *P. saiqui*, another species that presents diapause, was also different in the two phases (Table 4). These differences may be caused by environmental factors, probably temperature, as discussed later. Another factor that can influence the foraging activity of bees is the variation in the quantity and quality of food resources between days or seasons [2].

In the reproductive phase, the nectar collection was nearly constant along the day, as well as the general foraging pattern of the colonies. This is expected because most of the foraging activity of the colonies was of nectar bringing foragers. Other stingless bee species also present this pattern (Table 6). In the reproductive diapause, nectar was collected more in the middle of the day (11:00–13:00). Another bee species also showed this peak of nectar collection (Table 6).

TABLE 6: Peaks of the different foraging activities of different species of stingless bees (Meliponini) that present or do not present reproductive diapause.

Species	Reproductive diapause	General foraging peak	Nectar foraging peak	Pollen foraging peak	Resin foraging peak	Detritus removal	Reference
<i>Tetragonisca angustula</i>	No	—	None	—	End of morning/beginning of afternoon	—	[27]
<i>Melipona fasciata</i>	No	—	Middle of the day	—	Beginning of morning/after 15:00	—	[27]
<i>Melipona beecheii</i>	No	—	Middle of the day	—	Beginning of morning/after 15:00	—	[27]
<i>Melipona favosa</i>	No	—	Middle of the day	—	—	—	[27]
<i>Melipona bicolor bicolor</i>	No	—	—	Beginning of morning	End of afternoon	End of afternoon	[28]
<i>Melipona scutellaris</i>	No	—	None	Beginning of morning	None	Morning	[29]
<i>Melipona marginata obscurior</i>	No	—	Middle of the day	—	—	—	[30]
<i>Melipona asilvai</i>	No	—	Middle of the day	—	—	—	[31]
<i>Meliponula ferruginea</i>	No	—	None	—	—	Morning	[32]
<i>Meliponula nebulata</i>	No	—	None	—	—	Morning	[32]
<i>Plebeia pugnax</i>	No	—	Middle of the day	Beginning of morning	None	S: 11:00–13:00 W/A: 15:00–17:00	[5, 33]
<i>Plebeia saiqui</i>	Yes	RP: 11:00-12:00 RD: 13:00-14:00	—	RP: beginning of morning RD: none	—	—	[11, 12]
<i>Plebeia remota</i>	Yes	RP: none RD: around 12:00	RP: none; RD: 11:00–13:00	RP: beginning of morning RD: no	None	End of afternoon	This study

Removal of detritus from the colonies occurred mainly at the end of the afternoon. We did not find a pattern in relation to the number of foragers performing this activity in the different colony phases (reproductive and diapause), because each colony behaved in a different way. This activity might be influenced more by the internal conditions than by external factors, like season of the year and climatic factors. Souza et al. [34] found a positive correlation between nectar and pollen income and garbage removal, and suggested that the growth of the colony influences directly the amount of garbage produced by the colonies. The peak of this activity occurs at different times of the day in different species (Table 6). The resin foraging is also different in distinct species (Table 6). In *P. remota* this activity occurred along the day, in both phases.

Besides the distinct daily foraging patterns between the reproductive period and diapause, we observed differential foraging efforts according to the resource, a new finding

for the species studied. A similar number of bees bringing nectar to the colony was observed in the two phases, but the percentage of nectar in relation to the other resources collected was higher in the diapause. This might be a reflection of the differential allocation of foragers among different tasks. In the diapause the number of bees collecting pollen was lower compared to the reproductive phase, so the foragers concentrate their activity in nectar collection. On the other hand, nectar is the sugar source that provides energy for the bees. During diapause bees stay still inside the colony [17], but a large quantity of this resource may be needed when all colonies' activities restart. Although we did not quantify, we observed an increase in the number of storage pots with honey in the diapause and we did not observe pollen stored in the colonies. However, this increase in nectar storage may occur due to the decrease in consumption by the bees. In this phase there is a decrease in the colonies' population over time, since after the emergency

of all the remaining brood, no bees will emerge until the end of the reproductive diapause and the development of the first brood when the reproductive phase begins again.

Nectar could also be needed in greater quantity during diapause because of thermoregulation. *Apis mellifera* individuals can maintain their corporal temperature when they have sugar in their crop [35]. We do not know whether this is true for *P. remota* or not, but if it is, honey is more needed during diapause, when it is colder (autumn and winter). These hypotheses need to be tested. Also it is possible that bees need nectar as a source of energy to forage [36].

We observed quantitative differences in pollen collection of the two phases. A greater number of incomes of this resource was observed in the reproductive phase and the percentage of bees doing this activity increased in relation to the other resources, although nectar incomes made the greatest percentage of the incomes in both phases. This difference in the number of bees foraging for pollen was also observed in *P. saiqui* [13].

Most bees rely on pollen as the main source of nitrogen and it is collected mainly to feed the larvae [3]. In *A. mellifera*, the quantity of brood and stored pollen influences the pollen foraging [37]. This might be the case of *P. remota*. In the diapause there is no cell construction and provisioning of these cells for queen oviposition. This might influence the decision of the foragers, as a higher percentage of pollen foragers was found in the reproductive phase.

Furthermore, there is the possibility that, as in *A. mellifera* [38], winter bees eat less pollen than summer bees. Hrassnigg and Crailsheim [39] state that *A. mellifera* workers respond to different quantities of brood adjusting their behavior and physiology, eating more or less pollen and altering their flight activity. The authors state that this allows that the winter bees live longer and work when there is brood in the colony again. This reduced need for pollen in *P. remota* during diapause may influence the pollen foraging in this species in a similar way that happens in honey bees and maybe this mechanism of regulating the life span is also present in this species, whereas the bees lived longer in the reproductive diapause, which occurs in winter.

Another similarity between *P. remota* and *P. saiqui* pollen foraging was the daily peak of this activity in the reproductive phase. In both species it occurred in the beginning of the morning until 10 am, when it started to decrease until the end of the day [13]. In other stingless bee species a peak of pollen collection also occurred in the beginning of the morning (examples: *M. scutellaris* [13]; *M. bicolor bicolor* [10]; *P. pugnax* [7]).

Climatic factors such as air temperature and relative humidity influence the flight activity of bees, along internal conditions of the colony. The differences found in air temperature and relative humidity between the two phases may be one of the reasons of the distinct patterns observed in the colonies of *P. remota*. Generally, the flight activity of stingless bees is positively correlated with the air temperature and negatively correlated with relative humidity (*P. saiqui* [13]; *M. marginata obscurior* [33, 40]; *M. asilvai* [34]; *Meliponula ferruginea* and *Meliponula nebulata* [34]; *Tetragonisca angustula* [41]; *M. marginata marginata* [40]).

The same relationship was found by Hilário [23] for *P. remota*. However, we found weak relationships between these climatic factors and the different foraging activities of *P. remota*, when they were significant. This might be due to the type of analyses we made. We used partial correlation to describe these relationships. Partial correlation statistically corrects for the effect of a third variable which influences the variables involved in the original correlation [27]. We wanted to evaluate the effect of air temperature and relative humidity in the flight activity of *P. remota*, but these two climatic factors are highly correlated and with simple correlation it is not possible separate the effects of air temperature and relative humidity on this activity.

As Hilário [23], we found weaker relationships in summer (reproductive phase) than in winter (diapause). This might be because in the summer bees forage along the day (all temperatures registered), but in winter this activity occurs mainly in the middle of the day, when the temperatures are higher. Other climatic factors as wind [24] and rain [25] are also responsible for shaping the flight activity of the colonies.

The air temperature is a constraining factor. Bees must warm up before going out for foraging and waste removing. The minimum temperature that occurred during observations was in the diapause: 11.3°C for colony 1 and 11.6°C for colony 2. Flight activity was observed under 14.7°C for colony 1 and under 14.3°C for colony 2, indicating that temperatures under 14°C limits the flight activity of *P. remota*. This temperature is lower than the temperature found by Imperatriz-Fonseca et al. [29] (16°C, but not in winter). However, Hilário [23] observed flight activity under 10.2°C, indicating that the restraining temperature for the flight activity of this species is around 10°C. Other species from these genera presented similar low temperatures for foraging (*P. pugnax*: 14°C [7]; *P. saiqui*: 11°C [14]).

Kleinert-Giovannini and Imperatriz-Fonseca [40] observed that even under optimal climatic conditions for the flight activity of *M. marginata marginata* and *M. marginata obscurior* a decrease in this activity can occur, which indicates that there is a daily rhythm in it under favorable environmental conditions.

P. remota, as other eusocial bee species, presents an age dependent division of labor (age polyethism). Foraging is the final stage of the life of a worker and it begins around 30 days of life [13]. Van Benthem et al. [15] observed 46% of the marked workers performing foraging in autumn and winter. In this study, a similar percentage of bees (32.75%) in colony 1 was observed performing this activity, though in colony 2 a lower percentage (8%) was observed. The percentage of observed marked bees varies among studies (*M. bicolor bicolor*, 60% [42]; *Scaptotrigona postica*, 40% [43]; *Friesella* sp, 34% [44]; *M. compressipes fasciculata*, 88.3% [45]).

The age that the workers of *P. remota* became foragers varied from 43 to 90 days in the reproductive phase and from 42 to 107 days in the reproductive diapause. Van Benthem et al. [15] observed foragers of 30 to 87 days of age in the reproductive diapause, similar to our observations. The difference in the age of foragers of the two phases might be a reflection of the differential longevity of the bees in these

phases, as they live longer, they start foraging later. *P. remota* winter bees live from 25 to 100% more than summer bees [15]. *P. droryana* workers were also observed for more than 100 days in the reproductive diapause and began to forage after 35 days of age [18].

In general *Melipona* species start to forage earlier than *P. remota* (*M. compressipes fasciculata*: 15 to 85 days of age [45]; *M. beecheii*: 16 to over 60 days of age [28]). *S. postica* workers became foragers on 20 to 60 days of age [43] and *Friesella* workers on 17 to 41 days of age [44].

M. beecheii nectar foragers foraged for three days (from two to four days) [28]. Generally, *P. remota* foragers collected nectar for a similar number of days in the reproductive phase and diapause, but the variation (up to 13 days, colony 2) in the reproductive diapause was greater than in this *Melipona* species. The pollen and resin foraging was performed in a shorter period when compared to *M. beecheii* [28]. Based on the previous studies and our results (*M. bicolor bicolor*: around 6 days [42]; *M. compressipes fasciculata*: mean of 10 days [44]; *S. postica*: 3 to 7 days [43]), in general stingless bee foragers perform their activities for less than 10 days.

In stingless bees, the foraging of the colony is based on the individual decision of the workers. Each has to decide when to start or to stop foraging. These decisions are taken using intrinsic information, as genetic information, memory, development and hormones, and extrinsic information, which comes from inside the colony (stored resources, information from other forager, odors, among others) or outside (flower availability and competition, for example) [9–11]. Although there are no studies on the influence of these factors on the foraging of *P. remota*, these factors might influence this activity in this species as well. Besides, the absence of the provisioning and oviposition process, one of the extrinsic information from inside the colony, shall be a key factor in the organization of this behavior in the reproductive diapause, as in this phase there are behavioral changes in the behavior of workers [17, 22] and, as we observed, of the foragers, noticed despite the difference in the proportion of nectar and pollen foragers between the phases.

Diel rhythms were found in the foraging behavior of the *P. remota* colonies, and when we compared the acrophase of nectar collection detected in this study with the acrophase of flight activity detected by Hilário [23], they are very similar. *M. bicolor* also present daily rhythms in flight activity [30]. *Scaptotrigona aff depilis* [46], and *Apis mellifera* [47] also present circadian rhythms, as the studies were done under controlled environmental conditions.

The nectar foraging is a partitioned task in *P. remota*, as in other stingless bee species (*Melipona beecheii* [29, 48], *M. fasciata*, *M. favosa*, *Tetragonisca angustula* [30], *Trigona nigra*, *Plebeia frontalis*, *Scaptotrigona pectoralis* and *Nannotrigona perilampoides* [48]). We also observed task partitioning in garbage removal, but it is almost unknown in stingless bees.

Acknowledgment

The authors thank CNPq (135074/2005-3 and 140169/2000-8) for financial support.

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Research Article

Using Estimated On-Site Ambient Temperature Has Uncertain Benefit When Estimating Postmortem Interval

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Received 4 September 2009; Accepted 21 March 2010

Academic Editor: David Denlinger

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The forensic entomologist uses weather station data as part of the calculation when estimating the postmortem interval (PMI). To reduce the potential inaccuracies of this method caused by the distance between the crime scene and the meteorological station, temperature correlation data from the site of the corpse may be used. This experiment simulated the impact of retrospective weather data correction using linear regression between seven stations and sites in three climatic exposure groups during three different seasons as part of the accumulated degree days calculation for three necrophagous species (Diptera: Calliphoridae). No consistent benefit in the use of correlation or the original data from the meteorological stations was observed. In nine cases out of 12, the data from the weather station network limited the risk of a deviation from reality. The forensic entomologist should be cautious when using this correlation model.

1. Introduction

Knowledge of the postmortem interval (PMI) is of crucial importance in criminal investigations. When the limits of traditional legal medicine are reached for putrefied corpses colonized by insects, forensic entomology can provide the only means for estimating the time since death, often calculated with the thermal summation model [1]. The identification of necrophagous fauna collected on the cadaver [2] and its surroundings, together with analysis of reliable environmental data from the crime scene, enables the entomologist to determine the age of the immature insects and, consequently, estimation of the PMI [3–5]. However, there could be a delay between death and initial insect laying on the body caused by burial, freezing, or confinement in a sealed place, for example.

When insects are used for intelligence or evidential purposes in criminal investigations [6], it is important to assess the factors required for their growth and development. Environmental conditions and climatic parameters, mainly temperature, have an impact on the rate of tissue decay [7] and affect the adult behaviour, larval development, and

insect succession rate [8, 9]. Therefore knowledge of the microclimates under which insects develop on a body is fundamental to forensic entomology [5].

More often than not, the crime scene is located some distance away from the nearest weather station. Therefore, the microclimates experienced at the two locations will differ from each other, and the temperature data will not be directly comparable [6, 10]. These differences may affect the PMI calculation. Among 725 cases treated by the entomology department of the Forensic Science Institute of the French Gendarmerie [11, 12] between 1992 and 2007, 80.5% required accurate studies of climatic data. These cases were general when the corpse was situated outdoors or inside a location which was open to the elements. The other cases dealt with body discovery indoors, where other variables had to be considered such as heating systems, openings, and others parameters which might influence the prevailing conditions.

One of the solutions proposed by forensic entomologists is the use of a correction factor, which is calculated by comparing the data collected from the weather station with the body deposition site for some days after the corpse

has been recovered [7, 13–17]. However, limitations of this method have to be considered. Archer [18], through the use of temperatures correlations between six hypothetical body discovery sites and a single weather station, emphasized the difficulty in the application of retrospective regression when the climatic patterns differ greatly from those that occurred while the body was still in situ.

The following series of experiments introduce the limits of the linear regression method in its application to judiciary casework. The present paper measures the impact, expressed in insect time development, of weather data corrections in different environmental conditions and different correlation durations.

2. Material and Methods

2.1. Location of Experiments. Three groups (exposed, partially protected, and protected) were defined according to their climatic exposure to the following criteria: direct radiance, precipitations, wind, and dew. Some of the characteristics of these three groups are illustrated in Figure 1. Seven sites were chosen based on these exposure groups. The experimental period was spread out across several different seasons for a number of the sites (Table 1).

2.2. Collection of Ambient Temperature Data. Ambient temperature data were recorded with one Testo 175-T1 data logger per site (Table 1). These waterproof data loggers measure temperatures from -35°C to $+70^{\circ}\text{C}$ with a resolution of 0.1°C and an accuracy of $\pm 0.5^{\circ}\text{C}$. All of the data loggers were calibrated with a standard thermometer before the experiment. The relative error was measured at 0.07% to 0.24% before the experiment and 0.32% to 0.70% after. This deviation was due to the natural drift of the equipment with time. The data loggers were set to record at three hour intervals, to assure a sufficient range (some of them stayed several months on the sites), with a start time of midnight, which was synchronized with the Météo France station. The data loggers were placed between 0.2 and 1.50 meters in height from the ground. The data were subsequently analysed with Testo Comsoft software.

Weather data were obtained from the French national meteorology office, Météo France, which has 3725 weather stations throughout the French metropolitan territory. The Météo France temperature data are recorded with international Norma (World Meteorological Organization), which means that it is recorded under sheltered conditions at 1.5 meters in height with grassland surroundings. There was no significant difference in altitude between the locations of the data loggers and the weather stations.

2.3. Selection of Fly Species. Three species of fly (Diptera-Calliphoridae), which are known to colonize corpses soon after death [1, 3] were selected according to their frequency among 725 forensic caseworks carried out by the department and their developmental threshold (low, middle, and high value—Table 2) [1, 19]. *Calliphora vicina* Robineau-Desvoidy was encountered in 33% of cases. It is a common species of fly widely distributed throughout the Holarctic

region and reputed to be a very common urban species. Adults are attracted to faeces, decaying meat, and fruit whereas the larvae develop in carrion. *Lucilia sericata* (Meigen), present in 15.6% of the cases, is widespread throughout the major zoogeographical regions. Adults are attracted by carrion, open wounds, and faeces. The larvae can complete their development in all of these substrates. Finally, *Protophormia terraenovae* (Robineau-Desvoidy) encountered in 8% of cases has a Holarctic distribution. It is most common in spring and in summer where higher temperatures occur. The larvae develop in carrion.

2.4. Simulations from Retrospective Correlations. For each site the period of study was 40 days. At sites S2 and S7, three different times of the year were chosen and two different times of year were selected for site S6 (Table 3).

The temperatures shown in the table correspond to the following:

- (i) the daily average of temperature minima and maxima from Météo France data,
- (ii) the daily average of the temperatures recorded every three hours by the data loggers.

The day of cadaver discovery was termed $D = 0$. From $D = 0$, a linear regression curve was calculated between the temperature data from the data loggers (T_d) and from the Météo France station (T_{ws}) for three different durations: 5, 10, and 15 days (C_5 , C_{10} , and C_{15}) (Figure 2). The selection of the three durations has to be considered as a simulation (from $D = 0$) for a way to build a more representative working model for the PMI calculation in real casework and its judicial constraints (time limits, costs, and availability of data).

For 25 days before $D = 0$, we applied the linear regressions on T_{ws} . We obtained an estimation of temperatures (T_{est}) for C_5 , C_{10} , and C_{15} (Figure 2). Then the summation of the difference between T_d and T_{ws} for a period of 25 days was calculated:

$$\sum (T_d - T_{ws}). \quad (1)$$

The same calculation was done between T_d and each T_{est} obtained for the 25 days:

$$\sum (T_d - T_{est}). \quad (2)$$

The choice of time period for correlation calculations overlapped imposes a direct comparison between T_{est} (with C_5 , C_{10} , or C_{15}) and T_d and/or T_{ws} and not between T_{est} themselves.

2.5. Insect Development. In forensic entomology, the PMI estimation is based on the study of the duration of insect development. There is a direct relationship between time of development and the ambient temperatures experienced by the insect. Marchenko [19] reported that the development of insects could be described using temperature summation, that is, the accumulated degree day (ADD) model.

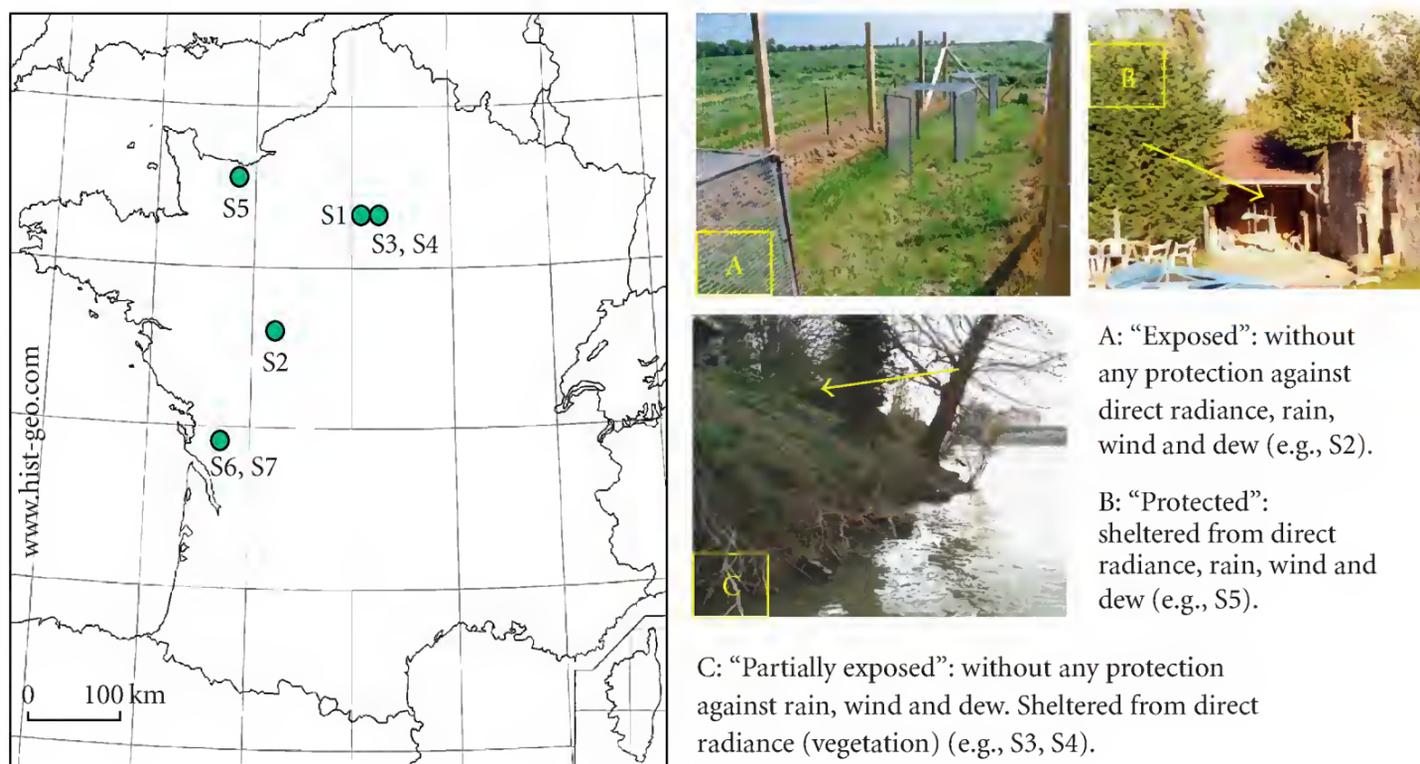


FIGURE 1: Location and general characteristics of the sites.

TABLE 1: Characteristics of the sites and data loggers’ climatic exposure; comparison with their meteorological station associated (A, B, or C: periods of experimentation for a same spot).

Spot (s)	Period of experiment	Town/Characteristic of location (S)	Data loggers climatic exposure	Distance Meteorological station (MS)—S	Altitude MS/(Altitude MS-Altitude S)
S1	03/10/2002 to 11/11/2002	Rosny sous Bois/On lawn	exposed	0.03 km	112 m/0 m
S2 (A, B, C)	A: 13/05/2004 to 21/06/2004 B: 27/09/2004 to 05/11/2004 C: 04/03/2005 to 12/04/2005	Nouzilly/Fallow field—on the ground	exposed	15.10 km	148 m/−2 m
S3	03/10/2002 to 11/11/2002	Joinville Le Pont/Under cover of vegetation	partially protected	0.20 km	37 m/0 m
S4	03/10/2002 to 11/11/2002	Joinville Le Pont/Banks of Marne river—Under cover of vegetation −1.8 m from the river	partially protected	0.20 km	37 m/0 m
S5	15/04/2004 to 24/05/2004	Cairon/Stone shelter with roof in tile −30 m ² —totally open on its South façade	protected	6.60 km	62 m/26 m
S6 (A, B)	A: 20/04/2004 to 29/05/2004 B: 22/07/2004 to 30/08/2004	Fontenet/Breeze block shelter with roof in cement −2 m ² —Partially open-Facing West	protected	12.20 km	148 m/−2 m
S7 (A, B, C)	A: 28/08/2005 to 06/10/2005 B: 28/03/2006 to 06/05/2006 C: 08/06/2006 to 17/07/2006	Fontenet/Breeze block shelter with roof in corrugated iron roof −2 m ² —totally open on its North East façade	protected	12.20 km	148 m/−2 m

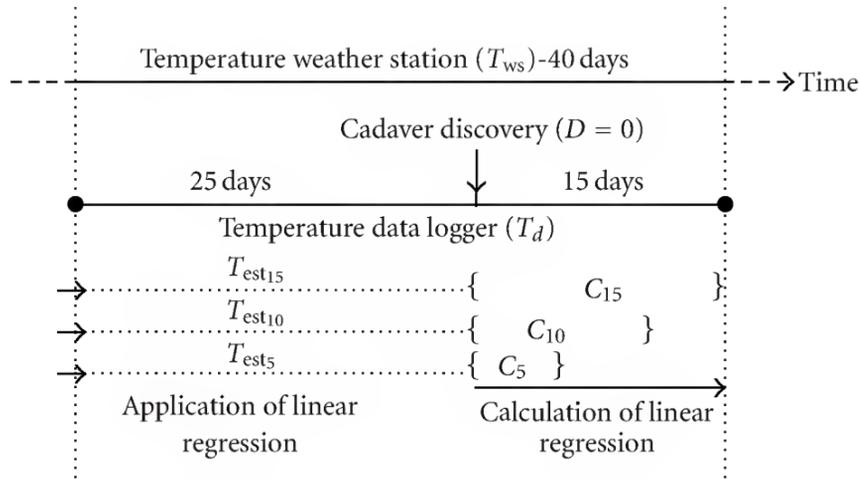


FIGURE 2: Calculation and application of linear regression.

TABLE 2: Thermal parameters ($^{\circ}\text{C}$) regulating development of flies. Data are from Marchenko [19] (ADD: accumulated degree day).

Species	ADD from egg to adult	Minimum development threshold
<i>Calliphora vicina</i> R.-D.	388	2.0
<i>Protophormia terraenovae</i> (R.-D.)	251	7.8
<i>Lucilia sericata</i> (M.)	207	9.0

To determine an estimate of the date of oviposition, the IRCGN Entomology department mainly utilises ADD model. Other published insect developmental data recorded under constant temperature conditions, as Kamal's work [8], are also used at the department in casework. The total ADD needed for insect development at one constant rearing temperature (ADD_i) was calculated from the equation: $\text{ADD}_i = n(T_i - T_s)$, with n being the number of development days, T_i the rearing temperature, and T_s the development threshold. This estimation of ovipositions period is expressed as a time interval.

In this study, we used the ADD model for all of the data from the three species of Diptera. Zero was used to replace all of the temperature values recorded below the developmental threshold.

T_d , which is the temperature recorded at the site, was used as a reference. There were two ways to express the results.

- (1) When the total temperature summation needed to complete development (with T_d) was not reached after 25 days, the total temperature summations were compared at 25 days.
- (2) When the total temperature summation needed to complete development (with T_d) was reached before 25 days, the total temperature summations were compared at this date, which was the closest value required for total development.

Comparisons between ADD T_d and the other ADD values obtained from T_{ws} and the correlations were produced in temperature format (degrees Celsius) and then transformed

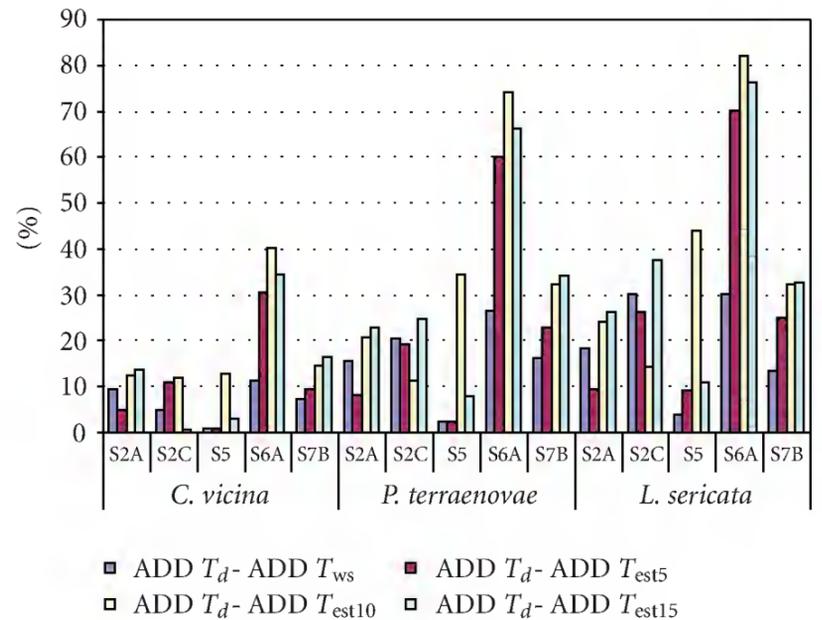


FIGURE 3: Differences between ADD T_d and ADD values using T_{ws} and correlations compared to ADD T_d , expressed as percentages ($[\text{ADD}_x - \text{ADD } T_d] / \text{ADD } T_d \times 100$) for data registered in spring.

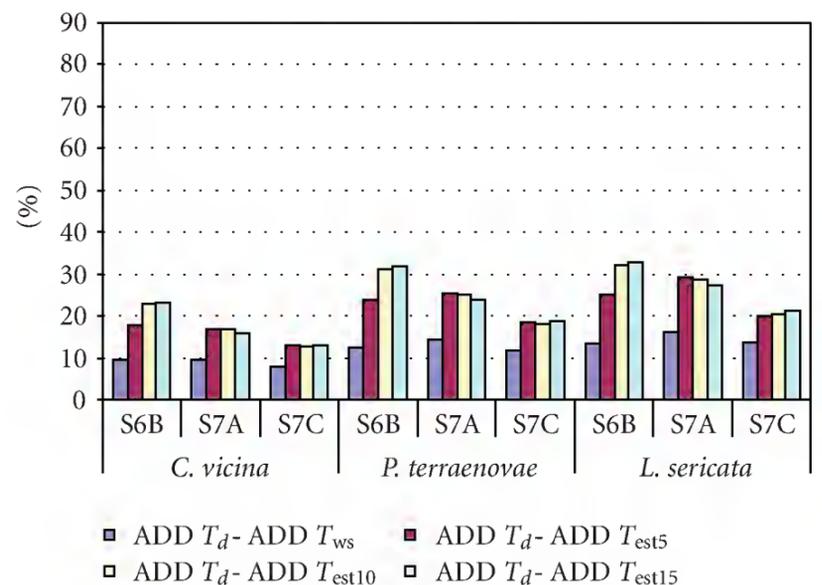


FIGURE 4: Differences between ADD T_d and ADD values using T_{ws} and correlations compared to ADD T_d , expressed as percentages ($[\text{ADD}_x - \text{ADD } T_d] / \text{ADD } T_d \times 100$) for data registered in summer.

into percentage values for ease of understanding (Figures 3, 4, and 5).

3. Results

Results are shown in Table 3 and in Figures 3–5. Overall, there was no consistent benefit in the use of correlation or the original data from the meteorological stations. In 9 cases out of 12, the data obtained from Météo France stations are closer to the local situation (T_d). In the three remaining cases, the best fitting results were obtained with C_5 or C_{15} .

For the sites under different climatic exposures, the best fitting temperature data originated from different sources. For S1, the most suitable temperature data was obtained with C_{15} and with an ADD shorter no matter which species was considered. For S2, depending on the time of year and the species considered, it was difficult to determine the most

TABLE 3: Summation of the difference between T_d and others temperatures ($^{\circ}\text{C}$) data for 25 days.

Spot	$D = 0$	Linear regression curve equation; R^2	$\sum(T_d - T_{ws})$	$\sum(T_d - T_{est_5})$	$\sum(T_d - T_{est_{10}})$	$\sum(T_d - T_{est_{15}})$
S1	28/10/2002	$C_5: y = 1.0172x - 0.4713; R^2 = 0.9068$	-16.16	-9.87	-14.28	4.67
		$C_{10}: y = 1.0682x - 0.9465; R^2 = 0.9149$				
		$C_{15}: y = 0.841x + 1.199; R^2 = 0.8468$				
S2 A	07/06/2004	$C_5: y = 0.6941x + 5.3294; R^2 = 0.5810$	34.60	17.82	45.63	50.12
		$C_{10}: y = 0.8332x + 2.0987; R^2 = 0.9067$				
		$C_{15}: y = 0.8773x + 1.2475; R^2 = 0.9375$				
S2 B	22/10/2004	$C_5: y = 0.9038x + 0.0263; R^2 = 0.9165$	11.70	41.92	35.11	36.20
		$C_{10}: y = 0.7369x + 2.4418; R^2 = 0.8691$				
		$C_{15}: y = 0.7373x + 2.3932; R^2 = 0.897$				
S2 C	29/03/2005	$C_5: y = 0.8418x + 1.8087; R^2 = 0.3797$	-5.45	-19.95	-22.18	15.84
		$C_{10}: y = 0.7554x + 2.5692; R^2 = 0.2638$				
		$C_{15}: y = 1,2407x - 2.7212; R^2 = 0.7564$				
S3	28/10/2002	$C_5: y = 0,9831x + 0,6616; R^2 = 0,9404$	-14.23	-25.12	-21.60	-17.16
		$C_{10}: y = 1,0683x - 0,6191; R^2 = 0,9524$				
		$C_{15}: y = 0,9848x + 0,3204; R^2 = 0,9186$				
S4	28/10/2002	$C_5: y = 1,0923x - 0,8681; R^2 = 0,94$	-6.37	-15.53	-10.30	-7.25
		$C_{10}: y = 1,1116x - 1,3356; R^2 = 0,9492$				
		$C_{15}: y = 1,0227x - 0,2685; R^2 = 0,9163$				
S5	10/05/2004	$C_5: y = 1,3852x - 4,35; R^2 = 0,9665$	-1.88	1.69	28.47	-6.49
		$C_{10}: y = 0,954x + 0,7115; R^2 = 0,9915$				
		$C_{15}: y = 0,9438x + 0,7984; R^2 = 0,9894$				
S6 A	15/05/2004	$C_5: y = 1,0567x - 2,7928; R^2 = 0,9894$	30.81	83.96	110.98	94.97
		$C_{10}: y = 1,1332x - 4,7736; R^2 = 0,9679$				
		$C_{15}: y = 1,078x - 3,4839; R^2 = 0,9486$				
S6 B	16/08/2004	$C_5: y = 0,6028x + 6,8452; R^2 = 0,3515$	55.22	97.83	123.89	125.42
		$C_{10}: y = -0,0693x + 20,269; R^2 = 0,0029$				
		$C_{15}: y = -0,3659x + 26,592; R^2 = 0,1363$				
S7 A	22/09/2005	$C_5: y = 1,1488x - 3,8955; R^2 = 0,9189$	40.27	69.81	71.17	65.54
		$C_{10}: y = 1,0032x - 1,2942; R^2 = 0,8203$				
		$C_{15}: y = 1,1275x - 3,3363; R^2 = 0,9275$				
S7 B	22/04/2006	$C_5: y = 0,5683x + 5,9323; R^2 = 0,8663$	16.13	-21.37	32.49	36.85
		$C_{10}: y = 0,9635x - 0,28; R^2 = 0,925$				
		$C_{15}: y = 1,0345x - 1,1833; R^2 = 0,9594$				
S7 C	03/07/2006	$C_5: y = 0,699x + 5,2275; R^2 = 0,9899$	36.29	66.15	61.57	62.03
		$C_{10}: y = 0,8556x + 2,0696; R^2 = 0,9413$				
		$C_{15}: y = 0,9345x + 0,368; R^2 = 0,9654$				

suitable values for use in the calculation: C_5 for S2A, T_{ws} for S2B, and C_{10} and C_{15} for S2C.

In partially protected conditions (S3, S4), the T_{ws} were the nearest to T_d , and in the ADD model, the lowest deviation was obtained with T_{ws} . However, for S3, the most suitable data for the estimation of the developmental time of *P. terraenovae* were obtained using C_5 . For the others species, the use of C_5 data resulted in the most significant ADD deviation.

In protected conditions, T_{ws} were the most appropriated data to use for all sites, except for S5 where a correlation based on 5 days gave a similar estimate for *C. vicina* and *P. terraenovae*. However, for S5, the differences with T_{ws} were negligible when compared with $T_{est_{10}}$ and $T_{est_{15}}$. For

S6 (A and B), T_{ws} was the closest and the deviation could be considered as significant in comparison with the ADD obtained by T_d . This difference was very marked in the temperature obtained by all the linear regressions.

For S7, none of linear regressions could be applied for the three periods of study; T_{ws} gave the best result for all species and in all cases. For S7A and S7C, the results obtained with linear regressions were very close. For S7B, the situation was less homogeneous; temperatures were overestimated with C_5 for all species and with C_{15} for *P. terraenovae*.

Finally, for all the species and temperatures considered, the higher the developmental threshold is, the greater the deviation became. Therefore, for a species with a low developmental threshold, for example, *C. vicina*, the PMI was

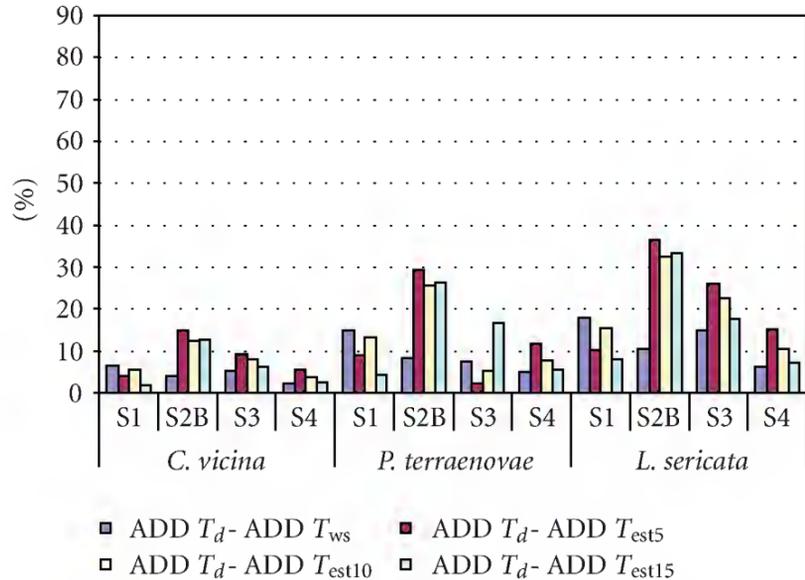


FIGURE 5: Differences between ADD T_d and ADD values using T_{ws} and correlations compared to ADD T_d , expressed as percentages ($[(ADDx-ADD T_d)/ADD T_d] \times 100$) for data registered in autumn.

closer to reality compared to a species with a higher threshold. Moreover, whatever the origin of temperatures (whether from weather station or estimated by linear regression), there existed a difference (positive or negative) with temperatures recorded on the site by the data loggers.

4. Discussion

In forensic entomology technique, one solution to obtaining an estimate of the temperature data that the body might have been exposed to would be the use of a mathematical tool to obtain an estimate based on temperatures recorded at the site post body discovery and weather station temperature data. However, neither temperatures from linear correlations nor weather station data could provide a truly accurate representation of temperatures experienced at a crime scene.

4.1. Effect of Linear Regression. If we compare all of the results obtained for the three different time intervals (5, 10, and 15 days) for the linear regressions used in the 12 “cases”, none of these were more suitable than the others for describing the situation recorded by data loggers. Moreover, consequences on the time duration were not standardized and could increase the deviation significantly. For example, for S2B and *Lucilia sericata*, development time deviation obtained with linear regressions was three times greater than when using weather station. For S6A, development time deviation with T_{est10} reached 82.2% of the total development time obtained with T_d for the same species whereas this deviation was 30.3% with T_{ws} . Thus, a positive deviation from temperatures registered by data loggers caused an overestimation of the local temperatures and led to reduce estimate of the length of time for insect development. A negative deviation caused an underestimation of the local temperatures and thus led to increase estimate of the length of time for insect development.

Furthermore, in ADD calculation, insect time developments could be affected when ambient temperature is closed to their theoretical development threshold [4, 18, 20]. This

is especially true when temperatures estimated by linear regression are lower than the threshold whereas temperatures recorder on the site are upper.

4.2. Effect of External Parameters. Within the same site, it was difficult to estimate the impact of the time of year on the method to use. For example, for S2, three times of year were selected and the linear regression for 5 days was more representative in June whereas T_{ws} was the most appropriate for October and C_{10} or C_{15} in March. This situation, at similar times of year, was not the same at sites S6 and S7. Here, T_{ws} was the most suitable value to use in calculations.

Additionally, neither the correlation data nor the data from Météo France were linked to the distance between the site and the weather station locations. At site S1, the distance from the station was 30 meters and the best result was obtained using C_{15} . For more distant stations (12.2 kilometers for S6 and S7) T_{ws} were the most suitable data to use.

Regarding the three different types of location (Figure 1), it appeared that T_{ws} was more accurate for describing the situation for partially protected and protected exposures (except for S5 which had a small difference between T_{ws} and C_5). For S1 and S2 (in exposed conditions), T_{ws} values were not the most suitable. This could be explained by the Météo France protocol whereby ambient temperatures are recorded under sheltered conditions.

4.3. PMI Estimation. The forensic entomologist has to use the most reliable data to provide an accurate PMI. At first glance, a statistical model based on correlations would appear to be a logical and useful method. However, the situation is more complex and depends on many external factors which are difficult to quantify. Regression (i.e., linear), sometime used in casework, is useful but there is risk of obtaining a significant deviation from the temperatures actually experienced at the crime scene. Correlations are obtained with data recorded after the body has been discovered and insect development has already occurred. Rain, direct sun exposure on the corpse and the state of the vegetation in the vicinity are some parameters which may directly affect the local temperature experienced by the necrophagous insects [5]. Therefore, care needs to be taken when using estimates obtained from linear regression calculations for temperatures at a crime scene and climatic variations during that occurred during the period of the body being present should be considered is possible.

Regarding this study and the extent of the meteorological station network available in France, the department of forensic entomology from IRCGN will continue to use data from Météo France directly in its forensic casework. Despite the fact that weather station data are likely to be different from conditions experienced at the crime scene, they should be considered, by default, as the most ideal data to use for PMI estimations, bearing in mind the possibility of any deviations. The risk of any deviations is considered to be acceptable for the purposes of forensic entomology as long as they are taken into account when providing PMI estimations and an appropriate range is given.

At least, it is necessary to realize that this study was performed with Marchenko data (ADD model). However, numerous authors displayed disparities in the threshold value for a same species depending, for example, on protocols of the study or the geographical strain origin [21–23]. These different parameters affect the postmortem interval calculation.

Thus, a deeper understanding of insect development rates, their physiological activity close to the developmental threshold, and the succession mechanisms of necrophagous insects may be a better way of improving the accuracy of PMI estimates in forensic entomology.

Acknowledgments

The authors are very grateful to Bernard Chauvet, Fabrice Lefèbvre, and Jean-Bernard Myskowiak for their great help to carry out these experiments. They thanks also Gérard Mayençon from Météo France for his availability and his technical advises. Special thanks to Andrew Hart for his precious help.

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Research Article

First Record of Trophobiotic Interaction between a Ponerine Ant and a Cicadelid Bug

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Received 16 March 2010; Accepted 28 April 2010

Academic Editor: Martin H. Villet

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The interactions of the ant *Odontomachus bauri* with nymphs of the sap-sucking bug *Xedreota tuberculata* (Cicadellidae: Ledrinae) were studied on *Sipanea* aff. (Rubiaceae) along a trail in an upland forest in the Ecological Reserve of Anavilhanas, AM, Brazil. Five complete interactions at day and at night (about 60 minutes) were analyzed. The care of cicadelid nymphs ranged between 12 and 961 seconds.

1. Introduction

Ants are the main arthropod predators in tropical forests [1–3] with many species using plants as shelters or foraging areas [4–7]. Some of these interactions are obligatory and several other facultative with plants attracting ants opportunistically. Extrafloral nectaries (EFNs) which are present in at least 66 angiosperm families [8, 9] or food bodies, present in at least 20 angiosperm families, are the main attractive agent and consequently, promote myrmecophily [10]. The presence of some insects such as bugs (Insecta: Hemiptera) on a plant is virtually a guaranty of the presence of ants which attend them [5, 11–13]. These insects suck the phloem and secrete honeydew from the anus which is fed on by some ant species [14–17].

Recent revisions on Hemiptera-Formicidae interactions include [18–20]. Ponerine ants are mainly solitary predators [1, 11], and some of them live in plants with EFNs or bearing sap-sucking bugs [1, 21].

Sap-sucking bugs generally secrete honeydew by ejecting high pressure drops some distance from insect body. This action avoids the attraction of predators and the development of mold in the colony and reduces the chance of colony drowning. Ants inhibit this behavior [22] eliciting the bug body with their antennas to obtain a perfect extruded drop which they then collect [23, 24].

Historically trophobiotic interactions between ponerine ants and cicadelid bugs have been little known [25]; the goal of this work was to describe our observations made in the Amazon on the interaction between workers of the ant *Odontomachus bauri* (Hymenoptera: Formicidae, Ponerinae) and nymphs of *Xedreota tuberculata* (Hemiptera: Cicadellidae).

2. Materials and Methods

The ant was identified following [26, 27] and the hemiptera following [28–30] and confirmed by Dr. Chris Dietrich (Center for Biodiversity, Illinois Natural History Survey) with vouchers deposited in the collections of MZUSP (USP) and MHNU (UNICAMP).

The observations were made on two plants of *Sipanea* aff. (Rubiaceae; Figure 2(a)), the first with 35 leaves, a height of 110 cm, and distant 140 cm away from the second with 15 leaves and a height of 60 cm, which were located in a trail behind Base de Pesquisas Dois on the Ecological Reserve of Anavilhanas, Brazil (2°32′01″ S; 60°50′03″ W). The higher plant grew next to a decomposing trunk which was diagonally placed in the trail with the nest of *Odontomachus bauri* being located at the base of this plant.

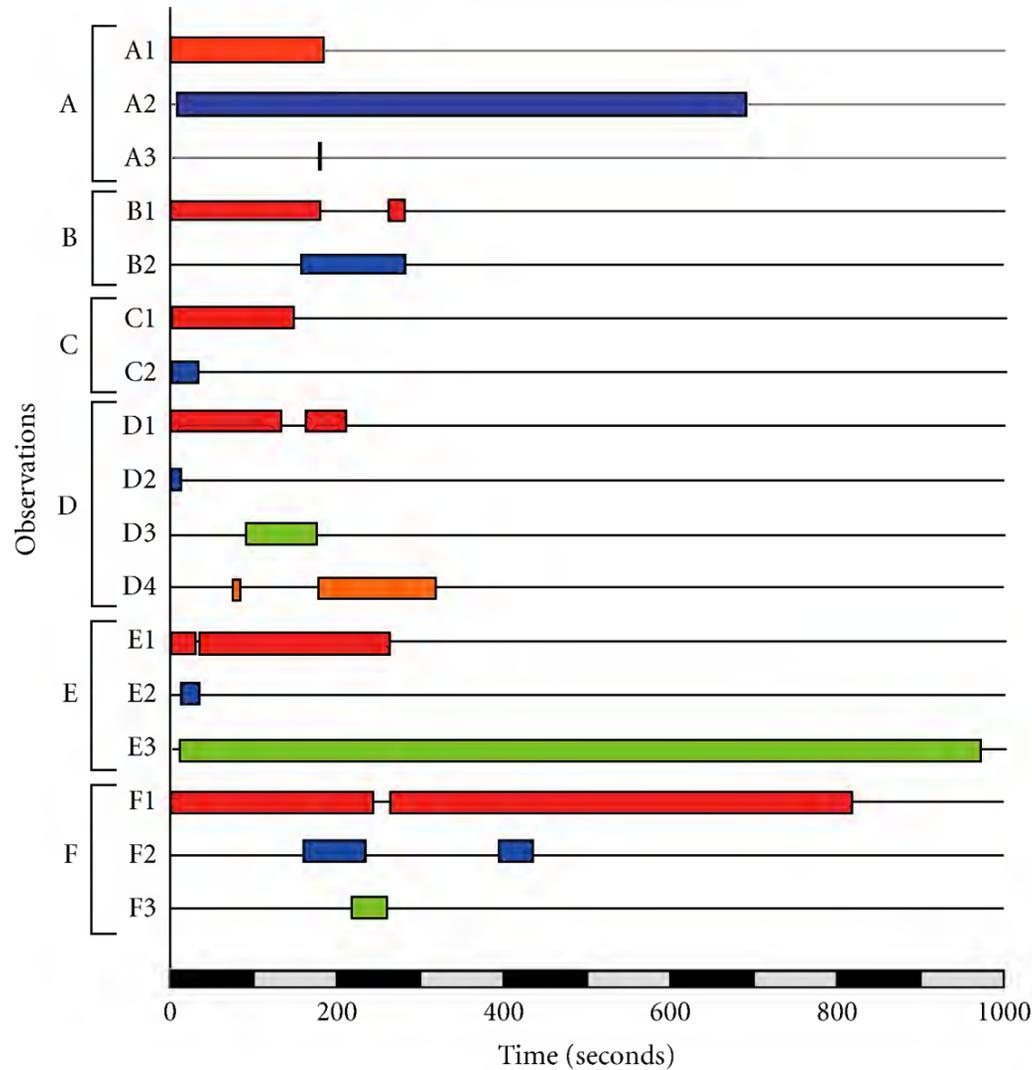


FIGURE 1: Duration of six video sessions of the interaction between different individuals of *Odontomachus bauri* attending nymphs of *Xedreota tuberculata*. The different colors represent different individuals of *Odontomachus bauri* observed simultaneously.

From July 13th to July 19th, we observed the interactions between the cicadelid nymphs and ants during the day and night. A total of 240 minutes of ant-cicadelid interaction were recorded on video, comprising six sequences of approximately 60 minutes each. We considered a sequence as the moment of arrival of an ant at the nymph location, its interactions with the nymphs, and the departure of ant from the group of nymphs.

3. Results and Discussion

This was the first time that *X. tuberculata* was found on *Sipanea* aff. (Rubiaceae) although [31] reported this species as a generalist, found on plants of the families Lauraceae, Leguminosae, Caesalpiniaceae, and Bombacaceae.

The workers of *O. bauri* attended the nymphs of *X. tuberculata* both during the day (morning and afternoon) and at night. The nymphs of *X. tuberculata* focussed on feeding on the more apical stems of the plant, where the stem or leaves are red. The care of nymphs ranged between 12 and 961 seconds not fitting a normal distribution (mean = 196.2 seconds; variance = 74674.32 seconds; kurtosis = 3.20; skewness = 1.97) (Figure 1A–F). Between 2 and 4 ants were observed interacting with a group of nymphs (Figure 1D). During the longest video sequence, one ant spent 961 seconds in the presence of two other ants (Figure 1E). When

eliciting a nymph the ants vibrated their antennae on the anterior region of the bug's abdomen (Figure 2(a)). The ant would consume the drop either directly from bug's anus with open jaws (Figure 2(b)) or by capillary action in the space between the closed or partially closed jaws. When full replenished the ant would return to the nest. Occasionally, ants were observed patrolling the plants where the nymphs were located occasionally stopping to check for nymphs.

If the plant was disturbed, the ants would respond, descending to the base. An attempt to remove one stipule caused an ant to bite the tweezers used. Two species of ant (*Crematogaster* sp and an unidentified formicine) were observed in close proximity to the *Odontomachus* ant, without eliciting a response.

According to [11], *Odontomachus* species are solitary forest predators; however this research has shown that *O. bauri* showed a nonpredatory behavior known towards *X. tuberculata*, a cicadelid bug capable of producing honeydew. This behavior has also been observed among some Ponerinae species which are used to collect the honeydew produced by hemipterans including: *O. haematodus*, from west Africa [32], and the American species *O. troglodytes* [33] from Cuba, *Ectatomma tuberculatum* (Colombia) and *E. ruidum* [34] from Ecuador and Trinidad, and *E. sp.* [35] from Guyana. In addition, L. Passos and P. S. Oliveira [36] found *Odontomachus chelifer* as disperser of nonmyrmecophilous seeds present in the soil of tropical forests in the coastal areas



(a)



(b)

FIGURE 2: (a, b) interaction of a worker of *Odontomachus bauri* to obtain a drop of the sugar liquid secreted by the nymph.

of southeastern Brazil. Thus it would seem that ants of the genus *Odontomachus* are not strictly predators and are able to feed off both honeydew and seeds.

Acknowledgments

The authors would like to thank MsC Daniel de Paiva Silva (UNICAMP), Dr. Kleber Del-Claro (UFU), and Dr. Woodruff Whitman Benson (UNICAMP) for the detailed revision of the paper and Dr. Chris Dietrich (Center for Biodiversity, Illinois Natural History Survey) for the identification of the cicadellid bug. They also are grateful to IBAMA, for the authorization to perform this study in the Reserva Ecológica de Anavilhanas and Ms. Bruno Marchena Tardio for the help in the field.

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Review Article

Intraspecific Variation in a Scorpionfly Newly Recorded from Texas and the State of Taxonomy of North American Panorpidae (Mecoptera)

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Received 17 February 2010; Accepted 12 April 2010

Academic Editor: Michael S. Engel

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Panorpa vernalis Byers is recorded for the first time from Texas, and represents only the second species of *Panorpa* documented from the state. Intraspecific variations between the Texas specimens and Byers' original description are discussed. A synopsis of the principal modern keys for identification of North American *Panorpa* is provided, and an argument for a modern taxonomic review of the Panorpidae of North America is presented.

1. Introduction

Texas is the largest state within the continental United States. Its expanse, an area 773 miles (1244 kilometers) wide and 790 miles (1270 kilometers) long and covering 261,797 square miles (678,050 square kilometers), includes mountains, deserts, medium-elevation hill country, high- and low-elevation plains, extensive drainages, and a long coastline, all products of a complex geologic history [1]. The diversity of these regions comprises a vast array of habitats with highly varied floras and faunas. In the east, pine forests predominate. Mixed oak grasslands occupy much of the central portions of the state, while sage and mesquite blend with a host of woody shrubs and grasses in the panhandle, west and south. Ranches and farmland dot this landscape, and riverine forests course throughout [2].

While some of Texas' geologic regions are contained completely within the political boundaries of the state, several, with their respective floras and faunas, extend into Texas from adjacent states and regions [3, 4]. The latter distribution pattern ought to hold true for the scorpionfly genus *Panorpa* (Mecoptera: Panorpidae), a speciose taxon (84 North American species) [5] distributed broadly throughout the deciduous forests of eastern North America,

from Quebec to Mexico and from the East Coast westward to at least Kansas and Oklahoma. However, despite the presence of abundant habitat ideal for scorpionflies in the eastern portions of Texas, prior to this paper only a single species of *Panorpa* had been recorded from the state, *Panorpa nuptialis* Gerstaecker [5]. This large and conspicuous species, sometimes referred to as "the Texas scorpionfly," is fairly widespread in the midwestern and southeast US, having been recorded in the fall in Alabama, Arkansas, Kansas, Louisiana, Mississippi, Missouri, Oklahoma, Texas, and northeastern Mexico [5].

Numerous species of *Panorpa* have been recorded from the three states that form the northern and eastern borders of Texas: Oklahoma, Arkansas, and Louisiana. No species of *Panorpa* have been recorded from New Mexico, which abuts the entire western U.S. border of Texas. From Oklahoma have been recorded: *P. choctaw* Byers and *P. nuptialis*; from Arkansas: *P. anomala* Carpenter, *P. braueri* Carpenter, and *P. capillata* Byers; and from Louisiana: *P. americana* Swederus, *P. anomala*, *P. lugubris* Swederus, *P. nuptialis*, *P. rupeculana* Byers, and *P. vernalis* Byers [5]. Thus, it is highly unusual that no species of *Panorpa* beside *nuptialis* have previously been recorded in Texas, particularly near the state's northern and eastern borders.



FIGURE 1: Collection sites in Texas for *Panorpa vernalis* from material in the Texas A&M Insect Collection. Sites are marked with yellow stars. The upper star corresponds to collection events 1–3 and the lower star to events 4–5 (see the appendix).

2. Methods and Results

During recent surveys of Mecoptera in the Texas A&M University Insect Collection it was discovered that several short series of a *Panorpa* species new for Texas were deposited in the unsorted material. *Panorpa vernalis* Byers, a spring-emerging species known from Arkansas, Louisiana, and Mississippi, was collected from two counties in northeastern Texas. Three males and two females were collected from Angelina National Forest in Angelina Co., and two males and two females were collected from a private residence in Wood Co. All collections were made in March and April of 2000 and 2001, identified by W. Bicha in 2004, and reconfirmed by the author. A list of the specimens with their complete label information is provided in the appendix. A map displaying the collection sites can be seen in Figure 1.

3. Intraspecific Variation

The Texas specimens meet the morphological criteria laid out by Byers in his original description [6] for *P. vernalis* on most every point, but appear to exhibit variation in several aspects. For example, in Byers' illustrations of the male genitalia, the ventral paramere is displayed as being straight and its apex colinear with its stem, with the apicoventral barbs erect and slightly divergent. But in the Texas specimens, the apex of the paramere is strongly flexed dorsad, and the apicoventral

barbs are compressed and convergent (Figure 2(a)). The ventral parameres are also slightly divergent from one another, rather than straight or slightly convergent as shown in Byers' illustration. One useful detail of the Texas material in making these evaluations is that except for a single specimen, all the males were critical-point dried, the soft tissues retain their shape and color exceptionally well and the sclerotized tissues are easily visualized. As a result, in one specimen from Wood Co., what appears to be an eversible sac is well preserved at the base of the base of the ventral parameres (not shown), and in the males a single pore is conspicuous in the center of the face of each ventral valve. Further, the fleshy nonsclerotized membranes are all a brilliant white color and have a very fine and subtle texture to them that may indicate glandular tissue. These features are usually obscured in dried pinned specimens. There does not appear to be a difference in the form of the ventral parameres as described above as a result of preparation technique, based on comparison of the single Texas male that was not critical-point dried with the specimens that were.

Regarding the maculation of the wings, Byers [6] stated that the "pattern of small dark spots [of the wings is] highly variable" but that "no bands or major spots [are] present". But in the Texas specimens, the remnants of the basal band (Byers does mention a few spots as being conserved, and at least one conforms to the basal band in the Texas specimens examined) can be seen along the anterior and posterior wing

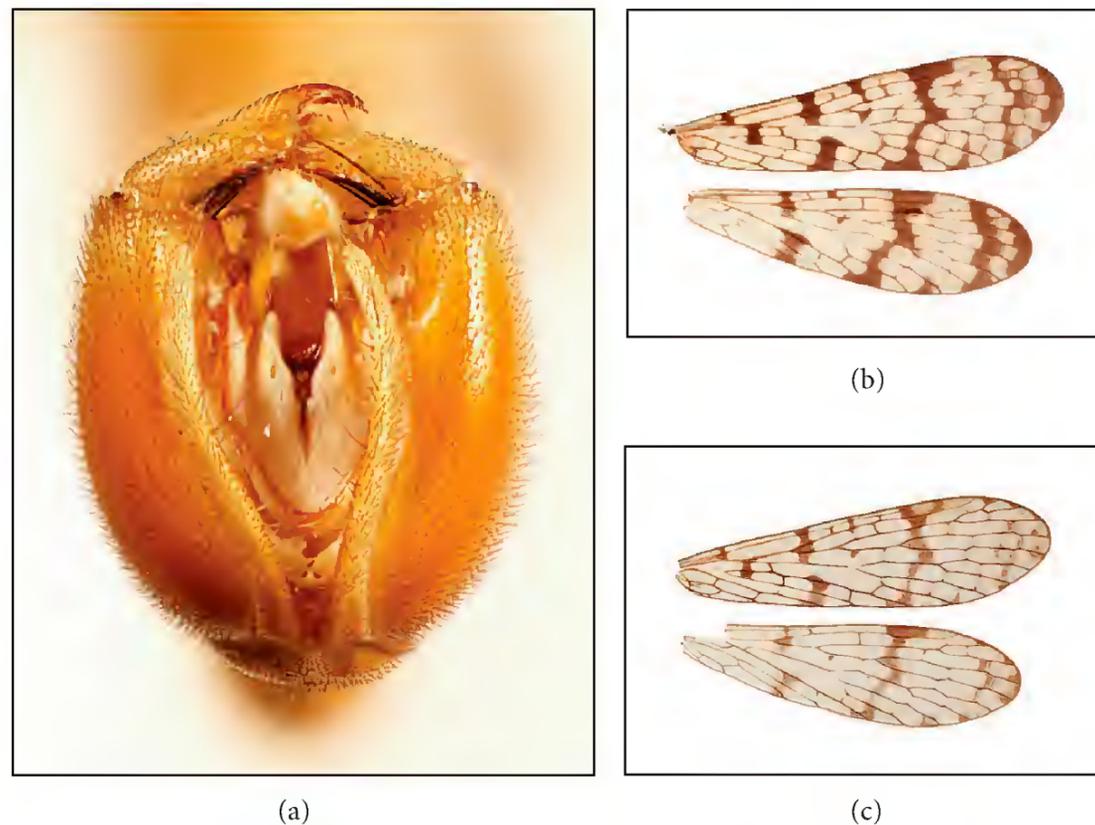


FIGURE 2: (a) Male genital bulb from Angelina Co. specimen demonstrating well preserved soft tissues, which appear white. The pores situated near the apices of the ventral valves can be seen clearly. (b)-(c) Wings of specimens of *Panorpa vernalis* from two counties in Texas displaying differences in expression of wing banding. (b) Specimen from Wood Co. (c) Specimen from Angelina Co.

margins in nearly every specimen, the marginal spot is often at least weakly expressed, the pterostigmal band is complete (see paragraph below) in all specimens, and extensive maculation in the area of the apical band is expressed in several specimens (Figures 2(b) and 2(c); see Byers [7] for wing maculation nomenclature). Further, Byers stated that “most crossveins [are] darkly margined”, and his illustration displays a forewing with well expressed margining on nearly all crossveins. But in the Texas specimens, while some crossveins are margined, the level of expression of the margining in various portions of wing is highly inconsistent from specimen to specimen and from crossvein to crossvein such that the margining of almost no specimen resembles that in Byers illustration. In some Texas specimens, only the outer rank of crossveins are darkly margined, while in others, only the innermost or median ranks are darkly margined; in some specimens many crossveins are margined, but not darkly; and in others still, margining is not well expressed in any part of the wing. Thus it may not be possible to say confidently for all specimens of *P. vernalis*, that “most crossveins [are] darkly margined” [6].

Additional differences exist between the Wood Co. and Angelina Co. series. In the two Wood Co. males, only three stout black spines appear on the posteroventral margin of each basistyle, rather than five to six as in the Angelina Co. males and in Byers’ description. There are also many differences in wing maculation. In all Wood Co. specimens (Figure 2(b)), the wing maculation is darker overall. The pterostigmal band is complete, slender and irregular, and the posterodistal branch is present but narrowly discontinuous, and obliquely spans two to three cells from the posterior margin. The pterostigmal maculation is dark, and eclipses the proximal half to three-fifths of the pterostigma, with no

spot enclosed. In the Angelina Co. specimens (Figure 2(c)), the wing maculation is paler overall. The pterostigmal band is complete, very slender and irregular, and the posterodistal branch is only very poorly expressed at the posterior margin of the wing or absent altogether. The pterostigmal maculation is medium dark, eclipses the proximal half of pterostigma only, and in the forewings encloses a well defined pale spot (which represents a portion of the stigma). This spot varies in size between wings of individuals, and on one wing of a single female is very small.

These variations observed in the Texas specimens are likely simply regional phenomena, but may also suggest genetic isolation and the early to middle stages of ongoing speciation events. However, it is difficult to draw strong conclusions because of the relatively small number of specimens collected from each Texas county and because specimens from western Louisiana, Arkansas and adjacent regions were not available for direct comparison.

4. Resources for Identification of North American *Panorpa* and the Current State of Taxonomy of North American *Panorpidae*

The information presented here suggests that there is a need to sample *Panorpidae* more aggressively within Texas. It is possible that accelerated efforts to do so, particularly in the eastern regions, will yield additional new distribution records of species of *Panorpa* for the state.

These data also serve to reinforce the reality that much work remains to be done in regards to determining the geographic limits of many, if not nearly all, North American *Panorpa* species. There is no doubt that many gaps in the

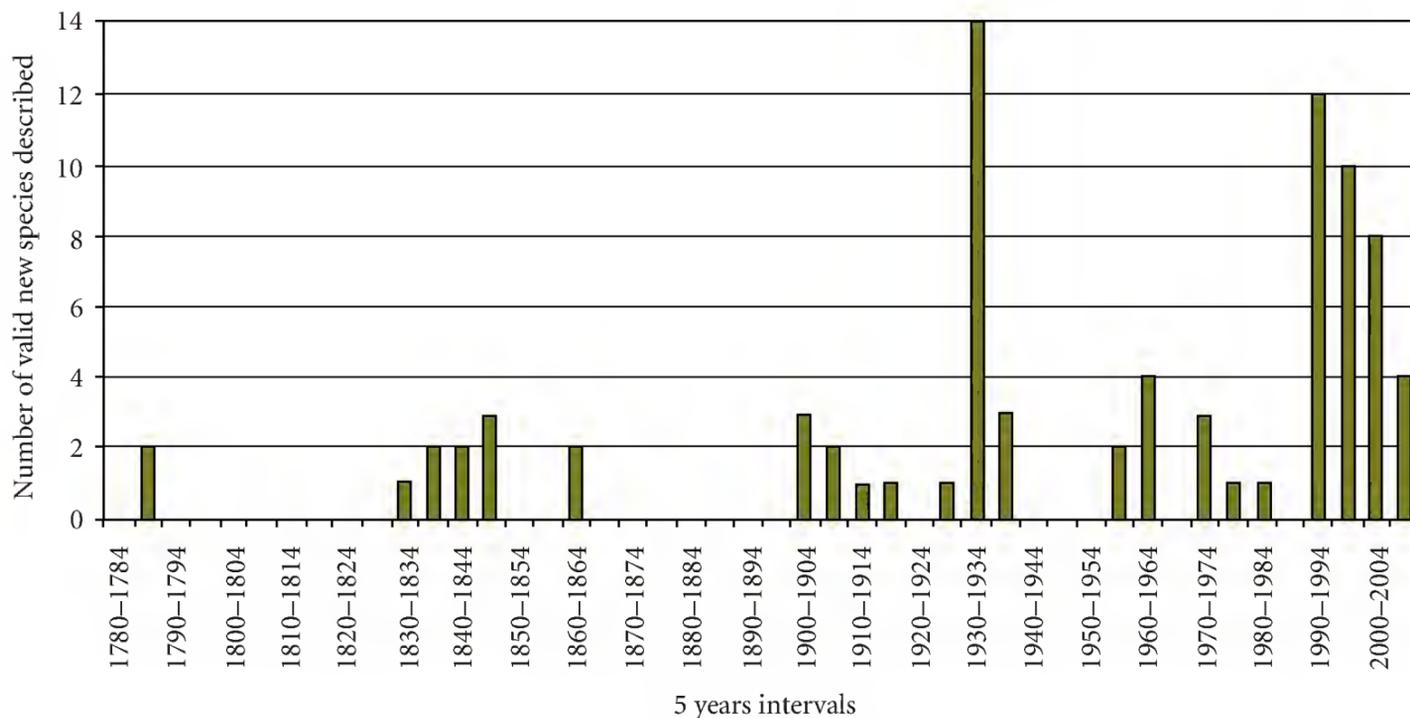


FIGURE 3: Progress in alpha-level taxonomy of North American *Panorpa*, 1780–2009. Since 1958, new species have been discovered at a rate of approximately nine every ten years.

distribution records for described species of North American *Panorpa* exist, and not only in Texas. The reasons for this are, primarily (1) the great void of expertise in the taxonomy of American Mecoptera that is being created as a result of the retirements of several important workers in the last decade or so; (2) the shortage of remaining knowledgeable workers (i) actively collecting scorpionflies in the field, (ii) making identifications of previously collected material retained in American natural history collections, and (iii) publishing new records from collections; and, perhaps most significantly, (3) the lack of a single, comprehensive, up-to-date key to species of *Panorpa* of North America (the three hindrances identified above constitute a phenomenon commonly now referred to as the taxonomic impediment, which is hardly unique to the Mecoptera [8]). Instead, several separate regional keys of varying utility exist. The most recent key, by Cheung et al. [9], treats 13 northeastern *Panorpa* species whose distributions extend into Ontario. Byers, in 1996 [10], provided a key to 6 Mexican species in the *involuta* species group. Byers' 1993 [7] key to the autumnal Mecoptera covers 33 fall-emerging *Panorpa* species from the southeast U.S. but uncomfortably omits species known to emerge only in the spring/early summer. Webb et al. [11] treated 23 Midwestern species in their important work from 1975 on the Mecoptera of Illinois. The only other broad-ranging treatment of American *Panorpa* species of continued usefulness is Carpenter's 1931 [12] revision of the Nearctic Mecoptera, which includes two species of *Panorpa* not covered in more recent keys. Collectively, these keys cover the majority of American species, but not all—at least four U.S. and twenty-two Mexican species of the 84 valid North American species are not included.

The time appears to be ripe for a revision, or at least a comprehensive review, of the North American Panorpidae. In the last 50 or so years, dozens of American *Panorpa* species have been described [6, 7, 10, 13–22] at a relatively steady pace (ca. 9 species every ten years—see Figure 3),

more than doubling—from 34—the number of species known at the time of the last major revision [12]. This indicates a possibility that at least some new species, perhaps those with more narrow distributions, yet remain to be discovered. Further, at present, no effort has been made to centralize knowledge of the recently described species, and descriptions are scattered throughout the literature. With the addition of all these new taxa it is clear that both a single, comprehensive, up-to-date key that treats the entire North American fauna, including the Mexican species, and a taxonomic synthesis summarizing modern knowledge of North American scorpionflies are now needed.

Appendix

Specimens of *Panorpa vernalis* Byers in the Texas A&M University Insect Collection

Label data are in quotes, and individual labels are separated by three slashes (“///”). Collection event series one (1) through four (4) retain an additional label on their pin, not included below, that reads: “*Panorpa vernalis* Byers 1973, Det. W. Bicha 2004”. Numbers of individuals in each series and their gender are given in *italics*.

- (1) “TEXAS: Angelina Co., Angelina Nat'l. For., Jct. Big Creek & USFS Rd. 302, 31°5'18'' N, 94°19'14'' W, III-24-2001, el. 70 m. /// Coll. J. B. Woolley, pan trap” *1 female*.
- (2) “TEXAS: Angelina Co., Angelina Nat'l. For., Jct. Big Creek & USFS Rd. 302, 31°5'18'' N, 94°19'14'' W, IV-6-2001, el. 70 m. /// Coll. R. S. Kirkpatrick, MV light” *2 males*.
- (3) “TEXAS: Angelina Co., Angelina Nat'l. For., Jct. Big Creek & USFS Rd. 302, 31°5'18'' N, 94°19'14'' W, IV-30-31-2001, el. 70 m. /// Coll. R. S. Kirkpatrick, Malaise trap” *1 male, 1 female*.

- (4) "TEXAS: Wood Co., Godwin Woods, 3.5 mi. SW Hainsville, IV-23-30-2000, 32°42'30" N, 95°24'36" W, M. Yoder, yellow pan trap" 1 male, 2 females.
- (5) "TEXAS: Wood Co., Godwin Woods, 3.5 mi. E Hainsville, IV-28-2000, 32°42'30" N, 95°24'36" W, Coll. M. Yoder" 1 male.

Acknowledgments

The author thanks L. Somma for providing information on Texas *Panorpa* in the Florida State Collection of Arthropods and for his support on this project, and N. Penny and J. Oswald for their input on an early version of the manuscript.

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Research Article

Distribution and Ecological Niches of Gamasid Mites (Acari: Mesostigmata) on Small Mammals in Southwest China

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Received 1 October 2009; Revised 2 February 2010; Accepted 22 April 2010

Academic Editor: Brian Forschler

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The ectoparasitic gamasid mites found on small mammals are important arthropods in the field of medical entomology. This paper studied the distribution and ecological niches of ectoparasitic gamasid mites on small mammal hosts in Yunnan Province of southwest China. Levins' niche breadth and Colwell-Futuyma's method were used to quantitatively evaluate host-specificity and similarity of host selection, and hierarchical analysis was used to illustrate niche overlap among gamasid mite species. Species diversity of both small mammals and gamasid mites was lower in indoor habitats than that in outdoor habitats. Most gamasid mite species were found on the body surface of the host species and niche breadths varied from species to species. A species with low niche breadth indicates high host specificity and most gamasid mites showed a relatively low niche overlap. The results suggest that a coevolutionary relationship may exist between some species of gamasid mites and their small mammal hosts.

1. Introduction

Ectoparasitic gamasid mites (Acari: Mesostigmata) on the body surface of small mammals (especially rodents and insectivores) are generally regarded as an important group of medical arthropods because some are suspected as potential vectors of more than 20 zoonoses. Besides dermatitis caused by feeding ectoparasitic gamasid mites, it has been proved that some gamasid mites could be vectors of rickettsial pox and hemorrhagic fever with renal syndrome (HFRS) [1–3]. Yunnan Province in southwest China (Figure 1) has been a persistent focal point for HFRS in recent years [4]. It is therefore deemed meaningful to investigate the distribution of ectoparasitic gamasid mites on small mammals in Yunnan Province. In recent years, Guo and his colleagues have made a series of studies on gamasid mites parasitic on small mammals in Yunnan, their research covered the fauna, geographical distribution, community structure, and other related issues concerning gamasid mites in that region [5–8]. Our intention was to expand on the distribution and ecological niches of ectoparasitic gamasid mites on small mammals ignored in Guo's former reports

by quantitatively evaluating host specificity and the possible coevolutionary relationship between ectoparasitic gamasid mites and their small mammal hosts. Mite ectoparasitism is a complicated phenomenon involving mutual adaptations between parasites and their hosts. As a result of long-term evolutionary and ecological processes, these complicated mutual interactions have important ecological and evolutionary implications [9, 10]. Parasitic species with high host specificity implies coevolution between parasites and hosts from an ecological view. Yet, host specificity is an ambiguous term that is difficult to quantitatively evaluate. We, therefore, introduce the concept of using the ecological niche to quantitatively evaluate host specificity of ectoparasitic gamasid mites [11–13]. On the basis of evaluating ecological niche and overlap, this paper also discusses co-evolution between selected, dominant species of ectoparasitic gamasid mites and their small mammal hosts in Yunnan Province.

2. Methods

2.1. Investigation Sites. The investigation compiled data came from 28 counties (28 investigation sites) in Yunnan

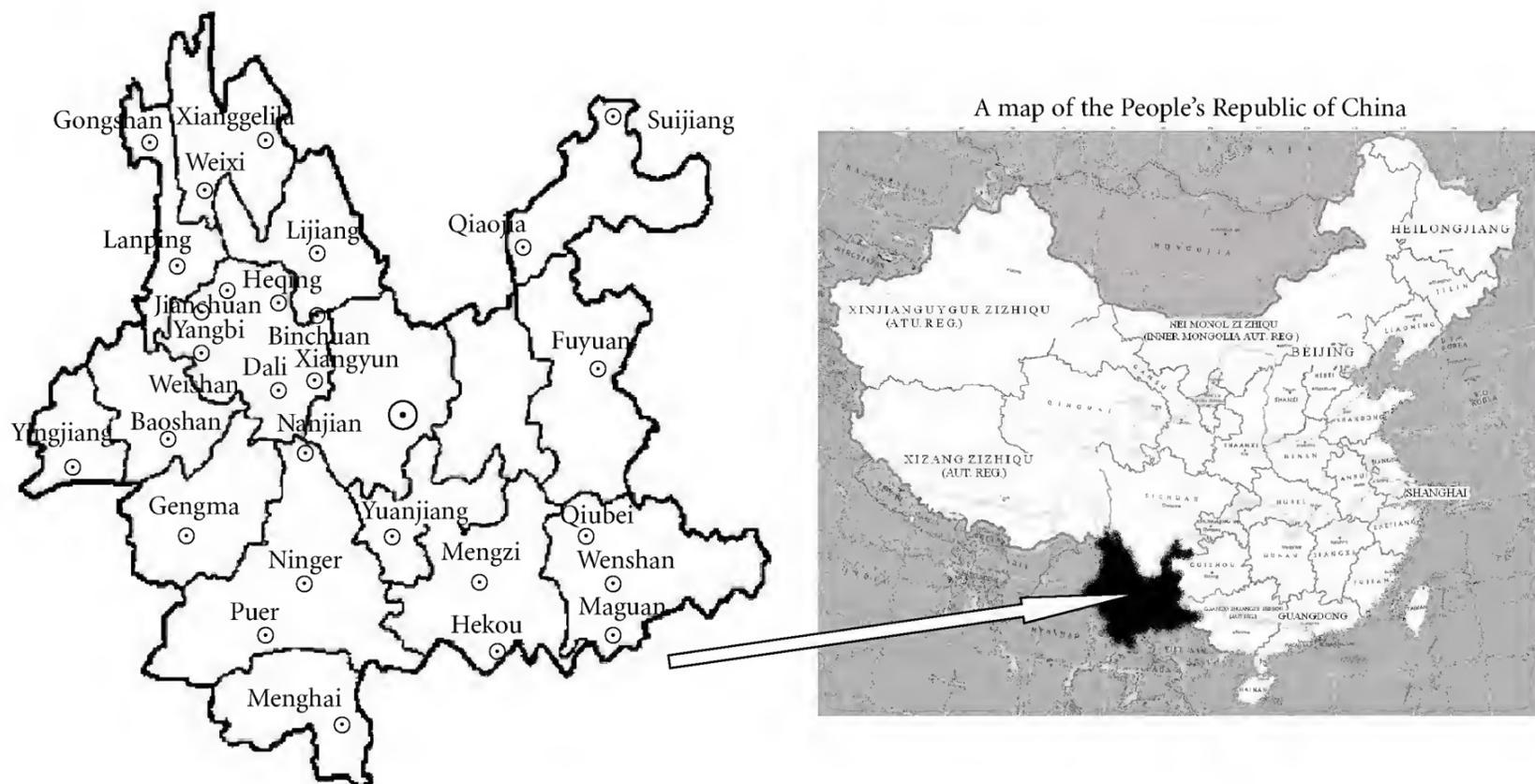


FIGURE 1: A map of Yunnan Province of China, showing the 28 investigated sites (28 counties).

Province (97°31'39" ~ 106°11'47" East longitude, 21°8'32" ~ 29°15'8" North latitude), China. In the field investigation, small mammals were sampled yearly from 1990 to 2008 and surveys were conducted mainly from June to August each year. The 28 investigated sites (the animals captured from each county) included the counties of Baoshan (107), Yangbi (132), Jianchuan (668), Lijiang (377), Heqing (61), Xianggelila (317), Gongshan (795), Weishan (210), Nanjian (201), Puer (634), Ninger (113), Weixi (1560), Lanping (587), Dali (4142), Binchuan (523), Xiangyun (325), Wenshan (111), Qiubei (306), Mengzi (274), Yuanjiang (692), Fuyuan (450), Qiaojia (172), Suijiang (24), Yingjiang (116), Gengma (475), Maguan (112), Hekou (65), and Menghai (995) (Figure 1).

2.2. Trapping, Collection and Identification of Small Mammals, and Gamasid Mites. Small mammals (rodents, shrews, moles, sciurids, and lagomorphs) were captured with mousetraps or mouse cages (10 cm × 11 cm × 24 cm) made by Guixi Mousetrap Apparatus Factory, Guixi, Jiangxi, China. In each investigated site, mousetraps were set in two different types of habitats, indoors (houses, stables, and stalls, etc.) and outdoors (garden, plowland, bush area, and forests). Each mousetrap was baited with a section cob of corn in the outdoors or a single oil-fried peanut in the indoors. The mousetraps randomly placed in a chosen habitat in the afternoon or evening and checked at dawn the next morning. Captured small mammal hosts were removed from traps, transferred to a white cloth bag in the field, and brought to the laboratory for mite inspection. In the laboratory, small mammals were inspected for mites after anesthetized with ether over a white tray. All gamasid mites found on the body surface of each host were collected and preserved in 70% ethanol. After gamasid mite inspection,

individual small mammal hosts were identified to species on the basis of morphological characteristics [14]. After the sample was processed, all instruments were cleaned with disposable paper towels to reduce the chance of cross contamination. After the investigation at one site, preserved individual mite samples were washed several times in water to remove the alcohol and mounted with Hoyer's medium on microscope slides. After clearing and drying, each mite specimen was identified to species under a microscope according to published keys [15].

2.3. Voucher Specimens. Representative voucher specimens of small mammal and gamasid mite were deposited in the specimen repository of Institute of Pathogens and Vectors, Dali University, China.

2.4. Distribution of Gamasid Mites. The constituent ratios (C_r) of every captured small mammal species and their associated gamasid mite species were calculated. We defined dominant species by the higher constituent ratio compared to common or rare species. Species that accounted for more than 0.1% of the constituent ratio in a community were determined as dominant. Together with the constituent ratio (C_r) of gamasid mites on a certain species of small mammal, mite infestation rates (the percentage of infested hosts with gamasid mites) and the mite abundance (MA , mean number of gamasid mites per host examined) were also calculated for each host species. The data were analyzed by using the Chi-square test.

2.5. Measurement of Ecological Niche and Overlap. Based on the constituent ratios of collected gamasid mites, 30 dominant mite species were chosen as the target mites for measurement of ecological niche and overlap. The total

constituent ratio of the 30 dominant mite species (target mite species) reached 97.68% and the rest 82 rare mite species were not considered because they were so rare. The 67 species of small mammals were regarded as 67 series of potential host resources. The individual distribution proportion (ratio) of each mite species on all 67 series of host resources was then calculated and regarded as the utilization proportion on host resources. Based on the utilization proportions, Levins' niche breadth was used to evaluate the host-specificity [16–18]:

$$B_i = \frac{1}{S \sum_{i=1}^s p_{in}^2}, \quad (1)$$

where B_i is Levins' niche breadth for mite species i while P_{in} is the utilization proportion of mite species i on host resource n (actually individual distribution proportion of mite species i on host resource n), and S the total series of host resources ($S = 67$ here, that is 67 species of small mammal hosts). A higher value of B_i for a certain gamasid mite species means a lower host specificity, and *vice versa*.

The following proportional similarity of niche by Colwell-Futuyma was used to measure niche overlap between two species of gamasid mites [19–21]:

$$C_{ij} = 1 - \frac{1}{2} \sum_{n=1}^S |P_{in} - P_{jn}|. \quad (2)$$

C_{ij} represents the proportional similarity of niche between every two species of gamasid mites (species i and j), P_{in} and P_{jn} are the utilization proportion of mite species i and j on host resource n , and S is the same as the previous formula. Values of C_{ij} range from 0 (no niche overlap) to 1 (complete overlap). Hierarchical analysis under SPSS 16.0 statistical software was used to illustrate the overall niche overlap among 30 gamasid mite species. Between-groups linkage method was used in the clustering process of hierarchical analysis, and the dendrogram was used to illustrate the clustering result.

All analyses were carried out in SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, 2006).

3. Results

3.1. Collected Small Mammals and Gamasid Mites. A total of 14,544 individual small mammals were captured from 1990 to 2008 in the 28 counties (28 sampled sites) and identified as representing 10 families, 35 genera, and 67 species in five orders (Rodentia, Insectivora, Scandentia, Lagomorpha, and Carnivora). We collected 80,791 individual gamasid mites that were identified as 10 families, 33 genera, and 112 species.

3.2. Habitat Distribution of Small Mammals and Gamasid Mites. Species diversities of small mammals were much lower indoors than outdoors; that is, much fewer species were found in indoors than in outdoors ($\chi^2 = 55.537$, $df = 1$, $P < .001$). The individual abundance of small mammal hosts and gamasid mites, however, was much higher indoors than in outdoor habitats (i.e., much more individuals were found indoors than outdoors). Of 67 species of small mammal

hosts captured, for instance, only three species, *Rattus tanezumi*, *Rattus norvegicus*, and *Mus musculus*, dominated the indoor habitat, but their constituent ratios are relatively high (especially in *Rattus tanezumi*). The remaining 64 species of small mammal hosts were mainly distributed in outdoor habitats, but most of them had a relatively low constituent ratio (Table 1).

3.3. Mite Infestation of Small Mammals. The number of mite species on mammals varies from host species to species (from 3 to 50 species, $\chi^2 = 286.1$, $df = 33$, $P < .001$) and most mite species can parasitize a very wide range of hosts (from 2 to 31 species, $\chi^2 = 109.0$, $df = 29$, $P < .001$). The mite abundance of different host species also showed significant difference ($\chi^2 = 575.3$, $df = 33$, $P < .001$). Some species of small mammals were infested with a great number of gamasid mite individuals (high individual abundance) but lacked rich mite species (low species richness). Other hosts, however, harbored large numbers of gamasid mite species (high species richness), but had low overall numbers of mites (low individual abundance). For example, 50 species of gamasid mites (high mite species richness) were collected from a rodent host, *Apodemus chevrieri*, but infested individuals displayed low mite abundance (1.61 individual mites per host). The opposite situation, relatively low species richness of gamasid mites (16, 11, 29, 18, and 4 species of the mites, resp.) with high individual abundance of mites, happened in the following small mammal hosts: *Dremomys pernyi*, *Niviventer excelsior*, *Niviventer fulvescens*, *Berylmys bowersi* and *Niviventer eha* (Table 1).

Although some small mammals harbored large numbers of mite species, most individuals had one or more mite species as the dominant ectoparasitics. For example, *Mus pahari* is usually infested with *Laelaps guizhouensis* (80.92%), *Laelaps paucisetosa* (49.64%), and *Laelaps xingyiensis* (43.47%), while *Mus caroli* is usually infested with *Laelaps algericus* (52.13%) and the genus *Eothenomys* often harbors *Laelaps chini* (Table 3).

3.4. Distribution and Host Selection of Gamasid Mites. In this paper, only 30 dominant species of gamasid mites were chosen as target species and they accounted for 97.68% of the total mite species collected. The distribution and host selection of gamasid mites varied from species to species. Some gamasid mite species often parasitized one or two species of mammal hosts and examples include the following mite species: *L. paucisetosa*, *L. xingyiensis*, *Dipolaelaps anourosorecis*, and *Laelaps liui*. Other mite species, however, tended to select a wide range of hosts, and *L. turkestanicus* and *L. nuttalli* are examples (Table 2).

In our study, we found that some species achieve maximum individual abundant on certain host species, that is *L. liui* (97.86%) on the host *Berylmys bowersi* and *L. algericus* (97.10%) on the host *Mus caroli*, while *L. guizhouensis*, *L. paucisetosa*, and *L. xingyiensis* (97.92%, 98.48%, and 95.71%, resp.) were found on the same host *M. pahari* (Table 4). The results suggest that the distribution of gamasid mite species among different host species is quite uneven. Although most

TABLE 1: Dominant small mammal hosts captured and number of gamasid mite species collected in Yunnan Province of southwest China.

Names of dominant small mammal hosts	Number of hosts collected	Constituent ratios of the hosts (%)	Number of mite species	Number of mite individuals	Mite abundance	Habitat distribution of the hosts
<i>Rattus tanezumi</i>	3859	26.53	43	18459	4.78	Indoor
<i>Apodemus chevrieri</i>	1870	12.86	50	3018	1.61	Outdoor
<i>Eothenomys miletus</i>	1802	12.39	41	2258	1.25	Outdoor
<i>Rattus norvegicus</i>	1262	8.68	31	6102	4.84	Indoor
<i>Mus pahari</i>	697	4.79	34	13559	19.45	Outdoor
<i>Rattus nitidus</i>	580	3.99	32	11765	20.28	Outdoor
<i>Apodemus draco</i>	540	3.71	26	562	1.04	Outdoor
<i>Niviventer confucianus</i>	464	3.19	44	5394	11.63	Outdoor
<i>Mus caroli</i>	376	2.59	33	2258	6.01	Outdoor
<i>Apodemus sylvaticus</i>	280	1.93	20	501	1.79	Outdoor
<i>Apodemus latronum</i>	278	1.91	18	204	0.73	Outdoor
<i>Suncus murinus</i>	254	1.75	25	196	0.77	Outdoor
<i>Rattus rattus slandeni</i>	253	1.74	37	842	3.33	Outdoor
<i>Tupaia belangeri</i>	237	1.63	19	159	0.67	Outdoor
<i>Crocidura attenuata</i>	235	1.62	22	139	0.59	Outdoor
<i>Mus musculus</i>	210	1.44	12	156	0.74	Indoor
<i>Niviventer fulvescens</i>	197	1.35	29	7058	35.83	Outdoor
<i>Anourosorex squamipes</i>	151	1.04	24	2445	16.19	Outdoor
<i>Apodemus peninsulae</i>	105	0.72	13	75	5.77	Outdoor
<i>Dremomys pernyi</i>	96	0.66	16	880	55.00	Outdoor
<i>Micromys minutus</i>	83	0.57	13	133	10.23	Outdoor
<i>Callosciurus erythraeus</i>	72	0.50	11	140	12.73	Outdoor
<i>Eothenomys eleusjs</i>	67	0.46	13	326	25.08	Outdoor
<i>Eothenomys sp</i>	64	0.44	5	28	5.60	Outdoor
<i>Niviventer excelsior</i>	54	0.37	11	594	54.00	Outdoor
<i>Berylmys bowersi</i>	45	0.31	18	603	33.50	Outdoor
<i>Eothenomys protidor</i>	44	0.30	3	12	4.00	Outdoor
<i>Eothenomys custos</i>	38	0.26	12	245	20.42	Outdoor
<i>Nasillus gracilis</i>	33	0.23	15	165	11.00	Outdoor
<i>Sorex excelsus</i>	28	0.19	4	4	1.00	Outdoor
<i>Niviventer eha</i>	26	0.18	4	126	31.50	Outdoor
<i>Sciurotamias forresti</i>	23	0.16	4	16	4.00	Outdoor

TABLE 1: Continued.

Names of dominant small mammal hosts	Number of hosts collected	Constituent ratios of the hosts (%)	Number of mite species	Number of mite individuals	Mite abundance	Habitat distribution of the hosts
<i>Tamiops swinhoei</i>	18	0.12	3	39	13.00	Outdoor
<i>Ochotona thibetana</i>	14	0.10	5	6	1.20	Outdoor

Annotation: The remaining small mammal hosts whose constituent ratios were lower than 0.1% and are not included in Table 1 and had an outdoor distribution. The hosts not included in Table 1 are *Ochotona forresti*, *Trogopterus xanthipes*, *Crocidura dracula*, *Petaurista elegans*, *Pteromys volans*, *Soriculus caudatus*, *Bandicota indica*, *Eothenomys melanogaster*, *Microtus clarkei*, *Ochotona gaoligongensis*, *Soriculus leucops*, *Sorex bedfordiae*, *Callosciurus quinquestriatus*, *Neotetracus sinensis*, *Apodemus agrarius*, *Pemurista atbiventer*, *Hylope alboniger*, *Arctonyx collaris*, *Parascaptor leucurus*, *Sorex cylindricauda*, *Mustela kathiah*, *Petaurista sp.*, *Niviventer andersoni*, *Belomys pearsoni*, *Petaurista xanthotis*, *Necmgale elegans*, *Dremomys lokriah*, *Tamias sibiricus*, *Ochotona Daurica*, *Vandeleuria oleracea*, *Vernaya fulva*, *Scaptonyx fuscicaudus* and Muridae spp.

TABLE 2: Host ranges and niche breadth of 30 dominant gamasid mites species on 67 species of small mammal hosts.

Dominant gamasid mite species	Codes of the mites species	Number of mite individuals	Constituent ratios of the mites (%)	Number of infested host species (host ranges)	Niche breadths
<i>Laelaps nuttalli</i>	1	20248	25.06	28	0.0397
<i>Laelaps echidninus</i>	2	15840	19.61	23	0.0622
<i>Laelaps guizhouensis</i>	3	10444	12.93	17	0.0156
<i>Laelaps turkestanicus</i>	4	6429	7.96	31	0.0402
<i>Laelaps traubi</i>	5	4165	5.16	25	0.0448
<i>Ornithonyssus bacoti</i>	6	3340	4.13	15	0.0360
<i>Laelaps chini</i>	7	2734	3.38	28	0.0450
<i>Dipolaelaps anourosorecis</i>	8	2358	2.92	15	0.0165
<i>Laelaps paucisetosa</i>	9	1979	2.45	9	0.0154
<i>Laelaps algericus</i>	10	1933	2.39	6	0.0158
<i>Hirstionyssus sunci</i>	11	1099	1.36	27	0.0880
<i>Laelaps xingyiensis</i>	12	955	1.18	8	0.0163
<i>Laelaps fukienensis</i>	13	923	1.14	10	0.0343
<i>Eulaelaps dremomydis</i>	14	832	1.03	13	0.0163
<i>Eulaelaps shanghaiensis</i>	15	815	1.01	9	0.0175
<i>Proctolaelaps pygmaeus</i>	16	689	0.85	24	0.0842
<i>Haemogamasus oliviformis</i>	17	651	0.81	23	0.1475
<i>Laelaps jettmari</i>	18	422	0.52	11	0.0186
<i>Laelaps jingdongensis</i>	19	410	0.51	12	0.0381
<i>Hypoaspis pavlovskii</i>	20	391	0.48	27	0.1646
<i>Laelaps liui</i>	21	374	0.46	2	0.0156
<i>Eulaelaps substabularis</i>	22	369	0.46	19	0.0606
<i>Haemolaelaps glasgowi</i>	23	290	0.36	14	0.1027
<i>Tricholaelaps myonyssognathus</i>	24	273	0.34	11	0.0230
<i>Liponyssoides muris</i>	25	244	0.30	7	0.0191
<i>Eulaelaps huzhuensis</i>	26	154	0.19	11	0.0731
<i>Haemogamasus dorsalis</i>	27	149	0.18	8	0.0429
<i>Hypoaspis miles</i>	28	138	0.17	17	0.0765
<i>Hypoaspis lubrica</i>	29	134	0.17	16	0.0420
<i>Androlaelaps singularis</i>	30	131	0.16	19	0.1566

of the gamasid mite species can parasitize many species of hosts, others have relatively fixed principal host specie.

3.5. Niche Breadth of Gamasid Mites. In the measurement of ecological niche, the individual distribution proportion (ratio) of each mite species on 67 series of host resources was used to calculate the breadth of gamasid mites. Most gamasid mite species could be found on the body surface of several host species (more than two host species at least) and niche breadths ranged from 0.0154 to 0.1646. Of the 30 mite species studied, *L. turkestanicus* was found on 31 species of small mammal hosts displaying the widest host range while *L. liui*, found on only two species of hosts, had the narrowest host range. The niche breadth of *H. pavlovskii* was the highest (0.1646) followed by *A. singularis* (0.1566) and *H. oliviformis* (0.1475). *L. paucisetosa* showed the narrowest niche breadth (0.0154). Niche breadth for the genus *Laelaps* was much narrower than the genus *Haemogamasus*. Although *L. turkestanicus* had the widest host range (found on 31 species of hosts), its niche breadth was relatively low (0.0402). In contrast, the host range of *A. singularis* was relatively narrow (on 18 host species), but its niche breadth was relatively high (0.1566). The niche breadth of gamasid mites does not seem to match their respective host range (Table 2).

3.6. Niche Overlap of Gamasid Mites. Three species of gamasid mites (*L. guizhouensis*, *L. paucisetosa*, and *L. xingyiensis*) tended to choose the same mammal species (*Mus pahari*) as their principle host. Those three mite species showed niche values with a high degree of overlap (from 0.96 to 0.99). Comparison of gamasid mites species showed a relatively low niche value overlap (≤ 0.50). The higher overlapping values beyond 0.50 only happened in 8.28% of the mite species. Some niche overlaps were almost zero, which happened in *D. anourosorecis*, *L. algericus*, *E. dremomydis*, *L. liui*, and so forth (Table 5). A low niche overlap usually indicates that the compared species have formed a niche separation in host selection. The complicated niche overlaps among 30 of the gamasid mite species we studied were illustrated by hierarchical clustering analysis. The 30 species of gamasid mites were classified into 15 niche overlapping groups when $\lambda = 5.0$ in the clustering dendrogram (Figure 2, Table 6). The gamasid mites within the same group tended to parasitize the same hosts (Table 6).

4. Discussion

4.1. Species of Ectoparasitic Gamasid Mites. Mite assemblages on small mammalian hosts are strongly influenced by the ecological habitat of their hosts [22]. Generally speaking, broad-ranging mammals should acquire more species of ectoparasites because a larger geographical range implies occupation of different habitats, a higher probability of contact with a larger number of other species, and this should lead to higher parasite species richness [23]. Additionally, from the parasite perspective, a large geographic range should indicate that a parasitic species has a larger number of

possible hosts, increasing the likelihood that more parasites become established [24]. Yunnan Province is a big province with accompanying altitude gradients and topographical variation providing complicated ecological landscapes and habitats. Plant and animal resources are abundant in Yunnan Province, which is often described as “the kingdom of plants and animals” in China. Although the field investigation in this paper involved 28 counties in Yunnan Province, it is impossible to cover all the complicated situations in all areas and habitats. As a broad-ranging investigation, we have accumulatively captured 67 species and 14,544 individual small mammals. From those 67 mammal species, 80,791 individual gamasid mites belonging to 10 families, 33 genera, and 112 species were collected. These numbers imply a high species diversity of gamasid mites in Yunnan Province. Thirty of the 112 gamasid mite were determined as dominant species. When the investigation is further extended, the individuals of some rare hosts will increased and therefore some rare species of gamasid mites on them will be probably found. The major dominant species of gamasid mites, however, should be stable and unchangeable because of the big host samples (14 544 individual small mammals). The results imply that Yunnan Province of China is rich in species of gamasid mites with high species diversity and it is a valuable research place. The outdoor habitats provided richer species diversity of both small mammals and gamasid mites compared to the indoor habitats. The species diversity of ectoparasitic gamasid mites is prominently influenced by the species diversity of their small mammal hosts.

4.2. Ecological Niche and Host Specificity of Gamasid Mites. Small mammals are the food resource of ectoparasitic gamasid mites that consume the blood or body fluids from their hosts. The host range and Levins’ niche breadth should provide values opposite host specificity for ectoparasitic gamasid mites that use the hosts as their principle food resource [11–13]. The host range is defined as the number of host species parasitized by a particular ectoparasitic gamasid mite species. The host range could reflect the host specificity to some degree, but it only reflects the number of host species and does not consider the distribution of mite individuals among host species, which can cause some bias in the evaluation of specificity. In comparison with the host range, Levins’ niche breadth is much more accurate for evaluation of ectoparasitic host specificity [12, 13]. A higher niche breadth usually indicates a lower host specificity, and *vice versa*. Ectoparasitic gamasid mites with low host specificity will naturally increase the opportunity of transmitting zoonoses assuming that they frequently change feeding sites (new host).

4.3. Ecological Niche and Coevolution. Host specificity is the result of co-evolution between the parasite and their host and a high specificity often indicates a high degree of co-evolution. Therefore the niche breadth can also be used to demonstrate co-evolution between ectoparasites and their hosts [25]. A narrow niche breadth indicates a higher degree of co-evolution between the mite and their host,

TABLE 3: The mite infestation rates of small mammals with 30 dominant gamasid mite species.

Dominant host species	Infestation rate of 30 dominant mite species (%)																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>Rattus tanezumi</i>	22.49	26.79	0.08	0.10	0.10	7.62	0.05	0.00	0.00	0.05	1.68	0.03	0.00	0.00	0.00	1.55	0.00	0.00	0.00	0.31	0.00	0.00	0.13	2.23	1.22	0.00	0.03	0.75	0.88	0.16
<i>Apodemus chevrieri</i>	1.66	1.39	0.43	0.96	0.96	0.16	1.18	0.32	0.21	0.00	6.04	0.05	0.00	0.00	4.33	1.98	3.37	5.99	2.57	2.19	0.00	2.94	0.64	0.16	0.00	0.70	0.00	0.43	0.37	0.43
<i>Eothenomys milletus</i>	0.39	0.44	0.55	0.61	1.28	0.06	28.25	0.00	0.39	0.00	0.89	0.00	0.06	0.06	0.44	1.55	5.38	0.22	0.50	2.55	0.00	0.78	1.17	0.00	0.00	1.78	0.33	0.39	0.06	0.28
<i>Rattus norvegicus</i>	16.64	20.44	0.16	0.32	0.32	10.70	0.48	0.00	0.08	0.32	3.25	0.16	0.00	0.00	0.08	2.14	0.24	0.08	0.16	0.55	0.00	0.16	0.24	0.63	0.24	0.08	0.00	0.08	0.16	0.40
<i>Mus pahari</i>	0.00	1.00	80.92	1.58	0.00	0.00	0.72	0.14	49.64	0.72	2.44	43.47	0.14	0.14	0.00	1.87	1.00	0.14	0.00	1.72	0.00	0.29	0.00	0.00	0.00	0.00	1.15	2.15	0.57	1.15
<i>Rattus nitidus</i>	47.07	67.76	0.17	1.03	0.34	0.52	0.69	0.00	0.17	0.00	8.97	0.00	0.17	0.00	0.00	8.28	0.34	0.00	0.00	2.24	0.00	0.17	0.34	1.38	0.52	0.00	0.00	0.34	1.21	
<i>Apodemus draco</i>	0.19	0.19	0.00	2.41	5.00	0.56	5.37	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.19	0.56	8.33	0.00	22.04	1.48	0.00	3.33	0.00	0.00	0.00	5.74	0.00	0.00	0.19	
<i>Niviventer confucianus</i>	1.29	26.08	0.65	50.43	40.95	0.22	1.94	0.22	0.00	0.00	1.51	0.22	4.09	0.43	0.22	2.37	3.88	0.00	1.72	5.17	0.65	0.65	0.22	0.43	0.00	1.29	0.00	0.43	0.65	1.29
<i>Mus caroli</i>	4.79	0.80	2.13	1.33	0.00	0.27	0.80	0.00	0.53	52.13	0.53	2.13	0.00	0.00	0.00	3.19	0.00	0.53	0.00	1.06	0.00	5.32	0.27	1.06	0.00	0.00	0.00	4.26	0.53	0.80
<i>Apodemus sylvaticus</i>	10.36	2.14	1.43	2.14	0.71	0.00	1.43	0.71	0.00	0.00	2.14	0.00	0.00	0.00	0.00	1.07	6.43	4.29	6.07	2.86	0.00	5.36	0.71	0.00	0.00	3.57	0.36	0.00	0.00	0.36
<i>Apodemus latronum</i>	3.96	0.36	0.00	0.00	0.36	0.00	1.08	0.00	0.36	0.00	3.60	0.00	0.00	0.36	1.44	0.00	5.76	1.08	10.43	1.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Suncus murinus</i>	9.06	1.97	0.39	0.39	0.00	2.36	0.79	1.18	0.00	0.00	1.57	0.00	0.00	0.00	0.00	3.54	0.39	0.00	0.00	2.36	0.00	0.00	0.00	1.57	0.00	0.00	0.00	2.36	2.36	2.36
<i>Rattus rattus slandeni</i>	20.16	15.02	1.19	6.32	1.98	2.77	0.40	0.40	0.00	0.00	8.30	0.00	0.00	0.00	0.79	3.16	1.98	0.00	0.00	3.16	0.00	1.19	0.79	0.40	0.79	0.00	0.00	2.37	1.58	3.56
<i>Tupaia belangeri</i>	0.84	1.69	0.42	0.84	1.69	0.84	0.42	0.42	0.00	0.00	2.11	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.84	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.42	0.42
<i>Crocidura attenuata</i>	3.83	2.13	0.43	0.43	0.00	2.98	0.43	0.85	0.00	0.00	3.40	0.00	0.00	0.00	0.85	0.85	0.00	0.43	0.00	0.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.43	0.43
<i>Mus musculus</i>	1.43	2.86	0.95	0.48	0.00	4.76	0.00	0.00	0.48	0.95	0.00	0.00	0.00	0.00	0.00	0.95	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.00	0.48	0.00	0.00	0.48	0.00	0.00
<i>Niviventer fulvescens</i>	5.08	56.35	0.00	67.01	40.61	0.00	0.00	0.00	0.51	0.00	4.57	0.51	5.08	0.00	0.00	2.54	1.02	0.00	0.00	1.52	0.00	1.02	0.51	0.00	0.00	0.00	0.00	0.51	0.51	0.00
<i>Anourosorex squamipes</i>	1.32	3.31	0.00	0.66	0.00	0.00	0.66	35.76	0.00	0.00	3.97	0.00	0.00	0.00	0.00	0.66	3.31	0.00	0.00	4.64	0.00	0.00	1.32	0.00	0.00	0.00	0.00	0.66	0.00	2.65
<i>Apodemus peninsulae</i>	0.95	0.00	0.00	2.86	0.00	0.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.95	0.95	4.76	12.38	0.00	10.48	0.95	0.00	2.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Drenomys pernyi</i>	0.00	0.00	0.00	4.17	5.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	53.13	0.00	0.00	1.04	0.00	2.08	1.04	0.00	0.00	0.00	0.00	0.00	0.00	3.13	0.00	1.04	1.04
<i>Micromys minutus</i>	4.82	7.23	3.61	2.41	0.00	0.00	2.41	0.00	0.00	1.20	1.20	10.84	0.00	0.00	2.41	1.20	1.20	2.41	0.00	0.00	0.00	0.00	0.00	4.82	0.00	0.00	0.00	0.00	0.00	0.00
<i>Callosciurus erythraeus</i>	1.39	0.00	0.00	0.00	12.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.56	0.00	0.00	2.78	0.00	0.00	1.39	0.00	0.00	0.00	0.00	0.00	0.00	1.39	0.00	0.00	0.00
<i>Eothenomys elujs</i>	1.49	0.00	0.00	0.00	1.49	0.00	43.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.48	1.49	2.99	1.49	2.99	0.00	4.48	0.00	0.00	0.00	0.00	0.00	1.49	1.49	0.00
<i>Eothenomys sp</i>	0.00	0.00	0.00	0.00	0.00	0.00	1.56	0.00	0.00	0.00	1.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.13	0.00	0.00	0.00	1.56	0.00	0.00	0.00	0.00	0.00	0.00
<i>Niviventer excelsior</i>	3.70	16.67	0.00	44.44	51.85	1.85	3.70	0.00	0.00	0.00	1.85	0.00	12.96	0.00	0.00	0.00	0.00	0.00	0.00	5.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.85	0.00	0.00
<i>Berytinys bowersi</i>	0.00	2.22	4.44	8.89	4.44	0.00	0.00	8.89	0.00	0.00	6.67	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	6.67	66.67	0.00	0.00	0.00	0.00	0.00	0.00	2.22	0.00	4.44
<i>Eothenomys protidor</i>	0.00	0.00	0.00	0.00	0.00	0.00	4.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Eothenomys custos</i>	2.63	0.00	0.00	0.00	0.00	0.00	52.63	2.63	0.00	0.00	2.63	0.00	0.00	0.00	0.00	0.00	15.79	0.00	0.00	0.00	2.63	0.00	0.00	0.00	0.00	0.00	13.16	0.00	0.00	0.00
<i>Nasillus gracilis</i>	6.06	0.00	0.00	0.00	0.00	0.00	3.03	9.09	0.00	0.00	3.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Sorex excelsus</i>	0.00	0.00	0.00	0.00	0.00	0.00	3.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Niviventer cha</i>	0.00	0.00	0.00	15.38	3.85	0.00	3.85	0.00	0.00	0.00	0.00	0.00	34.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Sciurotamias forresti</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.35	8.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.35
<i>Tamias swinhoei</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Ochotona thibetana</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.14	0.00	0.00	0.00	0.00	7.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Annotation: The rest small mammal hosts whose constituent ratios lower than 0.1% were not included in Table 2. The species codes of 30 dominant gamasid mite species are the same as those in Table 2.

TABLE 4: The distribution of 30 dominant species of gamasid mites on dominant small mammal hosts.

Dominant host species	Constituent ratios of 30 dominant mite species (%)																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
<i>Rattus tanezumi</i>	52.83	32.75	0.04	1.07	2.06	41.74	0.11	0.00	0.00	0.10	11.92	0.10	0.00	0.00	23.95	0.00	0.00	0.00	3.58	0.00	0.00	3.79	79.85	88.11	0.00	0.67	33.33	57.46	6.87		
<i>Apodemus chevrieri</i>	0.75	1.98	0.47	2.29	1.34	0.15	2.21	0.38	0.30	0.00	33.58	0.10	0.00	0.00	92.27	5.81	20.89	89.34	26.59	17.65	0.00	35.50	21.03	1.10	0.00	11.04	0.00	9.42	8.96	10.69	
<i>Eothenomys milletus</i>	0.03	0.39	0.05	0.23	0.89	0.03	55.72	0.00	0.15	0.00	2.09	0.00	0.33	0.48	2.45	2.03	20.28	1.18	3.90	16.88	0.00	0.54	12.07	0.00	0.00	24.68	55.70	4.35	0.75	5.34	
<i>Rattus norvegicus</i>	12.08	10.32	0.05	0.20	0.12	48.71	0.72	0.00	0.10	0.47	11.46	0.42	0.00	0.00	0.25	12.48	2.00	0.24	0.49	2.81	0.00	0.54	1.03	3.66	1.23	0.65	0.00	0.72	5.97	9.16	
<i>Mus pahari</i>	0.00	0.14	97.92	0.59	0.00	0.00	1.30	0.08	98.48	1.09	5.64	95.71	0.22	2.04	0.00	2.61	1.84	0.00	0.00	3.58	0.00	0.81	0.00	0.00	0.00	0.00	17.45	15.94	2.99	14.50	
<i>Rattus nitidus</i>	28.62	32.94	0.12	0.11	0.19	4.07	0.29	0.00	0.25	0.00	12.74	0.00	3.79	0.00	0.00	31.06	0.46	0.24	0.00	6.65	0.00	0.27	28.97	6.59	1.64	0.00	0.00	1.45	2.99	9.16	
<i>Apodemus draco</i>	0.04	0.01	0.00	0.44	0.96	0.30	1.30	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.37	0.87	8.76	0.00	53.66	3.07	0.00	10.84	0.00	0.00	0.00	32.47	0.00	0.00	0.00	0.76	
<i>Niviventer confucianus</i>	0.17	7.46	0.11	37.74	32.03	0.09	0.43	0.04	0.00	0.00	1.18	0.10	23.62	0.24	0.12	2.32	3.07	0.00	2.20	6.91	2.14	0.54	0.34	0.73	0.00	5.19	0.00	2.17	3.73	6.11	
<i>Mus caroli</i>	0.89	0.04	0.26	0.50	0.00	0.03	0.07	0.00	0.35	97.10	0.36	2.20	0.00	0.00	0.00	2.61	0.00	1.18	0.00	1.02	0.00	0.00	0.69	1.83	0.00	0.00	0.00	20.29	1.49	3.05	
<i>Apodemus sylvaticus</i>	0.60	0.28	0.48	0.48	0.05	0.00	0.00	0.21	0.00	0.00	0.55	0.00	0.00	0.00	0.00	0.44	4.61	5.45	9.76	3.07	0.00	31.71	0.69	0.00	0.00	0.67	0.00	0.00	0.00	0.76	
<i>Apodemus latronum</i>	0.14	0.01	0.00	0.00	0.05	0.00	0.18	0.00	0.05	0.00	1.82	0.00	0.00	0.12	1.23	0.00	5.22	0.95	13.90	1.02	0.00	8.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Suncus murinus</i>	0.23	0.06	0.01	0.03	0.00	0.39	0.11	0.38	0.00	0.00	0.91	0.00	0.00	0.00	0.00	2.03	0.15	0.00	0.00	2.81	0.00	0.00	0.00	5.13	0.00	0.00	0.00	4.35	8.21	6.11	
<i>Rattus rattus slandeni</i>	1.19	1.17	0.04	2.58	0.19	0.42	0.04	0.04	0.00	0.00	8.55	0.00	0.00	0.00	0.25	1.89	1.23	0.00	0.00	5.37	0.00	1.08	0.69	0.37	2.05	0.00	0.00	5.80	4.48	16.79	
<i>Tipaia belangeri</i>	0.01	0.03	0.01	0.08	0.58	0.24	0.04	0.00	0.00	0.00	1.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.77	0.00	0.00	0.00	0.00	0.00	6.15	0.00	0.00	0.00	0.75	0.76	
<i>Crocidura attenuata</i>	0.14	0.10	0.02	0.08	0.00	0.30	0.11	0.17	0.00	0.00	1.09	0.00	0.00	0.00	0.00	3.05	0.00	0.71	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.75	1.53	
<i>Mus musculus</i>	0.05	0.06	0.02	0.02	0.00	3.08	0.00	0.00	0.05	1.19	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.41	0.00	0.00	0.00	0.75	0.00	
<i>Niviventer fulvescens</i>	0.08	8.64	0.00	47.51	47.32	0.00	0.00	0.00	0.05	0.00	0.36	0.10	60.78	0.00	0.00	1.31	0.92	0.00	0.00	0.77	0.00	3.25	1.03	0.00	0.00	0.00	0.67	0.72	3.73	0.00	
<i>Anourosorex squamipes</i>	0.16	0.32	0.00	0.02	0.00	0.00	0.51	95.12	0.00	0.00	3.91	0.00	0.00	0.00	0.00	0.29	0.92	0.00	0.00	2.05	0.00	0.00	1.38	0.00	0.00	0.00	0.00	0.72	0.00	3.05	
<i>Apodemus peninsulae</i>	0.00	0.00	0.00	0.05	0.00	0.03	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.12	0.87	2.15	0.00	4.15	0.26	0.00	0.81	0.00	0.00	0.00	11.69	0.00	0.00	0.00	0.00	
<i>Drenomys pernyi</i>	0.00	0.00	0.00	0.20	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	95.79	0.00	0.00	0.15	0.00	2.05	0.00	0.00	0.00	0.00	0.00	0.00	2.68	0.00	0.75	1.53	
<i>Micromys minutus</i>	0.24	0.13	0.06	0.00	0.00	0.00	0.07	0.00	0.00	0.05	0.09	1.36	0.00	0.00	2.94	0.15	0.15	0.47	0.00	0.00	0.00	0.00	0.00	4.40	0.00	0.00	0.00	0.00	0.00	0.00	
<i>Callosciurus erythraeus</i>	0.01	0.00	0.00	0.03	1.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.84	0.00	0.00	0.31	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	
<i>Eothenomys eleusis</i>	0.00	0.00	0.00	0.00	0.05	0.00	9.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.15	0.24	0.24	0.51	0.00	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.75	0.00	
<i>Eothenomys sp</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.00	
<i>Niviventer excelsior</i>	0.03	0.14	0.00	3.92	6.87	0.00	0.36	0.04	0.00	0.00	0.09	0.00	0.65	0.00	0.00	0.00	0.00	0.00	1.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00	0.00	
<i>Berylnys bowersi</i>	0.00	0.17	0.02	0.22	0.05	0.00	0.00	0.68	0.00	0.00	0.64	0.00	0.00	0.00	0.00	0.58	0.00	0.00	10.74	97.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00	1.53	
<i>Eothenomys protidor</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
<i>Eothenomys custos</i>	0.00	0.00	0.00	0.00	0.00	0.00	6.44	0.55	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	3.38	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00	8.05	0.00	0.00	0.00	
<i>Nasillus gracilis</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.36	0.51	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
<i>Sorex excelsus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
<i>Niviventer eha</i>	0.00	0.00	0.00	0.61	0.02	0.00	0.04	0.00	0.00	0.00	0.00	0.00	9.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
<i>Sciurotamias forresti</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.76	
<i>Tamias swinhoi</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
<i>Ochotona thibetana</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

Annotation: The species codes of 30 dominant gamasid mite species are the same as those in Table 2.

TABLE 6: Niche overlapping groups of gamasid mites.

Niche overlapping groups	Species of gamasid mites (Code)	Corresponding main hosts
Group 1	<i>L. guizhouensis</i> (3), <i>L. paucisetosa</i> (9), <i>L. xingyiensis</i> (12)	<i>M. pahari</i>
Group 2	<i>D. anourosorecis</i> (8)	<i>A. squamipes</i>
Group 3	<i>E. dremomydis</i> (14)	<i>D. pernyi</i>
Group 4	<i>L. liui</i> (21)	<i>B. bowersi</i>
Group 5	<i>L. algericus</i> (10)	<i>M. caroli</i>
Group 6	<i>L. turkestanicus</i> (4), <i>L. traubi</i> (5), <i>L. fukienensis</i> (13)	<i>N. fulvescens</i> <i>N. confucianus</i>
Group 7	<i>L. echidninus</i> (2), <i>P. pygmaeus</i> (16), <i>L. nuttalli</i> (1)	<i>R. nitidus</i> <i>R. tanezumi</i> <i>R. norvegicus</i>
Group 8	<i>T. myonysoognathus</i> (24), <i>L. muris</i> (25), <i>H. lubrica</i> (29), <i>O. bacoti</i> (6)	<i>R. tanezumi</i>
Group 9	<i>H. oliviformis</i> (17), <i>H. pavlovskii</i> (20), <i>H. glasgowi</i> (23)	<i>A. chevrieri</i> <i>E. miletus</i>
Group 10	<i>H. sunci</i> (11), <i>A. singularis</i> (30)	<i>A. chevrieri</i>
Group 11	<i>H. miles</i> (28)	<i>R. tanezumi</i> <i>M. caroli</i>
Group 12	<i>L. chini</i> (7), <i>H. dorsalis</i> (27)	<i>E. miletus</i>
Group 13	<i>E. shanghaiensis</i> (15), <i>L. jettmari</i> (18)	<i>A. chevrieri</i>
Group 14	<i>L. jingdongensis</i> (19), <i>E. substabularis</i> (22)	Genus <i>Apodemus</i>
Group 15	<i>E. huzhuensis</i> (26)	<i>A. draco</i>

and *vice versa*. A few species of ectoparasitic gamasid mites have developed an adequate co-evolutionary relationship with their hosts because of the high host specificity. The specificity of most ectoparasitic gamasid mites, however, is relatively low and it suggests that the co-evolution between gamasid mites and their hosts has not well developed. Most gamasid mite species in genus *Laelaps* prefer to live on the body surface of the host while species in genus *Haemogamasus* tends to live in the host nests. The niche breadths of *Laelaps* were much narrower than those of *Haemogamasus*, suggesting a high degree of co-evolution between the host-living *Laelaps* compared with the nest dwelling *Haemogamasus*. Examples of nest dwelling mites in genus *Haemogamasus* are *H. pavlovskii*, *H. oliviformis*, and *H. glasgowi*, and they show broad niche breadths.

4.4. Niche Overlap and Host Selection. Niche overlap estimates can approximate the degree that certain species partition resources within a certain community. Niche

overlap measures the degree to which two different species share a particular resource and it reflects, in the case of gamasid mites, on small mammal hosts similarities of host resource utilization between two mites species in a certain community. When the host species are regarded as the food resource, a high niche overlap between any two mite species means that these species tend to choose the same or similar small mammal species, especially their dominant hosts. In contrast, a low niche overlap between any two mite species usually indicates a low similarity in host selection. The results showed that *L. guizhouensis*, *L. paucisetosa*, and *L. xingyiensis* had a high niche overlap values that indicated similar host selection. The common dominant host of *L. guizhouensis*, *L. paucisetosa*, and *L. xingyiensis* was *M. pahari*. The 30 species of gamasid mites were classified into 15 niche overlapping groups using the value of $\lambda = 5.0$ in the clustering dendrogram. Gamasid mites within the same group tended to parasitize the same hosts, especially the dominant ones. Most species of gamasid mites, however, showed relatively

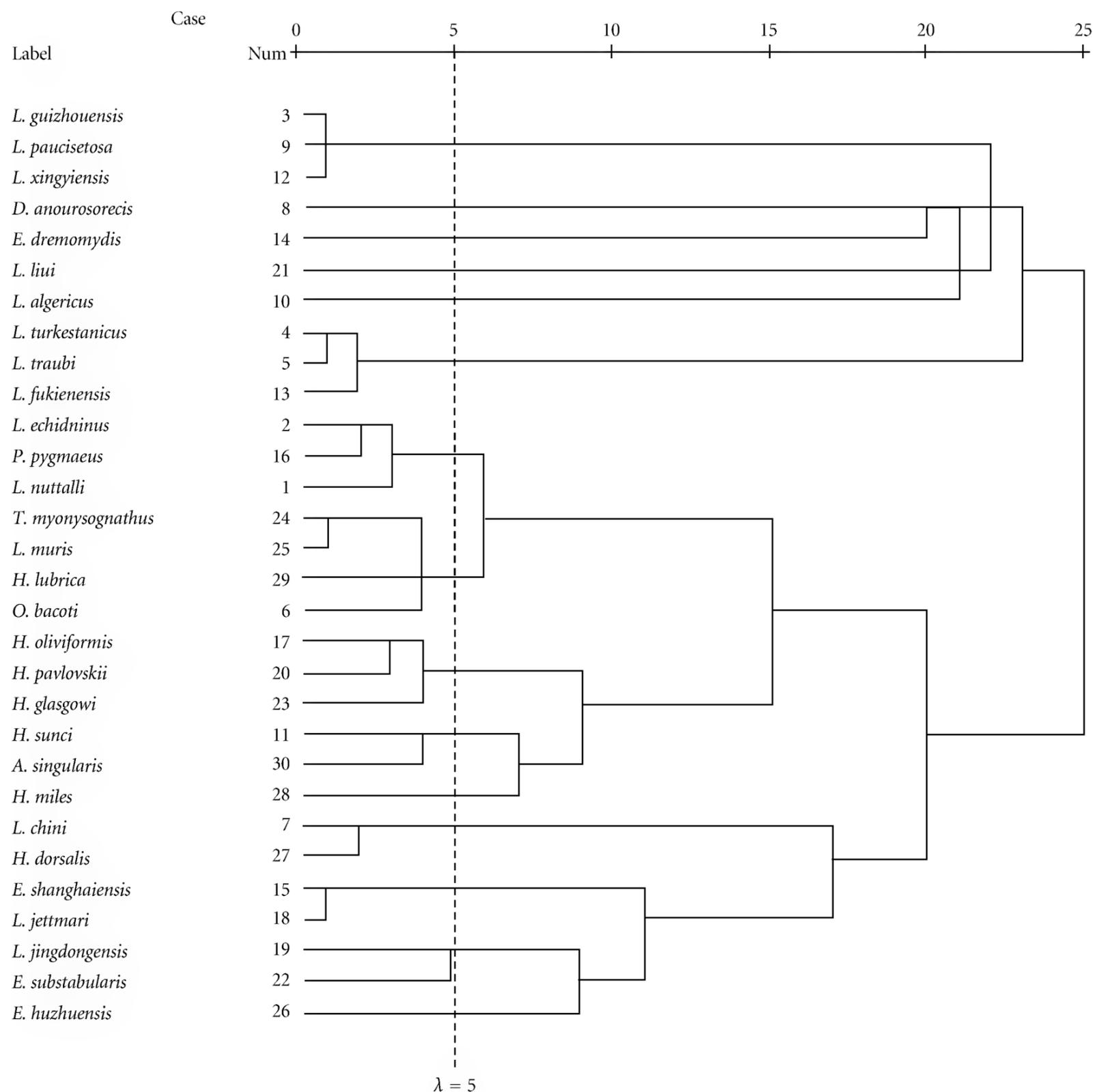


FIGURE 2: The hierarchical cluster dendrogram of 30 mite species based on the Euclidean distance.

low niche overlaps, and higher overlapping values (beyond 0.50) only happened in 8.28% of the mite species. Some niche overlaps were almost zero, as in *D. anourosorecis*, *L. algericus*, *E. dremomydis*, and *L. liui*. The results indicate that some species of gamasid mites have developed a mechanism of niche separation to avoid competition for the same host resources. Those gamasid mites tend to be parasitic on a distinct host species, leading to the niche separation. Niche separation is actually the process of natural selection, which drives competing species into using different hosts. High niche overlap often results from strong competition or repellency; yet the end result of niche separation can be an observed decrease in competition or avoidance. Some species with high overlap values should interact as competitors or intraguild predators, while other species with low pairwise overlap values are nonetheless vulnerable to the effects of

diffuse competition [26]. In considering the relationship between niche overlap and competition, niche overlap should not be taken as a sufficient condition for competition. Many factors may prevent or diminish competition between populations with similar resource utilization patterns. The typically opposing forces of intraspecific and interspecific competition need to be simultaneously considered, for it is the balance between them that in large part determines niche boundaries [27]. But what drives species to overlap or partition? The mechanism remains to be further studied.

Acknowledgments

Special thanks to Dr. Brian Forschler for editing language. We are grateful to the following people for their help during the

previous field investigation and laboratory work: Dong Wen-ge, Qian Ti-jun, Wang Qiao-hua, Li Wei, Men Xing-yuan, Zhang Sheng-yong, Meng Yan-fen, Ren Tian-guang, Jing Yong-guang, and some college students in Dali University. The authors thank Dong Wen-ge, Yan Yi, and Wang Qiao-Hua for their help in the mite identification. We also thank the CDCs (Center of Disease Prevention and Control) in the 28 investigated counties for their kind support, help, and contributions. The project was funded by the Natural Science Foundation of China (Grant no. 30760226).

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Research Article

Morphological Caste Differences in Three Species of the Neotropical Genus *Clypearia* (Hymenoptera: Polistinae: Epiponini)

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Received 12 February 2010; Accepted 29 April 2010

Academic Editor: James Traniello

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Clypearia is a rare genus of swarm-founding Neotropical wasp whose biology is very little known. Morphological castes differences, condition of ovaries, relative age, and color pattern differences were analyzed in three species of *Clypearia*. Physiological differences and low morphometric differentiation between queens and workers were present in all species studied, indicating that these species are characterized by “physiological caste only”. We suggest that caste determination in the three *Clypearia* species studied is postimaginal.

1. Introduction

Swarm-founding polistine wasps belong to the tribe Epiponini and are represented by 19 genera [1] with at least 229 species ecologically dominant in the Neotropical region [2]. The most remarkable characteristic of the Epiponini wasps is nest foundation by the swarming of a large number of individuals including workers and queens [1]. In some species there is slight differentiation between castes [3–6]. However, there are species in which queens are significantly larger or smaller than workers in some morphological characters [2, 7, 8]. Noll et al. [2] proposed the following groups: (1) casteless: no size or shape difference associated with reproduction, and all females largely develop ovaries; (2) physiological caste only: no morphometric differences, but ovarian condition unambiguous by the sterility of all workers; (3) queens larger but mostly the same shape; and (4) queens shaped differently with some measures smaller than workers.

In association with this variation in the patterns of caste distinction, there is the presence of uninseminated females which have developed ovaries named “intermediates” [3] because they usually occur more frequently in species with low caste differentiation [2]. According to Mateus [9], intermediates of *Parachartergus fraternus* are active workers “scouts” during the swarm, that build envelope, sometimes work as a foragers, and lay eggs during colony initiation. The presence, distribution, and occurrence (or not) of intermediate females in several levels of colony cycles organized the social wasps according to five different types of social regulation [8], and in some species the differences between females progressively increase during the colonies ontogenetic development. All these patterns provide to tribe Epiponini a status of prominence in sociobiology [10].

Clypearia was first described as subgenus of *Polybia* for the species *P. apicipennis* [11] and *apud* [12]. *Clypearia* occurs from Mexico to Brazil. In Brazil seven species are found and three are endemic. The cells of the nest are

constructed directly on the substrate and present hidden envelope. According to some authors [12–14] these nests are associated with ants of the genus *Azteca*. The nest of these species was described by Ducke [15], noting its similarity to that of the genus *Synoeca*. Ducke [16] raised *Clypearia* to genus [13], in part because of nest architecture, grouping *Clypearia* with *Synoeca* and *Metapolybia* rather than *Polybia* [12]. After some morphological analysis, Carpenter et al. [12] established that *Occipitalia* is a synonymy of *Clypearia*. According to Noda et al. [17], caste differences in *Synoeca cyanea* are clearly discriminated by physiology but not by morphology. Using morphometric analyses and multivariate statistics, it was found that caste differences in *Metapolybia docilis* are slight but more distinct in latter stages of the colony cycle [18]. Because *Clypearia* is related to these genera there is a possibility that caste syndrome for this genus is similar to that found in *Synoeca* and *Metapolybia*.

The species of genus *Clypearia* are rare [13], and little is known about its biology. This work intends to describe the caste patterns in three species of the genus and to show some possibilities of social organization in this group.

2. Material and Methods

For this study, five colonies of genus *Clypearia* were used: three of *C. sulcata*, de Saussure, 1854, one of *C. duckei*, Richards, 1978, associated with a nest of *Azteca*, both collected in municipal district of Presidente Figueiredo – Amazonas state, Brazil (S- 01° 48' 802"; W- 060° 07' 185") and one of *C. angustior*, Ducke, 1906 collected in the municipal district of Paraibúna – São Paulo state, Brazil (S- 23° 22'; W- 045° 39'). The nests were collected using plastic bags with paper towels soaked in ether. All adult wasps from each colony were preserved in ethanol 95% immediately after collection. Even though the study of several colonies is beneficial in terms of obtaining additional information on colony cycles [7], the information gained from examining a single colony provides important and useful information regarding caste syndromes and is an important starting point for future studies [2].

Castes were defined based on ovarian development and insemination of spermatheca. Queens were defined as ovarian-developed females bearing sperm in the spermatheca, and workers were defined as unmated females without ovarian development [10, 19].

In order to verify insemination, the spermatheca was removed and put on a slide bearing a drop of acid fuchsine solution (1 : 1), and the presence of sperm cells was detected under a microscope. According to Richards [20] and West-Eberhard [21], the relative age of all adult females can be estimated in three crescent classes, analyzing the pigmentation of the transverse apodeme across the hidden base of the fifth sternum, as follows: without pigmentation or incipient (1); light brown (2) and dark brown (3). For morphometric analysis, all females of each colony were studied, because these colonies are few in population. Measurements were taken for 7 morphometric variables in each female: head width (LC); minimum interorbital distance (DmI); gena

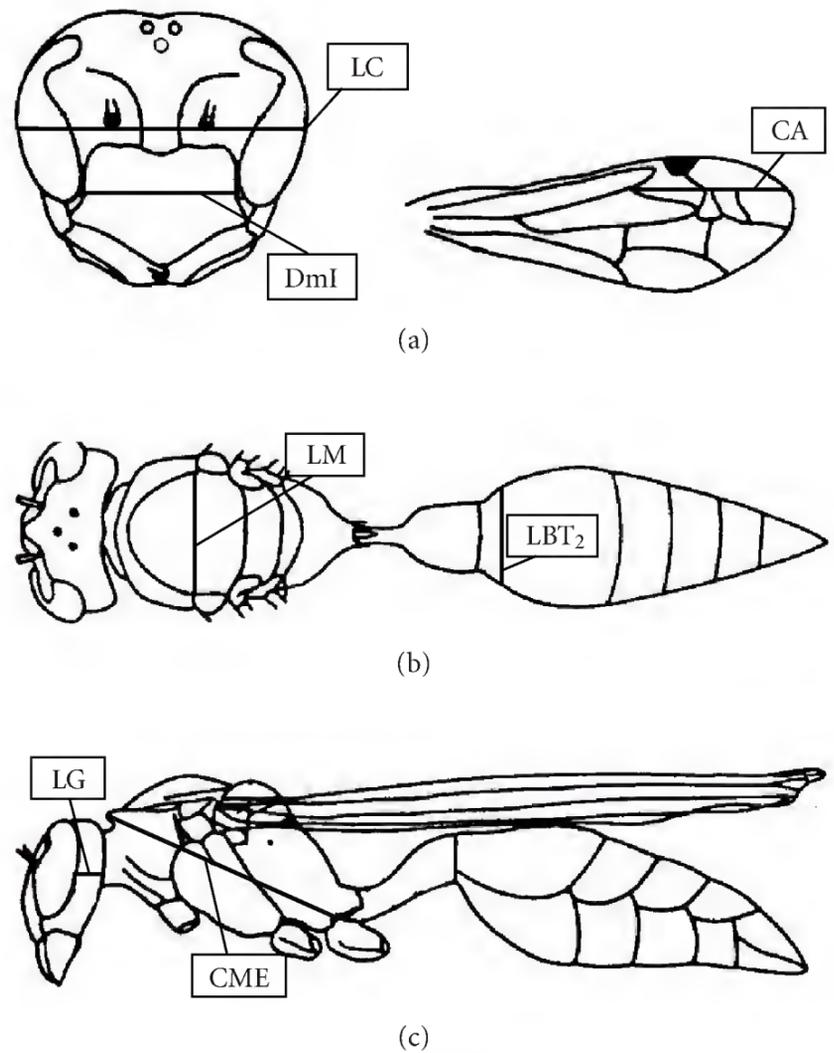


FIGURE 1: Representative measures for morphometric analyses: head width (LC); minimum interorbital distance (DmI); gena width (LG); mesoscutellar width (LM); alitrunk length (CME); basal width of tergite II (LBT₂); partial length of the forewing (CA). Modified by Shima et al. [22].

width (LG); mesoscutellar width (LM); alitrunk length (CME); basal width of tergite II (LBT₂); partial length of the forewing (CA) (Figure 1), using a stereomicroscope, digital camera, and measure software.

For the statistical analysis, females were divided in two groups: queens and workers. Means and standard deviations were calculated using one-way ANOVA. A stepwise discriminant analysis was used to identify the character most significant that contributes to caste distinction. After, the most discriminant characters were plotted for caste discrimination, the Wilks' Lambda was used to determine the degree to which separate measures contributed to final model. This is an alternative to the use of an *F to remove* at each step. Variables that appear in final model but do not have significant *F* ratios represent variance components that are explained by some combination of the other variables also in the model and therefore no longer contribute to discrimination itself. Wilks' Lambda varies from zero to one; the lower the value, the greater the significance. In order to check the test efficiency, a classification matrix test was used to check the number and percent of correctly classified cases in each group [8]. The data were computed using the "STATISTICA 6.0 Statsoft" software.

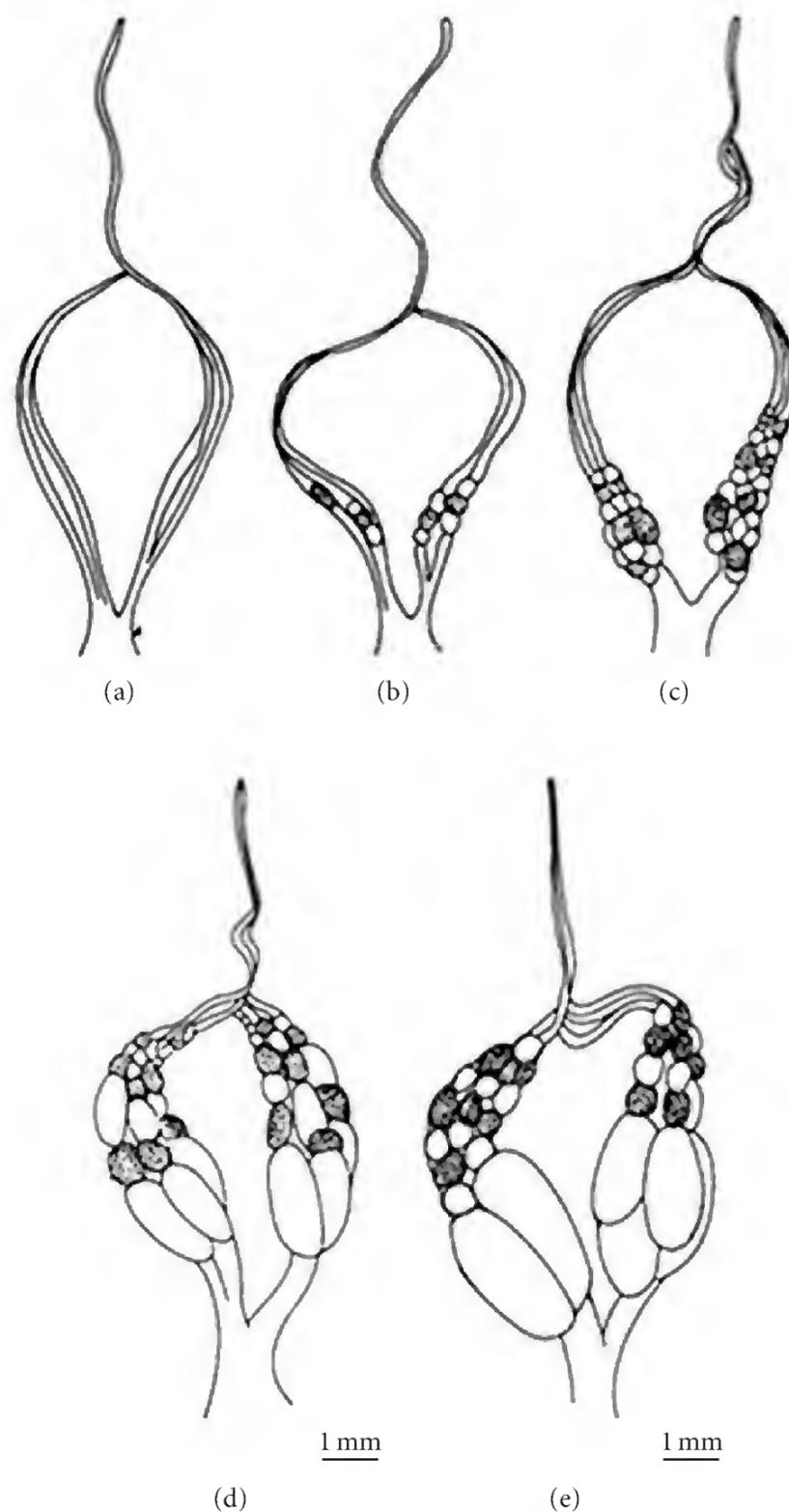


FIGURE 2: Grades of ovarian development found among females in colonies of *Clypearia*. Type (a), (b) and (c): workers; Type (d) and (e): queens.

3. Results

3.1. Ovarian Development and Insemination. The number of ovarioles was three in each ovary, as found in other Polistinae, and the ovarian development was classified according to five categories (Figure 2): Type (a): filamentous ovarioles bearing no visible oocytes, Type (b): possessed slightly developed oocytes, Type (c): small well-defined oocytes, Type (d): possessed at least one near mature oocyte, and Type (e): well-developed oocytes. In the *C. sulcata* colonies, we found all types of ovaries, except Type (d). In *C. duckei* all types of ovaries were found and in *C. angustior* there were found all types of ovaries, except Type (c) (Figure 2; Table 1). Insemination was found in females with Type (d) and (e) ovaries.

3.2. Relative Age. In relation to females age, colony I of *C. sulcata* presented workers in young and median age, and the single queen showed median age. In colony II of *C. sulcata*, queens showed median age, and the workers were found in all age patterns. Colony III of *C. sulcata* presented all queens as young females and workers as median and old females. In colony of *C. angustior*, queens and workers were old females, except one worker that had median age. In colony of *C. duckei* queens and workers females were found in all age patterns; however most queens were younger and most workers were older (Figure 3).

3.3. Morphometric Analysis. Among the colonies of *C. sulcata*, colony I showed two characters (LC and LG) statistically

TABLE 1: Ovary development for all species analyzed in the genus *Clypearia*.

Species	Colony characteristics	N Females	Ovaries (%)				
			A	B	C	D	E
<i>C. sulcata</i> I	Swarm	23	30.4	39.1	26.1	0	4.4
<i>C. sulcata</i> II	Work production	25	56	16	20	0	8
<i>C. sulcata</i> III	Work production	50	66	14	14	0	6
<i>C. angustior</i>	Male production	14	28.6	21.4	0	14.3	35.7
<i>C. duckei</i>	Work production	119	28.6	10.1	45.4	0	16

TABLE 2: Means, standard deviations, and *F* values of ANOVA for the analyzed colonies of the genus *Clypearia*.

(a)

Colony	<i>C. sulcata</i> I			<i>C. sulcata</i> II			<i>C. sulcata</i> III		
	Queen	Worker	Bonferroni <i>t</i> -test	Queen	Worker	Bonferroni <i>t</i> -test	Queen	Worker	Bonferroni <i>t</i> -test
Characters	(<i>N</i> = 1)	(<i>N</i> = 16)	Q/W	(<i>N</i> = 2)	(<i>N</i> = 23)	Q/W	(<i>N</i> = 3)	(<i>N</i> = 47)	Q/W
HW	2.97 ± 0.00	3.13 ± 0.05	**	3.19 ± 0.01	3.16 ± 0.03	#	3.12 ± 0.06	3.16 ± 0.06	#
IDm	1.19 ± 0.00	1.24 ± 0.03	#	1.28 ± 0.01	1.27 ± 0.02	#	1.26 ± 0.04	1.23 ± 0.03	#
GW	0.48 ± 0.00	0.55 ± 0.03	**	0.56 ± 0.00	0.59 ± 0.04	#	0.54 ± 0.03	0.55 ± 0.02	#
MSW	1.90 ± 0.00	2.00 ± 0.06	#	2.06 ± 0.01	2.04 ± 0.05	#	1.97 ± 0.03	2.00 ± 0.06	#
AL	3.86 ± 0.00	4.02 ± 0.11	#	4.18 ± 0.04	4.18 ± 0.07	#	4.06 ± 0.13	4.03 ± 0.10	#
T2BW	1.31 ± 0.00	1.37 ± 0.04	#	1.37 ± 0.03	1.37 ± 0.05	#	1.34 ± 0.03	1.34 ± 0.05	#
WL	1.50 ± 0.00	1.56 ± 0.11	#	1.65 ± 0.01	1.62 ± 0.08	#	1.58 ± 0.04	1.59 ± 0.05	#

(b)

Colony	<i>C. angustior</i>			<i>C. duckei</i>		
	Queen	Worker	Bonferroni <i>t</i> -test	Queen	Worker	Bonferroni <i>t</i> -test
Characters	(<i>N</i> = 7)	(<i>N</i> = 7)	Q/W	(<i>N</i> = 16)	(<i>N</i> = 112)	Q/W
HW	2.88 ± 0.04	2.78 ± 0.08	**	2.95 ± 0.05	2.95 ± 0.05	#
IDm	1.21 ± 0.02	1.14 ± 0.03	*	1.18 ± 0.03	1.15 ± 0.03	**
GW	0.42 ± 0.04	0.39 ± 0.03	#	0.34 ± 0.02	0.35 ± 0.03	#
MSW	2.01 ± 0.05	1.90 ± 0.10	***	2.26 ± 0.07	2.21 ± 0.06	**
AL	4.28 ± 0.09	4.07 ± 0.19	**	4.51 ± 0.19	4.43 ± 0.12	***
T2BW	1.34 ± 0.06	1.31 ± 0.03	#	2.03 ± 0.08	1.94 ± 0.06	*
WL	1.80 ± 0.06	1.69 ± 0.09	**	1.59 ± 0.10	1.58 ± 0.10	#

* $P < .001$; ** $P < .02$; *** $P < .05$; # not significant ($P > .05$).

smaller in queens compared with workers colonies II and III presented no significant different character (Table 2). The colony of *C. angustior* showed five characters (LC, DmI, LM, CME, and CA) statistically larger in queens compared to workers. Colony of *C. duckei* showed four characters (DmI, LM, CME, and LBT2) statistically larger in queens than in workers (Table 2).

According to multivariate analyses, LG, DmI, and LC were predominant in the discrimination models (Table 3). High values of Wilks' Lambda (above 0.8; Table 3) for colonies of *C. sulcata* II and III and *C. duckei* indicate low power of discrimination of castes, and in the colony of *C. angustior*, the Wilks' Lambda values were lower (below 0.4;

Table 3), that could be a better discriminator between castes. However, when we looked at the values of *P* for this colony, we observed that the values were not statistically significant. Thus, no single character could discriminate caste in these colonies. Such differences suggest that variation during the colony cycle occurs in other eiponines [7, 8].

Group comparisons after discriminant analysis showed queens and workers as well-defined groups in latter stages of the colony cycle as found in *Metapolybia docilis* [18], indicating a high overlap between castes in morphological characters (Table 4; Figure 4).

Color pattern differences were observed between queens and workers in areas of light coloration: in the gena, clypeus,

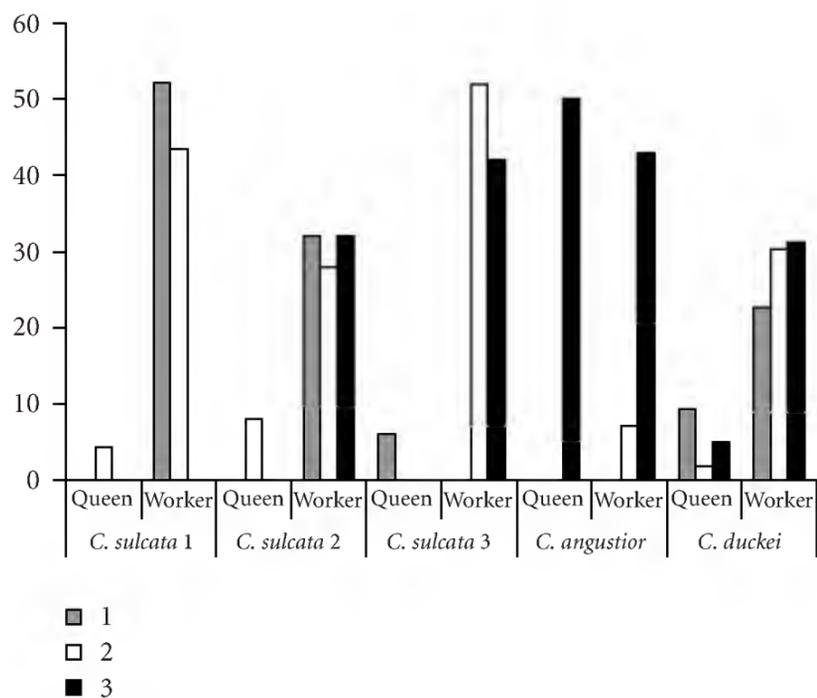


FIGURE 3: Relative age based on the pigmentation of the 5th gastric sternite of queens and workers in species of genus *Clypearia*. (1) Without pigmentation or incipient; (2) little brown; (3) dark brown.

TABLE 3: Discrimination between queens, workers, and intermediate females in all analyzed colonies of genus *Clypearia* after forward stepwise discriminant function analyses.

Colony	Character	Wilks' Lambda	<i>P</i>
<i>C. sulcata</i> II	GW	0.96	.08
	HW	0.94	.11
	IDm	0.98	*
<i>C. sulcata</i> III	HW	0.90	*
	MSW	0.77	.21
	IDm	0.60	*
<i>C. angustior</i>	T2BW	0.27	.42
	WL	0.35	.10
	GW	0.30	.26
	T2BW	0.87	*
<i>C. duckei</i>	IDm	0.78	*
	HW	0.75	*
	GW	0.71	.03

**P* < .01.

and hind margin of pronotum. In the workers these marks are strong yellow while in queens are light yellow to near white. These patterns are the same for all species studied (Figure 5).

4. Discussion

Size differentiation is considered as an initial step for the origin of morphological castes in the three main groups of social Hymenoptera. In social wasps, the differences are more conspicuous in Vespinae and more complex in Polistinae [2].

According to ovarian development, we observed that in colonies of *C. sulcata* and *C. duckei* Type (d) ovary was not found (Figure 2; Table 1). However, pattern (d) was found

TABLE 4: Classification matrix queens, workers, and intermediate females in all analyzed colonies of genus *Clypearia* after discriminant function analyses.

Colony	Observed classification	Predicted classification		Corrected classified females (%)
		Queen	Worker	
<i>C. sulcata</i> II	Queen (<i>n</i> = 2)	0	2	0
	Worker (<i>n</i> = 23)	1	22	95.7
<i>C. sulcata</i> III	Queen (<i>n</i> = 3)	2	1	66.7
	Worker (<i>n</i> = 47)	0	47	100
<i>C. angustior</i>	Queen (<i>n</i> = 7)	7	0	100
	Worker (<i>n</i> = 7)	1	6	85.7
<i>C. duckei</i>	Queen (<i>n</i> = 16)	9	7	56.3
	Worker (<i>n</i> = 112)	3	96	97

in *C. angustior*, and type (c) ovaries were not found, unlike all other colonies (Table 1). This may be due to phase of colony cycle for each colony. Indeed, in contrast to our data, Noll et al. [2] did not find females with Type (c) ovaries in colony *C. sulcata*, and perhaps this colony was in the phase of male and gyne production like the *C. angustior* colony studied here.

In all colonies, except colonies II and III of *C. sulcata*, at least two measures were statistically different between castes (Table 2). In *C. sulcata* II and *C. sulcata* III no character had statistical difference (Table 2). Because some colonies showed more significant differences among morphological characters, we suggest that the colonies studied here showed slight differences in morphology (Figure 4).

Based on discriminant analysis, small differences were found. The high values of Wilks' Lambda (Table 3) support this result. Indeed, *C. angustior* showed low Wilks' Lambda values (Table 3) perhaps due to phase of colony cycle. The absence of morphological and physiological caste differences was found in *Protopolybia acutiscutis* cited as *P. pumila* [23], *P. exigua exigua* [24], *Parachartergus smithii* [25], *Pseudopolybia vespiceps* [26], *Polybia chrysothorax*, *P. jurinei*, *Parachartergus fraternus*, *Angiopolybia spp*, *Chartergellus communis* [3, 10, 27], *Brachygastra scutellaris* [28], *B. lecheguana* [29, 30], *B. mellifica* [31], *Protopolybia chartergoides* [32], and *Metapolybia docilis* [18].

Based on relative age of adult females and absence of males (Figure 3), it is possible to suggest that all colonies of *C. sulcata* and the single colony of *C. duckei* were producing workers, and *C. angustior* colony was producing males and new queens (Figure 3; Table 1). According to group comparisons (Table 4), only *C. angustior* colony showed a

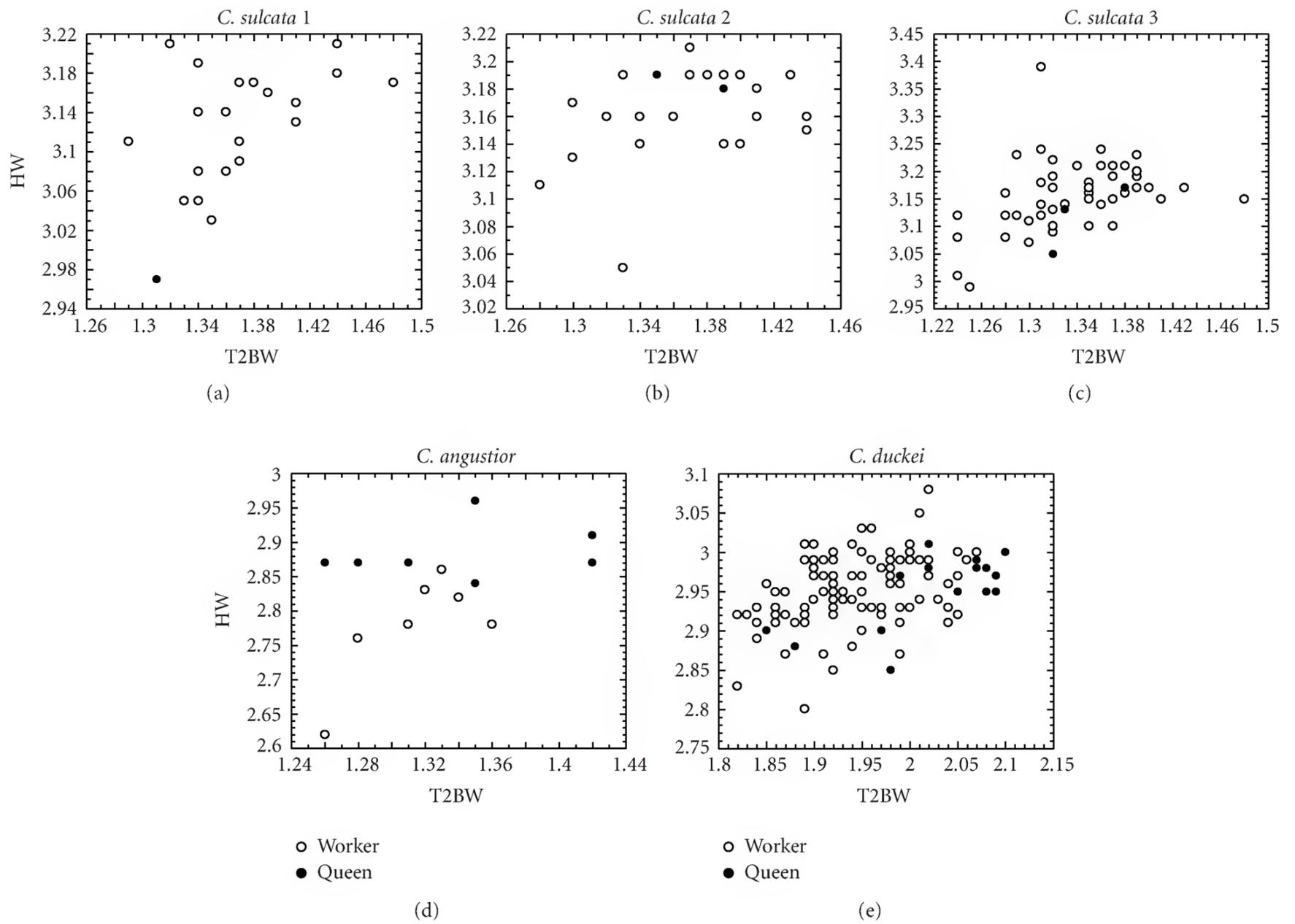


FIGURE 4: Caste discrimination in the analyzed colonies of *Clypearia* based on the multivariate analyses.

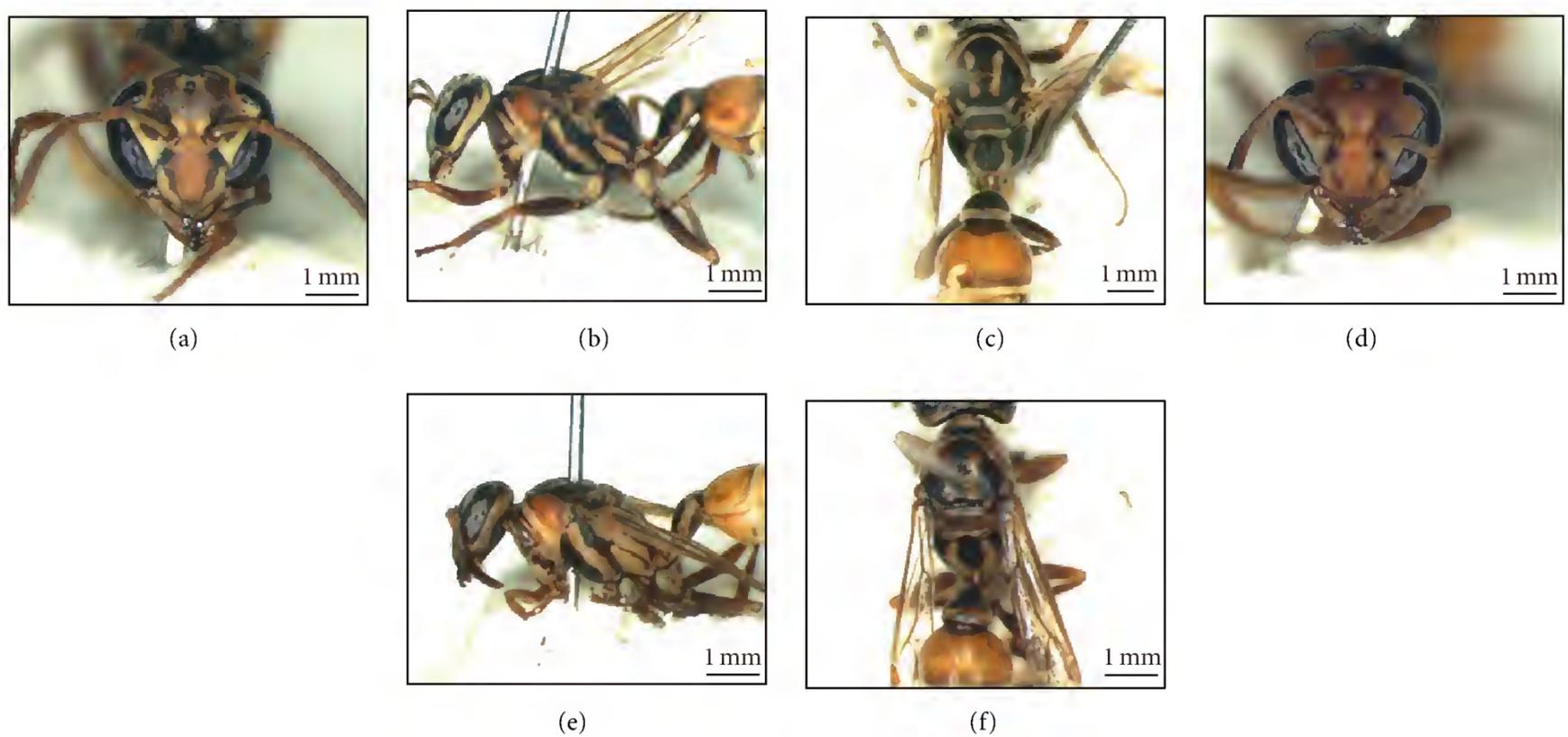


FIGURE 5: Color patterns: (a–c): view of worker; (d–f): view of queen.

good discrimination that could be because all queens were in a more advanced relative age (Figure 3).

The differentiation between queens and workers was found when color of head and mesosoma was compared (Figure 5). According to Shima et al. [22], color pattern differs between queens and workers of *Apoica flavissima*, as observed in *Polybia dimidiata* [33], *Parachartergus smithii* [25], *Chartergellus communis* [27], and various *Polybia* species of the subgenus *Myrapetra*, as *P. diguetama*, *P. occidentalis*, *P. platycephala sylvestris*, *P. scrobalis surinama*, and *P. scutellaris* [13].

The absence of morphological differences is shown to be ancestral condition for Epiponini [2, 34]. Noll et al. [2] grouped *Clypearia* with *Synoeca* and *Metapolybia* plus *Asteloeca* and proposed that these genera belonging to a pattern called “physiological caste only”, meaning that no morphometric differences but ovarian condition unambiguous by absence of type (c) females were present. Our data agree with these conditions. So, we can suggest that due to low morphological differentiation, the castes in species of *Clypearia* studied here are determined postimaginally [8, 10] probably due to reprogramming of growth parameters [35] and maybe the size has an important role during the process of queen elimination during the cyclical oligogyny [8].

Acknowledgments

This work was partially funded by Fapesp (07/086333-1). The authors acknowledge Elynton Alves do Nascimento for setting up the figures included in this paper.

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Research Article

Fruit Damage Patterns Caused by Ovipositing Females of *Conotrachelus dimidiatus* (Coleoptera: Curculionidae) in Guava Trees

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Received 3 December 2009; Accepted 17 May 2010

Academic Editor: Abraham Hefetz

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We evaluated the damage patterns produced by females of the guava weevil *Conotrachelus dimidiatus* Champion, 1904 (Coleoptera: Curculionidae), according to the position of the damaged fruit in guava trees *Psidium guajava* L. in Calvillo, Aguascalientes, Mexico. The trees were subdivided in eight zones, and during one year the level of fruit lesions due to oviposition was registered. Results showed a higher level of damage in the upper and external zone of the trees ($P \leq .05$). We found no significant differences in damage between the four cardinal points ($P \geq .05$). During the year, the level of damage was recorded and was higher in the months of August and September ($P \leq .05$) associated with rainfall (0.86, $P = .06$) and increase in temperature (0.84, $P = .03$). The most susceptible fruits were in the size range of 2.1–4.0 cm (polar diameter). The information from this study will be used to design and establish effective control strategies for the guava weevil, taking into account location of the most susceptible fruits, seasonality of the pest, and the abiotic factors.

1. Introduction

Guava *Psidium guajava* L. is a plant whose origin is tropical America, and is cultivated mainly for consumption as fresh fruit or for juices, jellies, or marmalades. It contains vitamin A, C, iron, calcium, and phosphorus [1]. In Mexico, the national guava production averages 300, 613 tons over the past five years [2]. Other factors associated with the crop are low technological standards, saturated internal consumption market, incipient exportations and damage from insect pests [3].

For the municipality of Calvillo, Aguascalientes, and Juchipila, Zacatecas, various species of the genus *Conotrachelus* are reported to be associated with the guava crop. The guava weevil *Conotrachelus dimidiatus* Champion is considered to be the species that causes the most damage [4]. When the adults emerge from soil they fly towards the tree

to feed on floral buds and tissues. After mating, the females oviposit preferably in the middle portion of green unripe fruits (2 cm diameter). Oviposition sites have a circular concave and cork-like appearance. Furthermore, the infected fruits develop prematurely and acquire a kidney shape that excludes them from commercialization. In a single season, the adult weevils can infest up to 70% of the cultivated orchards and cause losses of as much as 60% of production [4]. Until now chemical control has been predominant, which is applied when one weevil per tree is detected and the first fruits with oviposition are observed.

The damage pattern is the result of a variable behavior of the insects. This flexibility, according to the phenology of the crop and response to abiotic factors, increases the risk of damage. For example, for the bean borer *Cydia fabivora* Stansly and Sanchez [5] report a higher oviposition on the underside of the leaf prior to flowering (55%).

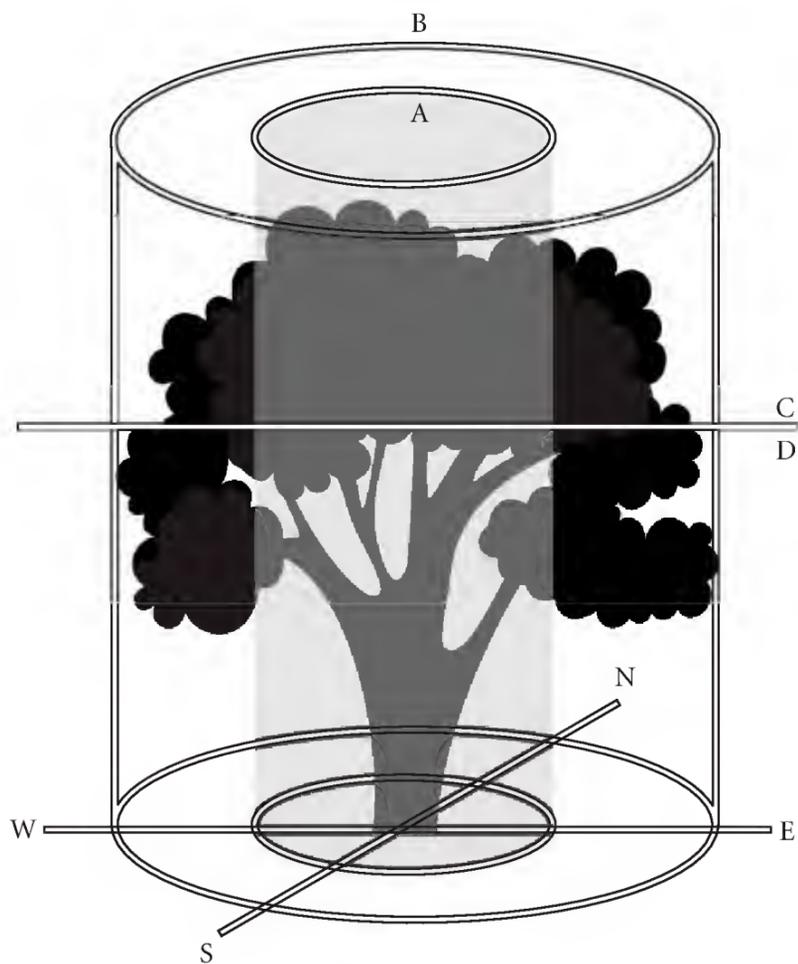


FIGURE 1: Division of the guava trees to determine the damage pattern of *Conotrachelus dimidiatus*. Internal zone (A) external zone (B). Upper zone (C) and lower zone (D). Cardinal points: North (N), South (S), East (E), and West (W).

However, when the pods emerge, they are preferred for oviposition (84%). A study of the biology pertaining to the oviposition of the beetle *Acanthocinus nodosus* reports a period of colonization of *Pinus taeda* with the totality of the ovipositions on the lower part of the trunk with an average of 3.3 eggs per hole [6]. By now the appearance of the oviposition of the guava weevil is well known [4, 7]; however, the pattern of ovipositions on the host and their distribution throughout the year are unknown. The objective of the present study was to establish the damage pattern of females of *C. dimidiatus* in different zones of guava trees, the distribution of damage throughout the year, the size of the most susceptible fruit, and the effect of abiotic factors.

2. Methods

This investigation was carried out from September of 2007 to September of 2008 in a leveled guava orchard (40 × 90 m) located in the municipality of Calvillo, Aguascalientes, Mexico (102° 43'W, 21° 51' N) and 1667 m altitude. The plantation was of the Media China variety with 10 years of age and free of pesticide applications.

2.1. Determination of the Damage Pattern. To determine the damage pattern on the host, the trees were divided in eight zones and three planes: internal and external zone (radial plane), upper and lower (horizontal plane), and the four cardinal points (cardinal plane) (Figure 1). To determine

TABLE 1: Average of damage from *Conotrachelus dimidiatus* in guava trees in Calvillo, Aguascalientes.

Plane	Sector	Mean oviposition ± SE	% damaged
Cardinal	North	0.78 ± 0.09a	27.02
	South	0.66 ± 0.08a	23.07
	East	0.66 ± 0.09a	23.07
	West	0.77 ± 0.08a	26.81
Radial	Interior	0.62 ± 0.09a	40.50
	Exterior	0.92 ± 0.07b	59.49
Horizontal	Superior	1.44 ± 0.19a	33.70
	Inferior	0.73 ± 0.13b	66.29

Means followed by the same letters inside planes were not significantly different (LSD, 0.05).

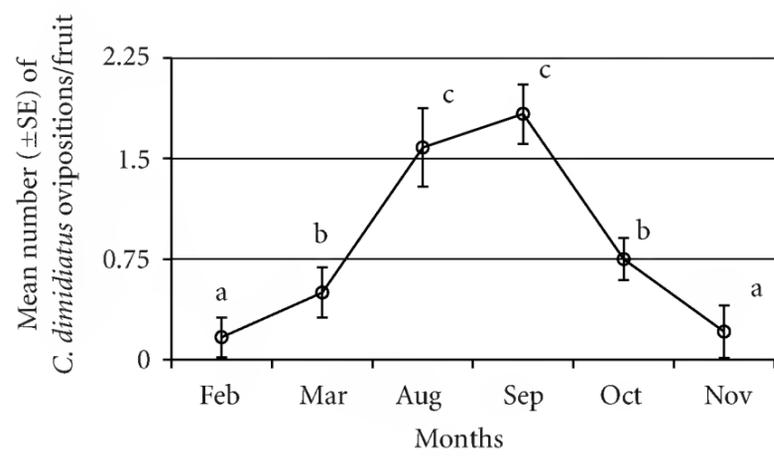


FIGURE 2: Temporal distribution of damage (\pm SE) by *C. dimidiatus* in guava trees in Aguascalientes, Mexico. Months with different letters are significantly different (LSD, 0.05).

the radial plane, the distance was measured from the trunk to the extreme end of the foliage, with the middle point being the external-internal division. For the horizontal plane, the total height of the tree was registered considering half of the height as the upper-lower division. With the help of a geopositioner (eTrex Summit; Garmin International), the four orientations of the cardinal plane were established. The distribution of damage on the fruits was registered throughout the year with samplings every fifteen days, where six different trees were randomly selected and their height determined. The damage pattern of the fruit was established by randomly selecting five fruits per sampling location of the eight established zones and registering the number of ovipositions per fruit. To establish the size of the most susceptible fruit, the polar diameter of each sampled fruit was determined with a Vernier caliper. The fruits remained on the tree after inspection.

2.2. Statistical analysis. For the evaluation of damage of the fruits in the trees, a completely randomized design was used with eight treatments (according to the zones) and six replicates. The averages of ovipositions per fruit and the total percentage of damaged fruit per zone of the trees were calculated. Means were square root transformed to stabilize

the variances. To determine if there was a difference in the damage among the eight zones at a significance level of 5%, we conducted an analysis of variance and the Fisher protected least significant difference test (LSD) using the statistical package SAS [8]. The assumptions of the ANOVA were verified [9]. The Pearson coefficients of correlation were also calculated among the total damage and the variables of temperature, rainfall, and relative humidity for the period of study.

3. Results

In total 83 trees were sampled with an average height of 3.09 ± 0.3 m. The average of damage produced by *C. dimidiatus* significantly differed according to the zones of the tree (Table 1). Within the radial plane, the external zone had higher damage with respect to the internal zone ($F = 5.79$; $df = 1$; $P = .01$). For the horizontal plane, the upper portion presented statistically higher damage than the lower portion ($F = 9.43$; $df = 1$; $P = .002$). Although there were variations in the percentages of damage among the four cardinal points, no statistical difference occurred among them ($F = 0.48$; $df = 3$; $P = .69$).

3.1. Temporal Damage Pattern. The mean fruit damage showed highly significant differences dependent on the month of sampling ($F = 16.37$; $df = 5$; $P = .0001$). The first indications of damage were observed in February (4), which were similar to what was observed in November (5). A higher incidence occurred in March (21) and October (18) (Figure 2). The peak damage occurred between the months of August (38) and September (44), which is during the rainy season. There was a significant correlation between the amount of damage by *C. dimidiatus* and temperature (0.84, $P = .03$) for the zone of Calvillo, Aguascalientes. There was no significant correlation between damage and the rainfall (0.86, $P = 0.06$) or the relative humidity (0.72, $P = .1$).

In our study the highest number of ovipositions (32) occurred in fruits of 3.3 cm polar diameter with a susceptibility range between 2.1 and 4.0 cm polar diameter (Figure 3). Of the total of fruits counted (2626), 17.9% presented signs of ovipositions of *C. dimidiatus* in the Calvillo region, Aguascalientes during the year of study.

4. Discussion

The damage pattern to the fruits of the guava trees indicates that the guava weevil initiates colonization of the plants from the outside to the inside, perhaps by means of short flights from other trees or from weeds and not by weevils climbing through the trunk. After colonization, higher damage occurred in the upper and inside zone of the trees. According to reports of damage patterns in Curculionids, Piñero et al. [10] did not find significant differences in the damage caused by plum curculio between the internal and external zones in apple trees. As in our study, there is evidence of higher damage by *Conotrachelus nenuphar* in fruits of the upper [11] and middle portion [12] of

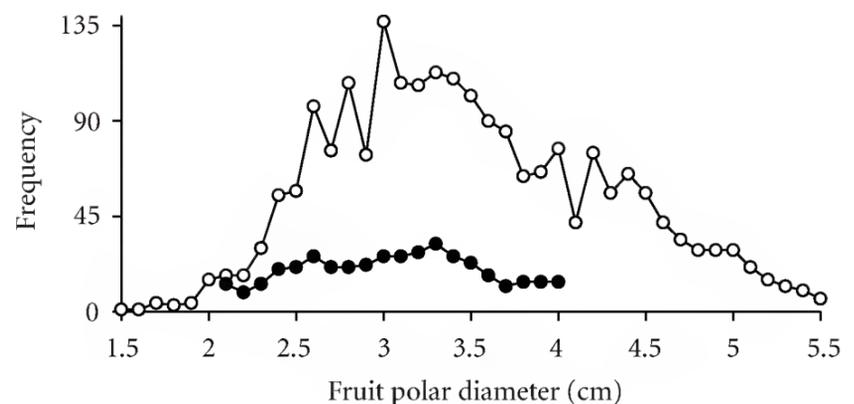


FIGURE 3: Distribution of damage (●) of *C. dimidiatus* with respect to polar diameter (○) in guava fruits in Aguascalientes, Mexico. Oviposition frequencies ≥ 10 are reported.

apple trees. We believe that differences in damage pattern are also closely related with meticulous procedures to scout the trees. Although we found no significant differences in damage between the four cardinal points, Piñero et al. [10] found higher levels of damage on the west side of the trees, given that the adults occupy this orientation during sunset. The peak damage during the rainy season of *C. dimidiatus* in Mexico is also reported in Venezuela by Boscan and Casares [13] for adults of *Conotrachelus psidii* Marshall which are present in guava trees from March to August, with a maximum in the month of May.

The appearance of the fruits damaged by ovipositions of *C. dimidiatus* was kidney shaped, with a cork-like concavity and early maturation. Gonzalez [4] mentions that oviposition occurs mainly in the middle portion of the fruit (72.3%) during the small fruit stage (2 cm diameter) which is similarly reported for *C. psidii* [14]. Kidney shape and premature abscission are caused by pectins. Levine and Hall [15] report several of these substances in plums and apples infested with plum curculio larvae *C. nenuphar* Herbst. We mostly found one oviposition per fruit, which indicated the presence of a signal compound in *C. dimidiatus* similar to natural antifeedants compounds reported in the feces of *Hylobius abietis* L. deposited adjacent to each egg, at which cavities were avoided by other pine weevils [16].

Although there was no significant correlation between the amount of damage and rainfall or relative humidity, Gonzalez [4] reported these abiotic factors as the cause of adult emergence. The capacity to remain in the soil after completed pupation also has been observed in *C. psidii* which can be underground for a further 34 ± 18 days under laboratory conditions [14]. All of this data indicates that abiotic factors need to be considered for guava weevil management.

According with our results of damage patterns and effect of abiotic factors, any control strategy for the adults, including pheromone traps, should be applied mainly in the upper and external portions of guava trees and there is no experimental evidence to apply pesticides during the rainy season. Following these directions, spraying a large amount of chemicals can be avoided. Additionally, low temperatures were a limitation for the presence of adults; therefore, control measures in the winter months are unnecessary.

Acknowledgments

The authors would like to express their thanks to Jose Carlos Ruvalcaba Camacho and Victor Manuel Ramirez Tapia for their assistance in the field work. This work received financial support from the Fondo Sectorial Sagarpa-Conacyt (registration code 2005-12161).

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Editorial

Foraging Biology of Neglected Bee Pollinators

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Received 12 April 2010; Accepted 12 April 2010

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Charles Darwin [1] was fascinated by bees, particularly bumble bees, and even recruited his children to trace their meandering paths through his garden [2]. Darwin's curiosity is part of a long history of interest in bee foraging biology. This attention is well deserved because bees are key pollinators in diverse ecosystems, exhibit complex adaptations such as recruitment communication, use foraging strategies that adapt to conditions inside and outside the nest, and have evolved elegant solutions to the challenges of gathering scattered floral resources. Much research has focused on honey bees, a remarkable model organism. However, the goal of this special issue is to explore new research on somewhat "neglected" bee pollinators. Many of these bees are not traditional model systems but have recently received attention because they are native pollinators whose diversity and numbers are in decline [3]. In addition, concern about the decline of honey bees (*Apis mellifera*), a frequently used agricultural pollinator, has led to increasing public awareness that non-*Apis* species can also pollinate crops. This has increased interest in alternative pollinators, many of which are native bee species.

This special issue therefore examines a wide range of bee species and is divided into three sections: (1) bee floral preferences in mixed landscapes, (2) the agricultural role of bees, and (3) influences on bee foraging activity. The first section, floral preferences, provides data on the abundance of different species and what they feed on. We then examine the role of diverse bee species in pollinating agricultural crops. Finally, we explore different factors that influence bee foraging activity inside and outside the nest.

Floral Preferences (C. Rasmussen). Single bee species never visit all of the different flowers in an area. Constraints on floral morphology or flowering phenology may prevent them from doing so, but even more interesting, most bees have a preference for pollen of certain plant species. This section traces the preferred floral resources for three different groups of bees: *Ceratina*, *Halictus*, and *Bombus*. Kobayashi-Kidokoro and Higashi examined pollen loads brought back to the nest by the small carpenter bee (*Ceratina flavipes*) and found that, within a bee population, such loads consist of 14 different pollen sources, although a single bee rarely exploits more than three different plants for pollen. While such preferences could be guided by local floral abundance, the authors found that preference for certain pollen sources persisted, even when the plant was uncommon. This phenomenon is termed flower constancy, where individual pollinators prefer flowers of the same species that they are already foraging at, thus bypassing other available flower species, even if the other flowers may be more rewarding. Polidori and collaborators likewise report a limited range of pollen sources for *Lasioglossum malachurum*, which can use from five to seven different pollen types but only visit one to two different plant species during each foraging flight. This species, however, exhibits large annual variation in the preferred pollen type. Lastly, Irene Konovalova compiled all known information about the bumblebees (*Bombus*) of Ukraine. Most of these bumblebees are polylectic with regional and seasonal preferences to the same flowering plant species, with the exception of *B. gerstaeckeri*, which almost exclusively forages from *Aconitum*.

Bees and Agriculture (J. Nieh). This section begins with papers examining the role of two solitary bee species in crop pollination. Güler and Sorkun report that *Andrena flavipes* (Andrenidae) is not an important pollinator of sweet cherry but does collect pollen from a wide variety of plants, particularly the Brassicaceae. Matsumoto and Maejima examine apple pollination by the megachilid bee, *Osmia cornifrons*. Interestingly, males and females of this solitary species collect nectar and, in net enclosure experiments, males contribute to apple pollination. Next, Keasar reviews the agricultural role of carpenter bees, which exhibit a wide range of solitary to quasisocial or communal organization. He describes the benefits and difficulties of using these bees as agricultural pollinators. Rao and Stephen then examine a thriving population of native bumble bees in western Oregon (USA) and suggest that cultivation of different crops blooming in succession may account for the diversity and strength of this population. Finally, Brunet and Steward study a guild of bee pollinators and report that some native bee species are more effective than honey bees at alfalfa pollination. Together, these studies highlight the agricultural importance of native bee species and suggest directions for future research, such as the role of solitary bee male pollinators and the need to determine the relative pollinating efficiencies of diverse bee species.

Influences on Foraging Activity (J. C. Biesmeijer). The “neglected pollinators” we are reporting on in this issue are primarily flower-visitors in search of food. Pollination is a side-effect of their foraging activities. The individual insects have to make decisions on when to forage, where to forage, and what to collect. The contributions in this section shed some more light on each of these three areas. Most bees have the difficult task of optimizing foraging and guarding their offspring in the nest. Lienhardt and collaborators show that, in the primitively eusocial bee *Halictus scabiosae*, the start of foraging in the morning depends on air temperature. Unexpectedly, however, they found that foraging ceases in the afternoon under excellent weather conditions, and bees even close the nest entrances. This is probably a response to the risk of nest parasitism by cleptoparasitic bees, whose activity patterns are largely asynchronous to that of the hosts. Eusocial bees do not have this optimization problem, because there are always some workers that remain in the colony and can defend the nest. In that case one would expect a more direct link between temperature and foraging activity. Couvillon et al. show that this is not the case for small and large bumblebee workers of the same colony. One might have expected larger bees to start foraging at lower temperatures than small worker and to cease foraging earlier at high temperatures, but this is clearly not the case for the North-American bumblebee, *Bombus impatiens*. Once foraging has started and flowers have been found, decisions have to be made on where to forage and how long to stay at flowers. Taneyhill presents a new more general model aiming to explain the decision of departure from a flower and tests the new model and a previous threshold model empirically. In addition to external factors, there are

internal factors influencing foraging activity, particularly in social bees. Nunes-Silva and colleagues report on a Brazilian stingless bee, *Plebeia remota*, where worker production stops during the colder winter months. Temperature and relative humidity affected foraging activity as expected. However, in addition they found that when brood production stopped, bees mainly collected nectar and rarely pollen. This has been reported before in honeybees but was not known from these tropical stingless bees.

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Review Article

Coleoptera Larval Fauna Associated with Termite Nests (Isoptera) with Emphasis on the “Bioluminescent Termite Nests” from Central Brazil

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Received 1 February 2010; Accepted 5 May 2010

Academic Editor: Michael Rust

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Beetle larvae that inhabit termite nests present modifications that allow them to cohabitate with the termites. Some are physogastric and bear special glands and different setae all over their bodies, whereas others are not physogastric. Both kinds of larvae may be termite predators. Some species usually live in the nest cabbage pan, feeding on organic matter, mushrooms, and excrements or eating the nest walls and sometimes causing the nest to be destroyed. Other species live in superficial galleries of the nest and feed on preys that live outside. However, all interactions between these inquiline beetles and their termite hosts are very complex and still little understood. Emphasis was done to the bioluminescent termite nests from Central Brazil and for this reason general aspects of the bioluminescence related to the elaterid fireflies were also given. The adaptations to live in nest environment and functional categories of association of all beetle larvae we have studied, including those not bioluminescent, to termite nests are discussed in this work.

1. Introduction

We present here a synthesis of our papers dealing with beetle larvae found inside termite nests in Brazil, Africa (Sudan, Tanzania, Guinea, and Ivory Coast) and Australia, pointing out some aspects not dealt with yet.

A numerous and diversified insect larval fauna including many beetle species is found in the interior of termite (Isoptera) nests. This fauna occurs in living colonies as well as in abandoned nests. The inside of the nest provides stable environment protected against climatic variations and enemies. In many cases the termite nest seems to constitute the main or the only place where these beetles can develop.

For many years we collected and studied Coleoptera larvae, associated with termite nests, mainly species of the families Elateridae, Passalidae, Melyridae, Scarabaeidae, Tenebrionidae, and Carabidae. As most of the studied species were described by us in several papers and in a book in

[1], and the species reported associated with the termite nests are of different taxa, we find it worthwhile presenting a synthesis of the gathered observations, pointing out some aspects not dealt with yet. We had had the opportunity to study larvae of Elateridae (Tetralobini) from Africa and Australia [2, 3] also collected from the inside of termite nests.

The phenomenon of the bioluminescent termite nest from Central Brazil is very impressive; it is the result of the luminescent activity of *Pyrearinus termitilluminans* Costa, 1982 (Elateridae, Agrypninae) larvae, which are found in old nests of *Cornitermes cumulans* (Kollar in Pohl, 1832) (Termitidae, Nasutitermitinae), one meter or more in height. These larvae excavate an intricate network of tunnels in the outer layers of the mounds leading outside, from where they stick out their head and their green shining luminous prothorax (in dorsal decubitus) to attract and catch flying preys, especially termites and ants (Hymenoptera, Formicidae).

2. Methods

The methodology included the collection of larvae, pupae, and living adults for correlating immatures and adults, and identifying the species. Depending on the species, the correlation larva/adult was made on the spot and then both were properly fixed. In other cases, depending on the larval instars, it was necessary to maintain them in the laboratory for no longer than two years. When a large larval series of the same species was collected and the laboratory rearing had been successful, it was possible to preserve larva, pupa, and adult. Being the number of larvae small, we generally photographed the pupa and preserved the larva and the adult. If there was a single larva, the pupa was photographed, the larva and pupa exuviae were preserved, and the adult was obtained.

The data presented in this paper were based on Brazilian species reared from larval to adult stage, deposited in the immature beetle collection of the Museu de Zoologia da Universidade de São Paulo. Australian specimens studied by us were provided by John Francis Lawrence (CSIRO) and reared specimens from Africa by Claude Girard (MNHNP).

The species studied in our papers are the following. Brazil. Mato Grosso, Taiamã Island, in arboreal nest of *Anoplotermes* sp. (Termitidae, Apicotermatinae)—*Veturius transversus* (Dalman, 1817) (Passalidae, Passalinae), and *Anchastus brunneofasciatus* Schwarz, 1906 (Elateridae, Elaterinae, Dicrepidini) [1]. Goiás. Parque Nacional das Emas, in *Cornitermes* sp. nest—*Odontocheila auripennis* Lucas, 1857 (Carabidae, Cicindelinae), *P. termitilluminans* (Elateridae, Agrypninae), and *Lemphus* sp. (Melyridae, Malachiinae) [1]. São Paulo. Itanhaém, Restinga Vegetation, in termite nest of *Microcerotermes* sp. (Termitidae, Termitinae)—*Homophileurus luederwaldti* (Ohaus, 1910) (Scarabaeidae, Dynastinae); in nests of Apicotermatinae and Nasutitermitinae—*Dilobitarsus abbreviatus* Candèze, 1857 (Agrypninae) [1]. Sudan. Kordopan, Khuei, from dead tree—*Tetralobus cavifrons* Fairmaire, 1887 (Agrypninae, Tetralobini) [2]. Tanzania. Lake Manyara National Park, in termite nest—*T. subsulcatus* Guérin-Méneville, 1847 [2]. Guinea. Mont Nimba, in dead termite nests of *Macrotermes* sp. (Termitidae, Macrotermatinae)—*T. arbonnieri* Girard, 2003 [3]. Ivory Coast, Savanna of Lamto near N'Douci, in termite nests—*T. gigas* (Fabricius, 1801) and *T. shuckhardi* (Hope, 1842) [3]. Australia. Victoria. Wodonga, in outer casing of *Coptotermes lacteus* (Froggatt, 1898) (Rhinotermitidae, Coptotermatinae) mound, in termite mound and in termite infested log—*Pseudotetralobus* compared with *murrayi* (Candèze, 1857) (Agrypninae, Tetralobini) [2].

3. Bioluminescence

The emission of cold light by living beings has been the object of curiosity and scientific interest. It occurs both in plants and animals as well. Bioluminescence appears in various species of bacteria, mushrooms, seaweed, coelenterates, clams, arthropods, annelids, echinoderms, and fishes [4]. It is the product of a chemical reaction, biologically functional, catalyzed by enzymes, resulting from highly exergonic

oxidations in which the energy is preferentially liberated in the form of light. The most representative bioluminescent beetle families in Brazil are Elateridae, Lampyridae, and Phengodidae (Elateroidea). They are popularly known as lightning bugs, fireflies, or glow worms.

In the Coleoptera, the luminescence can occur in adults of both sexes or is restricted to females; it can also be present in the larvae. There is much speculation on the subject of the origin and function of the luminescence in the Coleoptera, being still the cause of many unsolved questions, not deeply studied and not clearly understood.

The bioluminescence in animals can assume important functions in the intra- and interspecific communication such as sexual attraction, defense, camouflage, and attraction of the preys with feeding purposes [5, 6].

The bioluminescence in Coleoptera can develop the following relations.

(1) Mutual recognition between individuals. At least in the adults of the species that possess some degree of sexual dimorphism, the function of the luminescence would be to enable the recognition of the opposing sexes at a certain distance in the darkness [7]. The recognition can also occur between youths and adults of the same species.

(2) Defense, acting as warning signal to some predators. In many cases bioluminescence can only have evolved from a defensive function, since there are evidences that at least some fireflies are unpalatable to some vertebrate predators [8, 9]. It was demonstrated experimentally that a potentially important predator learns to avoid larval glowworms by using the light signals as aposematic cues [7] and it was proved experimentally that some Lampyridae species can be unpalatable to certain vertebrates, but not to toads and bats, nor to arthropods, like some spiders that attack and eat fireflies voraciously. On the other hand, some beetles of other families mimic the colorful patterns of some Lampyridae, which suggests them have repellent properties. The defensive function can be evidenced in larvae of Phengodidae, which increase the luminous intensity when stimulated or attacked by predators [10–13]). In Pyrophorini (Agrypninae) larvae increase of luminescence under stimulation was also observed [14]. Synchronized emission of light by several individuals, adults and larvae, would increase the signal of warning against possible predators [15].

(3) Attraction of preys. The use of bioluminescence by predators to attract preys is reported in a few animals. Some benthonic fish [5] possess a luminous organ in the dorsal fin which works as bait to attract them. Adult females of lampyrids of the genus *Photuris* LeConte, 1851 (Lampyridae, Photurinae) imitate the flashes of light of females of other species then attract males to be eaten [16]. This behavior is considered to be aggressive mimicry. Larvae of some dipteran species of the family Mycetophilidae that live in caves use the bioluminescence as lure to attract their prey [16]. Larvae of *P. termitilluminans* exhibit the behavior of entrapping them [17, 18]). Each larva lives inside a U-shaped gallery that opens on to the outside, where it shows its head and its green-shining prothorax to attract and catch winged preys [19].

4. Bioluminescence in Elateridae

The adults of these fireflies are easily identified as they have a pair of luminescent oval vesicles (yellowish when the light is extinct or the insect is dead) located at the base of the posterior angles of the prothorax that emit continuous light in the green region of the spectrum. There is a third luminescent organ located in the abdominal segment I, hidden by metaventrite and that is only activated during the flight [20], so producing light generally displaced to orange-yellow [6] or to red [21]. The luminescence in adult elaterids appears to be associated with sexual attraction, yet very little has been studied about their communication system.

In the larvae the luminescence is found mainly on the pronotum, and in some species, there are paired, lateral round organs or transverse dorsoventral zones on each abdominal segment [14]. Elateridae larvae only light up in the green area of spectrum. In the larval phase, bioluminescence may be related also with defense, but no detailed studies have been developed yet. Prepupae and pupae generally show luminescence over the entire body, with variations of intensity depending on the species. After 10–14 days of the pupal phase, it is easy to notice the outlines of the adult luminous organs. A newly emerged adult displays luminescence over the entire body, like in the pupa, but as the integument becomes darker, only the prothorax and abdominal organs emit light [14, 22].

5. The Bioluminescent Termite Nest from Central Brazil

5.1. Historical Review. In the natural world indescribable spectacles of beauty due to emission of light produced by living beings are known. Doubtlessly, in America, one of the most astonishing is the one offered by the “bioluminescent termite nest” that can be watched in the Open Cerrados of Central Brazil. Among the chroniclers, travelers, and naturalists who have written on the bioluminescent termite nests from Brazil, we can distinguish the report by Laporte [23] in Água Limpa (MT, Brazil). Laporte’s comments were very accurate and he correctly identified the causes of the phenomenon “small phosphorescent larvae” that from the inside of the galleries of the termite nest produced tiny pinpoints of light. He described: *Aux environs de la ferme d’Agoa-Limpa (sic), nous observâmes, durant la nuit du 15, une masse lumineuse située au milieu du Campo, et qui excita vivement notre curiosité. En approchant, nous reconnûmes que c’était une butte de termites, d’où sortaient une multitude de petits foyers lumineux. Ce phénomène est produit par la présence d’une infinité de petites larves phosphorescentes qui se retiraient dans les galeries qu’elles s’étaient ménagées dès qu’on tentait de les saisir.*

This phenomenon was also noticed in the Amazon region. It was reported that in the neighborhoods of Santarém, Pará, there were luminous termite nests whose light came from insects: *The Termites, on the contrary, live in large, irregular, conical mounds, hard as rock, and often ten feet and more high. In the daytime there is no sign of life, but if one*

enters the forest at night the sight is a beautiful and startling one —the darkness is intense. Here and there in the blackness may be seen clusters of glittering phosphorescent light; these are the Termite hills. No doubt, the light proceeds from the insects as the particles of the light mass move and change. The light is greenish and soft, and the effect is indescribable [24, 25].

Some explanations about the phenomenon of the luminous termite nests were given by other travelers. They realized that the light was originated from phosphorescent mushrooms, bacteria, or even from luminous insects [26–28]; also that the luminosity came from some insects, such as larvae of fireflies or dipterous that would live in the termite nests [29]. It is worth mentioning [27]: *I have just received authentic information to the effect that in the State of Matto Grosso (sic), in the low, swampy lands along streams, and especially in the rainy months beginning with October, myriads of fireflies are seen covering the ground. My informant, who has lately come from the upper part of Matto Grosso where it joins Bolivia, tells me that he has seen at night many of the nests of white ants that have been abandoned by the ants themselves entirely covered by fireflies that come from the small openings over the whole surface of the ant-hill. Is it possible that the fireflies select these abandoned ant-hills as places in which to grow their larvae?... Unfortunately I have never observed anything of the kind here about, though I have been interested in the subject in order to furnish you information.* Schaller [30, 31] observed luminous termite nest in Manaus (Ducke/INPA Reserve): *we found an abandoned termite nest which appeared illuminated like a Christmas tree. It soon became clear that hundreds of Hemirhipus sp (=Pyrearinus fragilis Costa, 1978) [20] larvae were living in superficial holes lying in wait for crawling or flying small animals.*

Kent Redford, when studying anteaters in the Parque Nacional das Emas, Goiás, Brazil, was very impressed with the phenomenon of the bioluminescent termite nests. In November 1981 in a letter to Costa, he wrote: *the landscape at the Park is covered with Cornitermes mounds in which live larvae which I believe are of the genus Pyrophorus. During the beginning of the rainy season, these larvae partially emerge from holes in the termite mounds, and, from the segment immediately behind the “head”, emit a bright light. It is possible to find several hundreds of these larvae in one mound and the view at night is breathtaking.* Costa studied the specimens sent by Redford and concluded that they were a new species, [17] *Pyrearinus termitilluminans*, which means “green fire that illuminates the termite nest”. Thanks are due to the works carried through by Redford, the origin of the light in the luminescent termite nests was finally elucidated and Laporte’s hypothesis [23] was confirmed. In the region of the Parque Nacional das Emas, there is great concentration of *C. cumulans* nests (Figure 1), being its mean density 55 nests/hectare. There are also some termiteinquilines of the genus *Paracornitermes* Emerson, 1949 (Nasutitermitinae), morphologically similar to *Cornitermes* Wasmann, 1897, that were surely mistaken by Redford for *Cornitermes*. The dynamics of settling of the great termite nests is related to the aging of the nests of *C. cumulans* (L. R. Fontes, personal communication) [21].



FIGURE 1: Parque Nacional das Emas, Goiás, Brazil, field with numerous termite nests, general view (photo: S. A. Vanin).

In August 1982, Erwin reported to Costa (pers. comm.) on his findings about another type of luminescent termite nest inside the forest in Puerto Maldonado (Rio Tambopata), Madre de Dios, Peru. He collected larvae and adults at the base of a dead tree, partially covered with soil. These larvae remained inside of orifices of the wood and shone at night, producing some points of greenish light on the trunk where they had been found. Erwin wrote: *In young terre (sic) firme Forest at the base of dead trees were uplifts of soil around the base. At night while walking near these trees I noted many little points of blue light. Upon investigating I found many small burrows like tiger beetle burrows. To my surprise I got a larva or adult elaterid from the holes.* These specimens were identified as *P. fragilis* and the larva was described in 1986 [14]. Although apparently less spectacular, the phenomena observed by Erwin in Peru and Schaller in Manaus are quite similar to that of the Parque Nacional das Emas.

5.2. *The Elaterid Genus PYREARINUS Costa, 1975.* This genus congregates 43 South American species, in seven species groups; 35 species being found in Brazil. *Pyrearinus termitilluminans*, a species of the *pumilus* group, is very abundant in the Parque Nacional das Emas. Invariably, larvae, pupae, and adults of that species were found in older nests of *C. cumulans*, about 1 m high or more, and invaded by other species of termites, mainly of the genera *Paracornitermes*, *Spinitermes* Wasmann, 1897 (Termitinae), and *Embiratermes* Fontes, 1985 (Nasutermitinae). In three broken up nests 142 larvae of *P. termitilluminans* were found on average. This is the first species of elaterid whose larvae were found in a network of galleries in termite nests opening on to the outside. In Brazil, the phenomenon of the luminescent termite nests (Figure 2) can be observed mainly from April to October. On the warm and humid nights of spring and summer, hundreds of larvae in each termite nest, soon after twilight, are positioned to exhibit its luminescent pronotum [19], and the termite fields look like miniature cities or countless illuminated Christmas trees. The female of *P. termitilluminans* lays its eggs at the base of the termite nests. After hatching, the larvae dig small galleries in the



FIGURE 2: Parque Nacional das Emas, Goiás, Brazil, luminous termite nest (photo: S. A. Vanin).

walls where they live. The nests provide a vertical surface or “tower,” causing the light produced by the larvae to be more visible and then making the prey attraction easier. The hunting position of the larva in the galleries seems to be very strategic. At night, the ventral part of its body is upturned in relation to the ground nest and the larva exposes its bending head and prothorax out of the gallery in such a way as to exhibit the luminescent pronotum to attract preys (Figure 3), generally butterflies, winged termites, and ants. Pupae and adults were also found inside sealed galleries with their shed larval skins. Adults (Figure 4) can also be collected flying at night in the neighborhood of the termite nests [17].

In internal chambers of one of the termite nests examined in the Parque Nacional das Emas, we found fragments of adults of *P. termitilluminans* (female) and scarab beetles. It is possible that some females enter the termite nest to lay their eggs and die there. Another probable oviposition place of *P. termitilluminans* is the base of the termite nest zone, where the first instars are always found brightly illuminating the area.

5.3. *The Pumilus Group.* The *pumilus* group includes, apart from *P. termitilluminans*, five more species: *P. flatus* Costa, 1975—Brazil (SP, MT) and Paraguay; *P. fragilis*—Brazil (PA, RS); *P. pumilus* (Candèze, 1863)—Brazil (GO, MG, SP); *P. scintillula* (Candèze, 1881)—Guyana, Ecuador, Brazil



FIGURE 3: *Pyrearinus termitilluminans* Costa, 1982 (Elateridae, Agrypninae), larva, head and prothorax, dorsal decubitus, in the outer opening of the gallery. Parque Nacional das Emas, Goiás, Brazil (photo: S. A. Vanin).

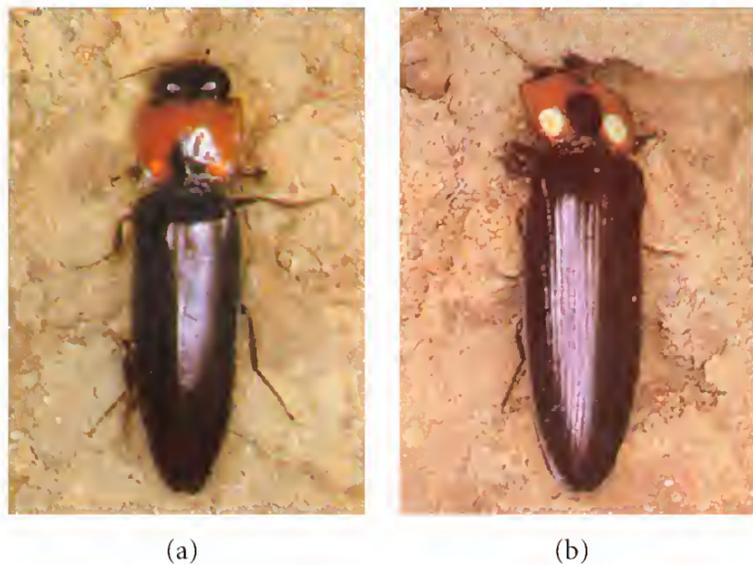


FIGURE 4: *Pyrearinus termitilluminans* Costa, 1982 (Elateridae, Agrypninae), male and female (note the large and salient eyes of male). Parque Nacional das Emas, Goiás, Brazil (photo: S. A. Vanin).

(AM, PA)—Peru, Bolivia; and *P. vitticollis* (Germar, 1841)—Brazil (ES) [20, 32]. The geographic distribution of that group [20] shows some species in the Brazilian open formations and others in the Amazonia forested region.

On the map (Figure 5), showing the localities where luminous termite nests were reported by earlier naturalist and travelers, there is certain overlapping between their distribution and that species of the *pumilus* group. For example, de Caumat de Laporte Castelnau [23] observed the phenomenon of luminous termite nests in “Água Limpa,” Mato Grosso; Smith [26] verified the occurrence of the phenomenon in a place close to Santarém (Tapajós Valley), Pará; Knab [24] reported the presence of luminous termites in Low Amazon; Knab [25] in Santarém, Pará; Branner, [33, 34], cited information on luminous termite nests received from Lustosa, in Mato Grosso, next to Bolivia; Neiva and Penna [28] mentioned the occurrence of the phenomenon in Goiás without specific locality and in the “Gerais Baianos,”

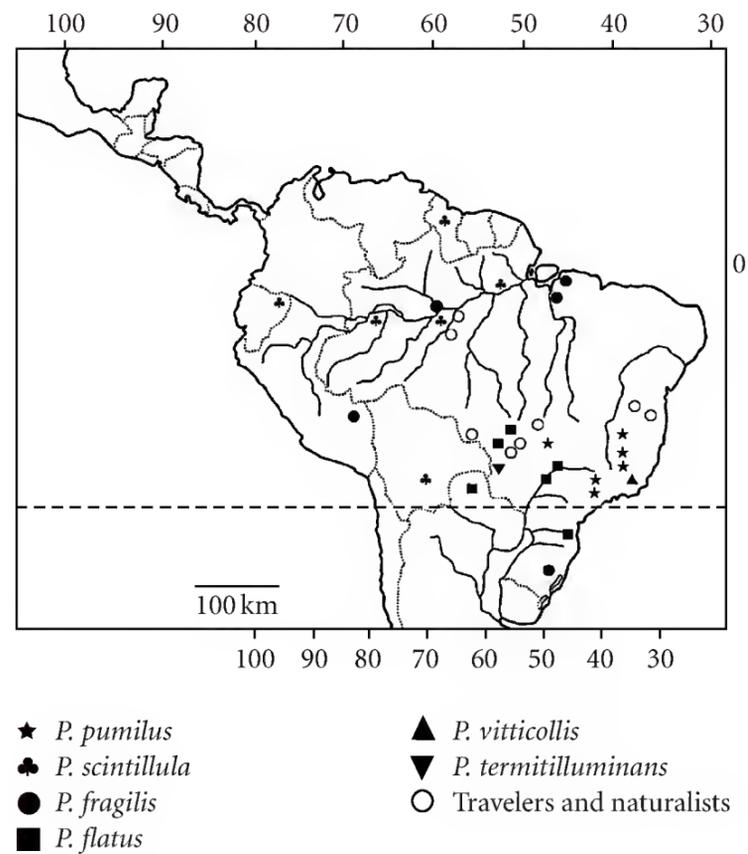


FIGURE 5: Map with distribution of *Pyrearinus*, group *pumilus* (Elateridae, Agrypninae) species, and records of occurrence of luminescent termite nests by naturalists and travelers.

Lage, São G. B. Raimundo Nonato municipality and in Jatobá Remanso municipality; Fonseca [35]) and da Fonseca and de Almeida [36] registered the occurrence of luminous termite nests in Rio Verde, Mato Grosso; Lenko and Papavero [37] and Marien [38], on the Raizama river, and Otero [39]) in Goiás. Costa [17] considered the distribution of species of the *pumilus* group associated with the termite nests' distribution.

Pyrearinus termitilluminans is very similar to *P. fragilis*. Larvae of the latter were collected inside luminous ground wooded termite nests from Peru [17], and Manaus, Amazonas, Brazil [30]. Specimens of *P. fragilis* from Rio Grande do Sul, Brazil were recorded to live inside termite nests, but there are no references if they were luminescent or not [20]. Larvae of *P. termitilluminans* (Figure 6) and of *P. fragilis* from Manaus were reared up to adults, while the larvae of *P. fragilis* from Peru were collected associated with the adults [17, 30]. The larval behavior of *P. fragilis* from Peru and Manaus is quite similar to those of *P. termitilluminans*; they remain inside of orifices of the wood infested by termites or small ground termite nest, with the anterior region emitting light and attracting preys [14, 30]. However, larvae of both species are quite different from the other known larvae of the same genus. They have bodies less dorsoventrally flattened, less sclerotized, and feebly pigmented; the abdominal segment IX is rounded, without tubercles; the anal hooks are less developed and the stemmata are dark pigmented. These characters seem to be adaptations to the life inside the galleries [14, 17].

The larvae of *P. termitilluminans* illuminate intensely the prothorax and the abdominal segment IX. The larval galleries form an intricate superficial mesh (Figure 7) of

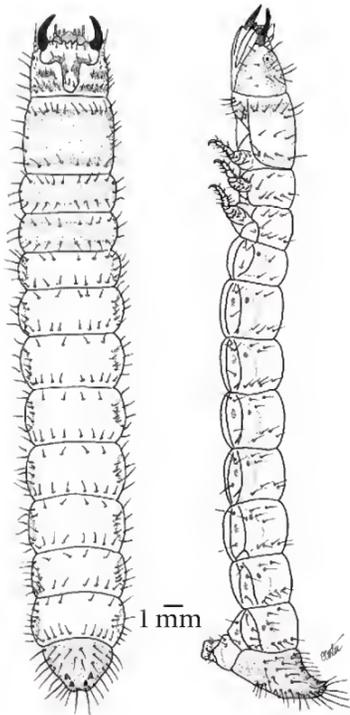


FIGURE 6: *Pyrearinus termitilluminans* Costa, 1982 (Elateridae, Agrypninae), larval drawings (dorsal, lateral). Parque Nacional das Emas, Goiás, Brazil (after Costa, 1982).

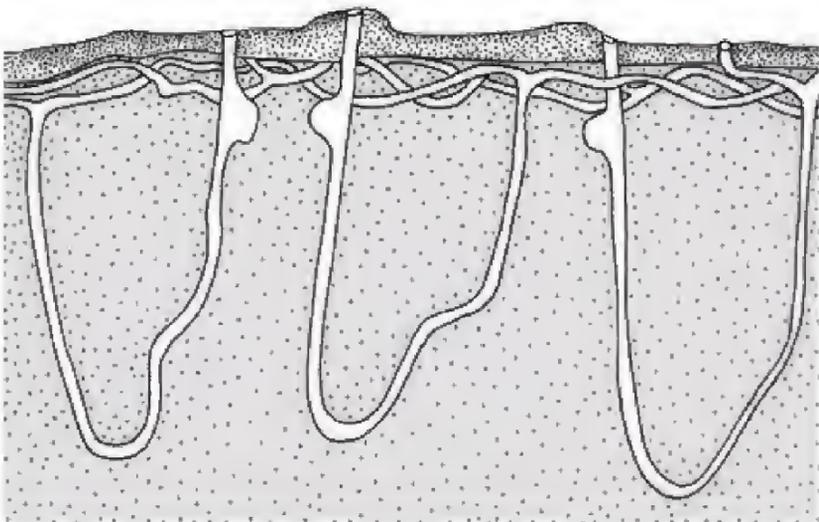


FIGURE 7: *Pyrearinus termitilluminans* Costa, 1982 (Elateridae, Agrypninae), scheme of galleries constructed by the larvae in the surface *Cornitermes cumulans* (Kollar in Pohl, 1932) (Termitidae, Nasutitermitinae) nests. Parque Nacional das Emas, Goiás, Brazil (after Bechara, 1988, modified).

tunnels with 0.1 to 0.3 cm in diameter, occupying almost the whole surface of the nest [21]. Each individual “U”-shaped gallery opens on to the outside; the arm of the U near the exit hole has a small atrium that seems to constitute a place for stockpiling predigested prey and also for facilitating the inversion of displacement of the larva in the gallery, as if it were a “marshaling yard”. It is possible that the lantern of the abdominal segment IX serves to signal the presence of the larva in the tunnel, for there are many larvae in each nest (one each 10 cm²), and thus to prevent the cannibalism, which is noticed in the laboratory when some larvae are kept together. The *in vivo* larvae bioluminescence increases sharply with the elevation of the temperature and this mechanism should be related to the hunting behavior [40].

Larvae of four different species of other *Pyrearinus* groups are known as *P. candelarius* Germar, 1841, *P. candens* (Germar, 1841), *P. janus* (Herbst, 1806), and *P. micatus* Costa, 1978. They differ from those of the *pumilus* group in the depressed, quite sclerotized and pigmented body, abdominal segment IX depressed and with many tubercles. Larvae of *P. candelarius* were collected in dead trunks and inside ground termite nests. The larvae of *P. candens* showed no luminescence, yet those of *P. candelarius* emitted light when stimulated, first in the prothorax and then in the lateral and dorsal regions of almost all abdominal segments. The larvae of *P. janus* and *P. micatus* illuminated intensely the prothorax, and almost all abdominal segments, in lateral and dorsal points [14, 32].

6. Other Larvae of Beetles Related to the Termite Nests

More than 1,500 species of termitophilous insects are known today [41]. The Brazilian Coleoptera families that have been studied and include species living associated with termite nests are Elateridae, Passalidae, Melyridae, Scarabaeidae, Tenebrionidae, and Carabidae. Considering the species of these families, the termite nest seems to be the ideal place for their whole development or important for part of their life cycles. In general, these beetles are so well adapted that, if removed from the termite nest, they do not resist and die in a short period of time [42]. The larvae have a greater dependence on the termite nest environment than adults, because larvae depend on the available food resources found inside the nests or upon preys that are attracted from the outside by light emission. These larvae can be saprophagous, mycetophagous, or predators, attacking the termites in the living colonies or other insect hosts, especially in dead colonies.

Some of these species present a curious structural modification in the abdomen, called physogastry. The abdomen is gradually enlarged or swollen, generally by possible growth of the interconnective membranes, expansion of the adipose body, and secondary sclerotization of the abdominal sternites. As a result, it resembles the abdomen of a termite queen. The physogastric abdomen was developed independently in some lineages of Coleoptera: in adults the species of the coroticines (Staphylinidae, Aleocharinae, Coroticini) and the Scarabaeidae presented it, in larvae the Carabidae and Elateridae did.

Physogastric larvae of the elaterid *D. abbreviatus* were collected inside epigeous nests of soil-feeding termite genera *Anoplotermes* Fr. Müller, 1837, *Araujotermes* Fontes, 1982, *Atlantitermes* Fontes, 1979, and *Subulitermes* Holmgren, 1910 (Nasutitermitinae), *Atlantitermes* Fontes, 1979 (Nasutitermitinae), and *Subulitermes* Holmgren, 1910 (Nasutitermitinae) in the Restinga Vegetation (6–12 m in height) in Itanhaém, a seaside town of the state of São Paulo [43].

Physogastric larvae of *Pseudotetralobus* spp. (Australia: Victoria, New South Wales, Queensland, and Northern Territory) were collected in varied environments: “in termite infested logs”, “in chambers next to termite galleries”, “in rotten logs”, and “in outer casing of *Coptotermes lacteus*

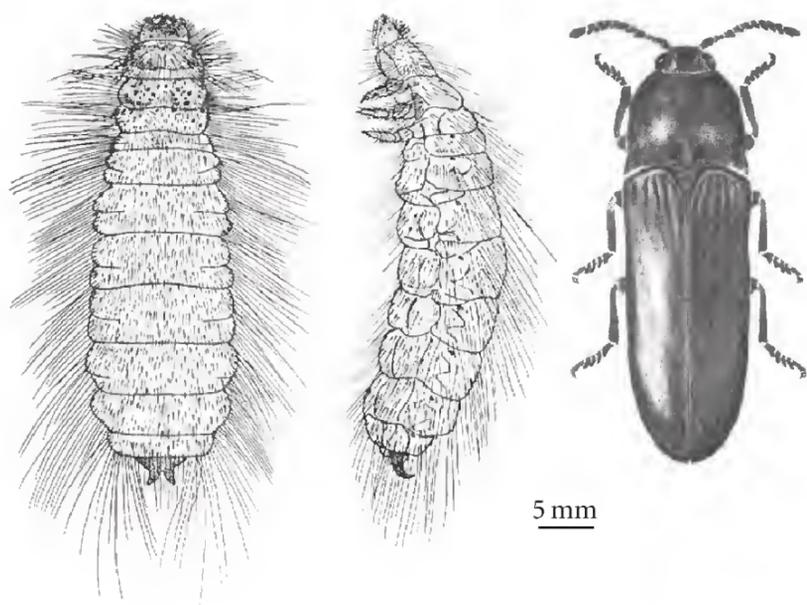


FIGURE 8: *Tetralobus cavifrons* Fairmaire, 1887 (Agrypninae, Tetralobini), larva (dorsal and lateral) and adult (dorsal), inside termite nest. Kordopan, Sudan (after Costa et al., 1992).

mounds”. The larva of *T. cavifrons* from Africa, Sudan (Figure 8), collected from the wood of a dead tree of *Acacia* sp. (Fabaceae), is also physogastric. These larvae resemble termite queens, but they have the body densely covered with a diverse kind of setae, and their heads are almost phragmotic (in resting position are obliquitous to body axis) [2]. They run actively over the ground, crawling very quickly and extending and contracting their body as they progress [44, 45]. Larvae of *T. arbonnieri*, *T. gigas*, and *T. shuckhardi* are widely distributed in Ivory Coast and Guinea and were collected inside galleries of the shield of dead nests of *Macrotermes* Holmgren, 1910, are also physogastric, and have phragmotic heads, which suggest that they are probably predators of termites and other small insects. Apparently, the galleries do not open in the nest outside. Their occurrence in termite nests seems regular. These nests represent a very *ad hoc* environment to complete their postembryonic development [3].

The development of glands in the abdomen or in other regions of the body is frequent, too. The glands secrete substances that surely influence the relations between the termitophilous beetles and the termite hosts. The larvae of *T. subsulcatus*, *T. arbonnieri*, and *T. shuckhardi* have glands in the dorsal area of abdominal segment VIII, but their function remains unknown [2, 3].

Immatures of *A. brunneofasciatus* (Figure 9), which inhabit epigeous termite nests in marshy areas, 10 km S. of the island of Taiaimã, the right bank of the Paraguay river, Mato Grosso, Brazil [43], of the passalid *V. transversus* (Figure 10) collected inside the same nest [46], and immatures of *Lemphus* sp. (Cleroidea, Melyridae) (Figure 11), collected inside termite nest in the Parque Nacional das Emas, are not physogastric. The larvae of *Lemphus* sp. are very conspicuous by having salmon color and a short velvety yellowish pilosity [1].

A detailed study on infestation of arborous carton nests of *Microcerotermes* sp. by the *H. luederwaldti* (Phileurini) [47] (Figure 12) demonstrated that the larvae of the

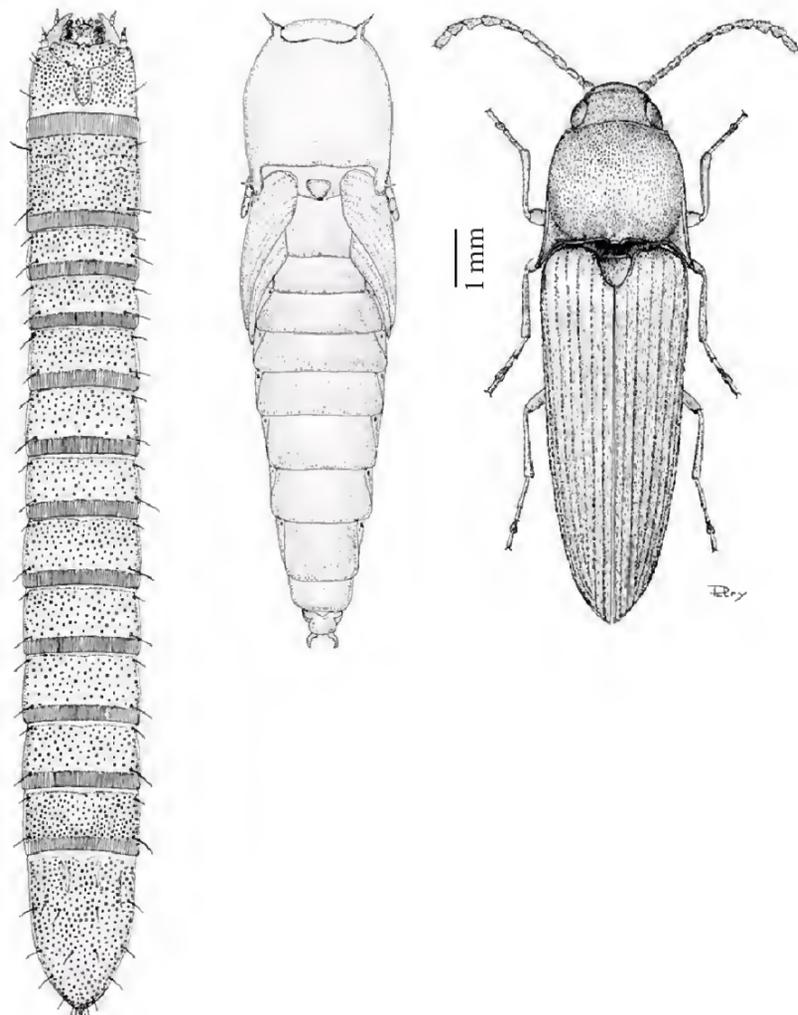


FIGURE 9: *Anchastus brunneofasciatus* Schwarz, 1906 (Elaterinae, Dicroptidiini) larva, pupa and adult (dorsal), inside *Cornitermes cumulans* (Kollar in Pohl, 1832) (Termitidae, Nasutitermitinae) nest., Parque Nacional das Emas, Goiás, Brazil (after Costa et al., 1988).

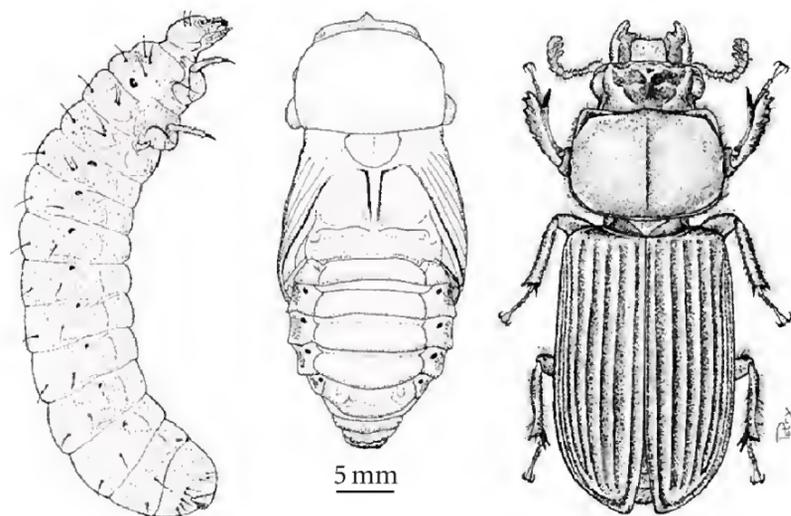


FIGURE 10: *Veturius transversus* (Dalman, 1817) (Passalidae, Passalinae), larva (lateral), pupa and adult (dorsal), inside *Cornitermes cumulans* (Kollar in Pohl, 1832) (Termitidae, Nasutitermitinae) nest. Parque Nacional das Emas, Goiás, Brazil (after Costa et al., 1988).

Scarabaeidae feed on the walls of the nest itself. The wall is constituted of remaining portions of chewed wood and termite excrements and provides necessary energy for the development of the larvae. Having completed the feeding phase, the larvae construct pupal chambers in the inner walls of the nest. After the metamorphosis, the adults leave

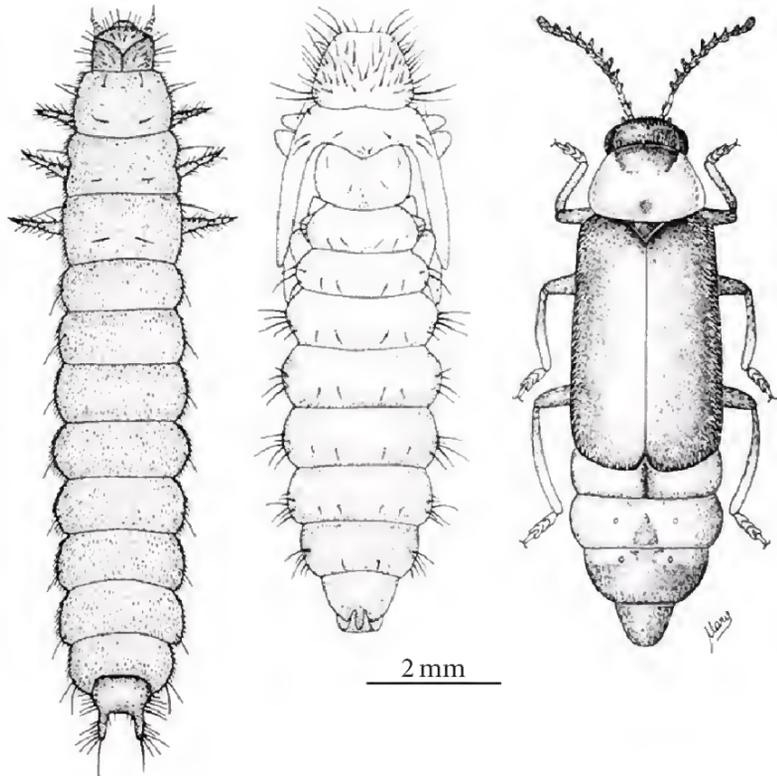


FIGURE 11: *Lemphus* sp. (Melyridae, Malachiinae), larva, pupa and adult (dorsal), inside *Cornitermes cumulans* (Kollar in Pohl, 1832) (Termitidae, Nasutitermitinae) nest. Parque Nacional das Emas, Goiás, Brazil (after Costa et al., 1988).

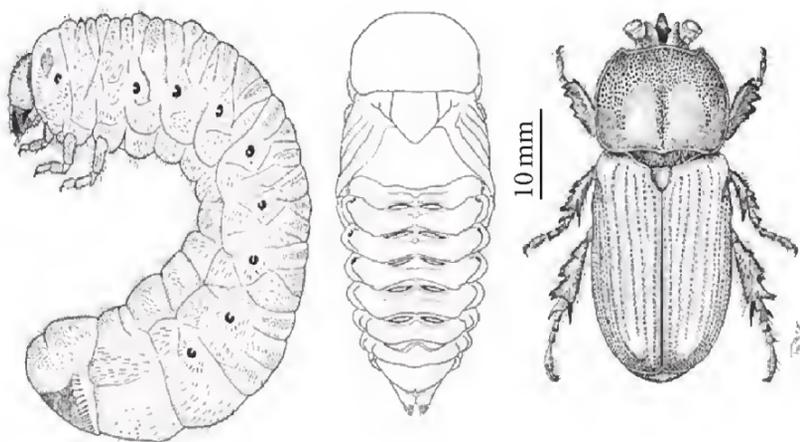


FIGURE 12: *Homophileurus luederwaldti* (Ohaus, 1910) (Scarabaeidae, Dynastinae), larva (lateral), pupa and adult (dorsal), inside *Microcerotermes* sp. (Termitidae, Termitinae) nest. Itanhaém, São Paulo, Brazil (after Vanin et al., 1983).

the nest by excavating a canal to the exterior (Figure 13). The activities of the larvae and adults of the Scarabaeidae can cause great damages to the termite nest, resulting sometimes in its destruction. It must be stressed that the termites do not attack living larvae, pupae, or adults and are not able to defend the nest against beetle's attacks, yet they occasionally enclose the beetles that die inside the colony with newly built nest material. It is interesting to notice that the fecal pellets produced by the larvae serve as food for hundreds of larvae of alleculine beetles (Tenebrionidae, Alleculinae) (Figure 14). Larvae of *H. luederwaldti* and of another Phileurini species, *Actinobolus trilobus* Luederwaldt, 1910, were observed inhabiting arborous nests of two species of *Nasutitermes* Dudley, 1890, suggesting an association between the Phileurini and the Nasutitermitinae [48].

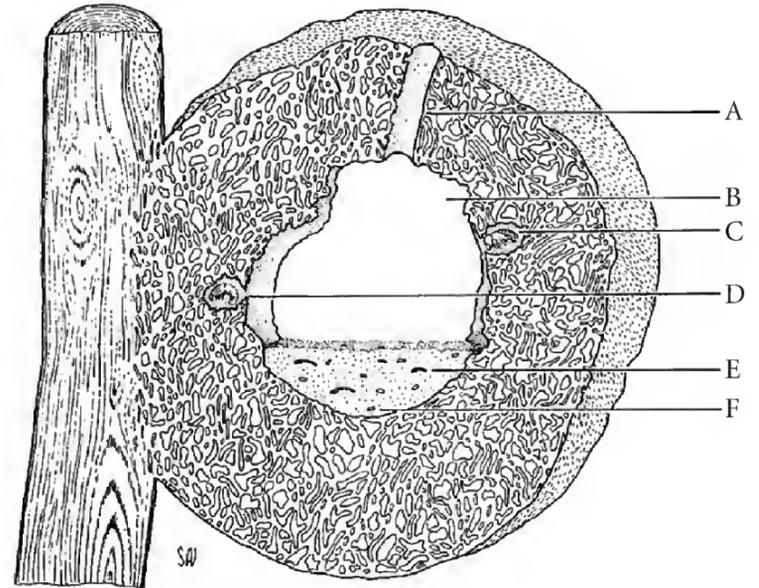


FIGURE 13: *Microcerotermes* sp. (Termitidae, Termitinae), transversal section of the nest: (a) the canal; (b) central chamber; (c) pupal chamber of *Homophileurus luederwaldti* (Ohaus, 1910) (Scarabaeidae, Dynastinae); (d) larva of *H. luederwaldti* feeding on nest walls; (e) *Lobopoda* sp. (Tenebrionidae, Alleculinae) larva; (f) fecal pellets of *H. luederwaldti* larvae. Itanhaém, São Paulo, Brazil (after Vanin et al., 1983).

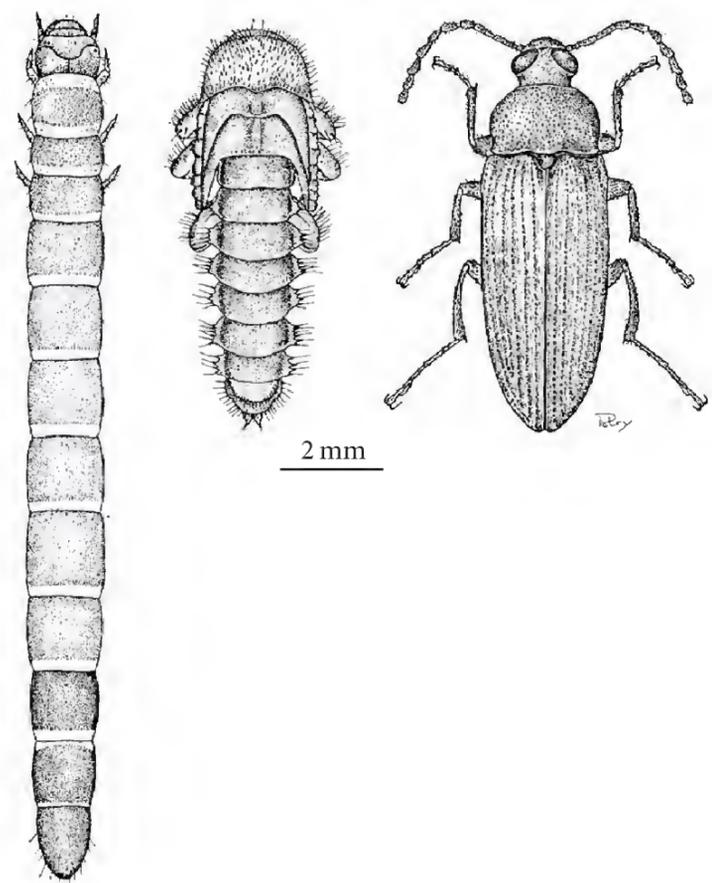


FIGURE 14: *Lobopoda* sp. (Tenebrionidae, Alleculinae), larva, pupa and adult (dorsal), inside nest of *Microcerotermes* sp. (Termitidae, Termitinae). Itanhaém, São Paulo, Brazil (after Vanin et al., 1983 and Costa et al., 1988).

Beetles of Cicindelinae (Carabidae) (Figure 15) and Tenebrionidae have been found associated with the luminescent termite nests of Central Brazil. The larva of *O. auripennis* is predator and lives in superficial galleries in the nests of *C. cumulans*. As other larvae of Cicindelinae, it possesses phragmotic head, which together with sclerotized pronotal plate, occludes the circular opening of the gallery.

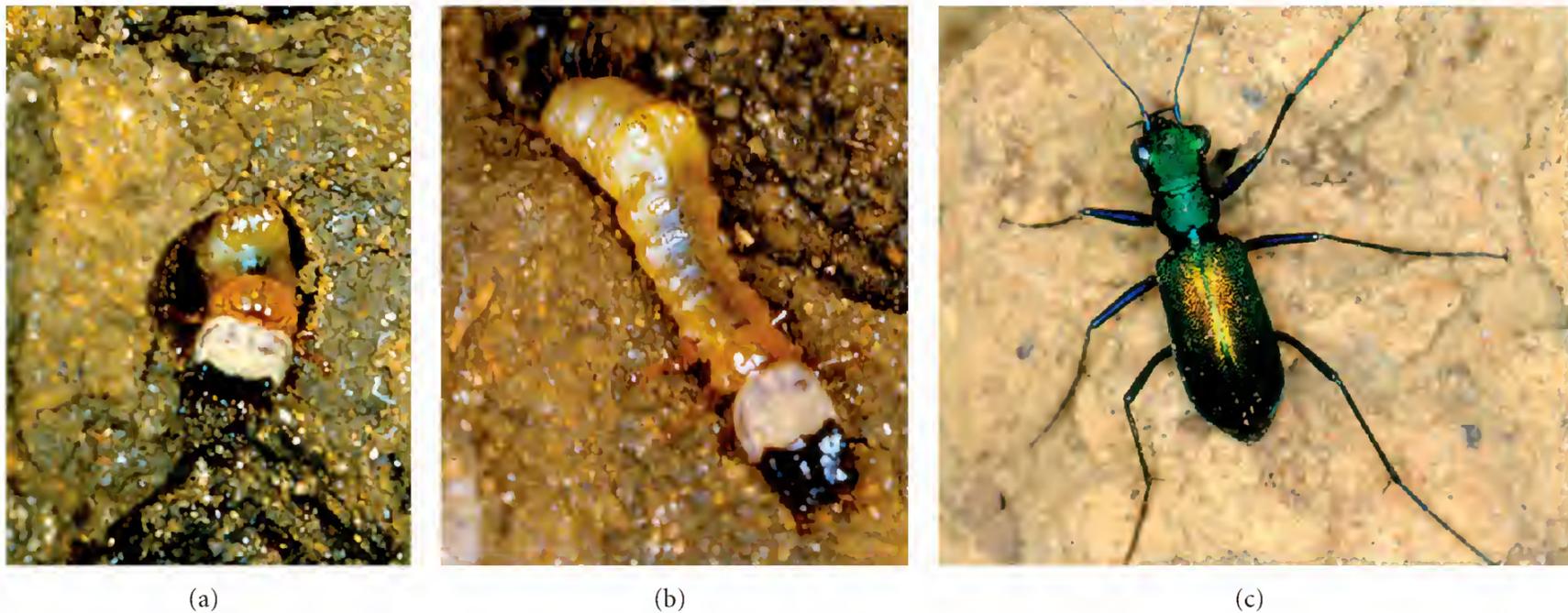


FIGURE 15: *Odontocheila auripennis* Lucas, 1857 (Carabidae, Cicindelinae) in the *Cornitermes cumulans* (Kollar in Pohl, 1832) (Termitidae, Nasutitermitinae) nest: larva, head, and prothorax in the external opening of the gallery; larva removed from the gallery (note the salient abdominal hooks); and adult in the external nest surface). Parque Nacional das Emas, Goiás, Brazil (photo: S. A. Vanin).

The preys are captured when they walk around the opening and are drawn to the interior of the gallery, where they are eaten. The larva has hooks in the dorsum of abdominal segment V that allow it to anchor firmly in the gallery wall, in the eventuality of confrontation with a larger prey that could drag it out of the gallery. It is interesting to notice that coinhabiting termite nests, where larvae of *P. termitilluminans* also occur, can be advantageous for the Cicindelinae, because both species can profit from the preys attracted by the luminescent larvae [1]. However, the adults are predators that stalk their preys in daylight. The larvae of Tenebrionidae probably dig galleries and can feed on existing organic substance in the walls of the nest itself or of any other organic substance found in its interior.

In the high termite nests of the genus *Macrotermes* (= *Bellicositermes* Emerson, 1925), called “cathedral termite nests” and abundant in African savannahs, many beetles are found mainly in those dead colonies. The African nests can exceed 3 m high by 2 to 3 m in diameter at the base [41]. The central structure of the nest (pan) is oval, about 1.3 m in diameter and 2 m high, and can contain from 30 to 50 kilos of organic substance, substratum for the culture of mushrooms. Four or five weeks after the colony’s death, while the pan still contains great amount of organic substance and mushrooms, hundreds of adults and immatures of insects, mainly dermapterans (Dermaptera), heteropterans (Hemiptera, Heteroptera), and coleopterans (especially tenebrionids) (Tenebrionidae) are pullulating there and feeding on this material [41]. A large number of other insects, belonging to diverse orders and families, exploit this trophic level and constitute excellent prey for carabids (Carabidae), the main predators found there. Girard and Lamotte [41] demonstrated that the colonization of the dead termite nests of Africans *Bellicositermes* is initially carried through by the mycetophagous and saprophagous species, followed by the predators. This fauna is frequently very diverse and special.

The community momentarily joined together is very abundant during the first weeks, and besides it is much diversified, including several tens of Coleoptera species (Carabidae, Elateridae, Staphylinidae-Pselaphinae, and others; Scarabaeidae, Scaphidiinae, Tenebrionidae), Dermaptera, Hemiptera, and also some Lepidoptera. All these species are considered rare. The dead termite nest also serves as a shelter, mainly in the dry season. The elaterid larvae of other subfamilies were found inside the termite nest prey on termites, but are not luminescent and only occur in abandoned nests.

Experimental observations on the behavior and life history of *Megaxenus* spp., (Aderidae) larvae from Australia, Papua New Guinea and the Philippines found in nests of *Microcerotermes* spp. revealed that these larvae are adapted not only to the nest environment, but also to the social system of the termites. These larvae have mastered the trophallactic code which results in their feeding by the termites [49]. Larva of Odontonychini (Elaterinae) from Zambia collected inside termite nest probably has similar behavior (Girard and Costa, in preparation).

7. Discussion and Conclusions

Kistner [50, 51] gave important information on inquilines of social insect nests. As many of these inquilines are obligatory predators, feeding on individuals of the colony, he preferred to call them symbionts. In the case of termites, these symbionts are called termitophilous. To him, termitophilia implies a compulsory or, at least, a long-term association. Larvae and adults of termitophilous species are accepted in the colonies, they are adapted “to decipher” and “to mimic” the “code” of the social insects. This code can be a volatile chemical substance, tactic exudates, or, still, stimulations. These substances or stimulations are, in general, responsible for the communication and the integration between the young and the adult termites of the colony. In some cases,

the symbiotic associations are complex enough to contain taxa of many species, which result in historical processes of coevolution, as in the case of the Coroticini symbiont of the Nasutitermitinae termites. The relations between these beetles and their hosts have not been adequately investigated yet. Some species are essentially commensals. Some can feed on regurgitated food of termites, as in the case of the physogastric staphylinids of the genus *Corotoca* Schiødte, 1853 (Coroticini). Others, still, can be predators, feeding on their hosts [6]. Kistner [52] quotes Wasmann's report of *Neoglyptus punctulatus* (Chaudoir, 1862) as one of the few known obligatory associations between termite and carabid beetles. In this species, the first instar larva is campodeiform but changes after entering the nest to a scarabaeiform physogastric larva. The adults may or may not develop physogastry by hypertrophy of the fat body.

According to Araújo [53], the term termitariophilia was coined by Berg [54] to designate no obligatory predators, associations of animals with termite nests rather than with the termites, as occurs in the cicindelid *Odontocheila auripennis*, reported above.

The species of the Coleoptera reported herein associated with the termite nests are very diverse in the number of different taxa involved. It is quite difficult to precise the character of all the interactions noticed between the inquilines and their hosts. Therefore, the classification is very complex as it involves all ecological relations between them. However, we can place them in some functional categories.

In general, all the species studied seem to use the nest as a place to rear their larvae and pupation can occur in galleries near the outer nest surfaces. The adult has its own life out of the nest where copulation occurs; the females can lay their eggs near the nest in the soil, inside the central area of the nest, or in the nest galleries. The larvae can be found inside the central part of the termite nest or in a superficial network of galleries with or without outside exits. The larvae can be physogastric or not; predators of preys ambushed or attracted from outside; feeding on diverse organic matter, mushrooms, and excrements that are found inside the nest, or the trophallaxis feeding behavior may be present.

(1) According to the dependence of the beetle species on the termite colonies.

- (1.1) Species adapted not only to the nest environment, since larvae and adults can also live inside dead logs or other similar habitats (e.g. *Veturius transversus*, *Anchastus* spp., and *Lobopoda* sp.).
- (1.2) Species that use the nest as a place for larval development. All phases of their life cycle but adulthood can occur inside the nest (e.g. *Homophileurus luederwaldti*). In some particular cases, larvae are adapted to live in galleries placed in the outer walls, from where they can attract (*P. termitilluminans*) or ambush (*O. auripennis*) preys from outside.
- (1.3) Species totally dependent on termites and the coleopterans included in the social system of the colonies as inquilines. There is production of mimic

pheromones by the coleopterans, and trophallaxis occurs between hosts and inquilines, and beetle larvae are mistaken for "termites" by the hosts (e.g. *Megaxenus* spp., and very likely *Odontonychus* spp.).

(2) According to attainment of food supply.

- (2.1) Species which feed on organic matter found inside the nest, mostly in the cabbage pan, for example, tenebrionids (*Lobopoda* sp.), elaterids (*Anchastus* spp.), passalids (*V. transversus*), and melyrids (*Lemphus* sp.). In some cases the species feed exclusively on the carton nest walls, as the Phileurini scarab *H. luederwaldti*.
- (2.2) Species that live in galleries built inside the outer walls of the termite mound and attract or ambush preys from the outside. They can occasionally prey on termites found inside the nest (e.g. *Odontocheila* sp. and *P. termitilluminans*).
- (2.3) Species which are predators of the hosts, such as elaterid larvae only found inside the nest and that prey on termites (e.g. *Dilobitarsus abbreviatus*, *Pseudotetralobus* spp., and *Tetralobus* spp.).
- (2.4) Species that exercise trophallaxis and are fed by hosts, being regarded as true termitophiles (*Megaxenus* spp.).

(3) According to the ecological and morphological adaptations of beetle larvae that live in termite nests.

- (3.1) Larvae found inside the central part of the termite nest.
 - (3.1.1) Physogastric larvae, bearing some special glands and different kinds of setae forming or not tufts. In some species inclined head, almost phragmotic. Termite predators (e.g.: *Dilobitarsus abbreviatus*, *Pseudotetralobus* spp., and *Tetralobus* spp.).
 - (3.1.2) Nonphysogastric larvae, glabrous body, with very small head, anterior legs directed forwards. Larvae fed by termite workers; trophallactic feeding behavior demonstrated in *Megaxenus* spp. and probably in *Odontonychus* sp..
 - (3.1.3) Non-physogastric larvae, with prognathous head, found mostly in the cabbage pan. Larvae eat many kinds of organic substances found in the nest interior (e.g. *Veturius transversus*, *Anchastus* spp., *Lemphus* sp., and *Lobopoda* sp.).
 - (3.1.4) Non-physogastric larvae, large head, feeding on the carton nest walls, which are rich in organic matter. The damage resulting from larvae feeding can cause the nest destruction (e.g. *Homophileurus luederwaldti*).
- (3.2) Larvae found in a superficial network of galleries leading outside.

(3.2.1) Non-physogastric larvae. Predators of preys ambushed or attracted from the outside of the termite nest (e.g. *O. auripennis* and *P. termitilluminans*). To *P. termitilluminans* larvae, the nests provide a vertical surface or “lighthouse” that extends the area reached by the light emitted, thus increasing the number of flying insects that can be attracted.

(3.3) Larvae found in galleries of the shield without openings to outside.

(3.3.1) Physogastric larvae. Probably predators of termites and other small insects (e. g. *Tetralobus arbonnieri*, *T. gigas*, and *T. shuckhardi*) with phragmotic head and bodies densely hairy.

Very little is known about the association of beetles larvae with termite nests, although the number of works published on the subject has increased in recent years. Many hanging questions are pending about the interactions that occur between the immature and adult individuals of beetles and termites in the colony. It is also important to assemble new data about behavior, chemical, and tactile stimulations responsible for the communication between termites and their guests, and possible substances produced by glands and used as simple nutrients, stimulants, or mimic attractant pheromones of the hosts. Other relevant questions are concerned with the possible functions of morphological features, seemingly adaptative, like the phragmotic head and physogastric hairy body of Tetralobini larvae that live in galleries inside the host nest.

The authors hope that the information and commentaries presented in this paper may stimulate other researchers, especially the young people, to search for such fascinating and instigating aspect of the biodiversity.

Acknowledgments

To Sergio Ide (Instituto Biológico, São Paulo) for the critical reading of the manuscript, Carlos E. Simonka and Simone P. Rosa (Museu de Zoologia, São Paulo) for help in the electronic treatment of the figures. To Luiz R. Fontes for information on termites and termite nests. Thanks are also given to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the Research Grant 302721/2007 – 0 to C. Costa.

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Research Article

Life-History Traits and Population Relative Fitness of Trichlorphon-Resistant and -Susceptible *Bactrocera dorsalis* (Diptera: Tephritidae)

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Received 21 August 2009; Revised 19 January 2010; Accepted 20 April 2010

Academic Editor: Coby Schal

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Life tables were established for trichlorphon-resistant and susceptible *Bactrocera dorsalis* strains based on the laboratory observations. Trichlorphon-resistant *B. dorsalis* strain had longer pupal and preoviposition periods, and mean generation time compared to the trichlorphon susceptible strain. Lower fecundity, emergence rate, and probability of standard fecundity P_F , and shorter female and male longevity also were apparent in the trichlorphon resistant strain. Based on the life tables, the life population trend index (I) of the resistant strain was 86.80, while that of the susceptible strain was 116.97. The net reproduction rate (R_0) and the intrinsic rate of increase (r_m) of the resistant strain were 1565.33 and 0.0164, while those of susceptible strain were 2184.00 and 0.0173, respectively. The results from this research revealed that the resistant strain was at a reproductive and developmental disadvantage relative to the susceptible strain.

1. Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is distributed throughout Southeast Asia and the Pacific [1]. Now it has also infested many areas of China and thus causes serious financial loss to orchards globally [2]. The loss cost incurred by *B. dorsalis* was estimated to be as high as 44.6~176.5 million U.S. dollars in California, and also led to 230 million U.S. dollars potential economic loss of stone fruit industry in California [3], 1.26 billion U.S. dollars in 1997 in Taiwan, and about 1.47 billion U.S. dollars in 2004 in the Fujian [4–6].

Using insecticides is the major way to control this pest, however, frequent use of common insecticides had resulted in insecticide resistance in several insecticides, including organophosphates [7]. Resistance to insecticides has become a major problem of many pest species, including the oriental fruit fly. Keiser used 73 insecticides to determine their toxicity to *B. dorsalis* in 1973 [8], and found most of these insecticides had high toxicity. Purcell tested 3 insecticides (Malathion, Benzyl Cypermethrin and Carbaryl) and found

that Malathion was the most toxic in 1994 [9]. Resistance of *B. dorsalis* populations to insecticides in the field has become more and more serious since 2003 [2]. In 2004, it was reported that fly populations in Taiwan had resistance to ten insecticides (Naled, Fenthion, Trichlorphon, Fenitrothion, Formothion, Malathion, Methomyl, Cyfluthrin, Cypermethrin, and Fenvalerate), and that resistance to Malathion increased at the fastest rate, while Naled had the lowest rate [7, 10, 11].

In South China, the oriental fruit fly is distributed widely and its population density is very high, resulting in tremendous yield loss to many fruits. Chemical control strategies still play an important role in depressing oriental fruit fly density and reducing economic loss, therefore, it is very important to monitor the resistance of *B. dorsalis* to insecticides in the field [12, 13]. The development of resistance to organophosphate-based insecticides is a current and growing problem for the management of this pest species. A wide range of studies have focused on the elucidation of the molecular basis of this resistance [14, 15], and showed that flies exhibiting high levels of organophosphate resistance

carried three specific mutations in the *ace* gene of this species. Trichlorphon has been used to control *B. dorsalis* in commercial orchards in China, and now there is evidence of high resistance to this insecticide as well [2, 16–18], however the biology and ecology of the resistant populations of *B. dorsalis* has not been well studied.

The ecology of resistant insects is essential to the selection of control methods and the understanding of resistance dynamics. This study presents population life tables and life-history traits of susceptible and resistant strains to determine whether the relative fitness of resistant strains increases or decreases [19]. In some resistant insects, because the relative fitness of resistant insects is decreased, rotation of different insecticides and temporarily discontinuing the use of certain insecticides can decrease the frequency of resistance genes, and restore their susceptibility to certain insecticides [20, 21]. However, if the relative fitness of resistant insects is not affected by the resistance, these practices can only delay resistance development, and it is difficult to restore their susceptibility to those insecticides [22].

Compared to susceptible insects, resistant insects usually have a lower fecundity [23]. To provide a scientific basis for resistance risk management and achieve successful control of resistant *B. dorsalis*, it is important to study the biological and ecological variations between the trichlorphon-resistant and -susceptible *B. dorsalis* populations. This study examined the following areas: (1) the basic susceptibility of *B. dorsalis* to trichlorphon; (2) the development of resistance to trichlorphon through exposure over several generations; (3) the biological and ecological features of trichlorphon resistant strain.

2. Materials and Methods

2.1. *B. dorsalis* Strains. The trichlorphon resistant strain of *B. dorsalis* was collected in March 2003 from damaged carambola fruits, in Yangtao Park, Guangzhou City, Guangdong Province, China. Few insecticides had been used at this location because bags are used to protect carambola fruits from the flies. We collected about 2000 aging larvae from the damaged carambola, then placed the larvae into humid sand for pupation. After pupa emergence, flies were kept in the laboratory for 33 generations before using in the study. Bioassays were conducted every 3 generations, and new 24-h LC_{50} values were obtained and guided the trichlorphon concentration which was used to treat the next 3 generations.

The susceptible strain was collected in August 2003 from the same place as the trichlorphon resistant strain, and maintained without any exposure to insecticides for 30 generations in the same laboratory as the resistant strain collected [2].

2.2. Method

2.2.1. Concentration-Response Bioassays. In order to make sure that the 24-h LC_{50} value of the trichlorphon-resistant and -susceptible strains was stable, the populations of two strains from the field were both divided into 3 groups,

and the 24-h LC_{50} value of each group was measured. Not only trichlorphon was used in the field, but also other organophosphorus pesticides which had systemic activity and the same mechanism as trichlorphon were used; so in the laboratory, we treated both larvae and adults with trichlorphon. The larvae of each generation were treated by topical application. In brief, aging larvae were treated with acetone diluted of trichlorphon whose concentration was the 24-h LC_{50} value of the last time measurement. Treated larvae were then placed in humid sand for pupation. Adult flies, 3–5 days old, were treated by vial residues of acetone diluted trichlorphon, whose concentration was the 24-h LC_{50} value of the last time measurement. Briefly, 5 mL of the acetone dilution was poured into a 250 mL triangular flask with constant shaking until the sides of the flask formed a uniform film. Excess diluent was discarded. When acetone evaporated, adults of *B. dorsalis* were then added to the flask, and supplied with a cotton ball saturated with 5% honey. The flask was sealed and placed at room temperature for $28 \pm 2^\circ\text{C}$ for 24 hours. Flies were considered dead when they could not crawl after continual prodding. The surviving adults were used for the next generation breeding [2, 24].

Two strains were both maintained at a temperature of $28 \pm 2^\circ\text{C}$ and in a photoperiod of 10:14 h [14]. The data from the regression equation, including 24-h LC_{50} values of treatments, 95% confidence intervals and correlation coefficient (r), were analyzed by probit analysis. For each of 5 concentrations (treatments), there were 5 replicates, with each replicate using 30 flies. From the number of dead flies, we calculated the mortality and corrected mortality, translated corrected mortality into mortality probit. Then according to the logarithm of the concentrations and relevant mortality probits, we made the toxicity regression equation (LC-P line). Finally, according to the regression equation, we calculated the 24-h LC_{50} value, the confidence interval, chi-square value, and correlation coefficient. If the 95% confidence intervals overlapped, we concluded that trichlorphon sensitivity was not statistically different between the strains [25]. The resistance ratios (RRs) are given as the values of the resistant LC_{50} to trichlorphon/the susceptible LC_{50} to trichlorphon in the same bioassay method [10, 11]. Microsoft Office Excel (2003) was used for data analysis, the test of significance (ANOVA: F test).

2.2.2. Duration of Life Stages. To determine the duration of the egg stage and hatching rate, we cut banana into small pieces and placed them in disposable plastic cups (150 mL), then we put the cups into the adult rearing cages ($60 \times 60 \times 60$ cm) of both strains to attract *B. dorsalis* female to lay eggs, respectively. The egg masses were removed after 2 hours, and 50 eggs from each strain were placed into a petri dish ($\varnothing 9$ cm) with filter paper containing banana juice. Hatched larvae were removed every two hours and the total number of larvae was counted.

Larval duration and pupation rate were determined from 50 susceptible and 50 resistant strain larvae that simultaneously eclosed. Banana was used to feed the larvae in a plastic box ($15 \times 15 \times 15$ cm). Dead and aging larvae were removed every 4 hours. The larval stage was from egg

hatch to aging, bouncing, larvae (larva of *B. dorsalis* have three instars, at the end of third-instar, they would bounce up 3–15 cm high and then fall to find a place to pupate). Total number of aging larvae was counted, and the duration of larvae and pupation rate were then calculated, respectively.

The duration of the pupal stage and the adult emergence rate was determined from 50 aging larvae from the susceptible and resistant strains. Bouncing larvae were placed into a box (15 × 15 × 15 cm) containing sand maintained at 70% RH. The emergence of pupae was observed every 8 hours until all pupa emerged. Newly emerged adults were removed every 8 hours. The duration of pupa was designated from the time aging, bouncing, larvae entered the sand to the time of adults emergence. The total number of adults was counted; the duration of pupa and emergence rate were then calculated.

2.2.3. Life-History Traits. Newly emerged adults (<8 h) were reared in a plastic cage (60 × 60 × 60 cm) with artificial feed and water. Before adults began to lay eggs (emerged about 10 d at 28 ± 2°C), 20 successful mating pairs were put into a new cage (60 × 60 × 60 cm) to observe egg laying every day until all female adults were dead. The total number of eggs laid by each female was recorded. Net reproduction rate (R_0 , the average fecundity of each adult by a generation), Intrinsic rate of increase (r_m , an important proliferation potential parameter of populations under certain environmental conditions, a composite indicator of the survival rate, fecundity, growth rate on the impact of population changes), and the index of population trend (I , the growth multiples of the next generation) were calculated using the following three formulas.

Formula of R_0 :

$$R_0 = \sum l_x m_x \quad (1)$$

(see [26]).

Formula of r_m :

$$\sum l_x m_x e^{-r_m x} = 1 \quad (2)$$

(see [27]), where x is age for insects; l_x is the population of x period m_x is the number of female at next generation of each female of x period.

Formula of I :

$$I = S_1 S_2 S_3 \cdots S_K F P_{\square} P_F \quad (3)$$

(see [28–30]), where $S_1 S_2 S_3 \cdots S_K$ is the survival rate at each acting stage, respectively; F is the standard egg number per female; P_F is probability of standard fecundity; P_{\square} is female rate.

2.2.4. Adults Survival Lines and Relative Fitness. We used age as an independent variable, the survival rate as the dependent variable to make a survival line, and fit a model based on the Weibull distribution. When shape parameter $c > 1$, then the age to which the vast majority of individuals in the population were able to achieve is its average life expectancy.

When shape parameter $c = 1$, then the population had the same mortality at different times. When shape parameter $c > 1$, then the population had very high mortality in the prophase, and most were dead before average life expectancy. The formula was as follows:

$$S_p(t) = e^{-(t/b)^c} \quad (4)$$

(see [31]), where S_p is survival rate of t age; b is scale parameter; c is shape parameter.

Fitness costs associated with resistance to insecticidal agents can have substantial negative effects on many life history traits and have been reported in Diptera [32, 33]. The formula of Relative fitness (R_f) was as follow: when $R_f > 1$, it suggested the fecundity of resistant strain was enhanced; when $R_f < 1$, it is suggested that the resistant strain possess a fitness defect

$$R_f = \frac{R_0 \text{ of resistant strain}}{R_0 \text{ of susceptible strain}} \quad (5)$$

(see [34, 35]).

3. Results

3.1. Establishment of Resistant Line. At the 8th generation, the resistant ratios (RRs) of the trichlorphon resistant strain exhibited up to a 41.42-fold increase (Table 1). After 14 generations of selection, the resistant ratios (RRs) rapidly went up to 84.55-fold. In order to understand the resistance stability, from the 14th generation to the 21st generation, no insecticide was used on the resistant strain, and the resistant ratios (RRs) rapidly declined to 19.49-fold; however, when trichlorphon was used again, after only 3 generations (i.e., the 24th generation in this experiment), the resistant ratios (RRs) went up to 54.60-fold. Then from 24th generation to 30th generation, the resistance of trichlorphon-resistant strain increased slowly, until the 33rd generation, the resistant ratios (RRs) reached 71.93-fold.

3.2. Growth and Development of Each Stage. Tables 2 and 3 showed that the duration of pupae, preoviposition period, longevity of female, longevity of male, fecundity per female, emergence rate, and probability of standard fecundity P_F of trichlorphon resistant strain all had a significant difference to that of the susceptible strain. Results also clearly showed that the duration of egg, duration of larvae, the pupation rate, mean generation time, hatching rate, pupation rate, and female rate P_{\square} had no significant differences between susceptible strain and trichlorphon-resistant strain.

3.3. Life-History Traits. Analyses of the population life tables, the influence of eggs, larvae, pupae, and adults of the susceptible and resistant strains, and the ratio of adult females indicated that the population tendency index of two strains had significant differences. The population tendency index of susceptible was 116.97, but that of the resistant strain was only 86.80, which suggested that the succeeding generation of the resistant strain was smaller than that of the susceptible strain, and had certain propagation disadvantages.

TABLE 1: Resistance ratios of the Trichlorphon-resistant line at selected generations.

Generation	No. of files	LC-P lines	Chi-square value (χ^2)	LC ₅₀ (mg · L ⁻¹) (95% FL)	RRs
Parental*	150	$y = 3.8072 + 5.8250x$	0.4924	1.6024 (1.3800–1.8600)	—
First	150	$y = 2.8577 + 3.7304x$	0.0883	3.7522 (2.8277–4.9788)	2.34
5th	150	$y = 2.0494 + 3.1722x$	1.8672	8.5142 (6.3843–11.3546)	5.31
8th	150	$y = -6.7862 + 6.9248x$	0.9521	50.3532 (21.9650–115.4311)	31.42
11th	150	$y = -1.0373 + 2.8953x$	0.9116	121.6741 (87.9158–168.3951)	75.93
14th	150	$y = 0.9795 + 1.8859x$	1.4533	135.4903 (85.4614–214.8062)	84.55
21th*	150	$y = 0.4228 + 3.0625x$	0.1085	31.2301 (23.2098–42.0217)	19.49
24th	150	$y = -1.7301 + 3.4657x$	4.0260	87.4861 (72.0158–106.2796)	54.60
27th	150	$y = -2.2498 + 3.5892x$	0.9287	104.6954 (89.0531–123.0853)	65.34
30th	150	$y = -4.4740 + 4.6329x$	7.0995	110.9027 (98.4476–124.9336)	69.21
33th	150	$y = -5.7631 + 5.2205x$	1.1999	115.2631 (103.6904–128.1274)	71.93

*Parental represented susceptible strain after 30 generations. the number of bioassays concentrations were 5.

Fecundity of the resistant and susceptible strains were both almost a parabolic shape; in the early oviposition period, the fecundity was small, and gradually increased, reaching a peak and then declining gradually. But there was a certain difference between the strains. The susceptible strain reached its peak oviposition time faster than that of the resistant strain. Fecundity at peak oviposition for the susceptible strain was more stable, while the resistant strain was more volatile. Based on the standard errors, the fluctuation range around the mean was larger in the resistant strain than that of susceptible strain (Figure 1).

3.4. Adults Survival Lines and Relative Fitness. The R_0 , r_m , and R_f of susceptible and resistant strains showed significant differences (Table 4), suggesting that the fecundity of the resistant strain declined significantly after the populations of *B. dorsalis* developed resistance to trichlorphon. The fitness study showed that the relative fitness of resistant strain obviously declined (only 0.7167 of the susceptible strain), which suggested that the survival and development of the resistant strain had been significantly poorer than the susceptible strain.

According to formula 2, the fitting of the adult survival curve equation of adults of susceptible and trichlorphon-resistant strains (Table 5) and survival curve (Figure 2) showed that the values of shape parameter (c) was 3.4637 and 2.6451. Both of them were greater than 1, in consistent with the type I of the basic model of survival curve, suggesting that the adults of both strains achieved their average life span and reached the inherent life to death. But the average survival rate of the susceptible strain was higher than that of resistant strain before 85 days.

4. Discussion

Insecticides can change the biology, ecology and other indicators of resistant insects. Many studies suggest that the resistant insects exhibit fitness costs or stimulate their proliferation [36, 37]. The variations of biology and ecology were closely related to different insecticides [38]. Studying the resistant population's relative fitness is the basis for

understanding and resolving the problem of resistance [39, 40]. Relative fitness refers to the relative capacity of biological survival and reproduction, compared to the susceptible strain. It is generally believed that the biological characteristics, such as declined fecundity and prolonged growth period, change relative fitness. However resistance increases the pest survival rate under selective pressure, which can often lead to a fitness decline. Resistant individuals have shown a survival competitive disadvantage in the absence of selective pressure agents [41, 42].

Our results in this study indicated that the resistant ratios (RRs) of trichlorphon-resistant strain of *B. dorsalis* quickly reached 84.55-fold after 14 generations under trichlorphon selected pressure. However, the resistance was not stable, with the resistant ratios (RRs) rapidly declining when insecticide applications ceased for 7 generations. After insecticide pressure was resumed, RRs went up rapidly only after 3 generations. A resistant strain of RRs 71.93-fold was obtained after 33 generations. These results showed that in *B. dorsalis*, the rising speed of RRs of trichlorphon-resistant strain was far smaller than that of spinosad-resistant strain; LD₅₀ of the selected line was 408 times greater compared with that of the untreated parental colony only after eight generations of selection [43]. And it was almost unanimous compared with rate of increase seen for other cases of insecticide resistance in *B. dorsalis*, including selection for resistance to six organophosphates, one carbamate, and three pyrethroids described previously in [7]. The results obtained here suggest that a rapid rise in trichlorphon-resistance may ultimately cause control failure after extended commercial use in the fields.

The life-history traits of *B. dorsalis* were significantly influenced by trichlorphon. The trichlorphon resistant strain had significant fitness costs when compared to the susceptible strain. The duration of pupa, preoviposition period, and mean generation time of resistant strain were all prolonged, while the longevity of female and longevity of male were shortened, and mean fecundity per female, the emergence rate, and probability of standard fecundity P_F were lower. The population tendency index of two strains had significant difference, the population tendency index of susceptible

TABLE 2: The development difference of each stage of two strains of *B. dorsalis*.

Insect stages	Susceptible strain ($\pm se$)	Trichlorphon-resistant strain ($\pm se$)
Duration of egg (h)	51.6220 \pm 0.3593	52.5060 \pm 0.1560
Duration of larva (h)	163.3520 \pm 0.8037	163.6500 \pm 1.2250
Duration of pupa (h)	267.7400 \pm 0.5931	272.1820 \pm 1.3398**
Preoviposition period (d)	16.0000 \pm 0.3162	20.6000 \pm 0.4000**
Longevity of female (d)	66.9600 \pm 1.3908	62.8300 \pm 1.2955**
Longevity of male (d)	72.1840 \pm 0.7263	60.8160 \pm 2.2512**
Fecundity/female	324.2460 \pm 0.6094	254.5500 \pm 0.8158**
Mean generation time (h)	481.9733 \pm 2.0076	488.1233 \pm 24636

Annotates: within the same row, * shows the significant differences between resistant strain and susceptible strain ($P < .05$) by ANOVA (F test), ** shows the significant differences between resistant strain and susceptible strain ($P < .01$) by ANOVA (F test). Tables 3–5 tables are the same.

TABLE 3: The life table of lab population of two strains of *B. dorsalis*.

stages	Effect factors	Survival rate (%)	
		Susceptible strain ($\pm se$)	Trichlorphon-resistant strain ($\pm se$)
Egg	Non-hatching	0.8360 \pm 0.0160	0.8200 \pm 0.0167
Larva	Recessive death	0.9240 \pm 0.0075	0.8960 \pm 0.0075
Pupa	Non-emergence	0.8960 \pm 0.0075	0.8520 \pm 0.0102*
	Female rate P_{\square}	0.5091 \pm 0.0169	0.5015 \pm 0.0109
Adult	Probability of standard fecundity P_F	0.1616 \pm 0.0010	0.1276 \pm 0.0011**
	Standard fecundity F	2000	2000
	Population tendency index I	116.97	86.8

Annotates: standard fecundity ($F = 2000$ eggs/per female) [13].

TABLE 4: The life-history traits and relative fitness of two strains of *B. dorsalis*.

Population Life Parameter	Susceptible strain ($\pm se$)	Trichlorphon resistant strain ($\pm se$)
R_0	2184.0000 \pm 50.3408	1565.3333 \pm 42.3365**
r_m	0.0173 \pm 0.0001	0.0164 \pm 0.0001**
R_f	1.0000	0.7167

TABLE 5: The adults survival lines of two strains of *B. dorsalis*.

Strains	Equation	Correlation coefficient (r)
Susceptible strain	$S_p(t) = e^{-(t/77.2504)^{3.4637}}$	0.9986
Trichlorphon-resistant strain	$S_p(t) = e^{-(t/68.0355)^{2.6451}}$	0.9972

was 116.97, but that of the resistant strain was only 86.80 (Table 2). The R_0 , r_m of resistant strains was smaller than that of susceptible strain, and R_f was only 0.7167 of the susceptible strain (Table 4). The average fecundity per female curves of two strains were both more or less a parabolic shape, but the susceptible strain reached its oviposition peak time in a shorter space of time than that of the resistant strain; the fecundity of oviposition peak time was more stable than that of resistant strains which was more volatile (Figure 1). Survival curves showed that the two strains were consistent with the I-type of the basic model of survival

curve, but the susceptible strain reached its oviposition peak time in a shorter space of time than that of the resistant strain; the fecundity of the oviposition peak time was more stable; than that of resistant strains which was more volatile (Figure 2). The values of shape parameter (c) was 3.4637 and 2.6451 (Table 5); they suggested that the adults of both strains achieved their average life span, and reached the inherent live to dead.

All the results could afford the important basics of resistance control of the resistant strain of *B. dorsalis*. In conclusion, the results obtained here show that in *B. dorsalis*, consistent with what has been seen in several species (*Culex pipiens*, *Helicoverpa armigera*, *Drosophila melanogaster*, *Cydia pomonella*) of insects in different orders, development of high levels of resistance to organophosphates will occur within a relatively short time after selection is applied, and had relative fitness costs [44–49]. The resistance gene had an adverse impact on the population reproduction and development. In general, arthropod resistance to insecticides often accompanies a fitness cost, which is the theoretical basis for using insecticide rotation, to govern pest resistance [50, 51]. For maintaining the effectiveness of this insecticide, any proposed management program must take into consideration the potential for resistance development seen here in the oriental fruit fly. If the major influencing factor of trichlorphon resistant strain was trichlorphon, when the resistance was found, it was proposed that trichlorphon and other organophosphate applications cease to allow the frequency of resistance genes to decrease. Because of the resistant strain's fecundity disadvantages and fitness costs,

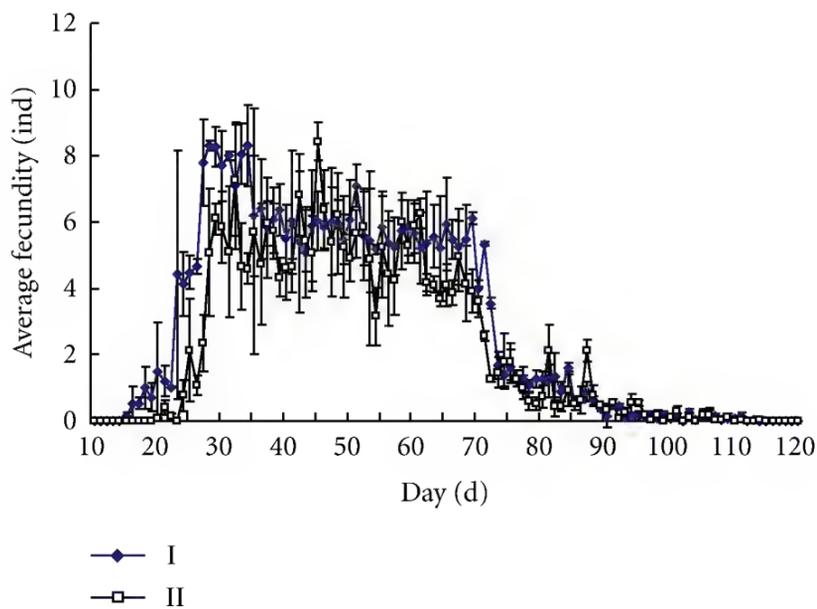


FIGURE 1: Oviposition lines of per female for two strains of *B. dorsalis*. I: Susceptible strain, II: Trichlorophon selected strain.

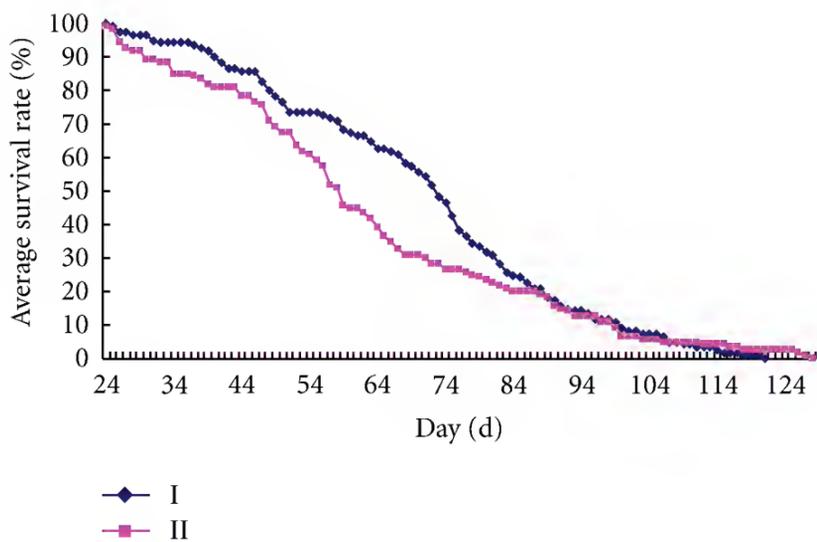


FIGURE 2: Survival curves of adults of two strains of *B. dorsalis*.

the population was relatively feeble, and without the presence of insecticides, the resistant population might slowly decline.

At the same time, in the nonresistant areas, rotation of trichlorophon and other different mechanism insecticides could thwart trichlorophon resistance development, increase the control effect, reduce environment pollution, and protect the environment, except for pyrethroids. The cross-resistance bioassays revealed, when the resistant ratios of trichlorophon resistant strain reached 69.21-fold, that some resistance to pyrethroids existed in trichlorophon resistant *B. dorsalis* strain. Pyrethroids showed about 30-fold cross-resistance to trichlorophon. So it is likely that oriental fruit flies already exhibiting higher resistance to trichlorophon also will develop middle-level resistance to pyrethroids. But abamectin showed low cross-resistance to trichlorophon, and we may use abamectin to rotate to trichlorophon (we will separately report this section study). Also we can use spinosad replace trichlorophon in the field where the population of *B. dorsalis* already had trichlorophon resistance, because according to the report by Hsu et al. [14], the results showed the spinosad did not exhibit cross-resistance to ten insecticides, including six organophosphates (naled, trichlorophon, fenitrothion, fenthion, formothion, and malathion), one

carbamate (methomyl), and three pyrethroids (cyfuthrin, cypermethrin, and fenvalerate) [43].

In this study, the biological and ecological indicators of two *B. dorsalis* strains were studied under laboratory conditions, although the dilution effect of gene flow to antagonistic alleles was avoided, the interference of other field factors outside was ignored, such as environment and natural enemies. So to be more accurate in our understanding of the dynamic resistance, and afford the most powerful evidence of resistance control, the biological and ecological indicators of trichlorophon resistant strain in the fields should be further studied.

Acknowledgments

The authors wish to acknowledge the significant improvements to this paper suggested by the editor and thank Dr. Julia W. Pridgeon (Agricultural Research Service, United States Department of Agriculture), Dr. Philip Leftwich; Dr. Gong HF (Oxitec Oxford Insect Technologies, Oxitec limited, England), and Professor Zeng FR (Plant Protection Institute of CAAS) for the correction of this paper. This paper was supported by the National Science and Technology Planning Project (no. 2006BAD08A14) and Scientific Research Project of Guangdong Province (no. 2004A20401002).

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Research Article

Additions to the Known Distribution of *Epipompilus aztecus* (Cresson, 1869) and *E. excelsus* (Bradley, 1944) (Hymenoptera: Pompilidae)

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Received 7 February 2010; Revised 28 May 2010; Accepted 15 June 2010

Academic Editor: David Roubik

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Knowledge about the species distribution of *Epipompilus* Kohl, 1884, is largely based on the records from the species description. Recent efforts in South American biodiversity studies indicate that knowledge about the distribution of *Epipompilus* species in the region is in an early stage. Two new records of *E. aztecus* were obtained for the semideciduous Atlantic Forest, in central Brazil, and one record for the Amazonian Forest in northern Brazil, indicating that its distribution extends between Central and South America. The new records of *E. excelsus* were obtained mainly from the Atlantic Forest highlands, indicating that this species is commonly found in the southeastern South American Central Plateau and restricted to forest ecosystem of this region.

1. Introduction

Epipompilus Kohl (1884) is a genus that occurs in the Americas and Australia [1]. Sixteen species are known in the Americas, one for the Nearctic Region and fifteen for the Neotropical Region, and 36 species in the Australian region. The actual knowledge about the distribution of the species indicates that *Epipompilus* arose possibly in the Paleocene, between 53 and 65 million years ago, after separation of Africa and South America + Australia + Antarctica. Knowledge about the biology of *Epipompilus* is based on a single species from Australia [2], which acts like a parasitoid koinobiont. This genus was studied mainly by Evans [3–8], who described most of the species. Its position is somewhat controversial because the species in this genus present several characteristics shared with other species classified in other subfamilies [9]. Recent phylogenetic analysis with morphological data indicates that *Epipompilus* must be classified in the Ctenocerinae [10] even though a more detailed study is necessary to corroborate this

hypothesis. Several species of the genus are described based only on one sex and known from restricted distributions. Although some species like *E. aztecus* and *E. excelsus* are morphologically distinct relative to other species of the genus [5], their distribution is not well known. The distribution of *E. aztecus* is based on efforts to document the biodiversity in Central America while the distribution of *E. excelsus* consists in the records of the specimens studied by Bradley [11] and Evans [6] from the 1940s to the 1970s, respectively. Evans [3] detailed morphological variations observed along the ranges of the two species. This paper presents new records of *E. aztecus* and *E. excelsus* from South America, discussing the observed morphological variations.

2. Material and Methods

The new records were obtained by three different inventory projects: “Dinâmica biológica e a conservação da Mata Atlântica do médio Rio Doce”, supported by the Conselho

Nacional de Desenvolvimento Científico e Tecnológico—PELD/MCT—CNPq (process: 520031/98-9); “Richness and diversity of Hymenoptera and Isoptera along a latitudinal gradient in the Mata Atlântica—the eastern Brazilian rain forest”, supported by Fundação de Amparo à Pesquisa do Estado de São Paulo—Fapesp (process: 98/05083-0); “Fauna and flora from forest fragments in the northwest region of São Paulo State: the basis of biodiversity conservation studies”, also supported by Fapesp (process: 04/04820-3). The specimens collected by the projects are deposited respectively in the scientific collections of the Universidade Federal de Minas Gerais (UFMG), of the Museu de Zoologia da Universidade de São Paulo (MZUSP), and of the Department of Zoology and Botany of the Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista (IBILCE). Some new records of *E. excelsus* were obtained from the American Museum of Natural History’s Hymenoptera (AMNH) collection and one specimen of *E. aztecus* was found in the Museu Paraense Emílio Goeldi (MPEG). To confirm the identification of *E. aztecus*, the South American specimens were compared to an identified specimen from Costa Rica, determined and made available by Dr. James Pitts. The *E. excelsus* specimens were compared to specimens identified by Dr. Marius Wasbauer and deposited in the AMNH.

The occurrences of the specimens were obtained from the literature (old records) and in the specimens’ labels (new records). Such data were used to obtain the geographical coordinates with the Google Web and Google Earth to construct distribution maps with the software PanMap [12]. To confirm the identification of both species, the specimens were compared with identified specimens deposited in the American Museum Natural History and in the Department of Biology’s Insect Collection. In this study, only females were considered because the taxonomy of males is based on detailed microscopic examination.

3. Results and Discussion

The new records are listed below.

Epipompilus aztecus:

- 1♀ São João de Pirabas, Boa Esperança, Pará, Brazil—0° 46′08″S 47°10′26″W—18–24.x.1990—Malaise trap (deposited in the MPEG collection),
- 1♀ Farm Fisher, in Onda Verde, São Paulo State, Brazil—20°32′54″S 49°14′34″W—29.x-04.xi.2009—Möericke trap (deposited in the IBILCE collection),
- 1♀ Matão, São Paulo State, Brazil—21°37′14″S 48°32′14″W—18.ix.2007—Malaise trap (deposited in the IBILCE collection).

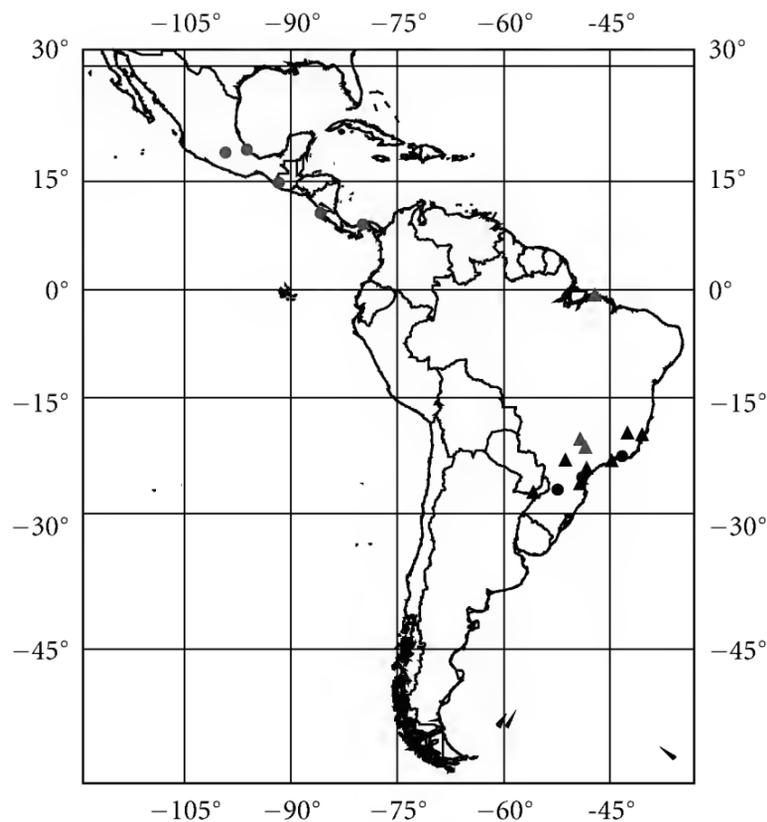
Epipompilus excelsus:

- 7♀ Rio Doce State Park, in Mariléria, Minas Gerais State, Brazil—19°42′35″S 42°36′00″W—720 meters above the sea level (a.s.l.)—02–09.xii.2003—Malaise trap—R. Parentoni & eq. col (deposited in the UFMG collection),

- 1♀ Estação Biológica Santa Lúcia, Santa Teresa, Espírito Santo State, Brazil—19°58′18.5″S 40°18′26.5″W—09–12.iv.2001–750 meters a.s.l.—Malaise and Möericke traps (yellow pans) (deposited in the MZUSP collection),
- 1♀ Rolandia, Paraná State, Brazil—23°19′25″S 51°21′15″W—vii.1948 (deposited in the AMNH collection),
- 3♀ Serra do Mar State Park, Ubatuba, São Paulo State, Brazil—1 specimen in 23°21′43.6″S 44°49′22″W around 50 meters a.s.l.—24–27.i.2002—Malaise trap; 2 specimens in 23°17′S 44°47′W—900 and 1001 meters a.s.l.—Möericke traps (1 specimen sampled by blue pan and 1 by yellow pan) (deposited in the MZUSP collection),
- 2♀ Ribeirão Grande, São Paulo State, Brazil—24°18′16″S 48°21′53″W—around 750 meters a.s.l.—11–14.xii.2000—Möericke traps (yellow pan) (deposited in the MZUSP collection),
- 1♀ Centro de Estudos e Pesquisas Ambientais Rugendas, São Bento do Sul, Santa Catarina State, Brazil—26°19′25.6″S 49°18′26.5″W—16–19.x.2001—Malaise trap (deposited in the MZUSP collection),
- 1♀ Encarnacion Peña, Paraguay—27°21′35″S 55°51′45″W—xii.1971 (deposited in the AMNH collection).

Comparing the specimens of *E. excelsus* showed the same morphological variation observed by Evans [6]. However, the *E. aztecus* specimens from Central and South America show some morphological differences. The South American specimens have darker clypeus than Central American specimens and fore legs fuscous with rufous maculations on the coxae and femur. The South American specimens’ mesosoma is rufoferruginous like in the specimen from Costa Rica, but slightly darker. Evans [6] describes that specimens from Barro Colorado, Panamá, are somewhat darkly colored. Other variation observed is in the propodeal rim; Central American species show whitish propodeal rim while the South American specimens’ propodeal rim is rufous like the rest of propodeum. Moreover, the *E. aztecus* from São João de Pirabas, Pará, shows long whitish streak on the eye margins, clypeus margin, and interantennal tubercle. On the other hand, the specimen from Matão does not show such whitish maculations on the head.

Figure 1 shows the range of the two species. In spite of the discontinuity between the occurrence records of *E. aztecus* for Central and South Americas, our records suggest that *E. aztecus* presents a wide distribution. Considering that some *E. aztecus* were sampled up to 1,000 meters a.s.l. and the geological structure of Northern Andes that shows similar elevations to *E. aztecus*’ areas of occurrence [13], the distribution may be continuous. Since the 1970s no researcher has studied the genus, and the discontinuity observed for *E. aztecus* distribution is possibly determined by the lack of studies in forest ecosystems along the range. *E. nigribasis* (Banks, 1925) shows a rather similar distribution,



Scale: 1:58224157 at latitude 0°

FIGURE 1: Occurrence records of *Epipompilus aztecus* (Cresson, 1869) (gray marks) and *Epipompilus excelsus* (Bradley, 1944) (black marks). The circles represent the old records of the species and the triangle represents the new records.

being recorded in Panama, Colombia, and southeastern Brazil [6]. A large sample effort is necessary, mainly for the Cerradão (forest type of Brazilian Savanna) and Amazonian Forests, to recognize the real distribution of these species.

Our data indicate that *E. excelsus* is restricted to the Atlantic Forest, occupying the biogeographical Atlantic and Paranaense Provinces [14], which consists respectively of the Atlantic Rain Forest at the Brazilian coast and the Atlantic Semideciduous Forest at the southeastern inside of Brazil, reaching eastern Paraguay [15, 16]. In the Atlantic Rain Forest, *E. excelsus* was recorded more commonly in the highlands, suggesting that its occurrence is linked to South American Central Plateau's forest ecosystems.

Acknowledgments

The authors are grateful to anonymous reviewers for suggestions and to Dr. John Wenzel for edition of the paper. They would like to thank Dr. Carlos Roberto F. Brandão, Dr. James Carpenter, Dr. James Pitts, and Dr. Rogério Parentoni for making the specimens and their occurrence data available and to workmate Otávio Laraia Capusso for helping them to collect the specimen from Onda Verde. Financial support was granted by Fapesp (04/04820-3; 07/08633-1).

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Research Article

Microstructural Characters of Lyctinae and Dinoderinae (Coleoptera: Bostrichidae)

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Received 21 March 2010; Revised 31 May 2010; Accepted 7 June 2010

Academic Editor: Arthur G. Appel

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Thirty-three species belonging to the bostrichid subfamilies Lyctinae and Dinoderinae were examined by low vacuum ESEM without sputtering. Eight types of microsculpture, five types of hair insertion, and 14 types of hair were found on the elytral disc and declivity. The different types of microstructure are described and illustrated with ESEM photos of each type. Surface microstructures provide additional taxonomically useful characters which can help to distinguish easily confused species. The study also showed that specimens can be examined under ESEM without any damage.

1. Introduction

The quality and number of characters is often a limiting factor in phylogenetic investigations. Thus, searching for new sets of characters is an important task in taxonomy. It is influenced both by detailed knowledge of the animals under investigation and by new techniques. In this study, the Environmental Scanning Electronic Microscope (ESEM) has been used to investigate microstructural characters of the cuticular surface of bostrichid beetles. Characters, such as hair, punctures, and tubercles, which are of significant value both to practical taxonomic work, and to further phylogenetic investigations of the Bostrichidae are illustrated.

The family Bostrichidae has a world-wide distribution but is mainly found in tropical and arid areas. There are more than 550 known species which vary from small to very large in size. The classification used in this paper is that of the recent catalogue of the family [1]. Bostrichidae mainly feed on and breed in bamboo, timber, rattan, stored grain, and products made from bamboo and timber. Their recorded plant hosts extend to at least 30 families [2], and probably almost any family with woody species can be attacked. In this paper, species in two of the subfamilies of Bostrichidae: Lyctinae and Dinoderinae are considered. A few species of lyctines have become important pests of timber, wooden objects and ancient structures [3–5]. Some

species of Dinoderinae are important pests of bamboo and stored grain [3, 4, 6].

On average, Lyctinae have the smallest body size of all bostrichids. Because of their small size, lyctines, as well as the taxonomically difficult genus *Dinoderus* (Dinoderinae) [7] are quite difficult subjects for classical investigation by light microscopy and deserve detailed morphological investigation with ESEM. Other genera of Dinoderinae are not as difficult to identify as *Dinoderus*, but to provide a more comprehensive concept of the microstructural characters of the subfamily Dinoderinae, some dinoderines in other genera have been examined.

A report on the use of microstructures to separate four bostrichid species in the genera *Minthea* and *Dinoderus* was presented by Liu et al. [8]. The present paper expands that study to include a greater range of genera and species.

2. Materials and Methods

In order to avoid any damage to the specimens, an FEI (model: Inspect-S) Environmental Scanning Electron Microscope (ESEM) was used to examine the elytral cuticle of specimens. Specimens were not removed from their mounts or pins, nor was the surface of the specimens coated with gold or other metals (i.e., the specimens were investigated

unspattered). The settings of the ESEM were beam spot from 2.5 nm to 4.0 nm, acceleration voltage between 15 kV and 30 kV, and low vacuum between 0.6 mbar and 0.8 mbar. Particular attention was paid to the middle part of the elytral disc.

Thirty-three species were examined as listed below. Specimens were loaned from the Natural History Museum, London or form part of the private collection of the author.

Lyctinae.

Lyctini

- Lyctus africanus* Lesne 1907
- Lyctus brunneus* (Stephens 1830)
- Lyctus carbonarius* Walth 1832
- Lyctus cinereus* Blanchard 1851
- Lyctus hipposideros* Lesne 1908
- Lyctus linearis* (Goeze 1777)
- Lyctus tomentosus* Reitter 1879
- Minthea bivestita* Lesne 1937
- Minthea obsita* (Wollaston 1867)
- Minthea reticulata* Lesne 1931
- Minthea rugicollis* (Walker 1858)
- Minthea squamigera* Pascoe 1866

Trogoxylini

- Cephalotoma perdepressa* Lesne 1937
- Trogoxylon aequale* (Wollaston 1867)
- Trogoxylon angulicollis* Santoro 1960
- Trogoxylon impressum* (Comolli 1837)
- Trogoxylon parallelipipedum* (Melsheimer 1846)
- Trogoxylon praeustum* (Erichson 1847)
- Trogoxylon punctipenne* (Fauvel 1904)

Dinoderinae.

- Dinoderus bifoveolatus* (Wollaston 1858)
- Dinoderus brevis* Horn 1878
- Dinoderus distinctus* Lesne 1897
- Dinoderus minutus* (Fabricius 1775)
- Dinoderus ocellaris* Stephens 1830
- Dinoderus porcellus* Lesne 1923
- Dinoderus punctatissimus* Lesne 1897
- Prostephanus punctatus* (Say 1826)
- Prostephanus sulcicollis* (Fairmaire & Germain 1861)
- Prostephanus truncatus* (Horn 1878)
- Rhyzopertha dominica* (Fabricius 1792)
- Stephanopachys quadricollis* (Fairmaire in Marseul 1878)
- Stephanopachys rugosus* (Olivier 1795)
- Stephanopachys substriatus* (Paykull 1800)

3. Results

The lyctines do not have a distinct elytral declivity, but dinoderines do. Usually the patterns of punctures, tubercles and hairs on the elytral disc are different from those on the declivity, so the morphological patterns of microstructures on the elytral disc and on the declivity will be discussed separately for the Dinoderinae.

There are two basically different types of punctures in Lyctinae, hair-bearing and nonhair-bearing punctures. Hair-bearing punctures always correspond in diameter to the root of the hair. Nonhair bearing punctures vary in size, depth and shape of the rim. Hence only nonhair bearing punctures of Lyctinae are discussed here.

Eight types of microsculpture, five types of hair insertion and 14 types of hairs were found on the elytral disc and declivity of the 33 species investigated. Short descriptions of the different types of microstructures observed are listed below. Each description corresponds to an ESEM photo (Figures 1, 2 and 3). In the captions to the figures, structures photographed on the elytral declivity are noted; all other photos are from the elytral disc.

Types of microsculpture:

- Type a. Small subcircular puncture, moderately deep (Figure 1(a)).
- Type b. Circular shallow puncture (Figure 1(b)).
- Type c. Small elliptical puncture, moderately deep (Figure 1(c)).
- Type d. Circular puncture with one or two deep pits inside (Figure 1(d)).
- Type e. Shallow subcircular puncture with corrugated rim (Figure 1(e)).
- Type f. Shallow subcircular puncture with corrugated rim and convex bottom (Figure 1(f)).
- Type g. Subcircular puncture with irregular rim, moderately deep (Figure 1(g)).
- Type h. Circular tubercle (Figure 1(h)).

Types of hair insertions:

- Type a. Hair inserted away from microsculpture (Figures 1(a)–1(d)).
- Type b. Hair inserted next to a puncture (Figure 2(a)).
- Type c. Hair inserted inside puncture and close to the rim (Figure 2(b)).
- Type d. Hair inserted on top of a tubercle (Figure 2(c)).
- Type e. Hair inserted on the side of a tubercle (Figure 2(d)).

Types of hairs:

- Type a. Long thin smooth hair (Figure 3(a)).
- Type b. Long smooth hair gradually tapering towards both base and apex (Figure 3(b)).

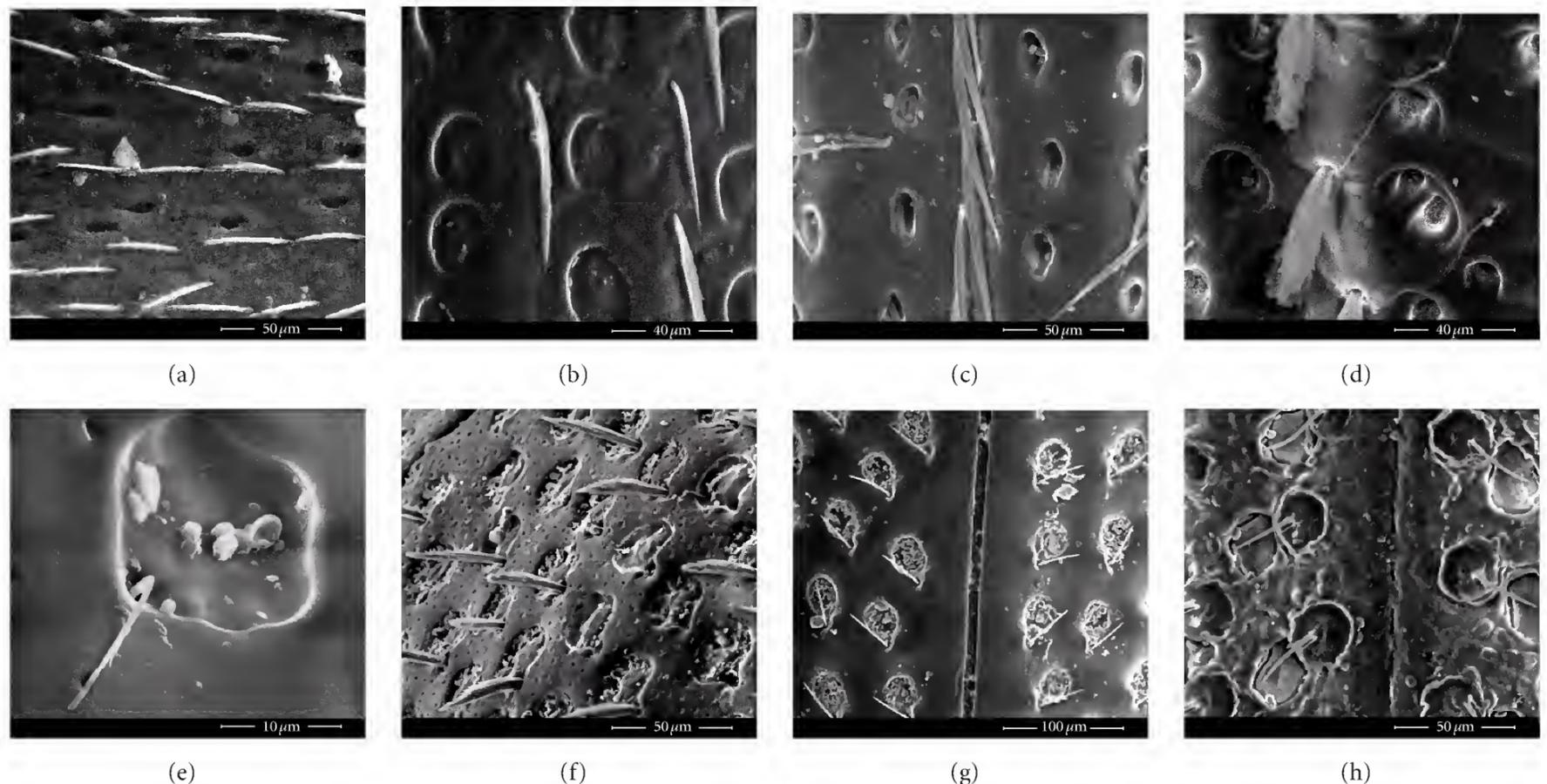


FIGURE 1: Microsculptures. (a) *Trogoxylon impressum* (Comolli 1837), (b) *Minthea bivestita* Lesne 1937, (c) *Lyctus carbonarius* Walzl 1832, (d) *Minthea squamigera* Pascoe 1866, (e) *Dinoderus minutus* (Fabricius, 1775), (f) declivity of *D. minutus*, (g) *Stephanopachys substriatus* (Paykull 1800), (h) *Stephanopachys rugosus* (Olivier 1795).

Type c. Long thin hair gradually tapering towards both base and apex, with one serrulate ridge (Figure 3(c)).

Type d. Moderately long and flat hair strongly tapering towards apex (Figure 3(d), arrows).

Type e. Short and flat hair strongly tapering towards apex (Figure 3(e), arrows).

Type f. Long thin hair feathered on the apical half of one side (Figure 3(f)).

Type g. Stout hair with serrulate ridge on one side (Figure 3(g)).

Type h. Long and thick hair with serrulate sides (Figure 3(h)).

Type i. Long thick hair gradually tapering towards both base and apex, with 4 serrulate ridges (Figure 3(i)).

Type j. Long thick hair with forklike end (Figure 3(j), top)

Type k. Simple short hair (Figure 3(j), arrow).

Type l. Stout hair with serrulate sides (Figure 3(k)).

Type m. Setae with an open brush-like end and 4–6 serrulate ribs (Figure 3(l)).

Type n. Lamelliform hair (Figure 3(l), arrow).

The distributions of the different types of microsculptures and hairs on the elytral disc and declivity vary greatly in the different species of lyctines and dinoderines. To give an overview, the distributions of the different types are presented in Tables 1 and 2.

In the subfamily Lyctinae, there are two types of punctures. The large shallow or deep one has no hair inside (microsculpture types a–d), the small one is hair-bearing.

The punctures which are nonhair-bearing are bigger, and in a more linear arrangement in the tribe Lyctini (*Lyctus* and *Minthea* in this study, Figures 1(b)–1(d)) than in the tribe Trogoxylini (*Trogoxylon* in this study, Figure 1(a)). *Cephalotoma* is the only lyctine genus studied which has no nonhair bearing punctures.

Within Lyctinae, the hairs are usually thicker and more densely serrulate in the tribe Lyctini (Figures 3(a), 3(b), 3(i), and 3(l)) than in the tribe Trogoxylini (Figures 3(c)–3(e)). In this subfamily, *Minthea* (Figure 3(l)) and *Cephalotoma* (Figures 3(d) and 3(e)) clearly have two types of hairs whereas both *Lyctus* and *Trogoxylon* have only one type (Figures 3(a)–3(c), and 3(i)).

Minthea usually has two types of interstitial hairs on the elytra and a stria separates the two rows of different types of hairs (Figures 3(d) and 3(l)). The large erect hairs (type m) are usually far more obvious than the fine and soft hairs on alternate interstriae (types k and n). Only in *M. bivestita*, the second type of hair is as large as the major one. In this species the hairs are thicker and have 4 serrulate longitudinal ridges (type i).

The morphology of the hairs on *Cephalotoma* is very different from other species. There are two types of hairs on *Cephalotoma*, one is short, the other one is long, and both are strongly flattened upward from the insertion of the hair (Figures 3(d) and 3(e)). Hence, the morphology of the hairs clearly distinguishes *Cephalotoma* from other lyctines and dinoderines. Figures 3(d) and 3(e) show both lateral and dorsal views of these special flat hairs.

In *Dinoderus*, usually the hair is inserted inside the puncture and close to the rim, or on a prominence on the rim

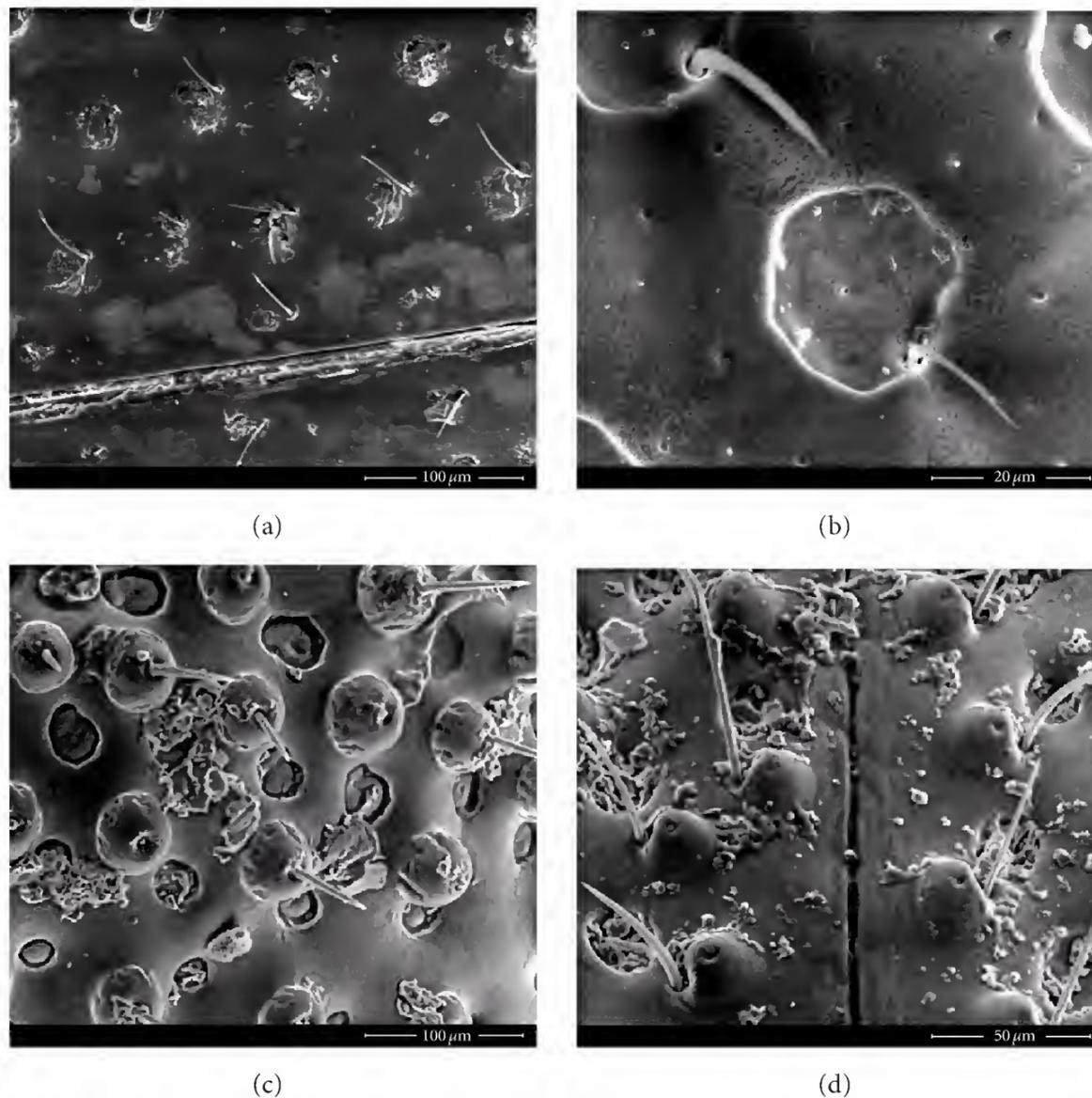


FIGURE 2: Insertions of hair. (a) *Rhyzopertha dominica* (Fabricius, 1792), (b) *D. minutus*, (c) declivity of *Stephanopachys rugosus*, (d) declivity of *Stephanopachys substriatus*.

of the puncture (hair insertion type c). *Prostephanus* has long simple hairs (hair type a) inserted on the rim of subcircular punctures (hair insertion type c). However, *Stephanopachys* has a tubercle next to each puncture (microsculpture type g and h) and a simple hair on the top or side of the tubercle (hair type a and k, and insertion of hair type d and e). This suggests that the tubercle may have evolved from the prominence of the rim of the puncture.

With regard to the way in which the hairs are inserted, type a (away from the microsculpture) is found throughout the lyctines except for *Cephalotoma*. In *C. perdepressa*, hair insertion type c (inside puncture and close to the rim) is found and this is also present in *Prostephanus* and *Dinoderus*. In *Rhyzopertha dominica*, the insertion of the hairs is next to a puncture (type b). It is on the side of a tubercle (type e) in *Stephanopachys quadricollis* and on the top of a tubercle (type d) in *S. rugosus*. *S. substriatus* is the only species with a different insertion of its hair on elytral disc and declivity, next to a puncture (type b) on disc and on the side of a tubercle (type e) on declivity.

4. Discussion

Lesne [9, 10] and Gerberg [11] both examined the elytral sculpture of bostrichids, and used it to provide

diagnostic characters for species. However, they did not examine the sculpture by ESEM, so they only found the larger microsculptural characters. Santoro [12] examined 31 species of Lyctinae under the light microscope by making the elytra transparent. He stated that the characters of punctures and hairs could be used to determine most of them at species level. However, his method requires the removal of the elytra and their treatment with potassium hydroxide before mounting, and is not suitable for rare or type specimens.

ESEM facilitates the examination of the microstructures on specimens. Not only the microsculpture, but also the shape of hairs, and the details of their insertion can be observed well under ESEM without any damage to the specimens. It is evident that microstructural characters can help to distinguish easily confused species and should be considered in future investigations.

4.1. Microsculptures on the Elytra of Lyctinae. In the subfamily Lyctinae, the nonhair bearing punctures are usually large and shallow apart from *Minthea squamigera*, which is the only species with very deep pits in the punctures (type d). Santoro [12] found there are two types of punctures on the elytra of *M. squamigera*, “single” and “double” in which two punctures seem to have fused. The ESEM picture of *M. squamigera* shows the two types of punctures (Figure 1(d)).

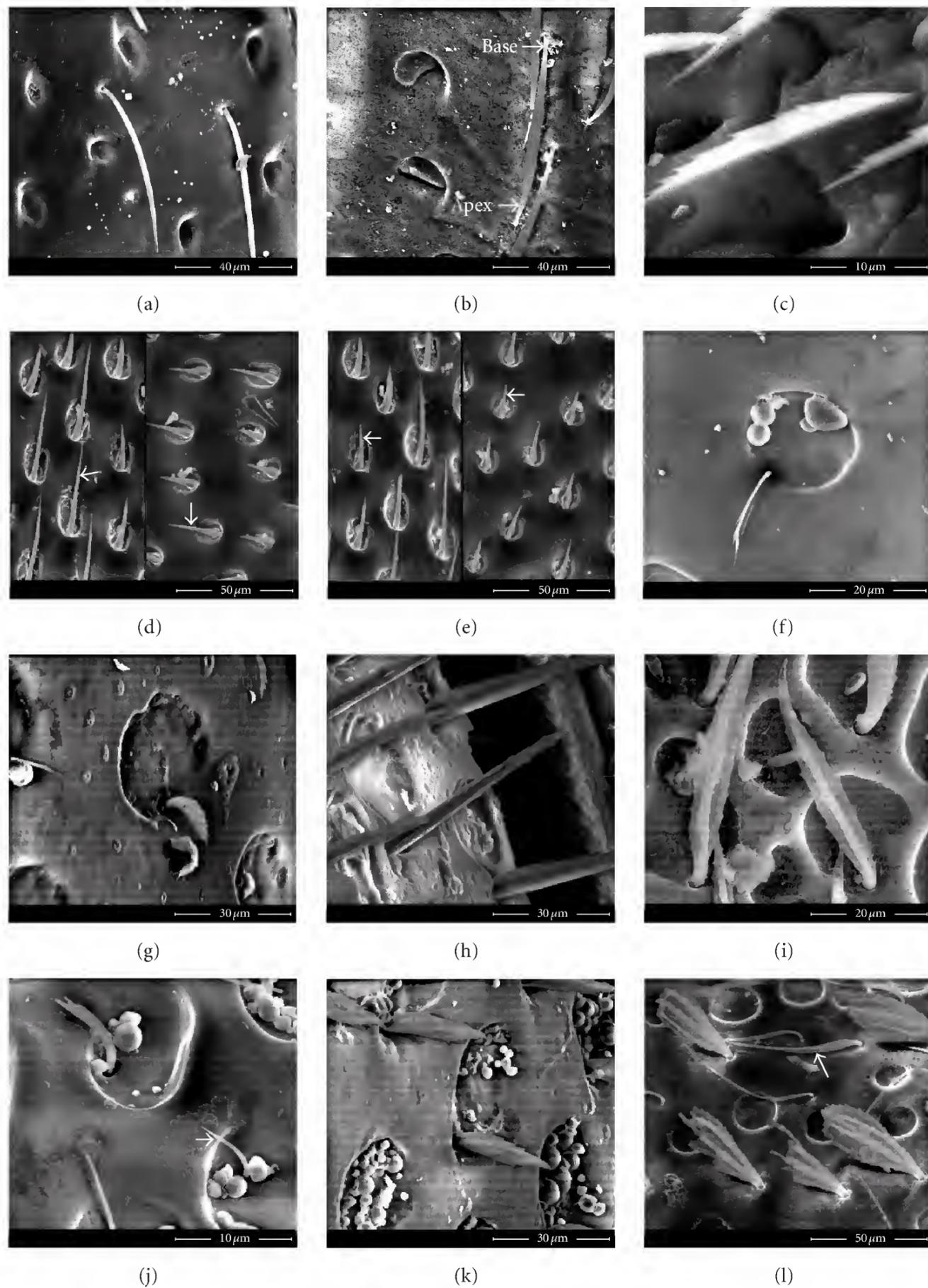


FIGURE 3: Hairs. (a) *Lyctus africanus* Lesne 1907, (b) *Lyctus cinereus* Blanchard 1851, (c) *Trogoxylon impressum*, (d) and (e) *Cephalotoma perdepressa* Lesne 1937 (left-lateral view, right-dorsal view), (f) *Dinoderus ocellaris* Stephens 1830, (g) *Dinoderus punctatissimus* Lesne 1897, (h) declivity of *Dinoderus distinctus* Lesne 1897, (i) *Lyctus tomentosus* Reitter 1879, (j) *Dinoderus bifoveolatus* (Wollaston 1858), (k) declivity of *D. bifoveolatus*, (l) *Minthea reticulata* Lesne 1931.

The double punctures occur only in *M. squamigera*, and not in other species of *Minthea* (cf. also [12]). To sum up, *M. squamigera* has three types of punctures: hair-bearing small punctures, nonhair bearing single deep punctures and nonhair bearing double deep punctures.

The shallow punctures shown on the ESEM pictures usually correspond to those shown with a blurred rim in

Santoro's [12] pictures. *M. squamigera* has the deepest punctures (type d) in Lyctinae according to this study, and shows the most distinct rim in Santoro's photographs. Santoro [12] suspected the structure under the punctures to be different in the shallow and deep ones. It would be interesting to study this further using a Scanning Transmission Electron Microscope (STEM).

TABLE 1: Distribution of the different types of microsculpture (*: appear on elytra, D: appear on decilivity).

Taxa	a.	b.	c.	d.	e.	f.	g.	h.
<i>L. africanus</i>			*					
<i>L. brunneus</i>			*					
<i>L. carbonarius</i>			*					
<i>L. cinereus</i>			*					
<i>L. hipposideros</i>		*						
<i>L. linearis</i>		*						
<i>L. tomentosus</i>		*						
<i>M. bivestita</i>		*						
<i>M. obstita</i>		*						
<i>M. reticulata</i>		*						
<i>M. rugicollis</i>		*						
<i>M. squamigera</i>				*				
<i>T. aequale</i>	*							
<i>T. angulicollis</i>	*							
<i>T. impressum</i>	*							
<i>T. parallelopipedum</i>	*							
<i>T. praeustum</i>	*							
<i>T. punctipenne</i>	*							
<i>C. perdepressa</i>			*					
<i>R. dominica</i>					*, D			
<i>S. quadricollis</i>							*, D	*, D
<i>S. rugosus</i>							*, D	*, D
<i>S. substriatus</i>							*, D	D
<i>P. punctatus</i>			D		*			
<i>P. sulcicollis</i>			D		*			
<i>P. truncatus</i>			D		*			
<i>D. bifoveolatus</i>					*, D			
<i>D. brevis</i>					*	D		
<i>D. distinctus</i>					*, D			
<i>D. minutus</i>					*	D		
<i>D. ocellaris</i>					*, D			
<i>D. porcellus</i>					*, D			
<i>D. punctatissimus</i>					*, D			

The complete scientific name of each species is given in the list of material.

4.2. *Hairs on the Elytra of Lyctinae.* In Lyctinae, usually only one type of hair is found on the elytral disc, but *Minthea* and *Cephalotoma* both have two types of hairs on the elytral disc. The small, slender hairs in particular have been overlooked by most previous authors. Gerberg [11] mentioned the rows of the small hair only when he described *Minthea obsita* and *M. rugicollis*, but not when describing *M. reticulata* and *M. squamigera*. So his description of the rows of hair—each row of erect hair separated by two rows of large, circular, and shallow punctures—was somewhat misleading.

The descriptions of the hair on the elytra of *Minthea* differ among previous authors. Kraus [13] described the hair as “erect bristles”, Lesne [14] described them as “erect claviform setae”, and Gerberg [11] described them as “erect, flattened, whitish hair”. Santoro [12] classified *Minthea* into the group of Lyctinae with lanceolate or claviform hair. The type species, *M. squamigera* is even named for its scale-like

hairs. The morphology of hairs on the elytra of *Minthea* becomes very clear under ESEM. The erect hair is not flattened at all. The major type of erect hair on the elytra of *Minthea* is the seta with an open brush-like end and 4–6 serrulate longitudinal ribs (type m). Hence, the shape of the major hair is similar to the stout claviform hair with a densely divided tip, not scale-like or lanceolate.

Only one species of *Cephalotoma* has been examined in this study. There are three species in the genus *Cephalotoma* and four species in the closely related genus, *Lyctoderma*. Even though the morphology of the hairs on the elytral disc of *C. perdepressa* distinguishes it from other lyctines very well, further studies are needed to clarify the morphology of the hairs in both genera. This will certainly help to evaluate the phylogenetic relationships between these two genera and other lyctines.

TABLE 2: Distribution of the different types of hairs (*: appear on elytra, D: appear on declivity).

Taxa	a.	b.	c.	d.	e.	f.	g.	h.	i.	j.	k.	l.	m.	n.
<i>L. africanus</i>	*													
<i>L. brunneus</i>	*													
<i>L. carbonarius</i>	*													
<i>L. cinereus</i>		*												
<i>L. hipposideros</i>	*													
<i>L. linearis</i>			*											
<i>L. tomentosus</i>									*					
<i>M. bivestita</i>									*				*	
<i>M. obstita</i>													*	*
<i>M. reticulata</i>													*	*
<i>M. rugicollis</i>											*		*	
<i>M. squamigera</i>											*		*	
<i>T. aequale</i>			*											
<i>T. angulicollis</i>			*											
<i>T. impressum</i>			*											
<i>T. parallelopipedum</i>		*												
<i>T. praeustum</i>	*													
<i>T. punctipenne</i>	*													
<i>C. perdepressa</i>				*	*									
<i>R. dominica</i>											*,D			
<i>S. quadricollis</i>											*,D			
<i>S. rugosus</i>	D										*			
<i>S. substriatus</i>	*,D													
<i>P. punctatus</i>	*,D													
<i>P. sulcicollis</i>	*,D													
<i>P. truncatus</i>	*,D													
<i>D. bifoveolatus</i>										*	*	D		
<i>D. brevis</i>							D			*	*			
<i>D. distinctus</i>	*						*	D						
<i>D. minutus</i>						*	D				*			
<i>D. ocellaris</i>						*		D						
<i>D. porcellus</i>							*	D			*			
<i>D. punctatissimus</i>							*,D			*				

The complete scientific name of each species is given in the list of material.

4.3. Microstructural Characters on the Elytra of *Dinoderinae*.

The type of puncture differs between the elytral disc and declivity in *Dinoderus brevis* and *D. minutus*. There are shallow subcircular punctures with a corrugated rim (type e) on the disc, and shallow subcircular punctures with corrugated rim and convex inside (type f) on the declivity. Other *Dinoderus* spp. all have the type e puncture on both elytral disc and declivity. The difference in the punctures was not discussed in Lesne's [15] paper, but is briefly mentioned in Fisher's [16] revision.

There are usually two different types of hair on the elytral disc and a third type of hairs on the declivity in *Dinoderus* (see Table 2). No previous author mentioned the difference in the types of hair on the disc and on the declivity.

Fisher [16] described the hairs of *Dinoderus* as "short, erect, rather stiff, yellowish hair", Lesne [15] simply described the hairs of *D. distinctus* as "very short". Of the two types of hair usually found on the elytral disc of *Dinoderus*, one type consists of fine simple hair (types a and j) as mentioned by Fisher [16], the other has a densely divided forklike end and/or is serrulate on one side (types f, g, and k), and is usually longer and of greater diameter. The latter were not found by Fisher [16], nor by Lesne [15]. Some *Dinoderus* species have no fine simple hair on elytral disc, but nevertheless have two different types of hair of types f, g, or k.

Except for *Stephanopachys*, all *dinoderines* have only hair-bearing punctures in which the hair is inserted inside

the puncture close to the rim. This character distinguishes *Dinoderinae* from *Lyctinae*.

5. Conclusion

The special advantages of the scanning electronic microscope are the possibility to depict three dimensional structures with a very large depth of focus, and the fact that the image is built up almost purely from the surface of the specimen (e.g., [17]). The apparently three-dimensional picture is very suitable to describe fine structures. For example, the broad hair of *Minthea* have often been described as scale-like hair before we examined them by ESEM. However, the ESEM showed the hair is not flat and scale-like at all, but a thick, ridged hair with an open brush-like end.

Usually biological specimens are sputtered with gold to produce a conducting coating on the surface to avoid charging, which is a well known problem to all those working with biological specimens using electron microscopy. It is usually not possible to sputter rare specimens or types, but the ESEM can be used to examine such specimens without coating them. The ESEM uses a low vacuum in the specimen chamber even though the acceleration voltage is not low. It produces clear images of the microstructures of the uncoated specimens. This study not only found additional taxonomically useful characters in the microstructures on the surface of specimens, but also showed that we can now examine specimens without any damage under ESEM.

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Review Article

A Review on the Fascinating World of Insect Resources: Reason for Thoughts

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Received 17 March 2010; Revised 20 May 2010; Accepted 10 June 2010

Academic Editor: Subba Reddy Palli

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Insect resources are vast and diverse due to their enormous diversity. The exploitation and utilization of insect resources is broadly classified into four different categories. The first category is the insects of industrial resources. This level includes the utilization of silk worm, honeybee, lac insect, dye insect, and aesthetic insect. The second category is the utilization of insects for edible and therapeutic purposes. Insects are high in protein and many are rich sources of vitamins and minerals. The third category is the use of insects in forensic investigation. By analyzing the stages of succession of insects at first, rough estimation of the postmortem intervals can be done. The fourth category is the insects of ecological importance. Many insect species act as potential predators and parasites of destructive pests of insect order Lepidoptera, Diptera, and Orthoptera. Insects are also used as bioindicator to assess the cumulative effects of environmental stressors such as pollutants. Despite these fascinating benefits, insect resources are often neglected in India due to lack of proper documentation, less expertise, and advance enterprises in these fields. Hence, the paper reviews the different fascinating facets of insect resources in order to explore and utilize it in a sustainable way with reference to Indian region.

1. Background of Insect Resources

Insects are one of the most successful groups of animal. They constitute about three-fourths of the total organisms present on earth [1]. Out of the 5.57–9.8 million estimated animals in the world, 4–8 million species are known to be insects [2, 3]. Approximately, 0.1 million species of insects occur in India [4]. However, a precise check listing of the insect fauna of India has not yet been done so far. Therefore, possibility of recording several new species in near future is very high. Insects are unique not only in diversity but also in number of individuals in each species. There are 200 million insects for every human, 40 million insects for every acre of land. In the Amazon, insect biomasses overweight all vertebrates at 4 : 1 ratio [5]. Depending upon the vast diversity, the resources from insects are also vast and diverse. With their multiple utilities, insects have been providing constant services to the mankind as other resources.

On the basis of their utility, insect resource is broadly classified into four different categories. The first category

is the insects of industrial resources. This level includes the utilization of silk worm, honeybee, lac insect, dye insect and aesthetic insect. The second category is the utilization of insects for edible and therapeutic purposes. Some important edible insects are grasshoppers, crickets, termites, ants, grubs, moths, caterpillars, and pupae. Insects are also an important natural source of food for many kinds of animals. The muscoid (Diptera) larvae and pupae from poultry manure or other organic wastes are used as a high protein source for broiler production. Usage of insects in traditional medicine was recorded since time immemorial. The therapeutic application of honeybee venom (bee venom therapy) has been used in traditional medicine to treat diseases like arthritis, rheumatism, back pain, cancerous tumors, and skin diseases. The third category is the use of insects in forensic investigation. By analyzing the stages of succession of insects at first, rough estimation of the postmortem intervals can be done. The fourth category is the insects of ecological importance. Many insect species act as potential predators and parasites of destructive pests of insect

order Lepidoptera (Butterflies and Moths), Diptera (Flies) and Orthoptera (Grasshoppers). As a biomass recycler, house fly larvae are used to recycle organic wastes to produce protein and fat. Insects are also used as bioindicator to assess the cumulative effects of environmental stressors such as pollutants.

People worldwide have been enjoying insect resources in diverse fields. The modern trends in the development of the utilization and industrialization of insect resources, including traditionally cultured industrial insects and newly developed industrialized species has been reviewed by Zhang et al. [6]. But in India, due to lack of proper documentation, less expertise and advance enterprises in these fields, their values do not get due recognition, as compare to insect resources utilization in different corners of the world. Though, India is having a rich diversity of insect [4], only known insect resources products like silk, honey, and lac are well utilized and developed, neglecting many other prospective fields. Considering these important facts, this paper is reviewed with an aim to explore and utilize the different fascinating facets of insect resources in a sustainable way with reference to Indian region.

2. Insects of Industrial Resources

2.1. Sericulture and Allied Purposes. The natural fibre silk is the product of insects that belong exclusively to the order Lepidoptera. India is home to variety of silk secreting fauna which includes an amazing diversity of silkmths. This has enabled India to achieve the unique identity of being producer of all the five commercially traded varieties of natural silks, namely, mulberry, tasar, oak tasar, eri, and muga [7] produced by silk moth species *Bombyx mori*, *Antheraea mylitta*, *A. proylei* (Figure 1), *Samia cynthia ricini*, and *A. assama*, respectively. As far as nonmulberry (tasar, oak tasar, eri, and muga) silk moth species are concerned, India alone recorded as many as 40 different species [8]. India also has native populations of wild silkmths such as *Theophila religiosa*, *B. mandrina*, and *Antheraea compta*. The North-eastern region of India makes ideal home for a number of wild sericigenous insects and is centre of wild silk culture including muga, eri, oak tasar, and mulberry silk [9]. There are still many species in the forests of this region of India that are yet to be explored [7].

Asia is the top producer of silk in the world contributing 95% of the total global output. Though there are over 40 countries on the world map of silk, bulk of it is produced in China and India, followed by Japan, Brazil, and Korea [10]. India, the world's second largest producer of silk after China, is also the largest consumer of silk. In India, mulberry silk is produced mainly in the states of Karnataka, Andhra Pradesh, Tamil Nadu, Jammu & Kashmir, and West Bengal, while nonmulberry silks are produced in the state of Jharkhand, Chattisgarh, Orissa, and north-eastern region [7]. In the north eastern states of India, Assam contributes almost 90% of Muga silk and 65% of Eri silk production [11]. Meghalaya and Manipur also appear in the map of silk producing states of this region (Table 1).

TABLE 1: Silk production in India (2005-06).

State	Mulberry (MT)	Vanya Silk (MT)			Total (MT)
		Tasar	Eri	Muga	
Karnataka	7471	0	0	0	7471
Andhra Pradesh	5375	20	27	0	5422
West Bengal	1552	34	4	0	1590
Assam	8	0	745	104	857
Tamil Nadu	739	0	0	0	739
Manipur	48	3	235	0	286
Meghalaya	3	0	280	5	283
Jharkhand	1	96	0	0	97
others	248	155	151	1	560
Total	15445	308	1442	110	17305

Source: Nagaraju [10].

Apart from silk, there are several other by products from sericulture which can be utilized as commercial input in many fields. The foliage of mulberry is used as a fodder for cattle [12]. Silkworm pupae were traditionally used as fertilizer, animal feed, food material, and medicine in some countries, such as China, Japan, Korea, India, and Thailand [13–15]. Human consumption of silkworm pupae has been practiced in China [16] and India by many tribal communities [10]. Recently, silkworm pupae have been put in the list of “Novel food resources managed as common food” by Ministry of Health PR China [15]. The waste liquor containing sericin, which is yielded through process of the degumming of silk fiber, is also regarded as another raw material for the production of sericin powder. Sericin powder is used in a variety of industries as a raw material in production of food, cosmetic, medicine, and so forth [17]. Thus sericulture not only provides silk for fashionable clothing, it also offers several useful by products to the human society [12].

2.2. Apiculture and Allied Purposes. Honey production has been proven as a promising profitable venture, which is a mean of low-cost or high-yield enterprise without requiring compulsory land ownership or capital investment. It has been used traditionally in various diet preparations, such as medicine, cosmetic, ointment, candle, and household bee-wax items [18]. The propolis of the bee hive is used in lip balms and tonics whereas royal jelly is used to strengthen the human body, for improving appetite, preventing ageing of skin, leukemia and for the treatment of other cancers. On an estimate, about 80% honey is used directly in medicines and 10% in Ayurvedic and pharmaceutical production. Honey bees during foraging for pollen and nectar from flowers of different plant species, enhance agricultural productivity to the tune of 30%–80% annually through cross-pollination [19]. Of the five honey bee species of the world, namely, *Apis florea*, *A. cerana*, *A. dorsata*, *A. mellifera*, and *Trigona iridipennis*, only two species, *A. cerana* and *A. mellifera* (Figure 2) are reared in India [20].

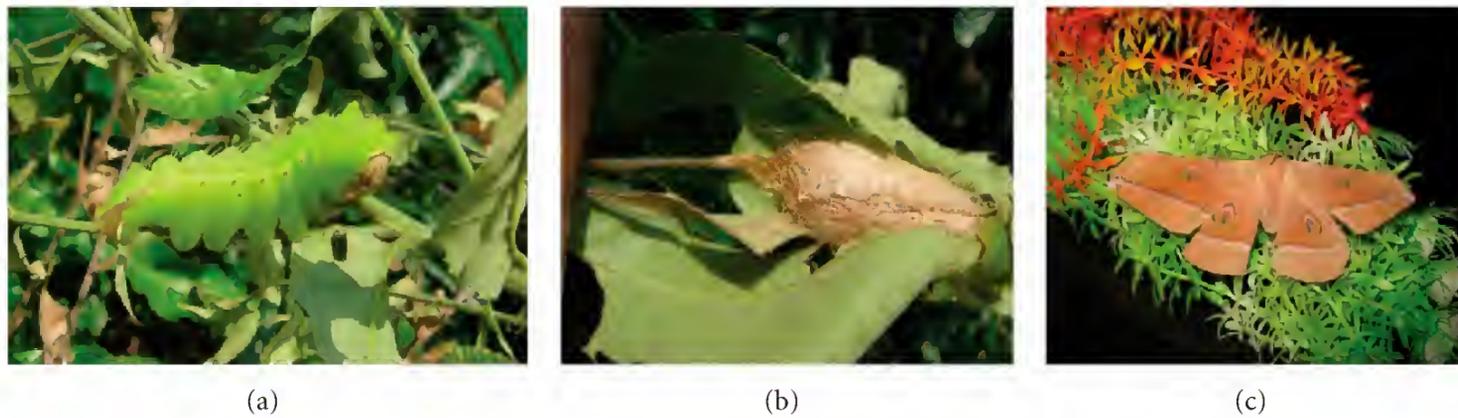


FIGURE 1: The native oak tasar silkworm of Manipur, North-East India, *Antheraea proylei* (a) larva, (b) pupa, and (c) adult moth.



FIGURE 2: Cultivated species of honeybee in India (a) *Apis mellifera*, and (b) *Apis cerana*.

There is a long history of honey hunting and traditional beekeeping by utilizing Asian wild honeybees. Asia has a suitable agro-climatic background for development of modern beekeeping. Currently, China captures 40% of the world market. The biggest importers of honey are Germany, Japan, and the United States. India produces about 70,000 tonnes of honey every year of which 25000–27000 tonnes is being exported to more than 42 countries. The major honey-producing states in India are Punjab, Haryana, Uttar Pradesh, Bihar, and West Bengal [21]. Study on honey and honeybee is a never ending venture in a vast country like India. The unexplored forests of the country especially those of north east may unfold the wealth of newer bee species in future. G. K. Ghosh and S. Ghosh [22] also cited the traditional importance and practices of apiculture in Manipur in their book, “Woman of Manipur.”

2.3. Lac Culture. Lac is a resinous substance produced by an insect popularly known as lac insect. Lac insects, the crowning glory of India’s rich insect fauna (representing 21.8% diversity of the known lac insect species) are exploited for their products of commerce, namely, resin, dye, and wax. The total numbers of lac insect species reported from the world are 87 species under nine genera, of which 19 species belonging to two genera are found in India [23]. Concerning the economic viewpoint, India is the largest producer of lac in the world, accounting for about 50%–60% of the total world lac production. India produces about 20,000 metric tones of raw lac every year. The major lac producing states are Jharkhand (57%), Chhattisgarh (23%), and West Bengal (12%) while Orissa, Gujarat,

Maharashtra, Uttar Pradesh, Andhra Pradesh, and Assam are minor producers. India fetches approximately Rs.120–130 crore of foreign exchange through export of lac every year. Lac resin being natural, biodegradable and nontoxic, finds applications in food, textiles, and pharmaceutical industries in addition to surface-coating, electrical, and other fields. It provides immense employment opportunities in the country [24]. Species belonging to genus *Paratachardina* produce a hard, horny substance, which is insoluble in alcohol. These are univoltine and are generally treated as parasites of economically important plant such as tea and sandal. Recently, *Paratachardina* spp. have been found to be potential biocontrol agents for managing weeds [25]. Of the 19 species of lac insects reported from India, *Kerria lacca* is mainly exploited for commercial production of lac. *K. chinensis* in the northeastern states and *K. sharda* in coastal regions of Orissa and West Bengal are also cultivated to a certain extent. Potential of other lac insect species reported from the country remains to be exploited [24] and also a persistent exploration of new species is required.

2.4. Natural Dye from Insect. The demand for natural dye is constantly increasing with an increase in awareness of the public on the ecological and environmental problems associated with synthetic dyes [26]. The effort of cultivating natural dye from insects has been suggested by Prasad [27] with a view to exploit it from India. The coccid, *Dactylopius coccus* (Hemiptera: Dactylopiidae) is the most important species due to its being used for the extraction of carmine acid, a natural red dye used in food, pharmaceutical, and cosmetic industries [28]. The coccid is an insect living

on cladodes of prickly pears (*Opuntia ficus indica*). Dried females are a source of red dyes widely utilized in food, textile, and pharmaceutical industries [29]. *D. opuntiae* is another wild species found in Mexico and has a shorter lifespan and reproduction cycles with a larger number of generations per year [30]. All the cochineal species have a high content of proteins and minerals. The residuals from coloring extraction can be used to enrich food for avian species or to prepare fertilizers [31]. Cochineal is used to produce scarlet, orange, and other red tints. The production and exploitation method of the dye was also studied by many workers in this field [32, 33]. The insects are killed by immersion in hot water or by exposure to sunlight, steam, or the heat of an oven. Each method produces a different colour which results in the varied appearance of commercial cochineal. It takes about 155,000 insects to make one kilogram of cochineal [34]. Likewise, oak galls were gathered and used commercially as a source of tannic acid. It was a principal ingredient in wool dyes and black hair colourants used during the Greek empire as early as the 5th century BC. It is still used commercially in the leather industry for tanning and dyeing and in manufacturing of some inks. Tannic acid was obtained from the Aleppo gall found on oak trees (*Quercus infectoria* Olivier) in Asia and Persia. The trees produce gall tissues in response to the chemical substance secreted by the larvae of tiny wasps (*Cynips gallae tinctoriae* Olivier; Hymenoptera: Cynipidae) that infest the trees. 50%–75% of gall's dry weight is composed of tannic acid [35]. The aspect of exploring as well as utilizing natural dye-producing insects is quite virgin in the India. North-eastern region being a main region of oak cultivated area, there is an absolute scope in this field and thus enthusiastic approach is required in near future.

2.5. Insect Trade for Aesthetic Purposes. The body colouration, beauty, and mode of life of the insects always attract us. Coloured wing and elytra of many coleopterans are used in jewellery, embroidery, pottery, and basket makings [27]. Among the insects of aesthetic value, butterfly attains maximum attention from museums and collectors for which it is established as one of valued items in market. For satiating the growing need of butterfly amongst the collectors, numerous butterfly farms have been developed in European countries [4]. In such butterfly farms like Brinckerhoffs, all the pupae are captives reared exclusively for sale as live insects, which yield \$100,000,000 annually [36]. Such view also captures thousands of income generating aspects utilizing insect resources.

3. Edible and Therapeutic Insects

3.1. Insects for Human Consumption and Animal Feed. Over 1,500 species of edible insects have been recorded in 300 ethnic groups from 113 countries. Many species of insects have served as traditional foods among indigenous peoples and the insects have played an important role in the history of human nutrition [37]. The insects are high in protein and many are rich sources of vitamins and minerals. DeFoliart

[38] provided a brief general overview of the nutritional quality of edible insects. In some ethnic groups, insects provide 5%–10% of animal protein input as well as fats, calories, vitamins, and minerals [39]. Edible insects have been reported to have more nutritional content than the other conventional foods (Table 2). Studies on nutrient analysis for various insects were conducted by many authors in different countries like Quin [40] in South Africa, Santos Oliveira et al. [41] in Angola, Malaisse and Parent [42] in Zaire, Gope and Prasad [43] in India, Sungpuag and Puwastien [44] in Thailand, and Ramos-Elorduy and Pino [45] in Mexico.

Some of the commonly eaten species of insects include grasshoppers, crickets, termites, ants, beetle larvae, moth caterpillars, and pupae. Insects generally have higher food conversion efficiency than other higher animals. For example, house cricket (*Acheta domesticus*) when reared at 30°C or more, and fed a diet of equal quality to the diet used to rear conventional livestock, they show a food conversion twice as efficient as pigs and boiler chicks, four times that of sheep, and six times higher than steer when losses in carcass trim and dressing percentage are counted [47]. Protein production from insects for human consumption would be more effective and consume fewer resources than vertebrate protein. This makes insect meat more ecological than vertebrate meat. The use of insects particularly locust and grasshopper (Figure 3(a)) as food have been a great significance not only from the nutritional value, but also controlling pests as many ethnic human societies believe. In Asia and Oceania increased consumption of grasshoppers and locusts has coincided with decreased pesticides use [48]. Insects are not used as emergency food to ward off starvation but are included as planned part of the diet whenever and wherever available. Among them, many of these organisms are taken for their flavor, for example, *Belostoma indica* (Figure 3(b)). The long history of human use suggests that the insects do not pose any significant health problem [49]. Some of the renowned works on edible insects from different parts of India are those of Singh et al. [49, 50], Alemla and Singh [51], and Singh and Chakravorty [52] (Table 3). This trend toward reducing the bias against insects as food is promising, by promoting nutritional value to stable diets and maximizing ecological benefits with edible insects. Despite these awesome benefits, the modernization have led indigenous population around the world away from this traditional food source, without providing nutritional equivalent substitutes [53].

Insects are also well known as an attractive and important natural source of food for many kinds of animals, including birds, lizards, snakes, amphibians, fish, insectivore, and other mammals [54–56]. The vast majority of studies in the west have dealt with the nutritional value of muscoid (Diptera) larvae or pupae used to recycle nutrients from poultry manure or other organic wastes as a high-protein source for broiler production [57]. According to Davis [58], there is no difference in taste of eggs from grub-fed hens and others, in fact, the former had better yolks. Cotton and George St. [59] also summarized the early use of the meal worm, *Tenebrio molitor* as animal feed.

TABLE 2: The nutritional content of edible insects and other animals based on 100 gram serving.

Animal	Energy (Kcal)	Protein (g)	Iron (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)
Termites (<i>Macrotermes subhyalinus</i>)	613	14.2	0.75	0.13	1.15	0.95
Caterpillar (<i>Usata terpsichore</i>)	370	28.2	35.5	3.67	1.91	5.2
Weevil (<i>Rhynchophorus phoenicis</i>)	562	6.7	13.1	3.02	2.24	7.8
Beef (lean ground)	219	27.4	3.5	0.09	0.23	6.0
Fish (Broiled cod)	170	28.5	1.0	0.08	0.11	3.0

Source: William [46].



FIGURE 3: (a) Traditional dish form of grasshopper, *Oxya hyla hyla* from North-East India, and (b) Edible giant water bug, *Belostoma indica*.

3.2. Insects in Medicine and Research. In many parts of the country, different sections of the society have been using the medico-entomological drugs in their day to day life [27]. Costo neto [61] termed entomotherapy for use of insects for therapeutic purposes. Some of the popular authors who have given the account of use of insects and the various stages in therapeutic activities are Antonio [62], Fosaranti [63], Alexiades [64], Zimian et al. [65], Green [66], Namba et al. [67], Maya [68], and Padamanbhan and Sujana [69]. One of the most commonly used insects in medicinal purposes is the blow fly larvae. During World War II, military surgeons noticed that wounds which were left untreated for several days healed better than noninfested wounds, when infested with the blow fly larvae maggots. It was later discovered that the larvae secreted a chemical called allantoin which had a curative effect. The therapeutic application of honeybee products has been used in traditional medicine to treat various diseases like diarrhoea, tuberculosis, impotency, asthma, exophthalmic goiter, and mouth galls. The practice of using honeybee products for medicinal purposes is coined as Apitherapy. One of the major peptides in the bee venom, called melittin, is used to treat inflammation in sufferers of rheumatoid arthritis and multiple sclerosis. Melittin blocks the expression of inflammation genes, thus reducing swelling and pain [70]. The therapeutic application of honeybee venom (bee venom therapy) has been used as a traditional medicine to treat a variety of conditions, such as arthritis,

rheumatism, back pain, cancerous tumors, and skin diseases [71]. The use of traditional knowledge could be extended further in modern medicine system by identifying the proactive biomolecules with pharmacological action [72–75]. Bee venom contains at least 18 active components, including enzymes, peptides, and biogenic amines, which have a wide variety of pharmaceutical properties (Table 4). Recently, it was reported that melittin inhibited the DNA-binding activity of NF- κ B, a critical transcriptional factor regulating inflammatory gene expression, by inhibiting I κ B phosphorylation [76]. Bee venom also has anticancer activity. Several cancer cells including renal, lung, liver, prostate, bladder, and mammary cancer cells as well as leukemia cells can be targets of melittin [77–80]. Pharmaceutical companies are currently funding extensive research into the potential of venom as the next generation of cancer fighting drugs. Thus, bee can be cited as a spectacular example as medicinal insects. Likewise, there may be many such insects having similar or superior medicinal properties. Cantharidin is another medicine obtained from blister beetle (Figure 4), an insect belonging to order Coleoptera and family meloidae. Its medical use dates back to description in Hippocrates (*ca.* 460–377 BC) [81]. It was administered as a diuretic and to alleviate epilepsy, asthma, rabies, and sterility. The eggs of red ants are said to be used as a constituent of medicine for the control of malaria. Extract of cocoons of mulberry silkworm is believed to check profuse menstruation and chronic

TABLE 3: Common edible insects in India.

Scientific name	Common name	Family	Edible form	References
<i>Cybister confusus</i> Shp.	Diving beetle	Coleoptera: Dytiscidae	Roasted, fried and curry forms of larva and adult	[50]
<i>Hydrophilus olivaceus</i> (Fabricius)	Water scavengers	Coleoptera: Hydrophilidae		
<i>Anoplophora glabripennis</i> (Motchulsky)	Asian long horned beetle	Coleoptera: Cerambycidae	Fried larva	[49]
<i>Acisoma panorpoides</i> Rambur	Dragonflies	Odonata: Libellulidae	Nymph are eaten in roasted or fried forms, prepared dishes by crushing with chilly and other spices	[52]
<i>Gryllotalpa africana</i> Palisot de Beauvois	Mole-cricket	Orthoptera: Gryllotalpidae	Roasted or fried body	[27]
<i>Belostoma indica</i> (Lep. & Serv.)	Giant water bug	Hemiptera: Belostomatidae	Roasted or fried body with edible herbs or spices.	[43]
<i>Laccotrephes maculatus</i> Fabr.	Nepa	Hemiptera: Nepidae	Fried body	[51]
<i>Oxya hyla hyla</i> Serville	Grasshopper	Orthoptera: Acridae	Steamed, fried and mixed with edible herbs	[60]



FIGURE 4: Blister beetle adult from India.

diarrhoea [26]. Pierisin, a protein from pupa of cabbage butterfly, *Pieris rapae* exhibit cytotoxic effects against human gasteris cancer. Extract of the body fluids of other cabbage butterflies, *P. brassicae* and *P. napi* also contains the same protein, pierisin [60]. Insect cell lines have been reported to be efficient expression hosts for the production of many glycoproteins, including monoclonal antibodies [82]. Tang et al. [83] proposed the idea that antimicrobial molecules from insects may serve as a potentially significant group of antibiotics. It was revealed from their experiment conducted on the Chinese traditional edible larvae of housefly, *Musca domestica*. Some of the insects used in Indian traditional medicine are highlighted in Table 5.

4. Forensic Entomology

Forensic entomology, the use of insects and other arthropods in forensic investigations, has gained a lot of importance during the past few decades [88]. Some of the recent literatures like, “The Manual of Forensic Entomology” by Smith [89], “The entomological review” by Catts and Goff

[90] and the installation of the International homepage of Forensic Entomology [91] may be cited as examples. Mende [92] listed a number of animals which feed on corpses. This list includes flies, beetles, and other insects. By analyzing the stages of succession of insects a first rough estimation of the postmortem intervals can be made [89]. Depending on the biogeographical region and ecological habitat, different species of necrophagous insects are involved in the decay of a corpse. For example, examinations on insect-succession from Canada are not applicable in the conditions of Germany [88]. However, the primary purpose of forensic entomology in today’s context is the use of insects in determining elapsed time since death [93]. Forensic entomology, therefore, holds a vital position in the arena of forensic science. The unique role played by insects in this field is overwhelming, whose place is nonreplaceable by other organisms.

5. Insects of Ecological Importance

5.1. Insects as Biological Control Agent. Biological control means the action of parasitoids, predators, and pathogens in maintaining other organism’s density at a lower average than would occur in their absence. In modern context, when we are becoming aware of the harmful effects of unilateral use of chemical insecticides in various agricultural field or ecosystem, the role of insect as biocontrol agent is immensely vital. The first dramatic example of deliberate biological control was the importation of vedalia ladybeetle, *Rodolia cardinalis* (Mulsant) in California, in 1888 to control the cottony-cushion scale insect, *Icerya purchasi* Maskell on citrus [94]. Many insects act as potential predators and parasites of destructive pests of insect-order Lepidoptera (Butterflies and Moths), Diptera (Flies), and Orthoptera (Grasshoppers) [27]. Predators are scattered in about 167

TABLE 4: Components of bee venom and their major characteristics.

Components	MW	Contents (% dry BV)	Major characteristics
<i>Peptides</i>			
Melittin	2840	40–50	26 amino acid Enhance of PLA ₂ activity Cytotoxic effects against cancer cells Anti-inflammatory and antiarthritic effects
Apamin	2036	2–3	10 amino acid Inhibition of Ca ²⁺ activated K ⁺ channel Cytotoxic effect against cancer Nociceptive effect Anti-inflammatory properties
MCD peptide	2588	2–3	22 amino acid Anti-inflammatory and analgesic effect Histamine release (low dose) Histamine release inhibition (high dose) Antiallergic effect
Adolapin	11,500	1	Inhibition of PLA ₂ and COX activity Anti-inflammatory activity Analgesic effect
Protease inhibitor	9000	<0.8	
Minimine	6000	2-3	
Procamine A,B		1.4	
Secarpin		0.5	
Tertiapin		0.1	
Melittin F		0.01	
Cardiopep		<0.7	
<i>Enzymes</i>			
PLA ₂	19,000	10–12	Cytotoxic effects against cancer cells Inflammatory Effects Antitumor effects
Hyaluronidase	38,000	1.5–2	Selectively attacks tissue hyaluronic acid polymers Increase the capillary permeability Immune response and tissue spread propertiesAntigenic
Glucosidase	170,000	0.6	
Acid phosphomonoesterase	55,000	1	
<i>Amines</i>			
Histamines	307.14	1.5	
Dopamine	189.64	0.13–1	
Norepinephrine	169.18	0.1–0.7	
<i>Others</i>			
Carbohydrates	307.14	1.5	
<i>r</i> -Aminobutyric acid	189.64	0.13–1	
<i>B</i> -Aminoisobutyric acid	169.18	0.1–0.7	

Source: Son et al. [84].

families of 14 orders of the class, Insecta [95]. Major groups of entomophagous parasites belong to the order Hymenoptera and family Tachinidae of the order Diptera. The well-known parasitoids acting as potential biocontrol agents are Ichneumonids, Chalcids, Proctotrupoids, and Evanoids. The Tachinid flies species of *Sturmia* and *Tachinia*

parasitize the insect pests like paddy armyworm and fruit moth larvae [27]. Biological control effort against noxious weed, *Parthenium hysterophorus* L. through the utilization of insect species, *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) has also been advocated by Gautam [96]. However, only few species are well established and employed

TABLE 5: Insects used in Indian traditional medicine.

Insect species	Disease cure	Mode of preparation & use	Practicing state	References
<i>Holochlora indica</i> (Orthoptera: Tettigoniidae)	Ulcer	Consumed as tonic	Manipur	[43]
<i>Diacrisia obliqua</i> (Lepidoptera: Arctiidae)	Dog bite	Freshly laid eggs are eaten as well as applied on the affected part	Chhatisgarh	[60]
<i>Stomphosistis thraustica</i> (Lepidoptera: Gracillariidae)	Common fever and to increase the flow of milk in lactating woman	Dried full grown larvae or powder are consumed in combination with herb mainly, <i>Amdrgraphes paniculata</i>		
<i>Hieroglyphus banian</i> (Orthoptera: Arcidae)	Liver disorder	Roasted nymph and adult are eaten	Nagaland	[51]
<i>Batocera titana</i> (Coleoptera: Cerambycidae)	Wound	Larvae are eaten alive		
<i>Periplaneta americana</i> (Dictyoptera: Blattidae)	Asthma and tuberculosis	Extraction of the roasted insects are consumed along with water	Arunachal Pradesh	[85]
<i>Apis indica, A. florae, A. mellifera</i> (Hymenoptera: Apidae)	Boil, snakebite and cough	Powder of the roasted insect is mix with honey and applied		
<i>Helicoverpa armigera</i> (Lepidoptera: Noctuidae)	Fever, nervous breakdown, eosinophilia and asthma	Dried powder consumed as tonic	Chhatisgarh	[86]
<i>Zonabris pustulata</i> (Coleoptera: Meloidae)	Problems in urinoginital system	Fresh extracts from larvae are consumed		[87]

in the field of biocontrol. Thus, further studies are required to explore successful insect species as biocontrol agents.

5.2. Insects in Biomass Recycling. Lindner [97] was the first to suggest the use of house fly larvae to recycle organic wastes, specifically human waste, to produce protein and fat as a useful byproduct. Although insect herbivory is common in terrestrial ecosystems, it has only recently been considered an important and persistent control on ecosystem processes and has not been included as a factor in most ecosystem models. Herbivore alteration of litter inputs may change litter decomposition rates and influence ecosystem nutrient cycling too [98].

5.3. Insects as Indicator of Water Pollution. Water quality researchers often sample insect populations to monitor changes in water bodies. The insects are monitored over time to assess the cumulative effects of environmental stressors such as pollutants. Environmental degradation resulting from pollution will likely decrease the density of insects found by eliminating those that are less tolerant to unfavourable conditions. Insects such as the mayfly, stonefly, and caddis fly larvae are sensitive or intolerant to changes in stream conditions brought about by pollutants [99]. Composition of species tells water conditions. Presence or absence of particular species like certain chironomid midge species is very specific in their environmental needs. For

example, specific species will survive only in pH 2, high-acidic conditions, high-nitrogen water, and so forth. Insects are effective indicators because they have a short generation time [100]. Trace metal contaminants can affect both the distribution and the abundance of aquatic insects. Insects have a largely unexploited potential as biomonitors of metal contamination in nature [101, 102]. *Lithocerus niloticum* (Hemiptera: Belostomatidae) was reported to be an efficient biomonitor for heavy metal pollution in lakes [103].

6. Perspective

There is no doubt that insects are potentially a more efficient source of many fascinating facets for mankind and others vertebrates. There is a need to link the potential of this bioresources to economic prosperity. Owing to the above facts, there is a debating question about the biomass availability of the insect species. In this regard, Benjo et al. [104] stated that wild harvest of insect pests in established crop or horticultural systems may be more practical. Collecting such pests would not only protect plant but it could benefit the environment by reducing the need to use pesticide [105]. Establishment of mass breeding insectaries with modern artifact such as raising them in artificial diet or through biotechnological intervention could provide a hope for golden aspects for income generation too. For instance, farmers may earn as much as \$1000 or more each year in

New Guinea by harvesting emerging adult of wild butterflies for trading [36]. Some interesting challenges also include the integration of mass rearing of insects into small scale farming ventures such as development of organic waste recycling systems using insects [37, 106]. Insects have long been significant dietary factor and remedy for illnesses in different regions of the world. Scientific validation and updating of traditional wisdom in bioprospecting has assumed greater significance. There is a need for more and more analysis of insect biodiversity for the development of virgin resources and their industrialization particularly in India. It is a high time that researchers recognize the manifold utilities of insects and begin to build on it.

Acknowledgments

The authors are indebted to Dr. N. C. Talukdar for his constant inspirations and suggestions in the preparation of this paper. Thanks are also due to Dr. W. Gusheinz for critically evaluating the paper for its improvement. Much of this work would not have been possible without the facilities of literatures from DBT's e-library Consortia (Delcon) in the institute. The authors are thankful to Department of Biotechnology (DBT), New Delhi for providing financial support and working facilities in the Inset Bioresources Division, Institute of Bioresources and Sustainable Development (IBSD), Manipur, India. Further support was also provided to the first author by DBT, through the DBT Research Associateship grant in Biotechnology and Life Sciences program.

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Research Article

Comparative Study of Spermatogenesis and Nucleolar Behavior in Testicular Lobes of *Euschistus heros* (Heteroptera: Pentatomidae)

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Received 23 March 2010; Accepted 17 June 2010

Academic Editor: Coby Schal

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In some testicular lobes of the Pentatomidae there may be occurrence of atypical spermatogenesis or polymegaly, leading to the production of nonfertile sperm. The comparative analysis of spermatogenesis and nucleolar behavior in testicular lobes of *Euschistus heros* showed cells with polymegaly in lobes 4 and 6. Generally, when these lobes are present in the same individual, there is also the formation of atypical cells in the flanking lobe. Such characteristic was not seen in *E. heros*. However, differences regarding the concentration of heteropyknotic chromatin and silver-positive bodies in this lobe deserve attention. This study explored the literature and demonstrated the prevalence of some lobes in the formation of differentiated cells. It was also found in the literature that there is an association of the chromocenter with the nucleolus in several species of Pentatomidae, but in *E. heros* this association does not appear to occur.

1. Introduction

The presence of testes formed by a number of compartments referred to as “lobes” is a characteristic of the Heteroptera. In some species, one of these lobes is of the *harlequin* type that differs from the other lobes by showing spermatogonial cells with meiotic pairing, nonspecific association of the autosomal bivalents, anomalous arrangement of the chromosomes in the metaphase plate, anomalous chromosome segregation, and cell fusion, resulting in the production of spermatozoa with highly variable chromosome numbers. There are reports of this type of lobe in 15 genera in three Pentatomidae subfamilies (Discocephalinae, Edessinae, and Pentatominae) [1].

Other lobes may also be associated with the formation of nonfertile sperm; for example, in *Antiteuchus tripterus* (Pentatomidae), lobes 4 and 6 show cells with polymegaly and significant intralobular metabolic differences [2, 3].

The aspects regarding the number of testicular lobes and the formation of atypical sperm are little known and explored. This information is found scattered in the literature

which makes it difficult to establish an evolutionary pattern among testicular lobes with regard to the formation of fertile and nonfertile sperm. For this reason, the data found in the literature are compiled in Table 1.

Recent studies have demonstrated the importance of examining the metabolic differences among species by the analysis of nucleolar bodies. It is known that the nucleolus or nucleolar bodies are related to the biosynthetic activity of the cell, so that the size and number of bodies depend on the functional characteristics of cells and may therefore reflect metabolic and functional differences [3–7].

Another aspect of nucleolar behavior in spermatogenesis is that, over time, several observations have suggested that the nucleolar granules or bodies which persist at the end of meiosis reorganize the nucleolus in early spermiogenesis and support protein synthesis in the process [8].

The study of metabolic differences between testicular lobes with analysis of nucleolar bodies has been proposed by Souza et al. [3], who found that the testicular lobes of *A. tripterus* showed significant differences in behavior and size of the nucleolar bodies, which may be due to

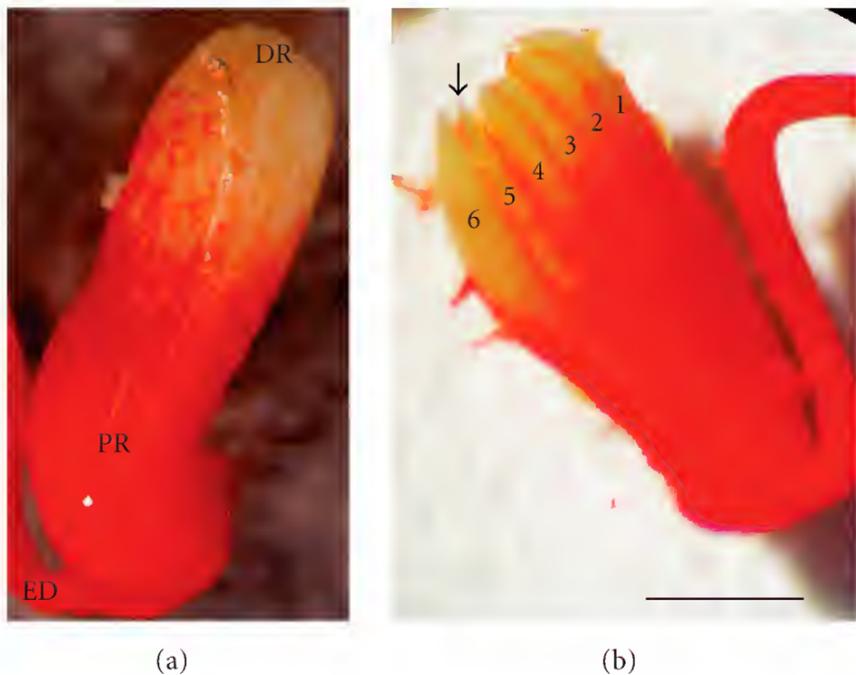


FIGURE 1: Testis of *Euschistus heros* enclosed by red membrane, where the proximal region (PR) of the ejaculatory duct (ED) is more intense red than the Distal Region (DR) (a). Observe in (b) the presence of six elongated lobes of approximately the same length, with lobe 5 narrower than the others (arrow). Bar = 1 mm.

differences in the formation of sperm with a nonfertile function.

The analysis of nucleolar organizer regions (NORs) during prophase has shown a close association of the chromocenter with the nucleolar body [9, 10]. The chromocenter in Heteroptera is characterized by its heteropyknotic nature, where it can be composed of sex chromosomes or even by heterochromatic autosomes [9, 11, 12]. In general, the nucleolar body is disorganized during diakinesis [13], reorganizing itself only in the beginning of spermiogenesis, supporting the initiation of protein synthesis [8]. However, the relationship between chromatin heteropyknotic and nucleolar bodies during spermiogenesis has not been previously explored, according to the literature.

Thus, the objective of this study was to analyze spermatogenesis in each lobe of *Euschistus heros*, comparing it with nucleolar behavior throughout spermatogenesis and to analyze in detail the distribution pattern of testicular lobes, described in the literature, with regard to differentiated spermatogenesis.

2. Material and Methods

Fifteen adult males of *Euschistus heros* Fabricius, 1794 (Heteroptera, Pentatomidae, Pentatominae, Pentatomini) were collected on soybean plants (*Glycine max* (L.), in the city of Sao Jose do Rio Preto (20°47'13" S, 49°21'38" W), SP, Brazil. The insects were fixed in methanol:acetic acid (3:1), and their testicular lobes were separated and submitted to the squash technique with lacto-acetic orcein staining, which is chromosome specific.

To study nucleolar behavior during spermatogenesis, the slides were submitted to the silver impregnation technique [47, with modifications] to stain argyrophilic proteins, which

are associated with rRNA and which can therefore localize the nucleolus or nucleolar bodies.

In the statistical analysis, measurements were taken of the diameter of 100 cells and their nuclei in the diffuse stage, that is, the stage after pachytene, which is characterized by its increased size and chromatin scattered throughout the nuclei, and of 100 cells nuclei in the stage of round spermatid in each lobe, randomly chosen. We used the program UTHSCSA Image Tool v.3.00 [48] and Minitab version 15.1 [49] for ANOVA (Tukey's comparison with 95% confidence interval) to compare the measurements of the cells between the testicular lobes. The best images were captured with a Zeiss microscope using the image analysis program AXIO VISION.

3. Results

3.1. Testis Morphology. The testes of *Euschistus heros* were enclosed by peritoneal sheath with red pigment, with the proximal region (PR) of the ejaculatory duct (ED) being more pigmented than the distal (DR) (Figure 1(a)). When the peritoneal sheath was removed in the distal region, we could observe the presence of 6 elongated lobes of approximately the same length, where lobe 5 was narrower than the others (Figure 1(b)).

3.2. Meiotic Behavior. The comparative analysis of meiotic cells of *Euschistus heros* stained with lacto-acetic orcein and silver impregnated showed that the behavior of cells in the six testicular lobes was quite similar, and therefore, the results are presented together.

During early prophase, a heteropyknotic body was observed at the periphery of the nucleus, and variation in cell diameter among the lobes was the only difference found. The diameter of the cells in lobes 1–3 (Figure 2(a)) was significantly smaller than that of lobe 5 cells (Figure 2(c)), which were smaller than cells in lobes 4 and 6 (Figure 2(b)). Due to these differences, an ANOVA test was performed, which showed that the lobes could be grouped by the size of the cells and their nuclei (Figures 2(a)–2(c)) into three different groups (group 1 = lobes 1, 2, and 3, group 2 = lobe 5, group 3 = lobe 4 and 6). Significant differences ($P < .0001$) were found between the groups, and the cells of group 1 (lobes 1–3) and their nuclei were smaller than those in group 2 (lobe 5) which, in turn, were smaller than those of group 3 (lobes 4 and 6) (Table 2).

During spermatogenesis, it was observed that the cells in diplotene/diakinesis showed chromosomes with chiasmata (Figure 2(d)). It was possible to determine in diakinesis and metaphase I the presence of a diploid number of $2n = 14$ (12A + XY) chromosomes (Figures 2(d), 2(e)). In anaphase/telophase I, it was observed that only autosomes undergo reductional segregation (Figures 2(f), 2(g)). During metaphase II, the autosomes were arranged in a ring with the sex chromosomes arranged inside (Figure 2(h)). During anaphase/telophase II, the sex chromosomes undergo equational division and it was possible to visualize lagging migration of the X chromosome (Figure 2(i)).

TABLE 1: All species of the family Pentatomidae in the literature where authors noted the number of testicular lobes and if they exhibited atypical meiosis and polymegaly. “No”: characteristic not found; “—”: information not found, “?”: author not sure about the presence of the characteristics.

Classification	No. lobes	Atypical meiosis	Polymegaly	References
Pentatomidae family				
Subfamily Asopinae				
<i>Apateticus crocatus</i>	7	No	No	[14]
<i>Euthyrhynchus floridanus</i> (Linnaeus)	6	No	No	[14]
<i>Perillus bioculatus</i> (Fabricius 1775) (as <i>Mineus bioculatus</i> , 1775)	7	No	No	[14, 15]
<i>Podisus maculiventris</i> (Say)	7	No	No	[14]
(as <i>P. modestus</i> (Dallas, 1851))				[15, 16]
(as <i>P. spinosus</i> (Dallas, 1851))				[15–19]
<i>Stiretrus anchorago</i> (Fabricius 1775)	7	No	No	[14, 16]
Subfamily Discocephalinae				
Tribe Discocephalini				
<i>Antiteuchus tripterus</i> (Fabricius, 1787) (as <i>Mecistorhinus tripterus</i>)	6	5	4 and 6	[2, 20–22]
<i>Dinocoris rufitarsus</i> (Ruckes, 1958)	8	5	4 and 6	[21, 23]
<i>Discocephalessa humilis</i> (Herrich-Schaeffer, 1843) (as <i>Platycarenum notulatus</i> (Stål, 1862))	4	No	—	[20]
<i>Platycarenum umbractulatus</i>	7	No	—	In press
Tribe Ochlerini				
<i>Alitocoris schraderi</i> (Sailer, 1950)	5	5	4 degenerate	[21, 24]
Subfamily Edessinae				
<i>Brachystethus rubromaculatus</i> (Dallas, 1851)	4	4	—	[20]
<i>Edessa bifida</i> (Say)	5	No	2 and 4	[14]
<i>E. meditabunda</i> (Fabricius, 1794)	4	No	—	In press, [25]
Subfamily Pentatominae				
Tribe Aeliini				
<i>Aelia americana</i>	7	No	No	[14]
Tribe Carpocorini				
<i>Carpocoris</i> sp.	6	No	3?	[14]
<i>Coenus delius</i> (Say, 1832)	6	5	4 and 6	[14, 15, 17–19]
<i>Cosmopepla bimaculata</i> (Distant)	5	No	?	[14]
<i>Euschistus euschistoides</i> , (Vollenhoven, 1868) (as <i>E. ssilis</i> Uhler, 1871)	6	5	4 and 6	[14, 15, 19]
<i>Euschistus heros</i> (Fabricius, 1798)	6	No	4 and 6	Present work
<i>E. ictericus</i> (Linnaeus, 1763)	6	5	4 and 6	[14, 15]
<i>E. inflatus</i>	6	5	4 and 6	[14]
<i>E. servus</i> (Say, 1832)	6	5	4 and 6	[14, 15, 25, 26]
<i>E. tristigma</i> (Say, 1832)	6	5?	4 and 6	[14, 15, 17, 18, 26]
<i>E. variolarius</i> (Palisot de Beauvois, 1805) (as <i>Pentatoma</i>)	6	5	4 and 6	[14, 15, 17, 18, 27–30]
<i>Holcostethus limbolarius</i> (Stål, 1872) (as <i>Peribalus</i>)	6	No	No	[14, 17, 18]
<i>Mormidea quinqueluteum</i> (Lichtenstien, 1796)	3	No	—	[1, 9]
<i>Oebalus poecilus</i>	4	No	—	[9]
<i>O.</i> (Fabricius, 1775) (as <i>Solubea pugnax</i>)	4	No	No	[14, 16, 26]
<i>O. ypsilongriseus</i> (De Geer, 1773)	4	No	—	[9]

TABLE 1: Continued.

Classification	No. lobes	Atypical meiosis	Polymegaly	References
<i>Trichopepla semivittata</i> (Say)	7	No	No	[14, 17, 18]
Tribe Chlorocorini				
<i>Arvelius albopunctatus</i> (De Geer, 1773)	6	4	3 and 5	[1, 14, 31]
<i>Chlorocoris complanatus</i>	7	5	4 and 6	In press
<i>Loxa flavicollis</i> (Drury, 1773) (as <i>L. florida</i> (Van Duzze))	7	5	4 and 6	[30, 32, 33]
<i>L. viridis</i> (Palisot de Beauvois, 1805) (as <i>L. picticornis</i> (Horvath, 1925))	7	5	4 and 6	[21, 32, 33]
Tribe Nezarini				
<i>Chlorochroa uhleri</i> (Stål)	6	No	3 and 5	[14]
<i>Nezara viridula</i> (Linnaeus, 1758)	6	4	3 and 5	[14, 34–39]
<i>Rhytidolomia saucia</i> (as <i>Chlorochroa saucia</i>) (Say, 1832)	6	No	3 and 5	[14, 40]
<i>R. senilis</i> (as <i>Chlorochroa senilis</i>) (Say, 1832)	6	3 and 5	No	[14, 40, 41]
Tribe Halyini				
<i>Brochymena quadripustulata</i> (Fabricius)	7	No	4 and 6	[14]
Tribe Pentatomini				
<i>Acedra hilare</i> (Say, 1832) (as <i>Nezara hilaris</i>)	6	No	No	[14, 15, 17–19, 34, 38]
<i>Adevoplitus longicomis</i> (Ruckes, 1958) (as <i>Pseudevoplitus longicomis</i>)	6	—	3 and 5	[21, 41]
<i>Banasa calva</i> (Say, 1832)	3	No	No	[14, 42–44]
<i>B. dimidiata</i> (Say, 1832)	3	No	No	[14, 43, 44]
<i>Thyanta calceata</i> (Say, 1832) [as <i>T. custator</i> (Fabricius, 1803)]	4	3?	No	[14, 31, 34]
<i>T. casta</i>	6	No	3 and 6	[14]
<i>T. custator</i>	4	3?	No	[14]
<i>T. perditor</i> (Fabricius, 1794)	3	No	—	[45]
Tribe Strachiini				
<i>Murgantia histriônica</i>	5	No	3 and 4	[14]
<i>M. histriônica</i> (var. <i>nigricans</i>)	5	No	3 and 4	[14]
Tribe Piezodorini				
<i>Piezodorus guildinii</i> (Westwood)	5?	No	No	[14, 46]

TABLE 2: Mean diameter of cells in diffuse stage and their respective nuclei and round spermatids of *Euschistus heros*, chosen randomly. The lateral bars indicate different groups with equality average. The values are given by Mean \pm standard deviation (Mean \pm SD). The unit utilized is micrometers (μm).

Lobes	Values (μm)		
	Cell	Nuclei	Spermatids
1	23.60 \pm 0.20	16.68 \pm 0.15	8.32 \pm 0.06
2	23.51 \pm 0.22	17.45 \pm 0.15	8.72 \pm 0.06
3	23.06 \pm 0.24	15.79 \pm 0.20	8.53 \pm 0.05
4	26.08 \pm 0.25	19.53 \pm 0.17	8.64 \pm 0.09
5	37.78 \pm 0.43	27.70 \pm 0.28	14.15 \pm 0.10
6	42.72 \pm 0.43	30.28 \pm 0.29	13.27 \pm 0.11
	$P < .0001$	$P < .0001$	$P < .0001$

When cells in prophase were silver impregnated, three round silver-positive bodies could be seen, two more impregnated with one bigger than the other, and the third lighter staining (Figures 2(j), 2(k)). It could also be observed that the lighter body may correspond to the heteropyknotic body revealed by the lacto-acetic orcein technique (Figures 2(a)–2(c)). In addition to the increased size of the cells in prophase of lobes 4 and 6, there were also several silver-positive bodies scattered throughout the nucleus (Figure 2(k)). With regard to metaphase I, two different behaviors were observed: lobes 1, 2, 3, 4, and 6 with impregnations in the cytoplasm and chromosomes (Figure 2(m)) and lobe 5 which showed negative silver impregnation (Figure 2(n)). During anaphase/telophase II, the positive silver impregnation behavior was similar for

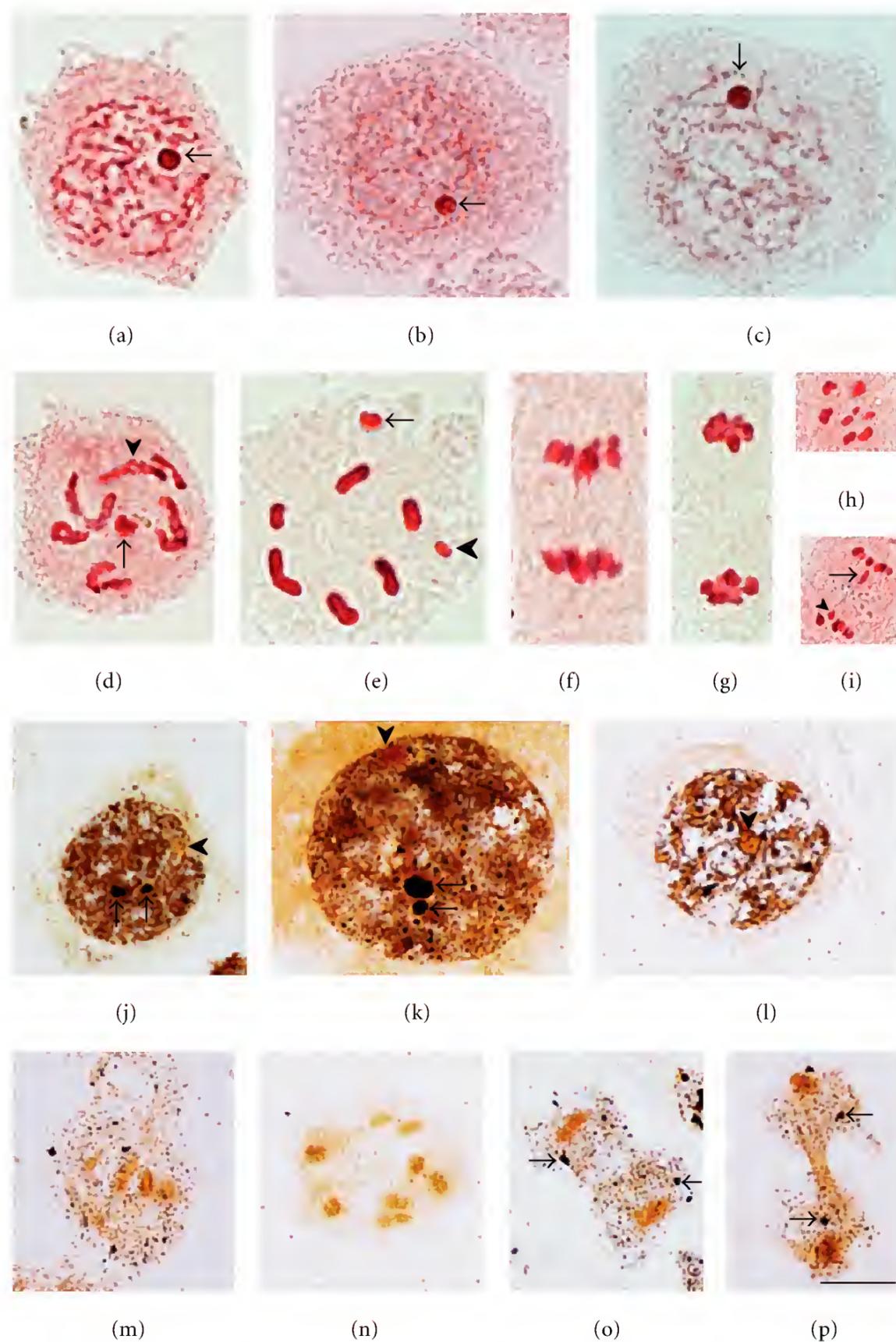


FIGURE 2: Meiotic cells stained with lacto-acetic orcein ((a)–(i)) and silver impregnated ((j)–(p)). ((a)–(c)) Early prophase I with heteropyknotic body in the periphery of the nucleus (arrows), with (a) belonging to lobes 1–3, (b) to 4 and 6 and (c) to 5; (d) diplotene/diakinesis showing a bivalent with interstitial chiasma (arrowhead) and heteropyknotic body (arrow); (e) final stage of diakinesis showing X and Y chromosome, respectively, arrow and arrowhead; ((f), (g)) anaphase/telophase I with regular segregation of chromosomes; (h) metaphase II showing the autosomes arranged in ring-shape and the sex chromosomes in the center; (i) anaphase/telophase II with lagging migration of the X chromosome (arrow) and the Y chromosome in the opposite part of the cell (arrowhead); ((j)–(l)) early prophase I showing two round silver-positive bodies (arrows) that are disorganized during the subsequent phases and one body less impregnated (arrowheads). Note that in prophase of lobes 4 and 6 (k), there are several silver-positive bodies scattered throughout the nucleus; (m), (n)) metaphase I with silver impregnation in the cytoplasm and chromosomes (m), differing from lobe 5, which has silver-negative metaphase (n); ((o), (p)) telophase II with nucleolar reorganization in both cell formations (arrows). Bar = 10 μ m.

all lobes, that is, nucleolar reorganization in both cells in formation (Figures 2(o), 2(p)).

3.3. Behavior of Cells during Spermiogenesis. A comparative analysis of the cells during the spermiogenesis of *E. heros*

stained with lacto-acetic orcein and silver impregnated showed that the behavior of cells in six testicular lobes was similar, and therefore, the results were presented together.

In the round spermatid, heteropyknotic staining was observed at the periphery and center of the nucleus, and

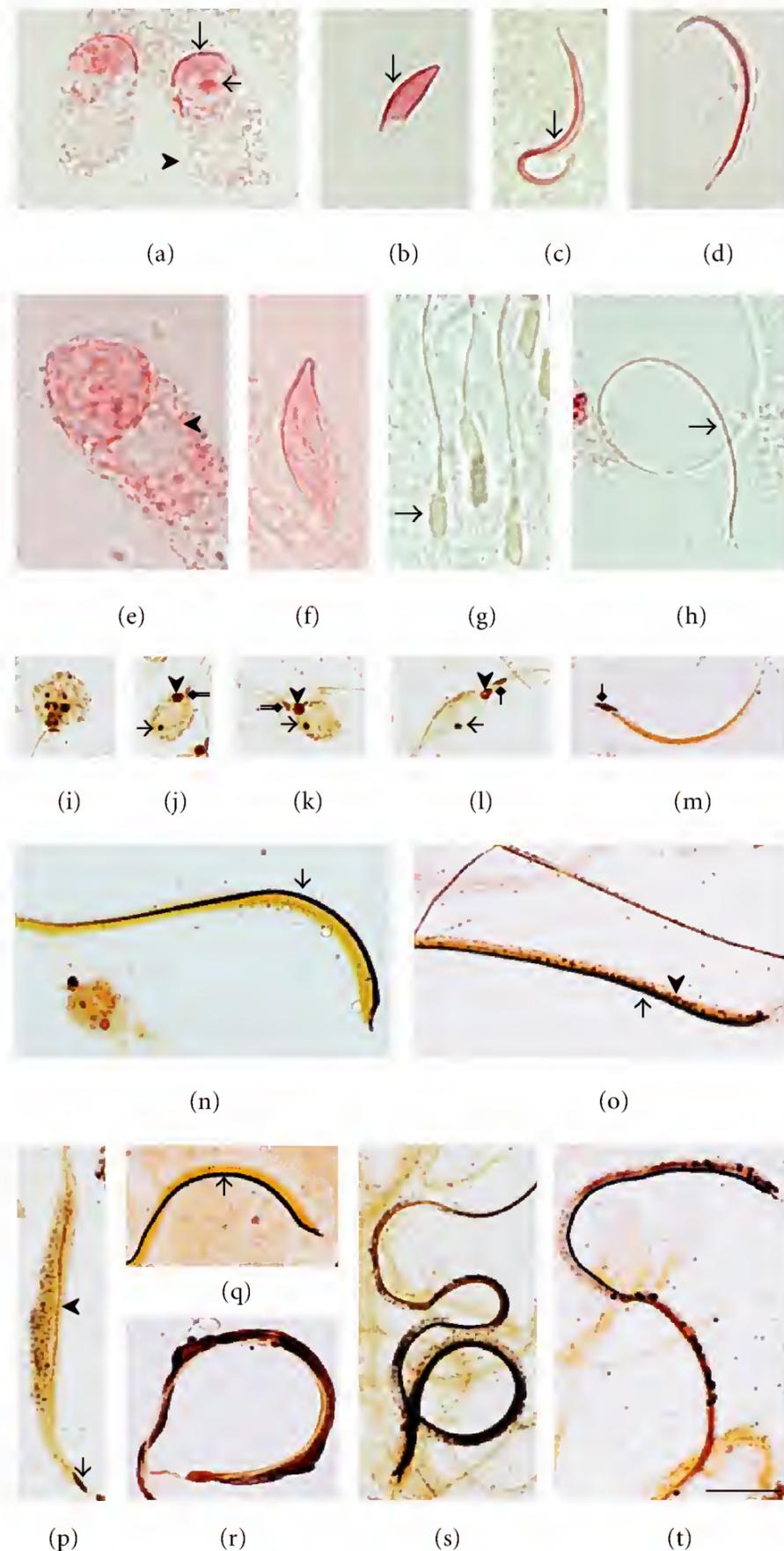


FIGURE 3: Spermio genesis cells stained with lacto-acetic orcein ((a)–(h)) and silver impregnated ((i)–(t)). (a)–(d) Development of spermatids in lobes 1–3 and 5. (a) Round spermatids showing heteropyknotic material at the periphery and center of the nucleus (arrows) and a vesicle in the posterior region (arrowhead); (b) spermatid in elongation with heteropyknotic material at the periphery of the nucleus (arrow); (c) in a following stage, the heteropyknotic chromatin is visualized on only one side (arrow); (d) in a later stage, it becomes indistinguishable; (e)–(h) spermatids of lobes 4 and 6; (e) round spermatids showing a vesicle in the posterior region (arrowhead); (f) spermatid in elongation; (g) spermatid at the later stage of development with chromatin stained weakly and in the posterior region with a protuberance (arrow); (h) sperm showing a thin heteropyknotic chromatin on one side of the head (arrow); ((i)–(m)) round spermatid with several silver-positive bodies; ((j)–(l)) during the subsequent phases were observed three silver-positive bodies: one rod-shape and lighter close to region of tail formation ((j), smaller arrow), one round intensely stained (arrowhead), both in the posterior region of the spermatid and another smaller than others (bigger arrow) that was visualized in the middle region of the spermatid ((k), (l)); (m) spermatid in development with only one silver-positive body in the posterior region of the nucleus (arrow); (n) spermatozoon of lobes 1–3 with continuous silver-positive staining in the posterior region of the head (arrow) and in lobe 5 (o) a continuous positive silver impregnation (arrow) and several small bodies in all the nucleus (arrowhead). ((p)–(t)) Spermiogenesis of lobes 4 and 6; (p) spermatid in development showing strong silver-positive body in the posterior region of the head (arrow), several small silver-positive bodies in the middle region and a line-shaped silver staining from middle to anterior region (arrowhead); ((q)–(t)) developed spermatids showing four silver-positive behaviors: (q) continuous and linear (arrow); (r) large and amorphous; (s) linear and throughout the extent of the head and several round and small silver-positive bodies and (t) with the same previous characteristics, but the silver-positive bodies are larger and smaller in number. Bar= 10 μ m.

a vesicle was seen in the posterior region (Figure 3(a)); in the elongated spermatid, the heteropyknotic material could be visualized at the periphery of the nucleus (Figure 3(b)). In a following stage of the development of the spermatid, the heteropyknotic chromatin could be observed on only one side (Figure 3(c)) while in a later stage becomes indistinguishable (Figure 3(d)). Despite the behavior of the spermatids in early development being similar among all six lobes, it was observed, in regard to the diameter of round spermatids, that the lobes could be grouped into two, by the size of cells (group 1= lobes 1–3 and lobe 5, group 2= lobes 4, and 6). The cells of group 1 (lobes 1–3 and 5, Figure 3(a)) were significantly smaller than those in group 2 (lobes 4 and 6, Figure 3(e)) ($P < .0001$) (results summarized in Table 2). The same development pattern of cells in spermiogenesis was observed of lobes 4 and 6 in relation to the other lobes; however, the heteropyknotic chromatin in early spermatids was less evident (Figures 3(e), 3(f)). In the developed spermatid, it is inconspicuous, where a protuberance appears in the posterior region of the nucleus (Figure 3(g)) and fine chromatin along the head of the sperm in formation (Figure 3(h)).

With the use of silver impregnation in the cells of spermiogenesis, round spermatids were observed with several round silver-positive bodies (Figure 3(i)), which moved to the posterior region of the nucleus, near the tail in formation (Figure 3(i)) and during the development of the spermatid (Figure 3(j)–3(l)), three silver-positive bodies were observed: one rod-shape and lighter located at the beginning of the formation of the tail, and two round ones of different sizes and intensities of impregnation. The largest and least impregnated was located in the posterior region of the spermatid's nucleus, close to the rod-shaped body, and the smaller and most impregnated was close to the anterior region of the spermatid's head (Figure 3(j)). With the development of the spermatid, apparently, these bodies remain in the same locations (Figure 3(j)–3(l)). During later stage of development there was only one silver-positive body in the posterior region of the nucleus (Figure 3(m)).

Differences were seen among the lobes in the pattern of silver impregnation in the developed spermatid. In lobes 1–3, this spermatid showed only a continuous silver impregnation in the posterior region of the head (Figure 3(n)), while in lobe 5, besides showing this continuous impregnation in the same region, also contained several small silver-positive bodies in the entire nucleus (Figure 3(o)). The developed spermatids in lobes 4 and 6 exhibited intense and elongated silver impregnation in the posterior region of the head, several small silver-positive dots in the middle region, and a line of silver staining from the middle region up to the anterior region of the head (Figure 3(p)). In the elongated spermatid, four different behaviors were noted in regard to distribution of silver impregnation: continuous and linear (Figure 3(q)); large and amorphous (Figure 3(r)); one linear region in the entire extent of head and several small dots, distributed throughout the head (Figure 3(s)); one linear region in the entire extent of head, several small dots, and larger round stained bodies distributed throughout the head (Figure 3(t)).

4. Discussion

The testes of Pentatomidae are divided by connective tissue into subunits called “lobes”. The most common number of lobes is seven, although there are variations among tribes and species [1, 50]. Based on the literature review regarding the number of lobes (Table 1), it could be seen that among the six species of the subfamily Asopinae, only one (*Euthyrhynchus floridanus*) has six testicular lobes, while the others have seven. In the other subfamilies, the distribution of lobes was found to be heterogeneous, ranging from four to eight lobes in the Discocephalinae, four to five in the Edessinae, and three to seven in the Pentatominae. At this moment, it is not possible to establish a direct relationship between the number of lobes and subfamily. Unfortunately, despite that the Heteroptera are composed of many species, very few have been studied in regard to this aspect. The subfamily Pentatominae, the most studied, showed wide variation with relation to the number of lobes. Therefore, a larger number of species should be analyzed to determine the ancestral number of lobes as well as to understand this diversity in the number of lobes.

Another feature found in species of the family Pentatomidae is the presence of a different lobe called *harlequin*. Schrader [21, 23, 32] was surprised that the forces of evolution have persisted with the *harlequin* lobes, a structure that produces heteropolyploid sperm not used in fertilization. They suggested that this sperm should provide additional nutrients, especially nucleoproteins for the development of eggs.

Atypical meiosis is another unusual feature that can be observed in the *harlequin* lobes. Table 1 shows the prevalence of these lobes in the subfamilies Discocephalinae, Edessinae, and Pentatominae. Among the six species of the subfamily Asopinae, no distinguishing characteristic was mentioned. However, there are no data in the literature with regard to the number of lobes and formation of atypical cells for the other subfamilies (Cyrtocorinae, Phyllocephalinae, Podopinae, and Serbaninae).

It could also be observed from the analysis of 50 species in Table 1 that 19 (38.0%) have atypical meiosis, of which 14 (73.7%) correspond to lobe 5, three (15.8%) to lobe 4, and three (15.8%) to lobe 3. It was found only one occurrence involving the lobes 3 and 5 in the same individual.

In addition to this atypical meiosis, the production of different-size sperm from the normal meiotic process also presents an evolutionary mystery. Although many species of insects have sperm showing polymegaly, which vary only in size and have a normal chromosome complement, all forms of sperm morphology may not be equally involved in fertilization [51]. The capacity of fertilization in sperm of different morphologies has not been demonstrated. At least for the large sperm, a nutrition argument similar to proposed to *harlequins* sperm [21] could be advantageous to the formation of multiple sperm morphology.

In Table 1, polymegaly was described in 25 (50.0%) species, of which 14 (56.0%) correspond to the lobes 4 and 6 simultaneously, that is, both lobes showed cells with polymegaly in the same individual, and only one (4.0%)

case not involving simultaneity in the formation of cells with polymegaly was found for lobes 3 and 4. These simultaneous events involving lobes 2 and 4, 3 and 4, 3 and 5 and 3 and 6 occurred in 1 (4.0%), 2 (8.0%), 5 (20.0%), and 1 (4.0%) cases, respectively.

When analyzing these two characteristics, the formation of atypical cells and polymegaly, it could be seen in Table 1, that when there are two lobes in the same species showing polymegaly, these lobes usually flank another lobe, as occurs in the species *Antiteuchus tripterus*. Especially for lobes 4 and 6, in which 12 simultaneous events were observed, only one did not show lobe 5 (flanking lobe) with the formation of atypical cells. This exception corresponds to species *Euschistus heros*, analyzed in this study. Although there was no formation of atypical cells in lobe 5, differences were found in the diameter of cells in prophase and in the concentration of silver impregnation, mainly in the sperm, suggesting the necessity to explore the true function of the sperm in this lobe, using other approaches.

Table 1 indicates that the minimum number of lobes found for the family Pentatomidae is three, as occurs in the species *Mormidae quinqueluteum* [1] and *Banasa calva* [43]. When a species possessed lobes forming differentiated cells (atypical or showing polymegaly), there was also the presence of at least three lobes demonstrating the production of fertile sperm, as in species *A. tripterus* [2] which has six lobes, three lobes responsible for producing typical sperm and the other three producing differentiated cells. Therefore, it could be inferred from the data obtained to date, that three is the minimum number of lobes needed to produce fertile sperm. Another feature that could be shown among these analyzed species described in Table 1, was that lobe 1 invariably produces fertile sperm, that is, with meiosis and production of typical sperm, while the only case of production of differentiated cells involving lobe 2 occurred in the species *Edessa bifida* [14].

When formation of differentiated cells occurred, this was related to lobes 4 to 6. This may suggest a preadaptation to the formation of nonfertile sperm in these lobes. However, with the data obtained so far, it has not been possible to characterize a distribution pattern of evolution of nonfertile sperm between the subfamilies or tribes.

The species *E. heros* showed cells in the diffuse stage in lobes 4 and 6, significantly larger than in other lobes, and therefore considered polymegaly. Morphologically, lobe 5 was narrow, in contrast to that in the species *A. tripterus* studied by Souza et al. [2]; lobes 4 and 6 produced cells with polymegaly, but lobe 4 was the narrowest in this species, while the lobe 5 (*harlequin* lobe) was disproportionately larger, forming a slightly twisted testis.

The differences found in regard to heteropyknotic material and silver impregnation in the lobes of *E. heros* showed that polymegaly is accompanied by differences in the behavior of these structures, especially when comparing spermiogenesis. Intensive silver impregnation and no condensed chromatin in spermatids and sperm may reflect intense metabolic activity and suggest a change in function in the sperm of these lobes.

Regarding cytogenetics, in Heteroptera, there is generally a close association of the chromocenter with the nucleolus in prophase I. Souza et al. [9] found an unusual morphology of the nucleolar body, called mushroom, which supports the association of the chromocenter, probably the more stained portion (the cap) with the nucleolus (the stem). The species of this study showed a heteropyknotic body when stained with lacto-acetic orcein, and when silver impregnated showed two darker bodies which were probably the nucleolar bodies, and a lighter one which was probably the chromocenter. It is interesting to note that the chromocenter may not be associated directly with the nucleolus in *E. heros*, diverging from other species of the Pentatomidae that show this association [9].

Thus, this paper provides insights into evolutionary process of the formation of nonfertile sperm, once this field lacks a more accurate exploration of such theme by different approaches.

Acknowledgments

Special thanks go to Dr. Sonia Maria Oliani of the Department of Biology of IBILCE/UNESP for the opportunity to capture cell images. Dr. Jocélia Grazia of the Department of Zoology of Universidade Federal do Rio Grande do Sul and Dr. Aline Barcellos of the Museu de Ciências Naturais of Porto Alegre, Brazil who helped with specimen identification. Research supported by Foundation for the Development of Sao Paulo State University (FUNDUNESP), Foundation for Research Support of the State of São Paulo (FAPESP) and National Counsel of Technological and Scientific Development CNPq.

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Research Article

Morphology of the Prosternal Glands of *Heliconius erato* (Lepidoptera: Nymphalidae)

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Received 9 April 2010; Accepted 22 June 2010

Academic Editor: Coby Schal

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Two types of exocrine glands, located midventrally on the prosternum, are described for the larval stage of *Heliconius erato* (Linnaeus) (Lepidoptera: Nymphalidae). The first type, formed by a single, flat secreting pouch, opens as a transverse slit on the anterior portion of the prosternum. The second, composed of a pair of ellipsoid secreting units, opens laterally by fine ducts on the distal portion of a cone-shaped sac, which is protruded by hemostatic pressure posteriorly between the prothoracic legs. The morphology of these glands is described and illustrated by light, scanning, and transmission electron microscopy. The varied terminologies adopted in the literature for describing these glands are discussed, and we propose a single term, prosternal glands.

1. Introduction

Heliconian butterflies have attracted the attention of biologists for many years, in particular regarding their close association with passion vines, their main host plants in the Neotropics (reviewed in [1–3]). All life stages of these butterflies are supposed to be unpalatable to vertebrates [3, 4]. Several cyanogenic glycosides have been associated with this toxicity, and could be either sequestered or modified from the host plants, or alternatively synthesized *de novo* by the larvae [4, 5]. The existence of specialized larval body structures, if any, where such chemicals are processed is largely unknown.

Chemicals associated with glandular secretions identified for these butterflies have been related to communication at mating [6–9]. The existence of exocrine glands has been reported for the adults, but not for the immature stages of heliconian butterflies. Adult males have modified scent

scales (androconia) located on the hind wings [10–12], as well as typical, multicellular exocrine glands within the genitalic valvae [13, 14]. Females have a pair of dorsal abdominal glands on the eighth tergum, which are usually associated with stink clubs (auxiliary glands) that are attached to a lateral fold on the posterior margin of the eighth sternum [10, 12–15]. These abdominal glands were originally presumed to be associated with defense in both sexes [10, 16]. Lately, however, they have also been related to the production (males) and storage and dispersal (females) of antiaphrosidiacs [8, 17, 18].

Prosternal glands are found in the larval stage in certain lepidopteran families, including Nymphalidae [19–22]. There is no consensus regarding their precise position in the larval body, except that they are located midventrally just posterior to the head, on either the cervix or prothorax. The terminology that has been adopted to describe these glands is also inconsistent. They show considerable variation

regarding their glandular units; and the corresponding homologies among lepidopteran families, if any, have not been established [21, 23]. Additionally, their function has been little explored; in some notodontid moths, these glands secrete a fluid of defensive nature [24–26]; and in some riodinid butterflies, they have been recently associated with larval-ant communication [27, 28]. Our observations suggest that they are frequently found in all instars of heliconian butterflies. Their description, which is the main objective of the present paper, is a prerequisite for future studies on the physiology and behavior involving these glandular structures, in order to fully understand their chemistry and function.

Heliconius erato (Linnaeus) (Lepidoptera: Nymphalidae) is one of the most common and well-studied heliconian butterflies in southern Brazil, where it has been used as a model in studies of evolutionary ecology (e.g., [18, 29–32], and references therein). The external morphology of its immature stages has been described in detail elsewhere [33], but the prosternal glands were not included in that study. Here, we describe and illustrate them based upon light, scanning, and transmission electron microscopy. We show that in *H. erato*, these glands are not simple eversible structures located within the integumentary infold, but are a glandular complex consisting of an assemblage of morphologically distinguishable glandular units. In addition, we discuss the limitations of the terminology that has been generally applied to these glands, and propose an appropriate unified term—prosternal glands.

2. Material and Methods

The study was conducted with larvae hatched from eggs collected from a *Heliconius erato phyllis* (Fabricius, 1775) outdoor rearing insectary at the Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS. The rearing procedures have been described in detail elsewhere [30]. Adults were fed daily with a mixture of commercially available honeybee pollen (AGA), honey (AGA), and distilled water (ratio 2 : 1 : 7). *Passiflora suberosa* (Linnaeus) (Passifloraceae) plants were grown within the insectary for oviposition. Under laboratory conditions, larvae were separately reared on intact *P. suberosa* shoots in bottles of water protected by a fine-mesh cloth [34]. Instars were identified by their head-capsule width [33]. To make sure that molts were not overlooked, larvae were gently marked with small dots of enamel paint (Testor) on the dorsal part of the ninth segment [35].

The gross morphology of the prosternal gland was studied primarily on fresh material. For dissections, the material was immersed in Ringer's solution and temporarily stained with methylene blue. Specimens previously fixed with Dietrich's fluid and preserved in 75% ethanol were also used. Prothoracic ventral portions (5 per instar) were dissected, cleared in a 10% potassium hydroxide solution (KOH), and slide-mounted in glycerin jelly. The structures were observed under a Leica M125 stereomicroscope, and

photographed with an attached Sony DSC-H10 digital camera. An attached ocular grid was used to aid in the drawings.

For histological and cellular studies by light microscopy, fresh prothoracic ventral portions ($n = 10$ per instar) were dissected and fixed with Bouin's fluid. For sectioning, a standard paraffin embedding method was employed. Sections $7\ \mu\text{m}$ thick were obtained with a Leica RM2155 microtome. The sections were stained with Gill's hematoxylin and eosin and mounted in Canada balsam.

The integumentary ultrastructure of the prosternal glands was studied at the UFRGS Electron Microscopy Center. For scanning electron microscope analyses, the specimens were dehydrated in a Bal-tec CPD030 critical-point dryer, mounted with double-sided tape on metal stubs, and coated with gold in a Bal-tec SCD050 sputter coater. Specimens were examined and photographed in a JEOL JSM5800 scanning electron microscope. For transmission electron microscopy, the specimens were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer. Next, the material was washed in the same buffer, postfixed with 1% osmium tetroxide for 1 h, dehydrated in an ascending series of alcohol and acetone, preembedded in epoxy resin and acetone (1 : 1), and finally embedded in epoxy resin (Durcupan ACM, Fluka). The material was then polymerized for 3 days at 68°C . Semithin sections ($1\ \mu\text{m}$) were cut with a Leica UCT ultramicrotome, using glass knives, and stained with 1% toluidine blue in 1% sodium tetraborate. Ultrathin sections ($70\ \text{nm}$) were obtained with the same ultramicrotome, employing a diamond knife (Diatome). These sections were stained with 2% uranyl acetate, followed by 1% lead citrate [36]. The ultrathin sections were examined using a JEM 1200 EX II transmission electron microscope.

3. Results

The glands are located ventrally on the prosternum (Figure 1). There are three units and two morphological types of glands, hereinafter called impair and paired glands (Figure 2(a)). The first type, composed of a single, flat secreting pouch, opens as a transverse slit in the anterior portion of the prosternum. The second, composed of a pair of ellipsoid secreting portions, opens laterally through fine ducts in the distal portion of each side of a conical integumentary sac (Figures 1(b) and 2(b)). By hemostatic pressure, the sac can be protruded posteriorly between the prothoracic legs (Figures 1(a) and 2(b)). The sac containing the attached paired glands is inverted and contracted back into the thoracic hemocoel by a pair of retractor muscles (Figure 2, Rp1).

Both types of glands are found in all larval instars, and apparently show negligible changes in shape during ontogeny. Except for the first instar, when they are small, the secretory portion of the impair gland is not everted (Figures 3(c) and 4(c)). The impair gland as a whole is pressed down by hemostatic pressure and pulled up by the action of an additional pair of retractor muscles (Figure 2; Rp2). When

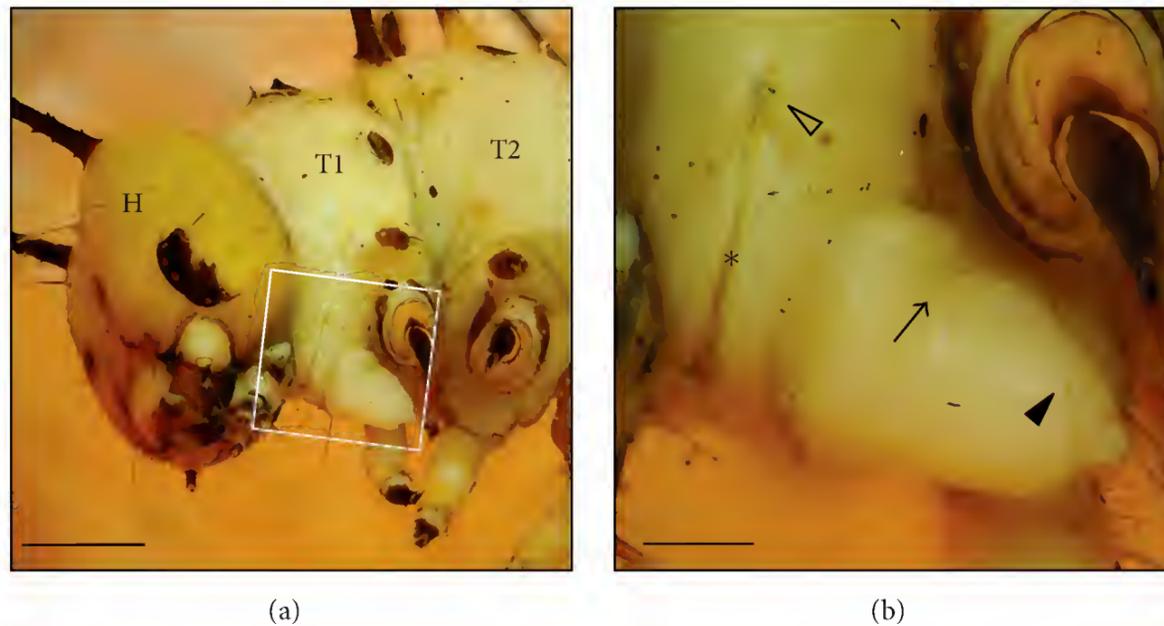


FIGURE 1: Latero-external view of the larval prosternal glands of the fifth instar of *Heliconius erato phyllis*, under light microscopy. (a) Midventral location in relation to the body (in rectangle). (b) Lateral view in detail (enlarged rectangle shown in (a)), indicating the openings of the impar (asterisk) and left paired (arrow) glands, and the insertion positions of the corresponding retractor muscles (open and closed arrowheads, respectively). (H) head; (T1) prothorax; (T2) mesothorax. Scale bars = 200, 100 μm , respectively.

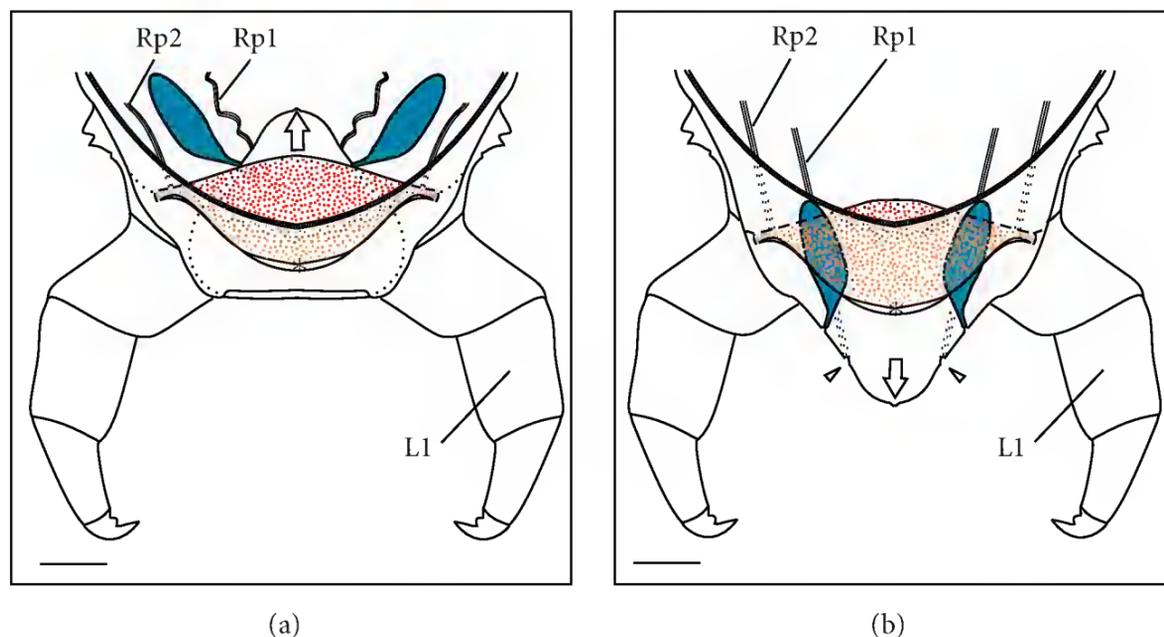


FIGURE 2: Schematic representation of fifth instar larval prosternal glands of *Heliconius erato phyllis*, from an antero-dorsal internal view, when in situ (a) and during extrusion of the prosternal sac (b). Impair and paired glands are shown in stippled red and solid blue, respectively. Open arrows indicate the direction of the internal hemostatic pressure and the respective movement of the prosternal sac. Impair and paired glandular openings are indicated by one asterisk and open arrowheads, respectively. (L1) prothoracic leg; (Rp1) proximal retractor muscle of the prosternal sac; (Rp2) distal retractor muscle of the prosternal sac. Scale bar = 50 μm .

protruded in the first instar, it appears as a bud, showing a medially located, little-differentiated slit that divides its secretory portion transversely into two lips (Figure 4(c)). The secretory portions of the paired glands are not everted from the sac itself in any instar (Figure 2(b)).

The openings of the paired glands are simple, each appearing from the outside of the everted sac as a small, delicate infold (Figure 1(b)). In contrast, the opening of the impar gland is proportionately large and elaborate. Its margin shows several sensillum-like structures (Figures 4(a) and 4(b); Se), which function remains unknown. In specimens fixed in Dietrich's fluid and preserved in ethanol, the secretion of the impar gland is yellowish, appearing solidified and in considerable amounts as small individual

fragments, on the cuticular surface of the secreting epithelium. Under scanning electron microscopy, this secretion appears as small spots that exude from many microcisterns that cover its cuticular surface (Figure 4(d)). The secretion of the paired glands is amorphous and acidophilous, and is stored in their central spherical lumen (Figures 3(e) and 3(f)).

The secreting nature of the two types of gland is clearly shown by the columnar shape of their epithelium cells (Se), which contrasts with the flat cells that form the remaining, nonsecreting epithelium (Ne) of the sac wall (Figure 3). The impar gland is formed by a simple, low-columnar, glandular epithelium. The secretion is expelled directly by the cuticle, through cisterns on its external

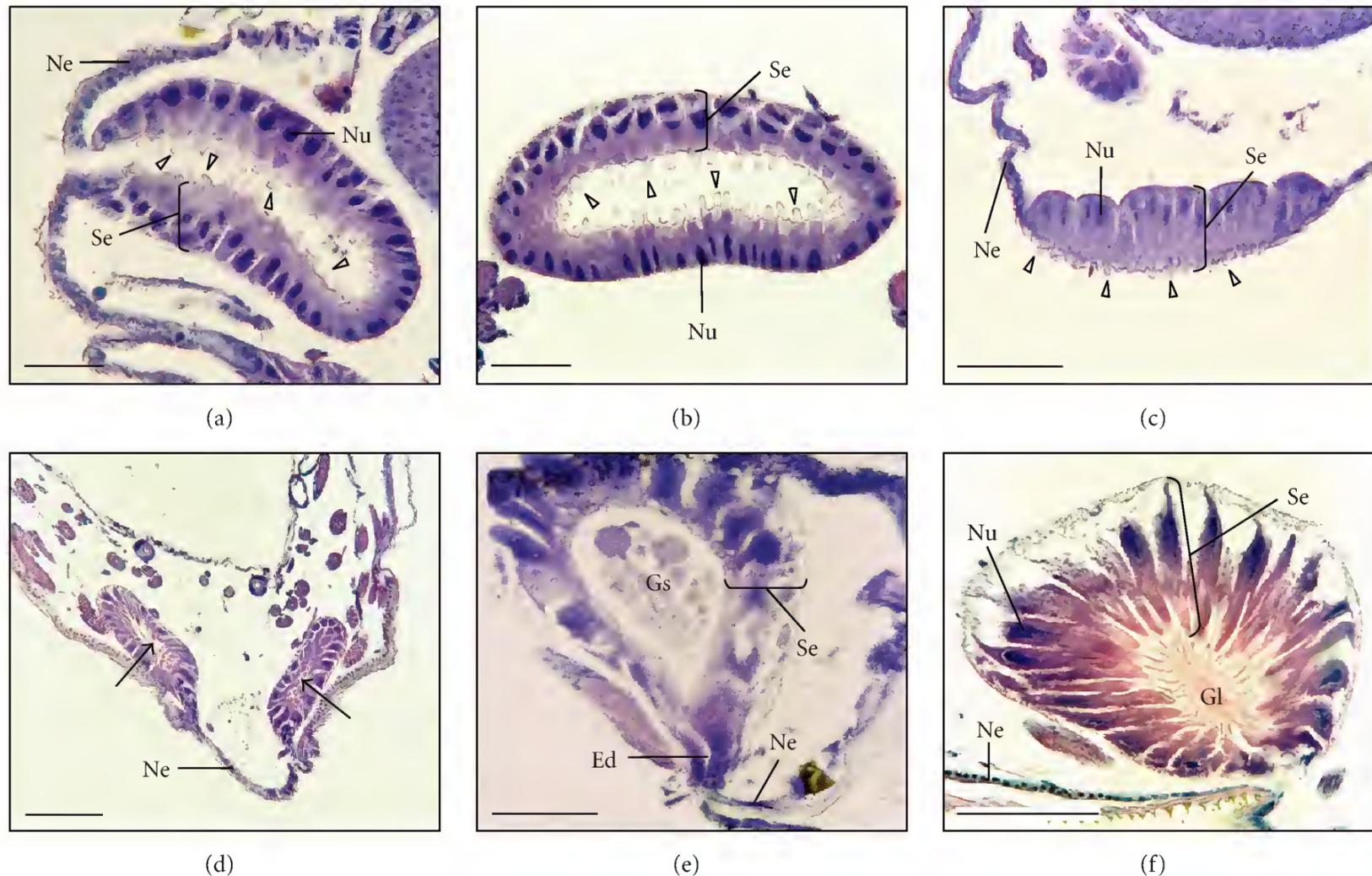


FIGURE 3: Histological sections of larval prosternal glands of *Heliconius erato phyllis* under light microscopy. (a) impair gland of second instar, longitudinal; (b) impair gland of third instar, cross section; (c) impair gland of first instar, longitudinal; (d) paired glands (arrows) of third instar, longitudinal; (e) paired gland of fourth instar, longitudinal, near the external opening, showing secretion in the lumen; (f) paired gland of fifth instar, cross section, at the middle of the secretory portion, showing the convergent distribution of secreting cells in relation to the lumen. Open arrowheads indicate the excretory cisterns in the impair gland. Ed: excretory duct; Gl: glandular lumen; Gs: glandular secretion; Ne: nonsecretory epithelium; Nu: nucleus; Se: secretory epithelium. Scale bars = 50, 50, 50, 150, 60, and 150 μm respectively.

surface (Figures 3(a), 3(b), 3(c), 4(c), and 4(d)). The apical portion of the epithelium secretory cells of this gland shows numerous infoldings (= microvilli) into which the secretion is conducted intracellularly prior to excretion (Figure 5(a)). The nuclei of the secretory cells are elongated and basally located, and contain evident heterochromatin and nucleoli. The distal portion of their cytoplasm shows a well-developed granular endoplasmic reticulum, Golgi apparatus with dilated cisterns, and abundant secretion-containing vesicles (Figures 5(c) and 5(d)). The cuticular surface of the impair gland cisterns is irregular, formed by microtrabeculae that delimit alveoli of variable size and shape (Figure 4(d)). The cisterns decrease in number and increase in size per cell in later instars (Figures 5(a) and 5(b)).

The paired prosternal glands are formed by a high-columnar, glandular epithelium with concentrically arranged cells (Figure 3(f)). The cells have an acidophilous cytoplasm containing a conspicuous, basally located nucleus with evident heterochromatin and nucleoli. An excretory duct (Ed) is formed in these glands (Figure 3(e)), through which the acidophilous secretion is excreted. The excretory ducts are formed by a cubical epithelium, which contrasts with that of the secretory portion of the gland and the flattened part that forms the sac wall as a whole.

4. Discussion

The general morphology of the prosternal glands described herein may not be unique. In the entomological literature, the prosternal glands found in lepidopteran larvae are usually poorly described, as single sacs that are everted by hemostatic pressure (e.g., [19, 20, 37, 38]). Our results clearly showed that they are not located within a single integumentary sac that contains a secreting epithelium that is everted by hemostatic pressure; in other words, the sac is not the gland itself. In *H. erato*, the prosternal glands form a glandular complex, composed of three glandular units of two distinct morphologies. The impair type is located outside the sac, and is not everted. In addition, we demonstrated that although the existing sac itself is eversible, the paired glands located inside are not. Percy and MacDonald [24] arrived at a similar conclusion regarding the internal complexity of these structures in *Schizura concinna* (Notodontidae). However, the two glandular units that are found in this species differ from each other in their general morphology, being interconnected by an interglandular neck, and thus their final product is a mixture of secretions. This is not the case for *H. erato*, where the impair and paired gland types open independently to the outside. Also, their excretions differ

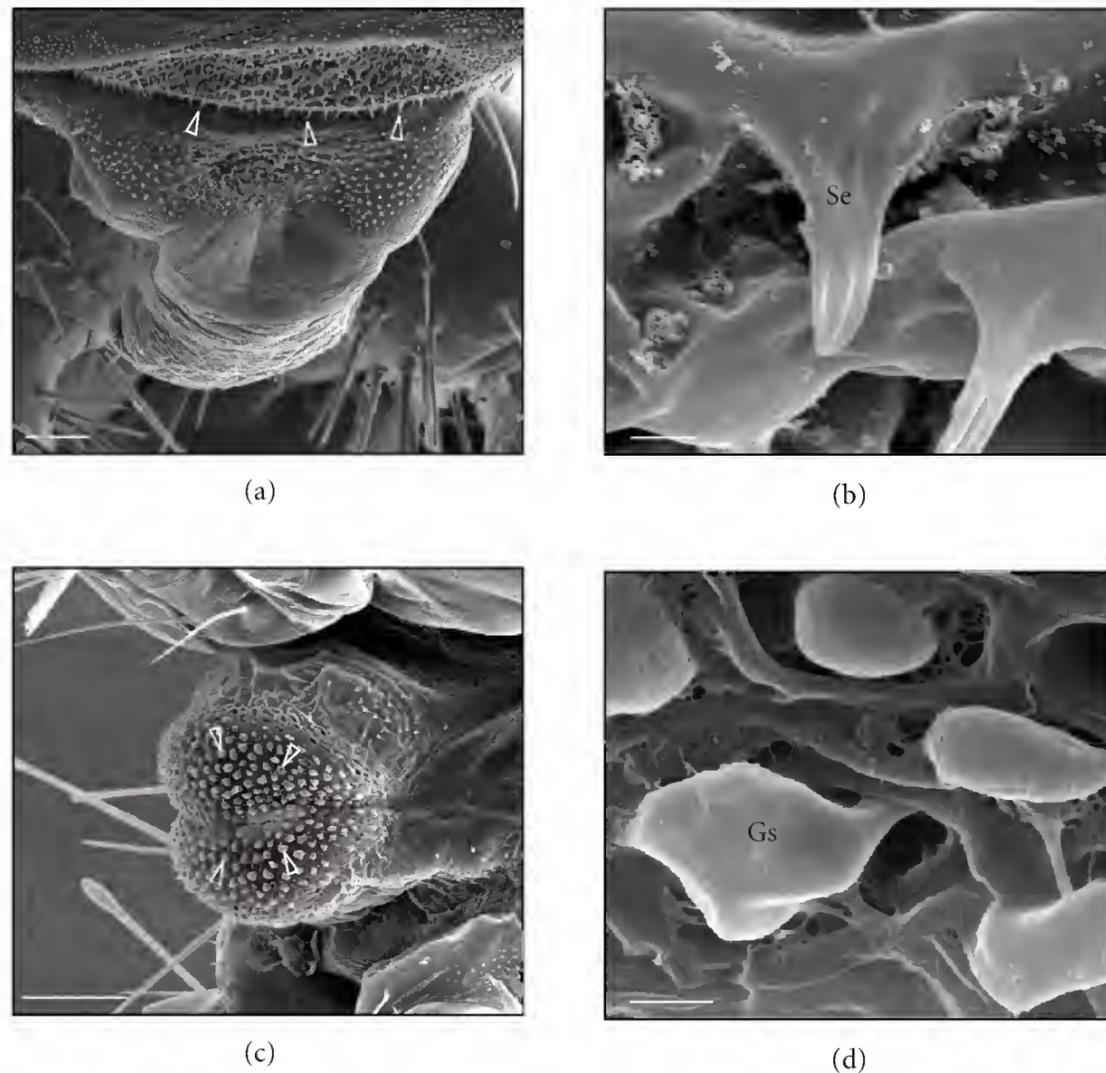


FIGURE 4: Impair prosternal gland of *Heliconius erato phyllis* under scanning electron microscopy. (a) Detail of the transverse opening slit in a fifth instar, anterior view; (b) sensilla on the opening margin (indicated by open arrowheads in (a)); (c) detail of a protruded gland in a first instar, latero-ventral view; (d) cisterns in detail, showing exudation (indicated by arrowheads in (c)). Gs: glandular secretion; Se: sensillum. Scale bars: 100, 5, 100, 2 μm , respectively.

in physical consistency and color, and probably also in the amount of secretion produced. The existence of noneversible units of the prosternal glands was previously detected in *Spodoptera frugiperda* (Noctuidae) [39]. Again, when the general description given for this species is compared with that of the present paper, it is clear that although the glands are also located in the prosternum, the tubular glands described are not homologous to the prosternal glands of *H. erato phyllis*, where such tubules are absent. These aspects should be taken into account in the search for homologies among the prosternal glands of different lepidopteran taxa, and equally importantly, in identifying their secretions. In the case of *H. erato phyllis*, the existence of differences in chemical constitution between the secretions of the impar and paired glands is very likely, since they differ in color and physical consistency.

The function of the prosternal glands also remains unknown for *H. erato phyllis*. They might be involved in defense, as previously suggested for notodontids [24–26]. Larvae of *H. erato phyllis* are solitary feeders, behave aggressively toward other heliconian larvae, and are cannibalistic [1, 40, 41]. The early stages of heliconians in general are preyed upon by ants, against which they have developed complex defense mechanisms [42–44]. When the anterior portion of its body is gently touched, the larva of *H. erato phyllis* assumes a defensive posture, moving its head and

elevating its front legs, and protruding the sac containing the paired prosternal glands.

At the microscopic level, the gland cells studied here are similar to those described for *S. concinna* [24] and *Abanante hylonome* (Nymphalidae) [26]. They fit into type I in the classification of Noirot and Quenedey [45, 46], where the gland cells are in direct contact with the cuticle. We found no perforations in the cuticular layers of cells of the impar type, and therefore we hypothesize that the secretion diffuses through the cuticle, as in the defensive glands of many other insects [24, 45]. The presence of microvilli on the apical surface of their cells, together with the abundant secretion vesicles, lends further support to this suggestion. Microvilli facilitate transport of secretions from the basal portion of the cell into the cuticle. In the case of *H. erato phyllis*, transport might be facilitated in the central area of the cisterns, where the corresponding cuticular layer is thinner and the secretion accumulates on the glandular surface.

Revised Terminology. Several terms have been used more or less interchangeably to identify the glands described herein, including “cervical” [21, 27, 28], “neck” [26], “ventral” [37], “thoracic” [24, 38], “prothoracic” [25, 47, 48], “eversible” [19, 20] gland(s), and “adenosma” [21, 49]; and also in combination (e.g., “ventral prothoracic” [50]). “Cervical”

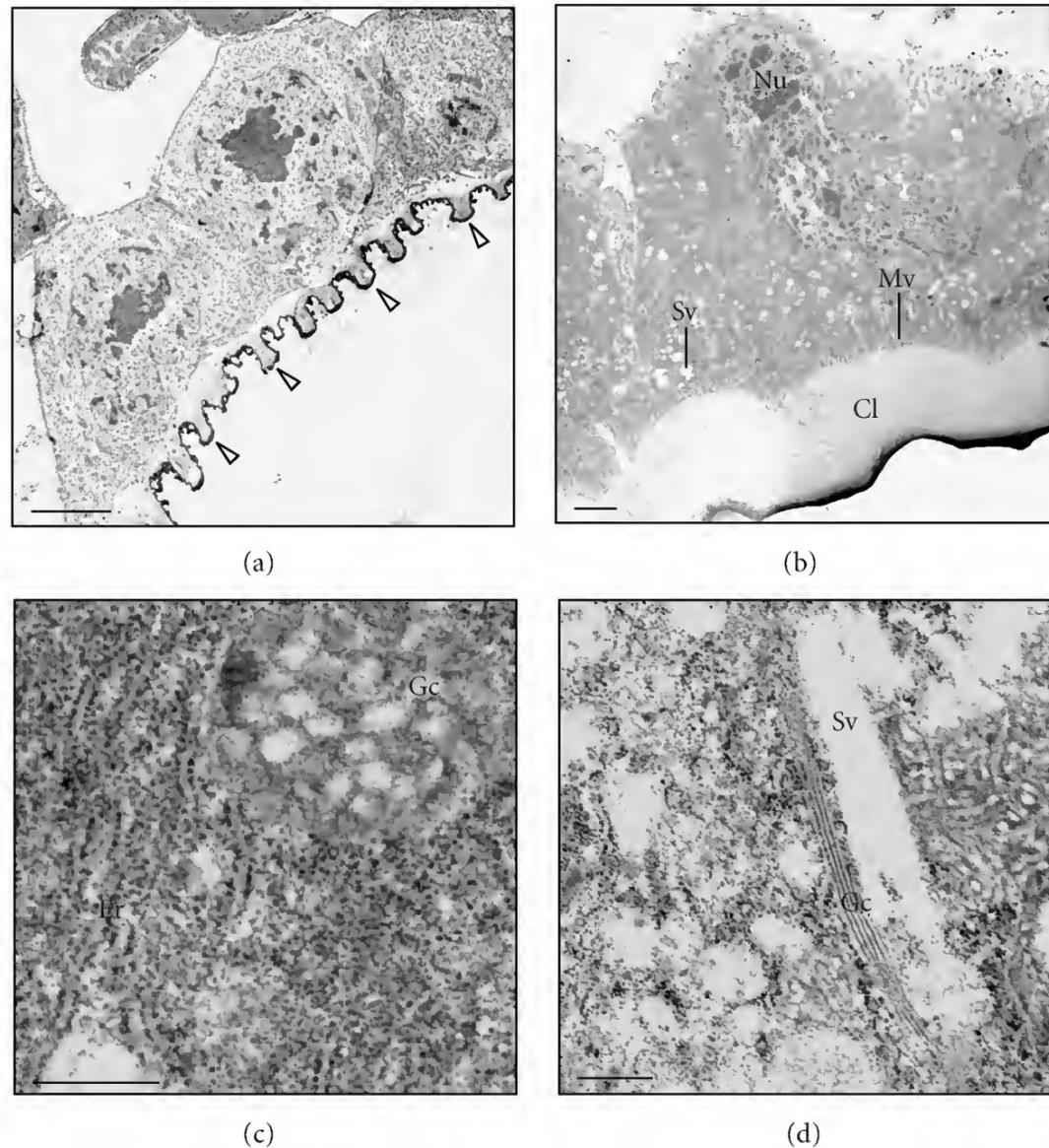


FIGURE 5: Transmission electron micrographs of the impair prosternal gland of *Heliconius erato phyllis*. (a) longitudinal section of secreting cells, showing irregular external sculpture of cuticle (open arrowheads) in a first instar; (b) longitudinal section of secreting cells of a fifth instar, with basal nucleus, several vesicles containing secretion in the cytoplasm, and abundant microvilli associated with the cuticular layers; (c) detail of the cytoplasm of cells from a fifth instar, showing well-developed rough endoplasmic reticulum and Golgi complex; (d) detail of the cytoplasm of cells from a fifth instar, showing numerous secretion vesicles. Cl: cuticular layers; Er: rough endoplasmic reticulum; Gc: Golgi complex; Nu: nucleus; Mv: microvilli; Sv: vesicle containing secretion. Scale bars: 2, 2, 0.4, 0.4 μm , respectively.

and “neck” are not appropriate to describe these glands, because they are situated within integumentary infolds that are located midventrally, not on the cervix (= neck, the membranous region located between the head and the prothorax [51]), but rather on the prothorax. “Ventral”, “thoracic”, and “prothoracic” are ambiguous, leading to confusion regarding the specific body site where these structures are located in relation to the body tagmata, thoracic segments, and prothoracic sclerites, respectively. In particular, the usage of “prothoracic” may lead to confusion with the “osmeterium” glands [52], which are also located on the prothorax, but dorsally on the tergum (= pronotum). Moreover, this term has been traditionally adopted in the entomological literature for the endocrine glands involved with hormone secretion (ecdysone), which are located on the same thoracic segment [53–55]. The term “eversible” is also inappropriate, because, as described in this paper, the secretory units of the glands themselves are not always everted. “Adenosma” suggests a gaseous nature of their secretion and associates the sense of smell with it (in Greek: adeno = gland; osma = odor), which cannot be generalized for all situations, for example in the case described herein. The use of the composite term

“ventral prothoracic” is redundant (= prosternal). Thus, we propose “prosternal glands” as best suited to describe this complex assemblage of glandular units (as a broad definition, *sensu* Noirot and Quenedey [42]). For *H. erato*, we propose the lexicon paired and impair prosternal glands to demarcate the two types. The term prosternal glands relates them to the prothoracic sternum (= prosternum [56]), the body site where they are in fact located. Also, this term does not imply any particular number or shape of their secretory units, nor the chemical nature and function of their secretion.

Acknowledgments

Thanks are due to Denis Santos Silva and Kim Ribeiro Barão (Universidade Federal do Rio Grande do Sul) for helping to edit the figures, and to Janet W. Reid for revising the English text. The authors also wish to thank two anonymous reviewers for significant improvements in the final version of the paper made possible by their comments. Part of this study was supported by CNPq (Grant no. 304458/2008-2 to Gilson Rudinei Pires Moreira).

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Research Article

Effect of Carcass Size on Feeding Modes of Larvae of *Nicrophorus quadripunctatus* Kraatz (Coleoptera: Silphidae)

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Received 11 March 2010; Revised 9 June 2010; Accepted 7 July 2010

Academic Editor: David Denlinger

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In the parental care of burying beetles of *Nicrophorus*, the role of males has not been clearly elucidated. To test our hypothesis that the investment in resource manipulation by males influences the feeding of larvae by males, we investigated parental efforts of *N. quadripunctatus*. On the small carcasses, the time spent on resource manipulation by males was short, and the males left the carcasses without feeding the larvae (maternal feeding). On the large carcasses, the males spent a long time on resource manipulation, and the male participated in the feeding of larvae (biparental feeding). This suggests that one of the reproductive roles of males in the absence of predators and/or competitors is resource manipulation, and the paternal efforts change depending on carcass size. A longer time spent on resource manipulation by males may be a trigger for the males to participate in the feeding of larvae on large carcasses.

1. Introduction

Burying beetles of *Nicrophorus* (Coleoptera: Silphidae) are subsocial insects exhibiting an elaborate system of biparental care. The parents prepare a small vertebrate carcass as a food resource for their offspring and defend the carcass from predators and/or competitors. The parents enter the carcass, remove its hair or feathers, shape the carcass into a brood ball, and deposit anal or oral secretions around it. This series of behaviors is known as resource manipulation. Additionally, the parents feed their larvae by regurgitating food and repair the crypt as needed [1–3].

The breeding schedule after pair formation is divided into the resource manipulation period and the feeding period. Although previous studies have focused on feeding behaviors to the brood, resource manipulation prior to feeding has been little examined to date.

It was thought that one important role of males in this biparental care was their defense against predators and/or competitors, supported by the fact that the presence of both parents on a carcass decreases the risk of a takeover by other beetles (*N. orbicollis*: [4, 5]; *N. defodiens*: [6]). However, many

studies have found that in the absence of competitors under benign conditions in the laboratory, there is no evidence that the participation of a male in the feeding of larvae confers any advantages on the survival or growth of the brood [1, 7–10]. Some studies reported that female burying beetles attacked males that remained during the feeding period [3, 11]. Thus, the adaptive significance of the presence of a male and the contribution of feeding by a male to larval development and growth have not yet to be fully elucidated.

Xu [11] reported that resource manipulation in *N. quadripunctatus* was carried out by both females and males but that thereafter, there appeared to be two modes of feeding: biparental feeding, in which larvae were fed by both a female and a male, and the uniparental (maternal) feeding, in which larvae were fed by only a female. To elucidate how these two modes of feeding occur, it is necessary to analyze male and female behaviors during the resource manipulation period prior to the feeding of larvae. We hypothesized that the investment in resource manipulation by males influences the feeding of larvae by males.

In the present study, to test our hypothesis, we analyzed the behaviors of *N. quadripunctatus* males and females in

the resource manipulation period under different amounts of resources, and the following questions in particular were addressed. (1) Do differences in the amount of resources influence the resource manipulation of males and females? (2) Do differences in resource manipulation behaviors influence a feeding mode? (3) Do female attacks against males influence the participation of a male in the feeding of larvae?

2. Materials and Methods

Bait trap surveys by Kouge [12] showed two peaks in the appearance of *N. quadripunctatus* adults in northern Kyushu, Japan, in April to May and September to October. Thus, the beetles were collected using traps baited with chicken at Mt. Hinokuma, Kanzaki, Saga (33°20'N, 130°21'E) and at Mt. Kinryu, Saga (33°20'N, 130°18'E), in May and September, 2004, 2005, 2006, and 2008. The collected beetles were reared individually in plastic cups (10 cm in diameter and 4 cm in depth) with a little soil in a laboratory at 20°C under a 12 hours light and 12 hours dark (12 L12D) photoperiod condition. Each beetle was supplied with approximately 0.2 g of chicken every 3 days as food.

All experiments were carried out in a laboratory at 20°C under a 12L12D photoperiod condition. We used chicken pieces because it was impossible to prepare exact amounts of mouse carcasses. The chicken pieces were wrapped in tissue paper in imitation of the skin of carcasses.

Eleven, 26, and 10 male-female pairs were individually released into 11, 26, and 10 containers (7 cm in diameter and 14 cm in depth, with soil about 5 cm in depth) with large (25 g), medium (10 g), and small (5 g) chicken pieces as carcasses, respectively. The reproductive behaviors of the males and females were examined using a video camera (SONY, CCD-TRV80) for one hour (09:00 to 10:00) every day from the day when the male-female pairs were released on the carcasses (Day 1) to the day when hatchlings first appeared on the carcasses.

In all cases in which a male did not appear on the video for one hour, the male did not later reappear on the video and was considered to have left the carcass. The period from the day when a male was released on the carcass (Day 1) to the day when the male disappeared from the carcass was regarded as the residence time of the male on the carcass.

Although the parent beetles manipulated the resource by conducting simultaneously both anal secretion and oral one, it was difficult to examine quantitatively both behaviors of anal secretion and oral one at the same time. Thus, we examined the depositing oral secretion as resource manipulation. Each pair copulated many times during resource manipulation. Furthermore, the females often showed aggressive behaviors in which they dashed up to males and bit the male's legs. We examined the time spent on resource manipulation by each male and each female, the frequency of copulations, and the frequency of aggressive behaviors by a female against a male for 1 hour every day.

Each carcass was manipulated by both a female and a male, but thereafter, there were two modes of feeding: biparental and maternal.

If the male was present when the hatchlings arrived at the carcass, we noted whether the male fed the larvae (biparental feeding) or not (maternal feeding).

The number and body weight of larvae were measured on the day when they dispersed from the carcasses for pupation.

2.1. Statistical Analysis. The arrival time of hatchlings on the carcass, the time spent of resource manipulation by males and females, the frequency of copulations, and the number and biomass of larvae dispersed from the carcasses were analyzed using the generalized linear model with a Gaussian distribution and an identity link function, following square-root transformed. Carcass size and feeding mode were included as covariates. We used Steel-Dwass test for multiple tests. The days elapsed after the commencement of the experiments were included as a covariate. The proportion of biparental feedings was compared among the carcass sizes, using Fisher's exact probability test. Change in the proportion of males remained on the small and medium carcasses where maternal feeding occurred was analyzed using Log-Lank-test. Correlation of the frequency of aggressive behaviors by a female against a male and the residence time of the male on the carcass was analyzed using Spearman's rank correlation. Using generalized linear models, we conducted analysis of deviance with a binomial error distribution and a logit link function to model a trigger for the males to participate in the feeding of larvae on the medium carcasses. We used five independent variables in the analysis: frequency of copulations, arrival time of hatchlings on the carcass, frequency of aggressive behaviors by a female against a male, time spent on resource manipulation by males, and time spent on resource manipulation by females.

3. Results

3.1. Frequency of Copulation. The frequency of copulations did not differ among carcass sizes ($P = .0503$) and feeding mode ($P = .2262$), but differed significantly among the first ($n = 46, 0.25 \pm 0.08$ (mean \pm SE)), second ($n = 39, 0.08 \pm 0.04$), and third days ($n = 27, 0.00 \pm 0.00$) (Friedman test, $P = .0018$).

3.2. Proportion of Biparental Feedings. On the large carcasses, maternal feeding was observed on only one carcass, which was then excluded from our analysis because parental behaviors were abnormal in this case. The parents spent an extremely short time on resource manipulation (about 17% of the time spent on resource manipulation by normal beetles), and the male was frail and died immediately following the experiments.

The proportions of biparental feedings on the small, medium and large carcasses were 0%, 31%, and 100%, respectively (Fisher's exact probability test, $P < .0001$).

3.3. Arrival Time of Hatchlings on the Carcass. The arrival time of the hatchlings on the carcass differed among carcass sizes ($P = .0487$) but did not differ among feeding modes ($P = .7106$) (small carcass: $n = 10, 3.9 \pm 0.3$ days (mean \pm SE), medium carcass: $n = 26, 4.0 \pm 0.1$ days, and large

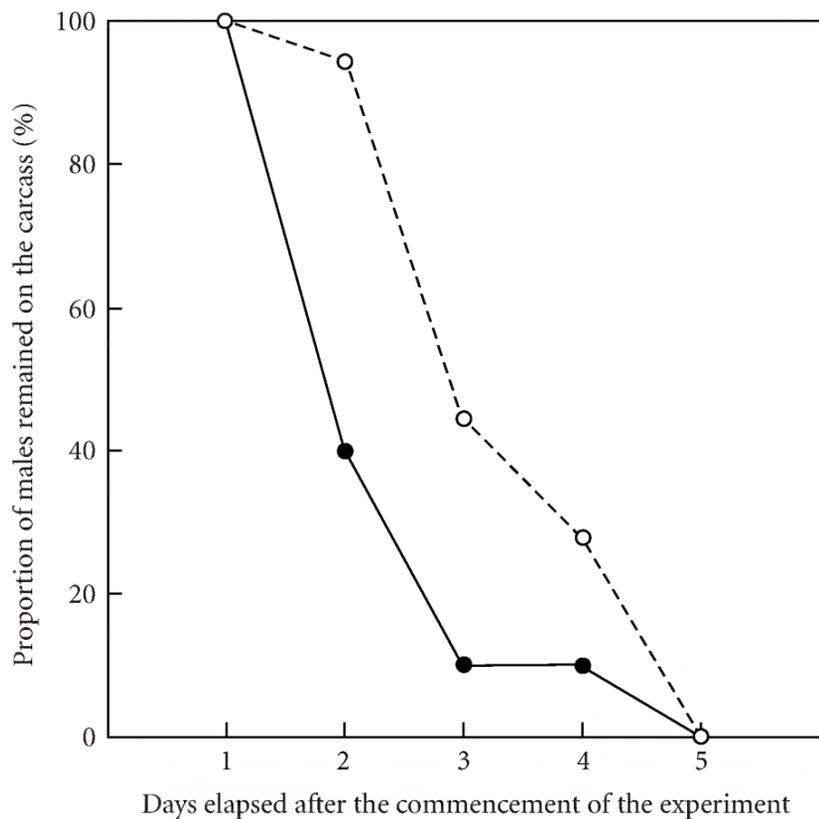


FIGURE 1: The proportion males of *N. quadripunctatus* remained on the small (solid circle) and medium carcasses (open circle) where the maternal feeding occurred.

carcass: $n = 10$, 4.5 ± 0.2 days). The arrival time of hatchlings on the medium carcasses was significantly earlier than that on the large carcasses, on which all feedings were biparental (Steel-Dwass test, $P = .0193$).

3.4. Leaving Time of Males from the Carcass. Changes in the proportion of males remained on the small and medium carcasses where maternal feeding occurred are shown in Figure 1. The males left small carcasses earlier than medium ones (Log-Lank-test, $P = .0106$). On the small and medium carcasses, 90% and 50% of males, respectively, left the carcasses before the hatchlings arrived. On the medium carcasses, furthermore, 38.5% of the males that were present when the hatchlings arrived left the carcasses without feeding the larvae.

3.5. Aggressive Behavior by a Female against a Male. The frequency of aggressive behaviors by a female against a male did not differ significantly among carcass sizes ($P = .3898$) and between feeding modes ($P = .1646$), and it did not influence the residence time of the male on the carcass (Spearman's rank correlation, $P = .5148$).

3.6. Time Spent on Resource Manipulation by Males. The time spent on resource manipulation by males depended significantly on carcass size ($P = .0051$) and feeding mode ($P < .0001$, Figure 2(a)). Although the time spent on resource manipulation by males did not differ significantly between maternal feedings on the small and medium carcasses (Steel-Dwass test: $P = .2387$) and between biparental feedings on the medium and large carcasses (Steel-Dwass test: $P = .0772$, Figure 2(a)), it differed significantly between maternal feedings on small carcasses and biparental feedings on large carcasses on medium (Steel-Dwass test: $P = .0099$) and large ones

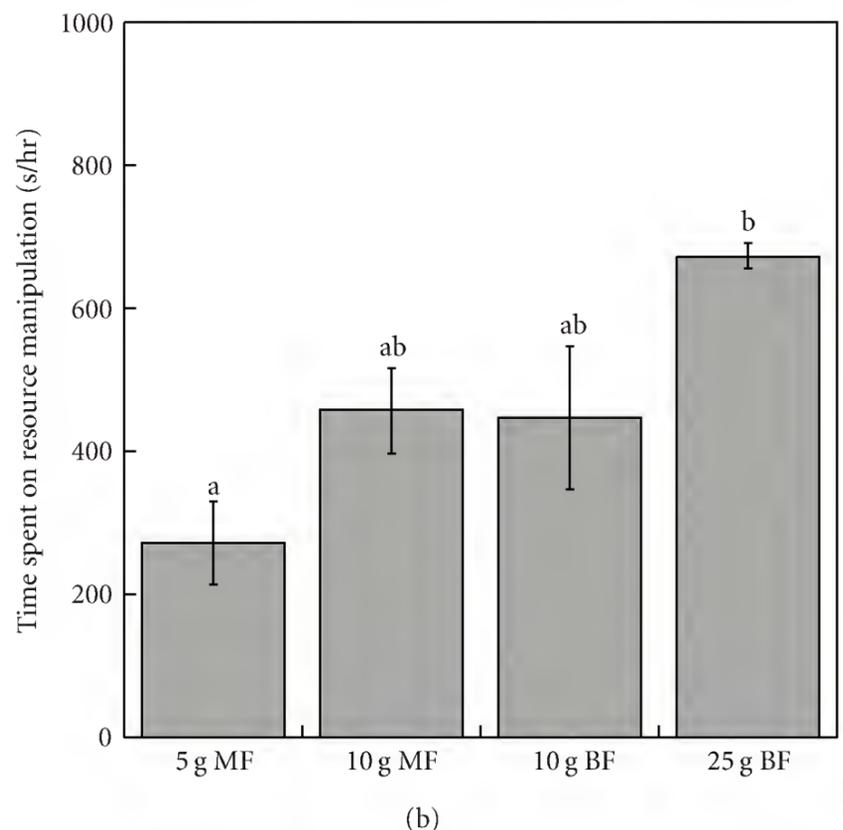
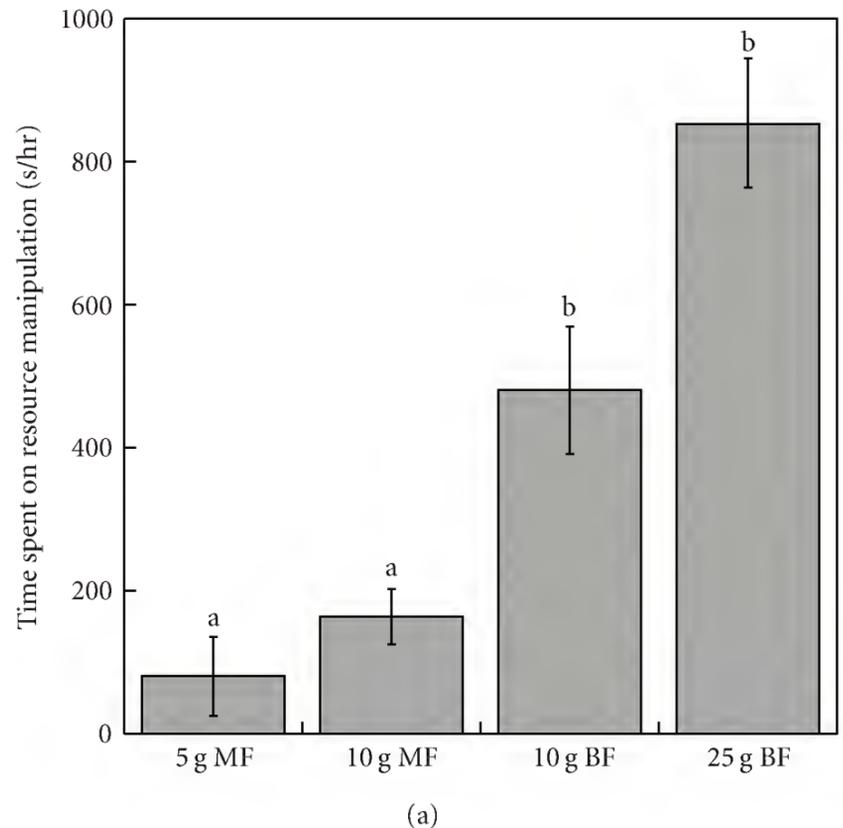


FIGURE 2: Time spent on resource manipulation by males (a) and females (b). Bars show SE. Different letters indicate a significant difference (Steel-Dwass test: $P < .05$). MF: Maternal feeding, BF: Biparental feeding.

(Steel-Dwass test: $P = .0012$), and between maternal feedings on medium carcasses and biparental feedings on medium (Steel-Dwass test: $P = .0278$) and large ones (Steel-Dwass test: $P < .0001$, Figure 2(a)).

3.7. Time Spent on Resource Manipulation by Females. The time spent on resource manipulation by females was found to depend significantly on carcass size ($P = .0268$) and feeding mode ($P = .0332$, Figure 2(b)), and it differed significantly between maternal feedings on small carcasses and biparental feedings on large carcasses (Steel-Dwass test: $P = .0133$, Figure 2(b)).

TABLE 1: Analysis of deviance from generalized linear model for the males to participate in the feeding of larvae.

Independent variable	<i>df</i>	Deviance	Resid. <i>df</i>	Resid. Dev	<i>P</i>
Frequency of aggressive behaviors by a female against a male	1	0.002	22	29.104	.968
Arrival time of hatchlings on the carcass	1	2.739	23	29.105	.098
Frequency of copulations	1	0.252	24	31.844	.615
Time spent on resource manipulation by females	1	0.003	21	29.1	.954
Time spent on resource manipulation by males	1	15.58	20	13.52	<.0001

3.8. *Number and Biomass of Larvae Dispersed from the Carcass.* The number of larvae dispersed from the carcasses depended significantly on carcass size ($P = .0007$) and feeding mode ($P = .0031$), and it differed significantly between the large and the medium carcasses (Steel-Dwass test: maternal feeding, $P = .0128$, biparental feeding, $P = .0127$) and between the large and the small ones (Steel-Dwass test: $P < .0001$, Figure 3(a)). The biomass of larvae dispersed from the carcasses differed significantly among carcass sizes ($P = .0250$) and between feeding modes ($P = .0425$, Figure 3(b)).

3.9. *Male's Decision to Participation in the Feeding.* The significant effect on the male's decision to participation in the feeding was found only in the time spent of resource manipulation by males (Table 1).

4. Discussion

The frequency of aggressive behaviors by a female against a male did not affect the residence time of the male on the carcass. Therefore, we consider that the male is not ejected from the carcass by the female, but rather chooses the time to leave the carcass for himself. Müller et al. [13] reported that females of *Nicrophorus vespilloides* Herbst were able to recognize their male partners. Then, when a male and a female collaborate in a breeding attempt, they usually do not exhibit aggressive behavior toward each other, but do attack newly intruding conspecifics that attempt to usurp the carcass [13]. In this study, however, female's aggressive behavior was observed. Although the reason why the females attack the males is unknown, their recognition to the partner may be incomplete in *N. quadripunctatus*.

This study showed short time spent on resource manipulation and no feeding of larvae by males on the small carcasses. On the medium carcasses, on the other hand, the time spent was longer on the carcasses with biparental feeding than on those with maternal feeding (Figure 2(a)). Furthermore, time spent on resource manipulation by males had a significant effect on male's decision to participation in the feeding (Table 1). This suggests that the male determines the time to be spent on resource manipulation and his residence time in response to carcass size.

As Smiseth and Moore [14] suggested, there is a possibility that on the large carcasses, the residence time of males elongates and males encounter the larvae that beg a feeding from the males, and then they participate in the feeding of

larvae. However, on the medium carcasses, approximately 40% of the males that were present when the hatchlings arrived left the carcasses without feeding the larvae, though the reason was unknown. These results may show that the presence of larvae on the carcasses and/or begging behavior by the larvae [15, 16] do not necessarily lead for males to participate in the feeding of larvae.

The present results indicate that one of the reproductive roles of *N. quadripunctatus* males in the absence of predators and/or competitors is resource manipulation, and that paternal efforts change depending on carcass size. Only the time spent of resource manipulation by males significantly affected on the male's decision to participation in the feeding (Table 1). A longer time spent on resource manipulation by males may be a trigger for the males to then participate in the feeding of larvae on large carcasses.

Although on the medium carcasses, the time spent on resource manipulation by males was shorter on the carcasses with maternal feeding than on those with biparental feeding, the time spent on resource manipulation by the female did not differ significantly between maternal feeding and biparental feeding. Rauter and Moore [17] reported that widowed males increased their effort on the feeding of larvae, whereas widowed females showing no change in their effort, and that the time spent of parental care by females increased slightly with increasing brood size. These suggest that although females are working at their maximum for parental activities, males may be able to adjust their care.

Therefore, we may consider that males are influenced more strongly than females by an immediate benefit (the number of offspring). If the benefit is great enough (enough offspring), the males stay; if not, they abandon the larvae and seek further mating opportunities [17]. We consider that on the large and small carcasses, the males can assess the number of larvae which correlates to the amount of resource, according to the information for the time spent on resource manipulation. On the medium carcasses, however, it may be hard to make a decision whether the males participate feeding larvae or do not. This suggests that males may be able to tune their reproductive efforts more finely than females based on carcass size.

Acknowledgment

The authors would like to thank the members of the Laboratory of System Ecology, Saga University, for providing valuable comments.

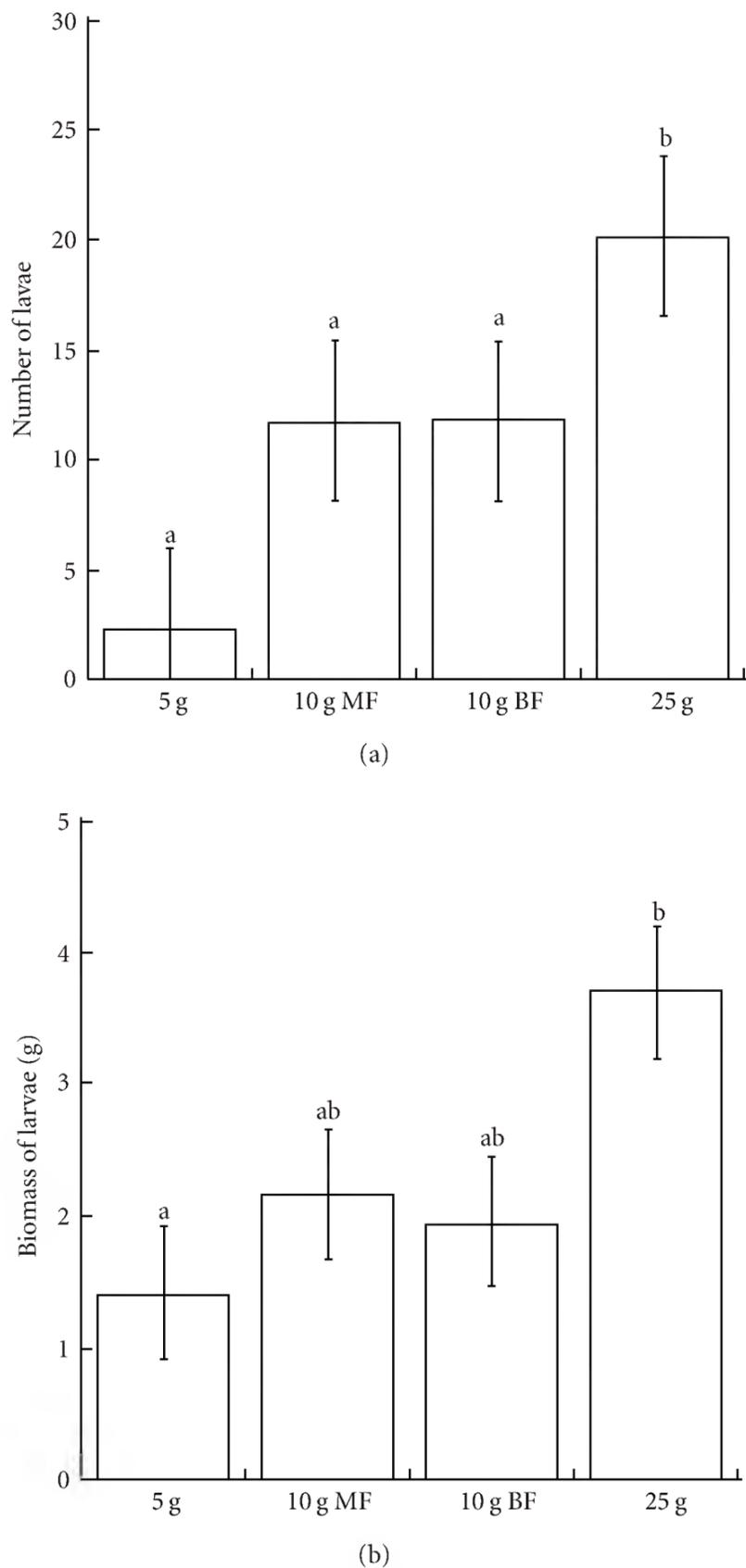


FIGURE 3: The number (a) and biomass (b) of larvae dispersed from the carcasses in relation to the carcass size and feeding mode. Bars show SE. Different letters indicate a significant difference (Steel-Dwass test: $P < .05$). MF: Maternal feeding, BF: Biparental feeding.

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Research Article

Bioactivity of Powder and Extracts from Garlic, *Allium sativum* L. (Alliaceae) and Spring Onion, *Allium fistulosum* L. (Alliaceae) against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) on Cowpea, *Vigna unguiculata* (L.) Walp (Leguminosae) Seeds

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Received 5 April 2010; Accepted 29 June 2010

Academic Editor: Arthur G. Appel

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Laboratory bioassays were conducted to investigate the bioactivity of powders, extracts, and essential oils from *Allium sativum* L. (Alliaceae) and *A. fistulosum* L. (Liliaceae) against adults, eggs, and larvae of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). On the basis of 48 hr median lethal toxicity (LC_{50}), test plant powders and extracts from *A. sativum* were more toxic to *C. maculatus* adults than those from *A. fistulosum*. The 48 hr LC_{50} values for the powder against the test insect species were 9.66 g/kg and 26.29 g/kg for *A. sativum* and *A. fistulosum*, respectively. Also the 48 hr LC_{50} values obtained show that aqueous extracts of the test plant species, 0.11 g/L (*A. sativum*) and 0.411 g/L (*A. fistulosum*) were more toxic to *C. maculatus* than the corresponding ethanol extracts. There was no significant difference in the toxicity of vapours from the two test plant species against *C. maculatus*, although *A. sativum* gave lower values. The study shows that *A. sativum* and *A. fistulosum* have potentials for protecting stored cowpea from damage by *C. maculatus*.

1. Introduction

Grain storage has often resulted in quantitative and qualitative losses due to physical, chemical, and most importantly biological factors such as pests which may be birds, rodents, fungi, or insects [1–3]. The most important among storage pests are insects because apart from their direct damage they create conditions that allow secondary infestation by rot organisms mainly fungi [1, 4].

Once infestation is established pest insects cause gradual and progressive damage leading to losses in weight, nutritional, organoleptic, and aesthetic quality of stored grains. Osuji [1] listed 40 insects affecting stored grains, the most important among which is the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera; Bruchidae) responsible for up to 100% infestation of cowpea, *Vigna unguiculata* (L.) Walp (Leguminosae) during storage [1, 3, 5]. These observations justify the control of insect pests like *C. maculatus* in order to reduce losses in stored cowpea.

Several methods are used in controlling insects in stored grains, including physical (smoking, sun-drying, heating), cultural, biological (male insect sterilization, natural enemies, resistant grain varieties), and chemical (synthetic and natural products) methods. The most common and widely used is the chemical method involving mainly the use of synthetic insecticides.

Several workers have reported the successful wide scale use of synthetic organic insecticides, commencing with the organochlorines in the middle 1940s, followed by the later use of organophosphates, carbamates, pyrethroids, avermectins, and others. Insecticides most commonly used to protect stored grains from insect pests include aluminium phosphide, lindane, methyl bromide, ethylene dibromide, edifenphos, pirimiphos methyl, permethrin, malathion, sumithion, chlorpyrifos methyl, chlorpyrifos, propoxur, fenithrothion, dichlorvos, bromophos, fenvalerate, bioresmethrin, phenothrin, and deltamethrin [3].

The observed overreliance on insecticides was mainly, due to their initial quick action, ease of use and general efficiency in reducing pest populations and damage. However, there are limitations to their use mainly the deleterious side-effects to nontarget species including humans and the development of resistant strains of pests [6, 7]. In addition to these limitations, there is also the problem of high cost of synthetic insecticides, which is a limiting factor particularly to the largely peasant farmers of Africa including Nigeria.

Due to the foregoing reasons, there has been a need to search for new insecticides with novel mechanism of action. In this regard, many scientists have reasoned that it is advantageous to investigate natural products as a source of degradable insecticides that may turn out to be safer to humans and the rest of the environment than the synthetics.

In the present study, garlic, *Allium sativum* (Alliaceae) and Spring onion, *A. fistulosum* (Alliaceae) were screened for their bioactivity against *C. maculatus*. Members of the genus *Allium* have been known to demonstrate repellent and insecticidal properties against medically important insect pest species [8] and a few workers including Stoll [9] and Oparaeke et al. [10] have reported their potency against other insects. However, there is a dearth of studies on the bioactivity of extracts and volatile oils from these plant species against *C. maculatus*, especially their eggs and larvae. Understanding the toxicity of compounds to adults and immature stages is very important as it would indicate the appropriate time to apply them for adequate control of the insect pests. The present study would therefore provide the needed information on the toxicity of the extracts and volatile essential oils from *A. sativum* and *A. fistulosum*, respectively, against adult, larva, and egg of *C. maculatus*.

2. Materials and Methods

2.1. Test Plant Materials. The cloves of garlic *A. sativum* and leaves of Spring Onion *A. fistulosum* obtained from Iyana Iba market, Lagos, were the test plant materials used.

Test plant materials were used against test insect species in four formulations, namely, powder, aqueous and ethanol extract of powders, and essential oils prepared as described below. To prepare the powder, plant parts were first dried slowly to constant weight in a wooden cabinet (1.0 m × 0.5 m × 1.0 m) fitted with 100 watts bulb, which provided an average temperature of about 42°C for 7–14 days before pulverization in a Binatone blender (model No. BLG 400). The powders were passed through sieve of 0.1 mm mesh size to standardize particles size.

Aqueous and ethanol extract were each prepared from the powder. In each case, 500 g of plant powder was steeped in 1 L of water or ethanol that served as solvent, for 24 hrs. The mixture was then passed through Whatman No. 1 filter paper (15 cm diameter). The filtrate in each case was stored in a labelled Kilner jar while the residue was reextracted with water or ethanol, respectively, and all filtrates combined for each treatment. Each of the combined filtrates was then dried over a water bath at 50°C temperature and the resultant residue used as crude active ingredient. Volatile essential

oil was extracted from 500 g of pulverised *A. sativum*, or *A. fistulosum* by hydrodistillation for 7–8 hrs in a Clavenger apparatus [11], collecting the volatile oil over hexane, which was removed by passing it over anhydrous sodium sulphate. Each of the essential oils was stored in glass vials kept in refrigerator at 4°C to reduce evaporative loss until when needed for bioassays.

2.2. *Callosobruchus Maculatus* Culture. Cowpea weevil, *C. maculatus* (F.) starter cultures obtained from the insectary of Nigerian Stored Product Research Institute (NSPRI), Abule-Oja, Lagos, where they have been held in cultures for decades unexposed to insecticide were used. Fresh experimental cultures were prepared from the original stocks and maintained at 30 ± 1°C temperature and 70 ± 4% relative humidity as described by Denloye et al. [12]. *Callosobruchus* was maintained on cowpea seeds. The grains were disinfested by picking those with damage holes and heating in the oven at 50°C for five hours. Disinfested grains were measured into clean 1 L Kilner jars with screw caps. Each jar contained 500 g of cowpea into which seven 0-1-d old adult *C. maculatus* (2 ♂, 5 ♀) were introduced. All adult *C. maculatus* were removed from the culture after seven days for oviposition to take place. Fresh cultures were made from this for subsequent tests.

3. Bioassays

3.1. Acute Toxicity of Plant Powders. Twenty active 0–3-day-old *C. maculatus* (mixed sexes) were exposed to disinfested cowpea grains admixed with powdered plant material at concentrations ranging between 5.0 g/kg and 320 g/kg or without plant material as control in disposable plastic cups covered with muslin.

3.2. Acute Toxicity of Aqueous and Ethanol Extracts. Similar sets of experiments as described above were carried out, but this time grains were treated by dipping them for approximately 30 secs in different concentrations (0.5–16 g/L) of each plant extract.

3.3. Fumigant Toxicity of Volatile Essential Oils

3.3.1. Adults. Fumigation bioassays were carried out in 1 L airtight Kilner jars using the method of Don Pedro [13, 14]. In this procedure, a 7 cm-diameter Whatmann No. 1 filter paper was always impregnated uniformly with a test essential oil at predetermined concentrations, and quickly hung with a thread in the fumigation chamber already holding 20 adult test insects. The chamber was then sealed with the cap, screwing the ring holding a glass lid tightly on to a rubber washer covered with aluminium foil to prevent reaction with essential oil. The cap remained tightly screwed to ensure fumigation in the airtight chamber for 24 hrs. In controls, insects were left in airtight sealed chambers without oil on the filter paper. There were four replicates per treatment. After the 24 hr fumigation the chambers were opened and the insects that were still alive transferred into recovery

TABLE 1: Acute (48 h) toxicity of test plant materials against *Callosobruchus maculatus*.

Formulation	Test plant species	LC ₅₀	LC ₉₅	Regression equation	DF	Slope (\pm SE)
		95% Confidence Limits	95% Confidence Limits			
Powder (g/kg)	<i>A. sativum</i>	9.661 (7.957–11.691)	70.143 (50.983–96.317)	$Y = -1.888 + 1.916x$	4	1.916 ± 0.031
	<i>A. fistulosum</i>	26.293 (20.485–33.632)	501.742 (293.804–854.42)	$Y = -1.829 + 1.288x$	4	1.288 ± 0.018
Aqueous extracts (g/l)	<i>A. sativum</i>	0.110 (0.087–0.137)	1.30 (0.80–2.17)	$Y = -1.475 + 1.583x$	3	1.538 ± 0.03
	<i>A. fistulosum</i>	0.411 (0.314–0.510)	4.017 (2.788–6.659)	$Y = 0.643 + 1.667x$	5	1.667 ± 0.035
Ethanol extracts (g/l)	<i>A. sativum</i>	0.219 (0.181–0.261)	1.297 (0.959–1.803)	$Y = 1.409 + 2.134x$	3	2.134 ± 0.046
	<i>A. fistulosum</i>	0.863 (0.687–1.072)	12.955 (7.624–28.913)	$Y = 0.089 + 1.403x$	3	1.403 ± 0.027

DF: Degree of Freedom; SE: Standard Error.

chambers. Mortality counts were taken in the recovery chambers every 24 hrs for seven days.

3.3.2. *Eggs*. Fumigation of *C. maculatus* eggs on cowpea was carried out in 1 L airtight Kilner jar using 0.5 mL of *A. sativum* or *A. fistulosum* oil, respectively. Twenty seeds bearing one egg each were assayed against each of the test oils and replicated four times. A control, also replicated four times, was set up similarly but the filter paper had no oil. The egg bearing cowpeas were transferred after 24 hours to ventilated plastic cups and later inspected for hatched (or unhatched) eggs under a stereomicroscope with X 8 objective after 12 days.

3.3.3. *Larvae*. Another similar experiment was set up with the arrangement described above using 6–8-day-old hatched eggs (i.e, 1-2-day-old larvae) since eggs hatch into larvae after 6 days of incubation. A batch of 20 cowpea seeds, each of which had one 6–8-day-old eggs were placed in fumigation chamber having 7 cm diameter filter paper impregnated with various concentrations of test oils. After 24 hours of fumigation, the cowpea seeds were transferred into ventilated plastic cups and left for 21 days. Each treatment and control was replicated four times. Mortality was assessed by dissecting each cowpea seeds to recover dead (or living) larvae.

4. Persistence of Test Plant Materials

4.1. *Extracts*. Forty undamaged cowpea grains were treated by dipping for approximately 30 secs in predetermined concentrations (0.5 to 8.0 g/L) of aqueous extracts of either *A. sativum* or *A. fistulosum*, and allowed to drain on filter paper for 5 minutes before transferring into bioassay containers. Several sets of treated seeds and two controls were prepared. For each set of treated seeds and controls, bioassays were started off by introducing 10 adult *C. maculatus* aged 0–3 days at preset times expressed as Hours After Treatment (HAT), namely, 0 (immediately after treatment), 12, 24, 96, 168, and 336 HAT. Each treatment and control was replicated four times. Each set of experiments was assessed by taking mortality of test insects every 12 hours for 336 hours.

4.2. *Essential Oils*. Similar experiments were carried out using concentrations (0.8 mL/L to 12.80 mL/L) of essential oil of *A. sativum* and *A. fistulosum*, respectively, instead of aqueous extracts.

4.3. *Assessment of Mortality*. In all bioassays insects were counted as dead when they failed to move any part of their body after prodding with fine brush bristle.

4.4. *Data Analyses*. Quantal responses (mortality) of *C. maculatus* were subjected to probit analysis [15] using computer software after correcting for mortality with Abbot formula [16]. From these analyses, LC₅₀ (the concentration at which 50% of test insects died at a given time) and LC₉₅ values of test plant materials were computed.

5. Results

5.1. *Acute Toxicity of Test Plant Powders to C. maculatus*. The 48 hr LC₅₀ values of *A. sativum* (9.66 g/kg) and *A. fistulosum* (26.29 g/kg) and their corresponding LC₉₅ values against *C. maculatus* are shown in Table 1. Powdered *A. sativum* was significantly more toxic to the test insect species than *A. fistulosum* (no overlap in 95% confidence limits).

5.2. *Acute Toxicity of Test Plant Extracts to C. maculatus*. The aqueous extracts were more toxic to *C. maculatus* than the ethanol extracts. Probit analysis show that the 48 h LC₅₀ values of the *A. sativum* aqueous extract was 0.11 g/l, a value lower than that of *A. fistulosum* (0.41 g/l). For the ethanol extracts, the *A. sativum* gave LC₅₀ value of 0.22 g/l which is 4X lower than the corresponding value for *A. fistulosum* shown in the Toxicity Factor column (Table 1).

5.3. *Fumigant Toxicity of Test Essential Oils to C. maculatus Adult, Eggs, and Larvae*. There was no significant difference in the toxicity of *A. sativum* essential oil when compared with that of *A. fistulosum* (no overlap in 95% confidence limits) although *A. sativum* gave lower LC₅₀ and LC₉₅ values relative to *A. fistulosum* essential oil (Table 2) against both the adults and the eggs, respectively. The essential oils of *A. sativum* and *A. fistulosum* resulted in mortality of the larvae of *C. maculatus* in the cowpea grains, though below 20%.

TABLE 2: Fumigant toxicity of test essential oils to *C. maculatus* adults and eggs.

Test insect species	Test plant species	LC ₅₀	LC ₉₅	Regression equation	DF	Slope (\pm SE)
		(95% Confidence limits)	(95% Confidence limits)			
Adults	<i>A. sativum</i>	15.46 (12.44–19.153)	157.122 (104.97–235.058)	$Y = -1.948 + 1.638x$	3	1.638 ± 0.024
	<i>A. fistulosum</i>	23.144 (18.403–29.059)	363.125 (205.718–643.59)	$Y = -1.883 + 1.38x$	3	1.38 ± 0.021
Eggs	<i>A. sativum</i>	14.536 (11.826–17.953)	142.789 (79.183–262.334)	$Y = -1.933 + 1.663x$	3	1.663 ± 0.032
	<i>A. fistulosum</i>	20.844 (15.589–28.232)	335.986 (137.429–858.69)	$Y = -1.802 + 1.367x$	3	1.367 ± 0.031

DF: Degree of Freedom; SE: Standard Error.

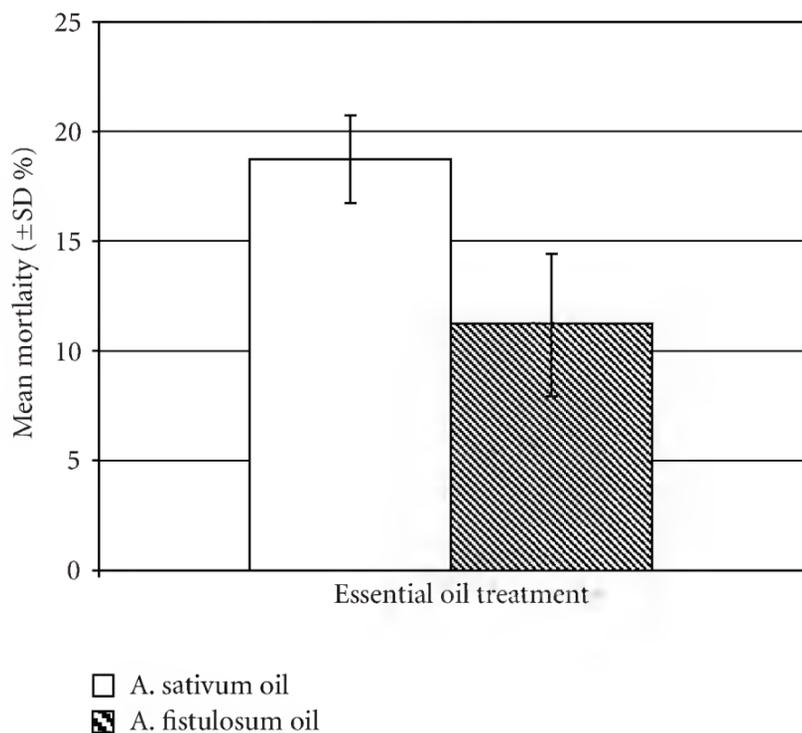


FIGURE 1: Fumigant toxicity of essential oil of *Allium* spp against *C. maculatus* larvae.

A. sativum resulted in a higher number of dead larvae than *A. fistulosum* oil (Figure 1).

6. Persistence of Plant Extracts and Oils for Bioactivity against *C. maculatus*

6.1. Extracts. The persistence of the toxicity of aqueous extracts of both test plant species is shown in Figure 2. The computed LC₅₀ values for the two test extracts increased slightly by 12 hrs and was maintained up to 24 hrs. The ethanol extract of *A. fistulosum* was less persistent than that of *A. sativum* (Figure 2).

6.2. Essential Oils. The potency of the oils from the two test plant species remained only for 12 hrs, after which it was lost rapidly. The potency of *A. fistulosum* oil was completely lost by 96 HAT (Figure 3).

7. Discussion

The results demonstrate that although *A. sativum* and *A. fistulosum* are of the same genus, they showed different potencies against the adults, eggs, and larvae of *C. maculatus*, respectively. The powder of *A. sativum* gave high toxicity

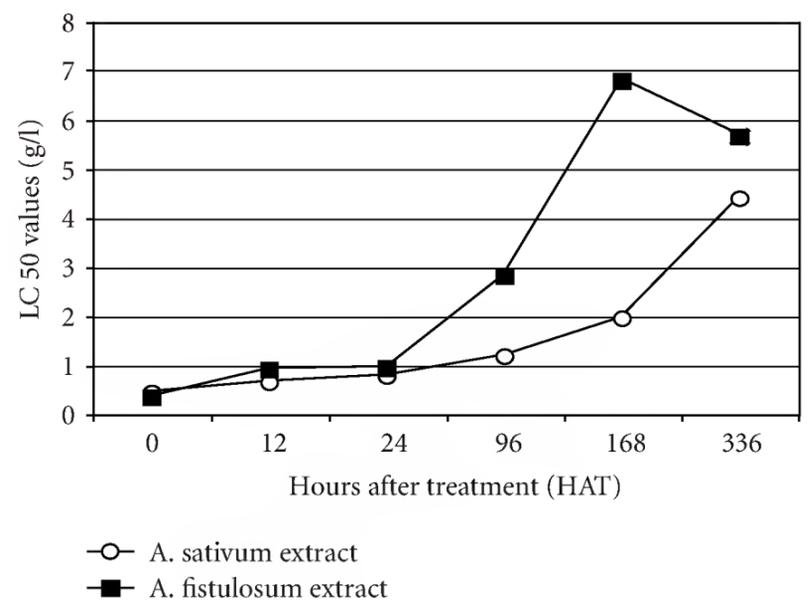


FIGURE 2: Persistence of test plant extracts against *C. maculatus* adult.

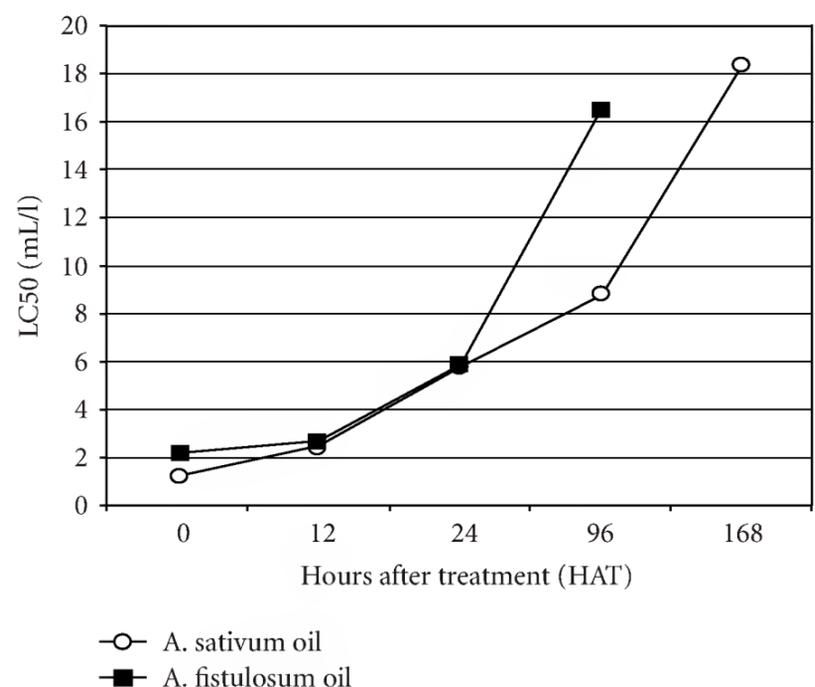


FIGURE 3: Persistence of test plant essential oils against *C. maculatus* adult.

against *C. maculatus* adults with LC₅₀ values of 9.66 g/Kg. This value show that powdered *A. sativum* was equally toxic to *C. maculatus* as Citrus species in studies carried out by Don-Pedro [17] and Kellouche and Soltan [18].

The extracts of *A. sativum* were more toxic to *C. maculatus* than those of *A. fistulosum* in this study. This may be because the active principles responsible for the activity of

the test extracts were present in higher quantities in the *A. sativum* than the *A. fistulosum*. In addition, *A. sativum* may contain other compounds not contained in *A. fistulosum*. Our study shows that the aqueous extracts were toxic to *C. maculatus*, thus reinforcing earlier observations that members of the genus *Allium* are potent against insects. Denloye and Makanjuola [19] and Denloye et al. [8, 20] have reported the insecticidal potency of the aqueous extracts of *A. sativum* against *Sitophilus zeamais* and *Anopheles* species. Our results from the present study agree with these earlier reports.

The solvent used in extracting plant materials for insecticidal potency is highly important as our present study shows. Ethanol extracts were less toxic than the aqueous extracts. This agrees with earlier reports that aqueous extracts of garlic *A. sativum* were more toxic to *S. zeamais* than the methanolic extract [20]. This could be because the active principles in the test plant materials are more soluble in water. Grieve [21] stated that the higher efficacy of aqueous extracts over that of ethanol is due to the fact that alkyl compounds present in the Alliacea family are readily obtained by distillation with water. Our results in the present study show that the effectiveness of a natural plant extracts increase with decreasing polarity of the solvent used for extraction in agreement with earlier reports by Denloye et al. [20] and Ojewole et al. [22].

The ovicidal action of the essential oils from test plant species have been demonstrated in this study. This indicates that *A. sativum* and *A. fistulosum*, like other plants with essential oils having ovicidal effects [13, 14, 23], may be exploited for the prevention and control of *C. maculatus* infestation of stored cowpea. Overall, the results obtained from this study portend greater usefulness for *A. sativum* as a source of bioactive formulations capable of protecting stored cowpea from infestation by *C. maculatus*.

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Research Article

Larval Feeding Habits of the Cuban Endemic Firefly *Alecton discoidalis* Laporte (Coleoptera: Lampyridae)

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Received 24 May 2010; Accepted 4 July 2010

Academic Editor: Martin H. Villet

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Alecton Laporte, 1833, with four known species is the only firefly genus endemic to Cuba. *Alecton discoidalis* Laporte, 1833, is its most common species, distributed in the western half of the country. Unfortunately, much of its life history remains unknown, as with the rest of Cuban representatives of the family Lampyridae. Larvae are associated with adults of *A. discoidalis* through rearing, and observations on larval feeding habits of this species are presented. Thirteen species belonging to seven gastropod families are reported for the first time as prey of *A. discoidalis* larvae. Our data suggest that these are generalist predators of terrestrial snails. A remarkably close association exists between this lampyrid and operculate species of snails. The later represents the most abundant and diverse group of molluscs in limestone landscapes, where the beetles are commonly found.

1. Introduction

The Lampyridae is one of the coleopteran families with many gaps regarding the knowledge of its taxonomy and ecology in the Caribbean region. Currently, 37 species in 8 genera of fireflies are reported in Cuba [1].

Alecton Laporte, 1833, with four known species is the only genus of the family endemic to Cuba. *Alecton discoidalis* Laporte, 1833, is its most common species, distributed in the western half of the country. All what is known about the genus natural history is that larvae prey on terrestrial snails of the family of Helicinidae [2, 3]. Ecological and ethological data are lacking for any Cuban Lampyridae, both for adults and larvae. As for the latter, there are some important contributions for lampyrids of other regions [4–7]. The entire literature on Cuban fireflies is represented by taxonomic works [8–11] or species lists [1, 12]. Data on *A. discoidalis* natural history, specifically the feeding habits of its larvae, are herein provided.

2. Materials and Methods

Specimens were collected throughout two nights in August 2009, at Pan de Matanzas ($n = 14$), one night in February 2010, at Bacunayagua ($n = 3$) and during the day of the same month at Escaleras de Jaruco, La Jaula ($n = 3$). All these areas are located in western Cuba (Figure 1(a)). Localities where immature stages were collected are characterized by outcrops of limestone and some degree of disturbance (Figures 1(b), 1(c), and 1(d)). Most larvae were captured while emitting light signals (from 20:00 to 22:00 hours approximately). Individuals of different instars were collected, most of them in the leaf litter and some under or even on rocks. Temperature and humidity of those nights were in the range of 26–32°C and 64–86%, respectively, and for Escaleras de Jaruco, during the day, were between 22–25°C and 80–93% respectively, recorded with a digital Control Company thermohygrometer (error = 1°C and 1% RH).

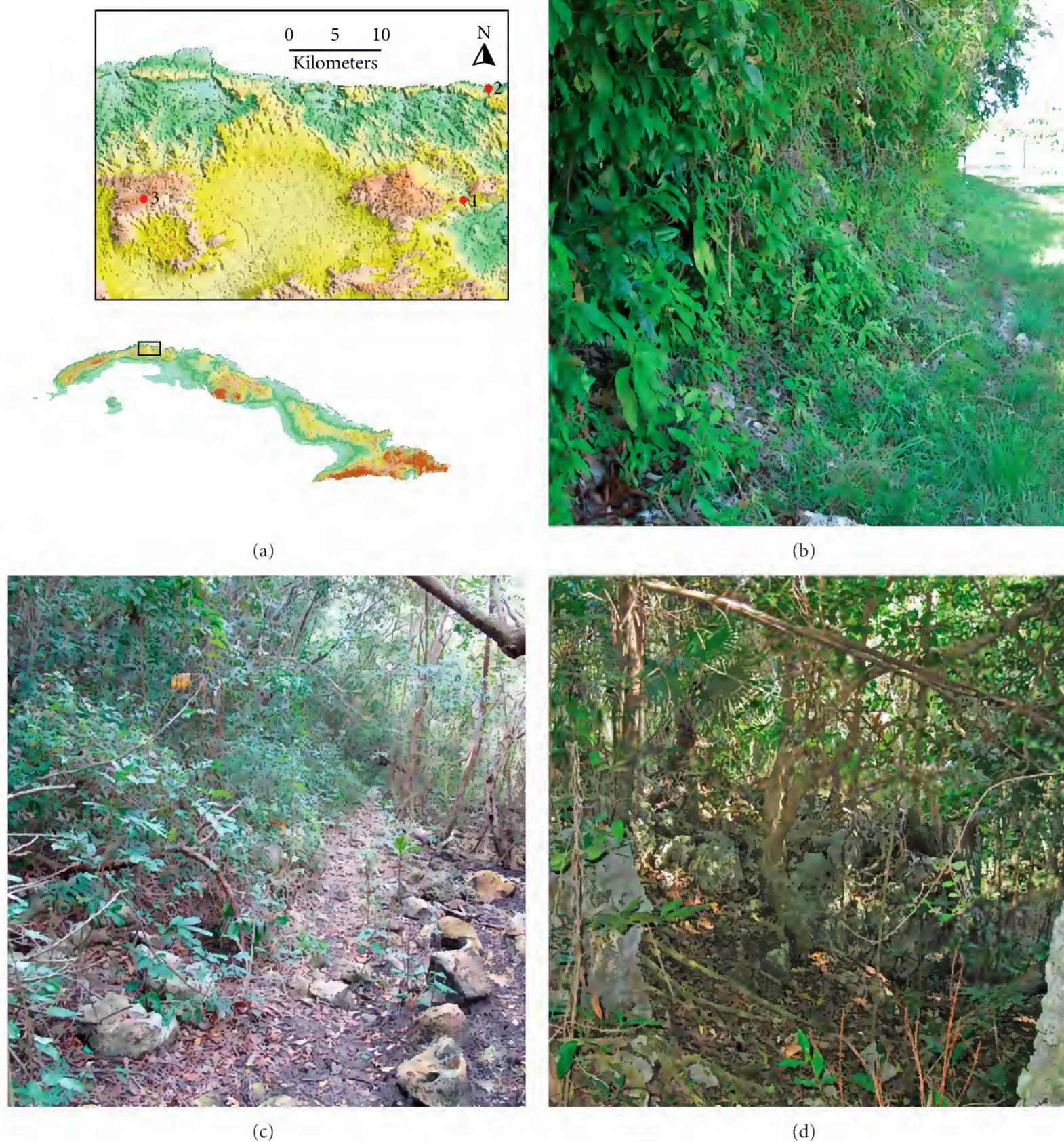


FIGURE 1: (a): Collection localities of *Alecton discoidalis* larvae in western Cuba: 1- Pan de Matanzas, 2- Bacunayagua, 3- Escaleras de Jaruco, La Jaula. (b): Habitat at Pan de Matanzas, Matanzas. (c): Habitat at Bacunayagua, Matanzas. (d): Habitat at Escaleras de Jaruco, La Habana.

Several species of terrestrial snails were also collected to feed lampyrid larvae in captivity. The snails were abundant on the ground, near the firefly larvae, on neighboring limestone walls, and on vegetation, less than 1m above ground. All possible representatives of the snails that could be potential prey items were collected. Also, snail species from Sierra del Rosario, where this firefly genus is present, were offered to the larvae. Preys offered were always snails, except for one earthworm.

Individuals were reared in Petri dishes of 9 cm diameter, with fragments of soil litter and mosses that were sprayed regularly with water to preserve humidity, the most critical

requirement for other lampyrid species [13]. In most cases they were individualized, but the smallest instars were kept together. Room temperature and humidity were daily monitored, and kept in the range of 22–33°C and 47–98%, respectively. The broad variation range of room humidity should not have any effect on larvae since the insides of the dishes were always damp.

3. Results and Discussion

The larva of *A. discoidalis* is associated with conspecific adults for the first time through rearing, the most reliable



FIGURE 2: Larval feeding habits of *Alecton discoidalis*. (a): Larva feeding on *Torrella immersa* in nature. (b): Two larvae of early stages feeding on *Torrella immersa* in captivity. (c): Predation on *Ustronia sloanei*, keeping the operculum at side in captivity. (d): Predation on *Chondropoma pictum*, with the operculum out, in captivity. (e): Predation on the carnivorous snail *Oleacina* sp. in captivity. (f): Foaming behaviour of *Helicina aspersa* while attacked.

association, sometimes very difficult to obtain in this group [14]. Two earlier publications [2, 3], both on gastropods, identified the larvae only as *Alecton* sp. A complete description of the larva is being prepared. Clench and Jacobson [2] mentioned predation of *Alecton* larvae on *Viana regina*, and González [3] on *Trochelviana* sp. Both snail genera are operculate (Subclass: Prosobranchia), as well as the

majority of prey items accepted in this study (Table 1). Helicinidae and Potamiidae species represented 71% and 92%, respectively, of those accepted by the larvae. These groups constitute the most abundant and diverse families in Cuban limestone landscapes. Particularly, the density of Potamiidae is very high in these areas, sometimes over 10 ind/m².

TABLE 1: List of snail species offered to *Alecton discoidalis* larvae. The most common substrate of each species is indicated, G: ground dwelling. R: rock dwelling. T: tree dwelling.

Family and species	Number of snail offered	Number of snail accepted
Helicinidae (Subclass Prosobranchia)		
<i>Helicina aspersa</i> (T)	15	12
<i>Ustronia sloanei</i> (R)	11	7
<i>Alcadia hispida</i> (G)	4	4
<i>Emoda sagraina</i> (G)	4	1
Potamiidae (Subclass Prosobranchia)		
<i>Chondropoma pictum</i> (G, R, T)	16	15
<i>C. auberianum</i> (T)	1	1
<i>C. irradians</i> (G, R)	5	5
<i>Eutudora jimenoii</i> (R)	8	7
<i>Torrella immersa</i> (R)	1	1
<i>Rhytidiopoma coronatum</i> (R)	3	3
Megalostomidae (Subclass Prosobranchia)		
<i>Farcimen tortum</i> (G)	5	2
Bulimulidae (Subclass Pulmonata)		
<i>Liguus fasciatus</i> (T)	1	0
Urocoptidae (Subclass Pulmonata)		
<i>Pycnoptychia</i> sp. (G)	1	0
Oleacinidae (Subclass Pulmonata)		
<i>Oleacina</i> sp. (G)	1	1
Polygyridae (Subclass Pulmonata)		
<i>Praticolella griseola</i> (T)	3	3

The snails most vulnerable to *A. discoidalis* larvae's attack seem to be ground and rock dwellers, followed by tree dwellers. The latter can fall to the ground with the leaf where they are resting or hibernating; it is even possible that lampyrid larvae climb up to the trees, as they do with rocks. Species of Urocoptidae (*Pycnoptychia* sp.) and Bulimulidae (*Liguus fasciatus*) were not eaten by *A. discoidalis* larvae. The former is a very spirally and elongated species, preventing access of the larva to the snail's body. It is therefore quite likely that this group of gastropods does not constitute prey of *A. discoidalis* larvae. The earthworm offered was not eaten either.

Larvae of *A. discoidalis* were also observed in nature feeding on three snail species: *Torrella immersa* (at Pan de Matanzas), *Chondropoma pictum* (Bacunayagua), and *Rhytidiopoma coronatum* (Escaleras de Jaruco). The first two observations were made at night while the third one was made during daylight (0900–1200 h) on three different occasions. Although McLean et al. [13] in photurid larvae, said that feeding is promoted by temperatures of 20–25°C and by darkness, *A. discoidalis* larvae could be so nocturnal as diurnal, since the attacks observed in captivity not always occurred at night. Nevertheless, according to our observations, the larvae may spend around 24 hours inside a single prey until finishing with it. Lampyrid larvae were seen

feeding only on both living and fresh terrestrial snails, either in nature or in captivity (Figure 2). On some occasions, that is, the early stages, several larvae (up to three) were seen consuming together a single snail (Figure 2(b)).

Clench and Jacobson [2] suggested that lampyrid larvae may wait for the *Viana* to relax the operculum and then attack. In this paper, we observed that they attack mostly active or recently active snails. When snails spend many days inactive with the opercula closed, they are seldom attacked. On some occasions, the snails (especially *H. aspersa* and *C. pictum*) begin to foam when lampyrid larvae attack them (Figure 2(f)). Performing such a specific behavior, they evaded the attack. Another behavior was observed in the field when *C. pictum* swung the shell forward when disturbed by us. Wang et al. [5] described this behaviour in other snails and interpreted it as a defense mechanism in order to avoid attacks by other larvae.

More detailed papers are needed for a better understanding of the natural history of this endemic firefly. Label data from collections of *A. discoidalis* are mostly from limestone landscapes. This may suggest an association with operculate gastropods, abundant in such places. Therefore, a food preference study and a biogeographical analysis of these two invertebrate taxa could show how closely related they are or even they may have had a coevolving relationship defined by their predator-prey connection.

Acknowledgments

The authors thank Gilberto Silva, Rayner Núñez, and Esteban Gutiérrez for their critical review of this paper, and Oraily Madruga for her corrections to it. The authors are grateful to Rayner, Annery, Anay, Jans, Maikel, and Aurora for their help in collecting firefly larvae. Author's gratitude is due to Annabelle Vidal and Joel Lastra from "Flora y Fauna" of Havana Territory for their collaboration at Escaleras de Jaruco Protected Area. The instruments used in this paper were donated by IDEA WILD. Two anonymous reviewers contributed to clarifying the submitted manuscript.

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Research Article

New Types of Behavioral Manipulation of Host Spiders by a Parasitoid Wasp

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Received 14 October 2009; Revised 9 June 2010; Accepted 15 July 2010

Academic Editor: Robert Matthews

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The larva of the parasitic wasp *Zatypota* sp. nr. *solanoi* induces its host spiders *Anelosimus* spp. to modify its web in ways not seen in normal webs of this species or in any other species, providing apparent protection and support for the wasp's cocoon by covering the entire web with a protective sheet and adding a central platform and opening a space below in the enclosed tangle, where the larva suspends its cocoon. These modifications differ qualitatively from those induced by a congeneric wasp. Parasitized spiders appeared to adjust modified web construction behavior to local conditions, implying that larval manipulations may occur at higher rather than lower levels of behavioral control (e.g., eliciting overall design decisions, rather than particular motor patterns).

1. Introduction

The behavior of some animals changes when they are parasitized. The possibility that such changes are adaptive for the parasite has been controversial, because some alterations may be incidental byproducts of the parasite's effects on its host [1]. Other changes, however, are well designed to promote the survival of the parasite, and are thought to represent manipulation of the host to the parasite's advantage [2–5].

Ichneumonid wasps in the tribe Polysphinctini are ectoparasites of spiders, [6, 7] and several species in the “*Polysphincta* clade” (hereafter “polysphinctine wasps”) modify the web construction behavior of their hosts [7, 8]. After growing slowly for a week or more as it feeds on the spider's hemolymph (and apparently not affecting its host's behavior), the larva induces the spider to build a modified, “cocoon” web. The larva then kills and devours its host, builds a cocoon attached to the cocoon web, and pupates. Cocoon webs built by spiders appear to improve the chances of survival of the wasp's cocoon [8–11]. Spiders never survive to reproduce once their behavior is altered, so cocoon web construction has probably evolved under selection on the larva rather than the spider.

Different wasp species show diverse and subtle effects on host behavior, and these effects consistently take advantage

of particular features of the web designs of unparasitized spiders of the different hosts to provide protective structures for their cocoons. Modifications include reducing an orb web to a few highly reinforced radial lines [8, 12], reinforcing a tangle of lines next to the orb and adding a vestigial hub at its center from which to suspend the cocoon [9], reinforcing the frame and anchor lines of an orb and building a reduced, strengthened orb-like web within these frames and adding camouflage for the cocoon [13–15], building or reinforcing a small protected silk chamber for the cocoon in the host's web [10, 11], and simply omitting the orb next to a resistant tangle web [16].

The present study concerns the wasp *Zatypota* nr. *solanoi* (Ichneumonidae), which parasitizes the spiders *Anelosimus* nr. *studiosus* and *A. octavius* (Theridiidae). This rare species (1.1% of the 374 *A. octavius* spiders sampled systematically were parasitized) induces still different behavioral changes; these changes are also adjusted to the biology and behavior of their hosts.

2. Material and Methods

Parasitized mature female spiders with an egg or larva on the abdomen were collected with their webs in early second growth in San José Province, at about 1500 m el. near

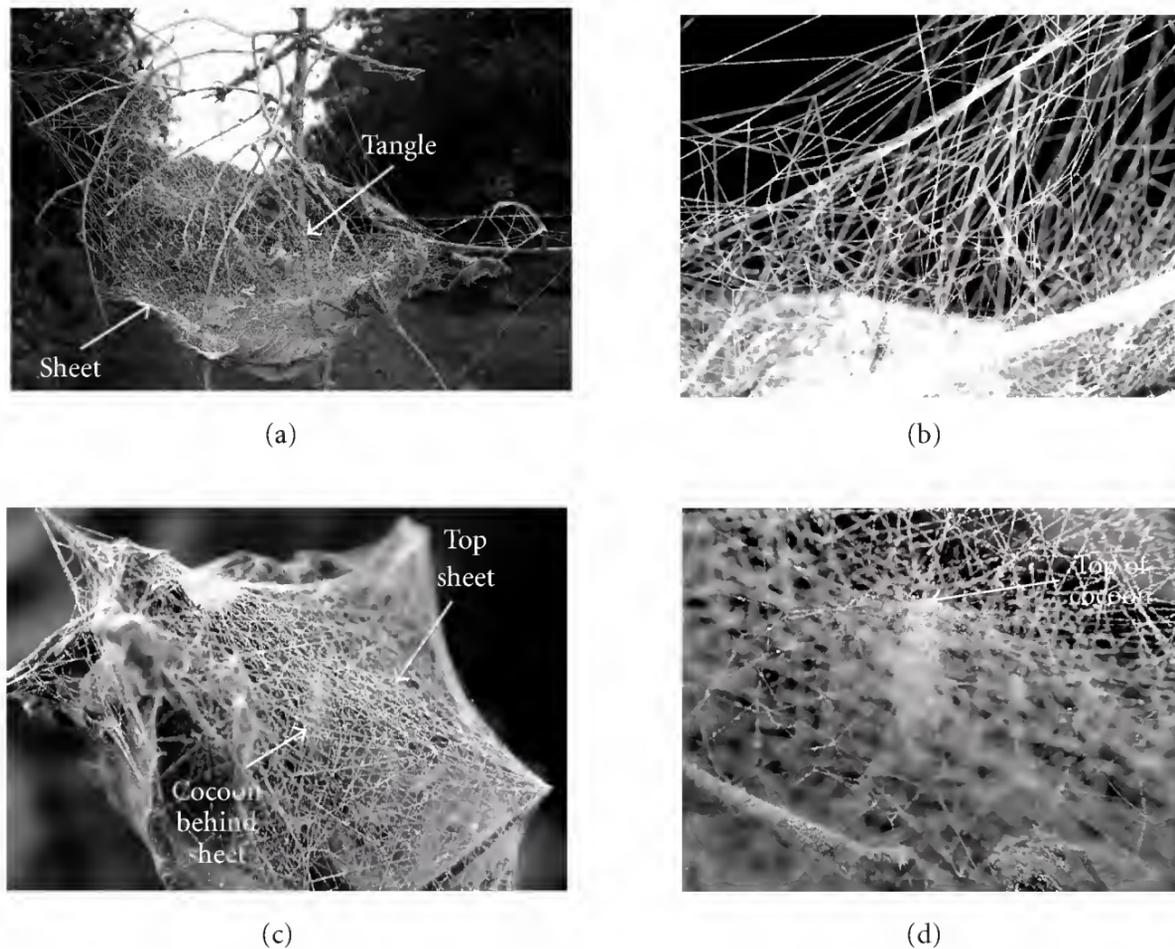


FIGURE 1: Lateral views of an entire typical web of a nonparasitized mature female *A. octavius* (a) and a closeup view of the tangle (b). Lateral views of a cocoon web of *A. nr. studiosus* with a sheet enclosing the entire web (c), and of the central platform in the tangle of this web to which the upper portion of a wasp cocoon is attached (d).

San Antonio de Escazú, and 1100 m el. in San Pedro de Montes de Oca (*A. nr. studiosus*), and at about 1650 m el. above Bebedero, Costa Rica (*A. octavius*). Cocoon webs with wasp cocoons built in the field were also collected intact, and examined under a dissecting microscope. Spiders in captivity were fed *Drosophila* sp. flies. Voucher specimens are deposited in the Museo de Zoología of the Universidad de Costa Rica (MZ) and the U. S. National Museum (spiders), and in the MZ and the Museum of Natural History, London (wasps). At each field site one of the two spider species was much more abundant than the other.

3. Results

3.1. Normal Webs. The webs of nonparasitized individuals of both *A. studiosus* and *A. octavius* were indistinguishable, and resembled those of other solitary *Anelosimus* species [17] in having a dense, somewhat concave sheet of irregularly oriented lines at the bottom of the web, and a moderately sparse tangle of lines above this sheet (Figures 1(a) and 1(b)). The degree to which the sheet extended upward at the edges varied; in extreme cases it formed a deep cup. Most webs were built near the end of a small branch. Many had leaves or other detritus in the tangle, and the spider hid under objects in the tangle during the day. Normal webs lasted for days and weeks at a time in the field, and were little damaged by moderate rain and wind.

3.2. Cocoon Webs. Seventeen cocoon webs were observed. Four in the field and one in captivity were made by

A. octavius; six in the field and six in captivity were made by *A. nr. studiosus*. In five cases the spider modified a web brought into captivity to produce a cocoon web, in all cases 1-2 days before the larva killed the spider. Two spiders abandoned their webs in captivity, and each built a complete cocoon web during the two nights before it was killed by the larva.

All cocoon webs in the field and all but one built in captivity were similar: the normal sheet was extended to enclose the entire web (Figure 1(c)); there was a small dense patch of more or less horizontal lines in the central portion of the tangle which had an approximately radial arrangement (Figures 1(d), 2(a), 2(c), and 2(d)); and just below this central patch there was a space that was free or nearly completely free of lines (Figure 2(b)). After completing the cocoon web: the spider rested at this exposed central area in the tangle during the day, instead of crouching as usual under a leaf or branch. The wasp larva then killed the spider, sucked its carcass dry and dropped it, and built a pupal cocoon attached at its upper end to the dense central patch of lines.

The exceptional web was built in captivity on the night of the same day the spider was collected (the field web sustained substantial damage when it was collected). The spider did not build a sheet over the tangle, but the tangle included a central area with a dense array of more or less radial lines and an open space below.

Of the two parasitized spiders that abandoned their previous webs in captivity, one built a small tangle on one night on a plant where it rested under a leaf, and then, on the night that the larva killed it, built silk sheets that joined the

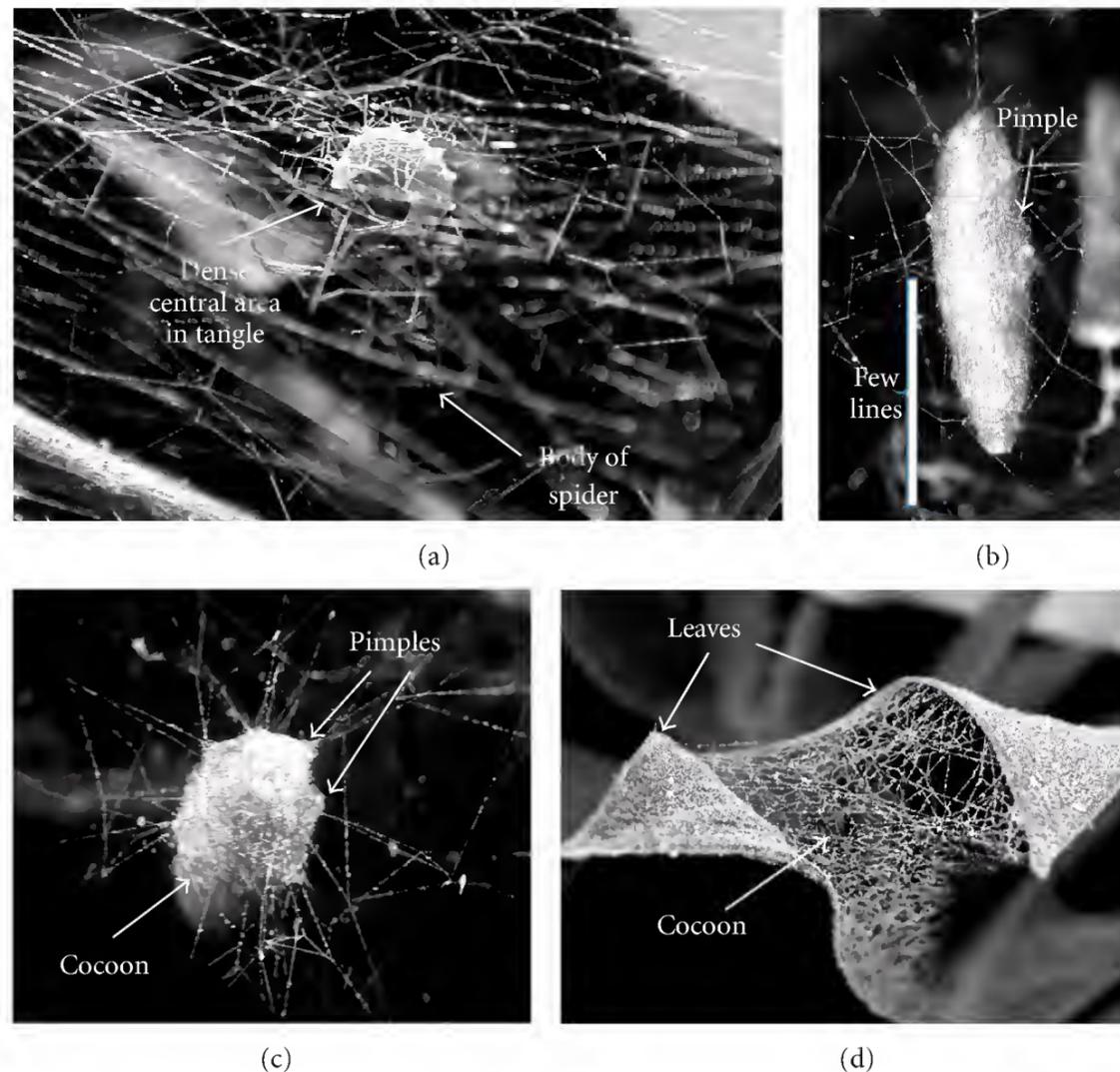


FIGURE 2: Cocoon web of *A. nr. studiosus* (a) in which the wasp larva holds onto the densely-meshed central area just after having discarded the corpse of the spider. Lateral (b) and dorsal (c) views of wasp cocoons in cocoon webs of *A. octavius*, showing the radial pattern of lines around the upper end of the cocoon (c), and that the lines intersecting the cocoon (indicated by small “pimples”) are in the upper portion of the cocoon, with an open space below in which the cocoon hangs free (bar in (b)). The cocoon web spun from scratch in captivity (d) incorporated flat leaves (covered with white dust in the photo) as parts of the sheet.

two edges of this leaf to the leaf below, thus enclosing itself on all sides (Figure 2(d)). The other spider built a tangle and a sheet that completely enclosed the tangle on the first night, then added further lines to the sheet the next night before the larva killed it early the next morning.

One spider observed while building a sheet in a cocoon web alternated between working on the sheets above and below the tangle; making multiple successive attachments to other lines in each sheet. It hung under the upper sheet while it worked there, and walked on top of the lower sheet while adding lines there. Each of two spiders observed during the final stages of cocoon web construction (these spiders were dead and being consumed only 5 and 4.5 hrs after observations ended) spent the entire time in a small section (about $2 \times 2 \times 4$ cm) in the central portion of the tangle, making forays downward and to the sides from the upper edge of this central volume and then returning to the central site. Each spider occasionally attached her dragline as she moved, and may have also broken lines; examination of one finished web revealed several lax, apparently broken lines in this area (and also a few in other parts of the web). Eventually the spider rested immobile at the central site, where the larva then killed and consumed it, and built its cocoon the next day.

4. Discussion

In all species of polysphinctine wasps known to modify the behavior of their web-spinning host spiders [8–14, 16], the manipulated spider performs fragments of behavior patterns that are used to build normal webs or retreats. In some spiders (*Plesiometa*, *Allocyclosa*, *Agelena*) these behavioral fragments are combined to produce structures that are apparently never produced by nonparasitized spiders [11], while in others (*Nephila*, perhaps *Theridion*) the resulting structures are at least somewhat similar to those of normal webs [9, 10]. At least some behavior elicited by *Z. nr. solanoi* produced web designs resembling those of normal host webs. The tightly meshed sheet that encloses the web is an extension of the lower sheet present in normal webs, and the construction behavior of the two sheets did not differ perceptibly except for the spiders’ orientation. However, a small platform in the tangle, with multiple radiating lines just above an open space, is not a recognizable component of normal *Anelosimus* webs. Breaking lines, which was used to open the space, may be part of normal web construction, as broken lines were also present at other sites in a newly built web.

The most surprising behavior in this study was that of spiders which built complete cocoon webs from scratch. The

normal webs of *Anelosimus* generally last for many weeks, and spiders apparently only rarely change sites and build new webs, so wasp larvae are probably seldom called upon to induce the construction of an entire cocoon web from scratch. One of these webs employed large flat leaves to substitute for parts of the upper and lower sheets of the cocoon web (Figure 2(d)). Although further observations are needed, this flexibility suggests that the wasp larva may manipulate the spider at higher rather than lower levels in the hierarchy of behavioral control mechanisms.

Despite its close relation with *Z. petronae*, the behavioral modifications induced by *Z. nr. solani* are qualitatively different [10], thus following the pattern seen in other polysphinctine wasps, in which behavior modifications tend to be more closely adjusted to the natural history of their hosts than to the phylogenetic relations of the wasps [7].

Acknowledgments

The author thanks Gilbert Barrantes and Pablo Guitierrez for spiders wasp larvae, Ingi Agnarsson and Paul Hanson for identifying spiders and wasps, and STRI and the Universidad de Costa Rica for financial support.

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Research Article

On the Breeding of Bivoltine Breeds of the Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), Tolerant to High Temperature and High Humidity Conditions of the Tropics

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Received 14 May 2010; Accepted 10 June 2010

Academic Editor: Subba Reddy Palli

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The hot climatic conditions of tropics prevailing particularly in summer are contributing to the poor performance of the bivoltine breeds and the most important aspect is that many quantitative characters such as viability and cocoon traits decline sharply when temperature is high. Hence, in a tropical country like India, it is very essential to develop bivoltine breeds/hybrids which can withstand the high temperature stress conditions. This has resulted in the development of CSR18 × CSR19, compatible hybrid for rearing throughout the year by utilizing Japanese thermotolerant hybrids as breeding resource material. Though, the introduction of CSR18 × CSR19 in the field during summer months had considerable impact, the productivity level and returns realized do not match that of other productive CSR hybrids. Therefore, the acceptance level of this hybrid with the farmers was not up to the expected level. This has necessitated the development of a temperature tolerant hybrid with better productivity traits than CSR18 × CSR19. Though, it was a difficult task to break the negative correlation associated with survival and productivity traits, attempts on this line had resulted in the development of CSR46 × CSR47, a temperature tolerant bivoltine hybrid with better productivity traits than CSR18 × CSR19. However, though, these hybrids are tolerant to high temperature environments, they are not tolerant to many of the silkworm diseases. Keeping this in view, an attempt is made to develop silkworm hybrids tolerant to high temperature environments.

1. Introduction

Silkworm breeding aims to achieve superior performances in respect of egg yield, cocoon raw silk yield, cocoon stability, and production followed by expansion to new areas besides others. Silkworm breeders continue to strive for an inherent gain in resistance by incorporating resistant genes into the genetic backgrounds of high yielding temperate bivoltines. Besides this, the cocoon crop stability also relies more on improving other production technologies which have to be explored. It is interesting to note that in inbreeding experiments, besides choice of parents, selection and inbreeding the hybrids are very important which have to be carefully executed since both inbreeding and hybridization are forms of nonrandom mating or selective mating, but operate in opposite ways. Inbreeding is a kind of genetic assortative

mating as compared with phenotypic assortative mating in hybridization. The major effect of inbreeding which is most apparent in the reduction of mean performance of the population is in question. While gene frequencies do not change on the whole, genotypic frequencies do change towards the production of more homozygotes and fewer heterozygotes. Thus, any change in the population mean as a result of inbreeding must be related to difference in genotype value between homozygote and heterozygote [1].

India enjoys the patronage of second position for the production of silk in the world next only to China. Sericulture in India is practiced predominantly in tropical environmental regions such as Karnataka, Tamil Nadu, Andhra Pradesh and West Bengal and to a limited extent in temperate environment of Jammu and Kashmir. The existing tropical situation provides scope for exploiting multivoltine

× bivoltine hybrid at commercial venture as they are hardy and have tremendous ability to survive and reproduce under varied or fluctuating environmental climatic conditions. But its quality is at low ebb when compared to the existing international standard.

Considering these drawbacks, adoption of bivoltine sericulture became imperative and imminent considering its potentiality even under Indian tropical conditions. Keeping this in view, breeding experiments were initiated at Central Sericultural Research and Training Institute, Mysore to evolve hardy bivoltine silkworm races suited to tropical conditions for achieving the primary objective of establishing bivoltine hybrids as a concept among sericulturists. Accordingly, many productive and qualitatively superior bivoltine hybrids have been developed by utilizing Japanese commercial hybrids as breeding resource material [2]. However, the hot climatic conditions prevailing particularly in summer are not conducive to rear these high yielding bivoltine hybrids throughout the year. It is a well-established fact that under tropical condition, unlike polyvoltines, bivoltines are more vulnerable to various stresses, that is, hot climatic conditions of tropics, poor leaf quality, and improper management during summer which are not conducive for bivoltine rearing. In order to select efficiently the breeds with high temperature tolerance, it is important to analyse the impact of high temperature on many silk yielding attributes of silkworm races and their heritability.

The success of sericulture industry depends upon several factors of which the impact of the environmental factors such as biotic and abiotic factors is of vital importance. Among the abiotic factors, temperature plays a major role on growth and productivity of silkworm, as it is a poikilothermic (cold blooded) insect [3]. It is also known that the late age silkworms prefer relatively lower temperature than young age and fluctuation of temperature during different stages of larval development was found to be more favourable for growth and development of larvae than constant temperature. There are ample literature stating that good quality cocoons are produced within a temperature range of 22–27°C and above these levels makes the cocoon quality poorer [4]. However, polyvoltine races reared in tropical countries are known to tolerate slightly higher temperature [5], which is also true with crossbreeds, that have been evolved specially for tropical climate.

The continued efforts for the improvement of cocoon characters of domesticated silkworm were aimed at increased quality silk production. The main objective of silkworm rearing is to produce qualitatively and quantitatively superior cocoons, which in turn will have a direct bearing on the raw silk production. Therefore, it becomes imperative or essential to develop bivoltine breeds/hybrids which can withstand high temperature stress conditions. Sericulture, the viable agro-based industry aptly matches the socioeconomic backdrop of rural India. One of the main aims of the breeders is to recommend silkworm breeds/hybrids to farmers that are stable under different environmental conditions and minimize the risk of falling below a certain yield level. Silkworm breeds that are reared over a series of environment exhibiting less variation are considered stable. The climatic

TABLE 1

Sl. No.	Breeding lines	Parentage	Breeding Plan
Dumbbell			
1	HH8	CSR19,	(CSR47 × CSR19) × CSR51
2	HH10	CSR47,	(CSR51 × CSR19) × CSR51
3	HH12	CSR51.	(CSR51 × CSR47) × CSR51

conditions prevailing in the tropics are most unpredictable and the problems of tropical sericulture are occurrence of aggravated silkworm diseases, unsuitable mulberry leaf for bivoltine silkworms, and lack of sustainable silkworm breeds for effective selection of desirable characters. In order to introduce bivoltine races in a tropical country like India, it is necessary to have stability in cocoon crop under high temperature environment. The prerequisite of summer hybrid is healthiness and adaptability to adverse conditions of high temperature, low food quality, relatively higher economic traits, with potential for increased cocoon production. Considering the poor performance of productive bivoltine hybrids during summer season, emphasis was given to evolve bivoltine silkworm breeds suitable to tropical conditions for achieving the primary objective of establishing bivoltine sericulture with quality raw silk among sericulturists. Thus a compatible robust bivoltine hybrid, CSR18 × CSR19 was evolved from a Japanese hybrid, B201 × BCS12 under high temperature ($36 \pm 1^\circ\text{C}$) and high humidity ($85 \pm 5\%$) conditions [6, 7] by taking clues from earlier experiments conducted by Japanese experts [8–10]. Though, this hybrid was authorized by Central Silk Board for commercialization, large-scale testing in the field is yet to take momentum due to its low productivity. Therefore, attempts are being made to develop bivoltine hybrids tolerant to high temperature conditions.

2. Materials and Methods

To initiate the breeding programme of high temperature ($40 \pm 1^\circ\text{C}$) and high humidity ($85 \pm 5\%$), six bivoltine breeds three each of oval, namely, CSR18, CSR46, and CSR50 and dumbbell, namely, CSR19, CSR47, and CSR51 were found to be temperature tolerant and selected as parental breeds for breeding programme. In oval lines, CSR46 and CSR50 are characterized by plain larvae while CSR18 is characterized by marked and plain (sex limited) larvae where female is marked and male is plain, similarly in dumbbell lines, CSR19 is characterized by sex limited (marked and plain) larvae and CSR47 and CSR51 are characterized by marked larvae.

For the development of breeds through appropriate techniques breeding programme was initiated with an objective to introduce the bivoltine breeds/hybrids for high temperature, that is, $40 \pm 1^\circ\text{C}$ and high relative humidity, that is, $85 \pm 5\%$. By utilising the breeding resource material three dumbbell lines, namely, HH8, HH10, HH12, tolerant to high temperature and high humidity conditions were developed, the parentage of which are depicted in Table 1.

Silkworm rearing was conducted following the standard method under the recommended temperature and relative humidity till 2nd day of 5th instar. During the process of breeding composite layings were prepared by utilized fifteen to twenty disease-free layings to ensure large population size with wide genetic base from F1 to F5, and progenies were raised by conducted mass rearing. Ten replicates of 100 larvae were counted after passing out 3rd moult and were kept in plastic trays and subjected for two different temperature treatments, that is, $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH and $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH in SERICATRON. (Environmental chamber with precise and automatic control facilities for uniform maintenance of temperature and humidity) from 3rd day of 5th instar and were fed fresh mulberry leaves twice a day. From F1 to F5 continuously thermal exposure was given, the larvae selected for two different high temperature treatments were exposed six hours per day (10 AM to 4 PM) till spinning which is appropriate time for exposure to high temperature [7–9]. As continuous exposure under high temperature conditions reduces quantitative traits drastically, recurrent backcrossing/outcrossing was given with one of the productive parental breeds as outlined in each breeding plan. From the base population of F1, larvae were also counted (300 larvae) and inbreeding was done for each breed and reared at room temperature up to F12, these room temperature reared batches were considered as control batches.

Cellular rearing was resorted to from F6 onwards to F12 with minimum five replications preceded by half sib/full sib mating for three different temperatures. At the time of brushing, the rich egg layings showing good hatching % were selected from each set of room temperature and two different high temperatures and reared. Owing the thermal effect in successive generations, it was observed that after 5th generation both qualitative and quantitative characters have declined sharply. So the experiment was modified in such a way that with every alternate generation from F6 onwards to F12 both high temperature lines were brought to room temperature conditions and reared continuously till spinning to recoup the lost vitality under stress conditions. The breeding plans of the three dumbbell lines are depicted in Figures 1 to 3.

3. Results

3.1. Performance of HH8 at Two Temperature Conditions

3.1.1. Rearing Performance. Generation wise mean performance for rearing of HH8 is presented in Table 2. Highest fecundity (598) was recorded at F6 and lowest (544) was recorded at F8 at $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH. At $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH, the *V* age larval duration ranged from 132 to 138 hours with the shortest of 132 being recorded at F4, F7, and F11. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH shortest *V* age larval span of 138 hrs was observed at F7 and it ranged from 138 hrs to 150 hrs (F5). The survival percentage in respect of HH8 at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH ranged from 76.3 to 89.3% with the highest of 89.3% recorded at F3 and the lowest of 76.3% at F5. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$

RH the survival percentage ranged from 90.9 to 94.1% with the highest of 94.1% at F1 and the lowest of 90.9% recorded at F6. At $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH, the highest cocoon yield/10000 larvae for HH8 was observed in F9 (15.79 kg) and the least (14.37 kg) at F2. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH8 was observed in F6 (18.58 kg) and the least (17.23 kg) at F2. The highest cocoon weight (1.607 g) for HH8 was recorded at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH in F7 and the lowest (1.562 g) at F7. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH3 was observed in F5 (1.904 g) and the lowest (1.727 g) in F3. The highest cocoon shell weight at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH for HH8 was observed in F9 (0.342 g) and the lowest (0.316 g) in F2. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH8 was observed in F5 (0.440 g) and the lowest (0.379 g) in F3. The highest cocoon shell percentage at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH for HH8 was observed in F9 (21.42%) and the lowest (20.26%) in F2. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH8 was observed in F2 (22.78%) and the lowest (22.19) in F1. Analysis of variance with regard to pupation rate recorded highly significant difference ($P > .001$) while Yield/10000 larvae recorded significant difference ($P > .01$) and fecundity recorded significant difference ($P > .05$) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH between generations. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, cocoon weight, shell weight recorded highly significant difference ($P > .01$) and Yield/10000 larvae recorded significant difference ($P > .05$) (Table 2).

3.1.2. Reeling Performance. Generation wise mean performance for reeling of HH8 is presented in Table 3. The reelability at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH ranged from 80% (F3) to 82% (F1 and F2). Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 84.67% at F2 to 86.67% at F3 and F12. Longest filament length of 997 m was recorded at F1 and the least of 851 m in F3 at $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the longest of 1106 m was recorded in F10 and least of 980 m in F2. Lowest renditta of 6.38 was observed at F9 and it ranged from 6.38 to 6.79 (F2) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 5.47 (F4) to 5.72 (F1). The highest raw silk percentage at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH was recorded in F9 (15.69%) and the lowest (14.76%) in F2. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest was observed in F4 (18.27%) and the lowest (17.48%) in F1. Thinner filament size of 2.29 was observed at F3 and it ranged from 2.29 to 2.50 d (F4) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 2.51 d (F2) to 3 d (F4). Highest neatness at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH was observed in F7 (90.67 p) and the lowest (85.0 p) in (F1). Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest was observed in F4 (92.67 p) and the lowest (90.67 p) in F5. Analysis of variance with regard to filament length (m), filament size (d) recorded highly significant difference ($P > .001$) while reelability recorded significant difference ($P > .01$) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH between generations. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, filament length (m) and filament size (d) recorded highly significant difference ($P > .001$) and reelability recorded significant difference ($P > .01$) was recorded (Table 3).

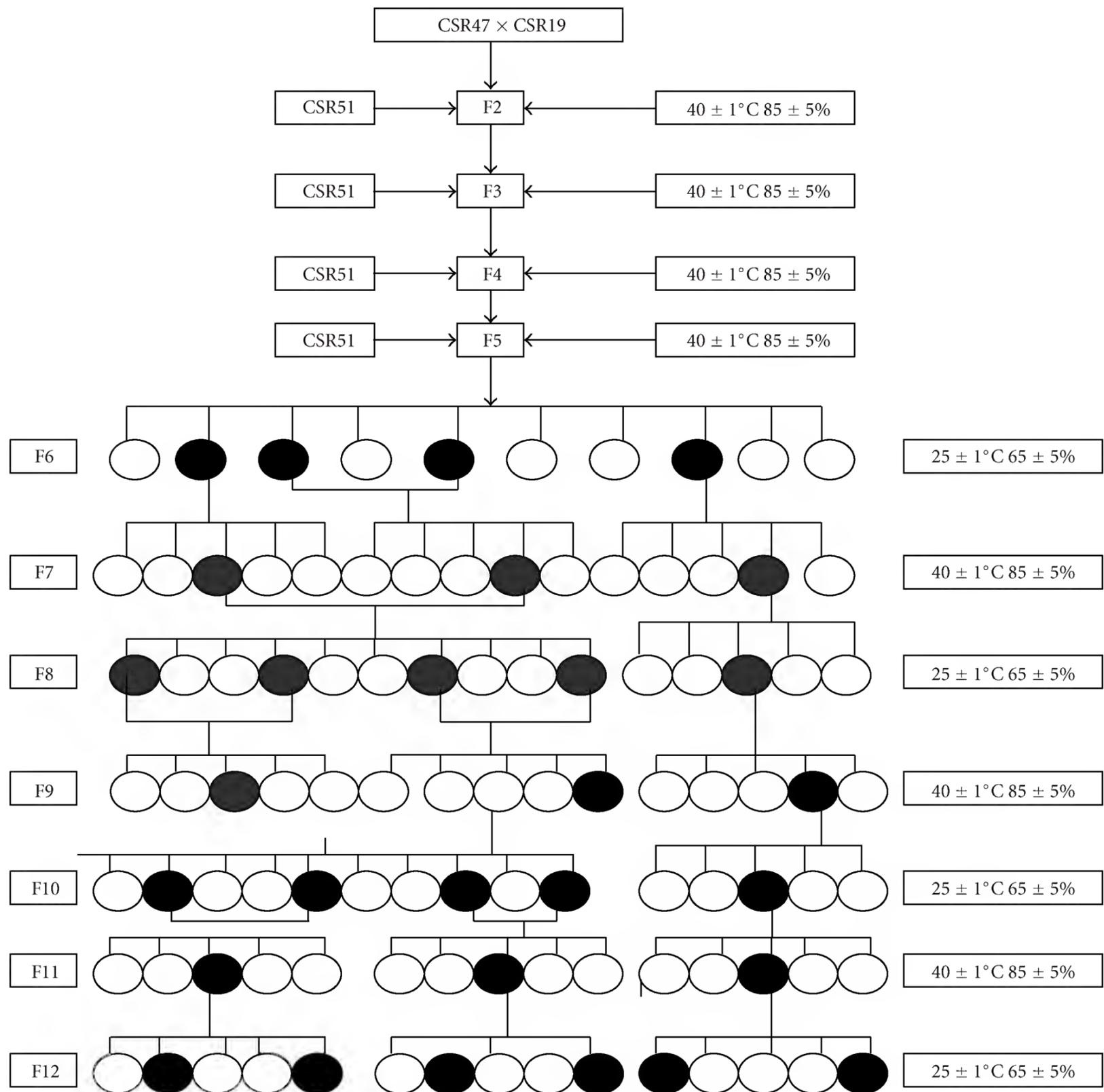


FIGURE 1: Breeding plan for HH8.

3.2. Performance of HH10 at Two Temperature Conditions

3.2.1. Rearing Performance. Generation wise mean performance for rearing of HH10 is presented in Table 4. Highest fecundity (597) at F3 and lowest (554) at F8 was recorded at $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH. At $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH, the *V* age larval duration ranged from 132 to 138 hours with the shortest of 132 was recorded at F4, F7, and F11. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH larval span of 138 hrs ranged from 144 hrs to 150 hrs (F5). The survival percentage in respect of HH10 at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH ranged from 77.7 to 87.7% with the highest of 87.7% recorded at F4 and the lowest of 77.7% at F7. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH the survival percentage ranged from 91.6 to 93.5% with the highest of 93.5% at F3 and the lowest of 91.6% recorded

at F4. At $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH, the highest cocoon yield/10000 larvae for HH10 was observed in F4 (16.39 kg) and the least (14.74 kg) at F2. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH10 was observed in F5 (19.41 kg) and the least (16.75 kg) at F4. The highest cocoon weight (1.601 g) for HH10 was recorded at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH in F1 and the lowest (1.521 g) at F2. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest (1.901 g) for HH10 was observed in F5 and the lowest (1.650 g) in F3. The highest cocoon shell weight at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH for HH10 was observed in F9 (0.342 g) and the lowest (0.312 g) in F2. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH10 was observed in F5 (0.408 g) and the lowest (0.364 g) in F3. The highest cocoon shell percentage at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH for HH10 was observed in F9 (21.74%) and the lowest

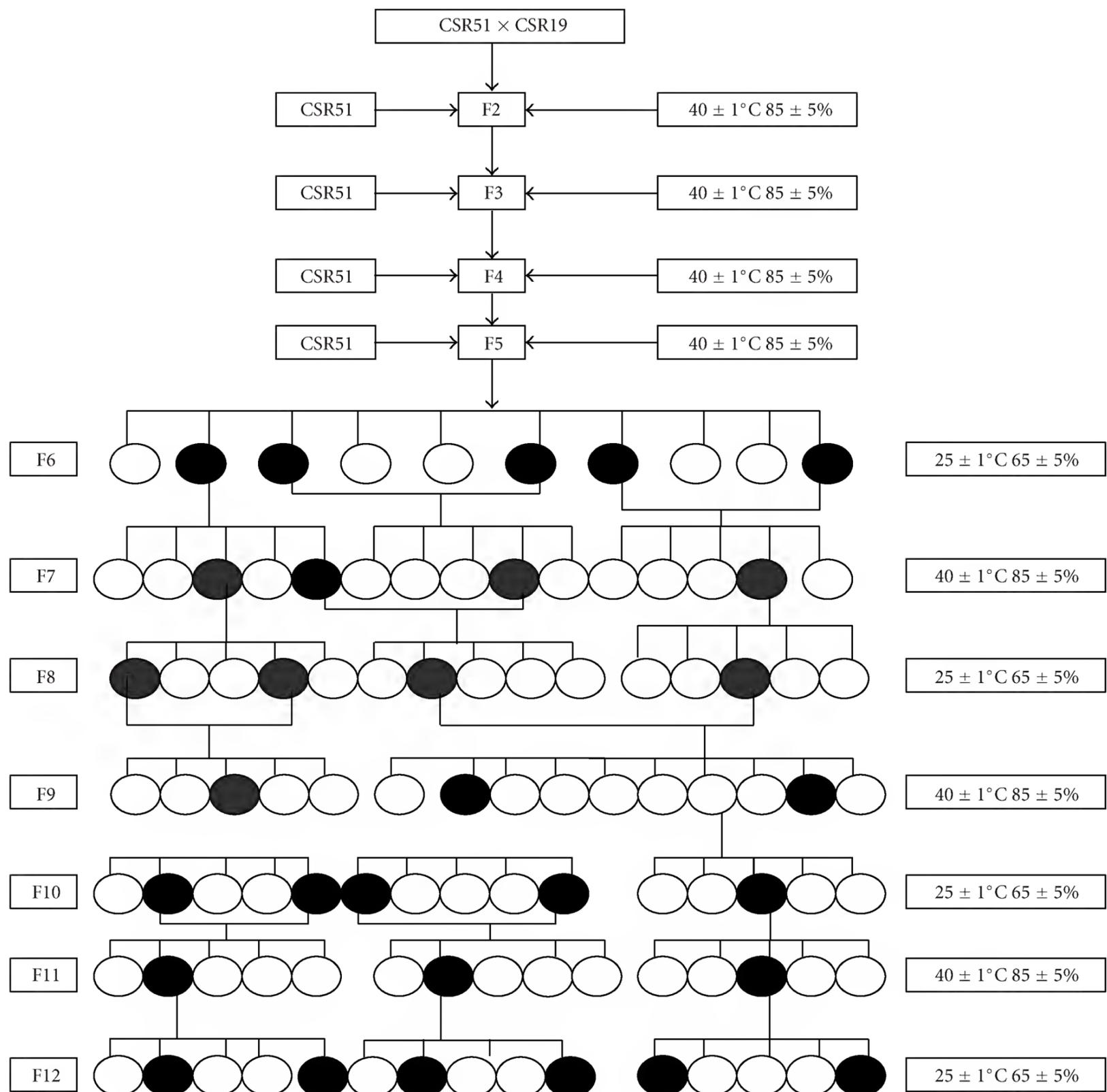


FIGURE 2: Breeding plan for HH10.

(20.42%) in F1. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH10 was observed in F12 (22.29%) and the lowest (21.39%) in F6. Analysis of variance with regard to Yield/10000 larvae recorded highly significant difference ($P > .01$) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH between generations. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, Yield/10000 larvae recorded significant difference ($P > .001$) and cocoon weight recorded significant difference ($P > .05$) (Table 4).

3.2.2. Reeling Performance. Generationwise mean performance for reeling of HH10 is presented in Table 5. The reelability at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH ranged from 80.33% (F11) to 82.33% (F1). Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 84.67% at F2 to 86.67% at F3 and F12. Longest filament length of 968 m was recorded at F1 and the

least of 582 m in F3 at $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the longest of 1106 m was recorded in F10 and least of 967 m in (F2). Lowest renditta of 6.30 was observed at F9 and it ranged from 6.30 to 6.73 (F7) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 5.65 (F10) to 5.86 (F6). The highest raw silk percentage at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH was recorded in F9 (15.87%) and the lowest (14.86%) in F7. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest was observed in F10 (17.70%) and the lowest (17.08%) in F6. Thinner filament size of 2.36 was observed at F9 and it ranged from 2.36 to 2.54 d (F4) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 2.54 d (F2) to 3 d (F5). Highest neatness at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH was observed in F7 and F11 (90.67 p) and the

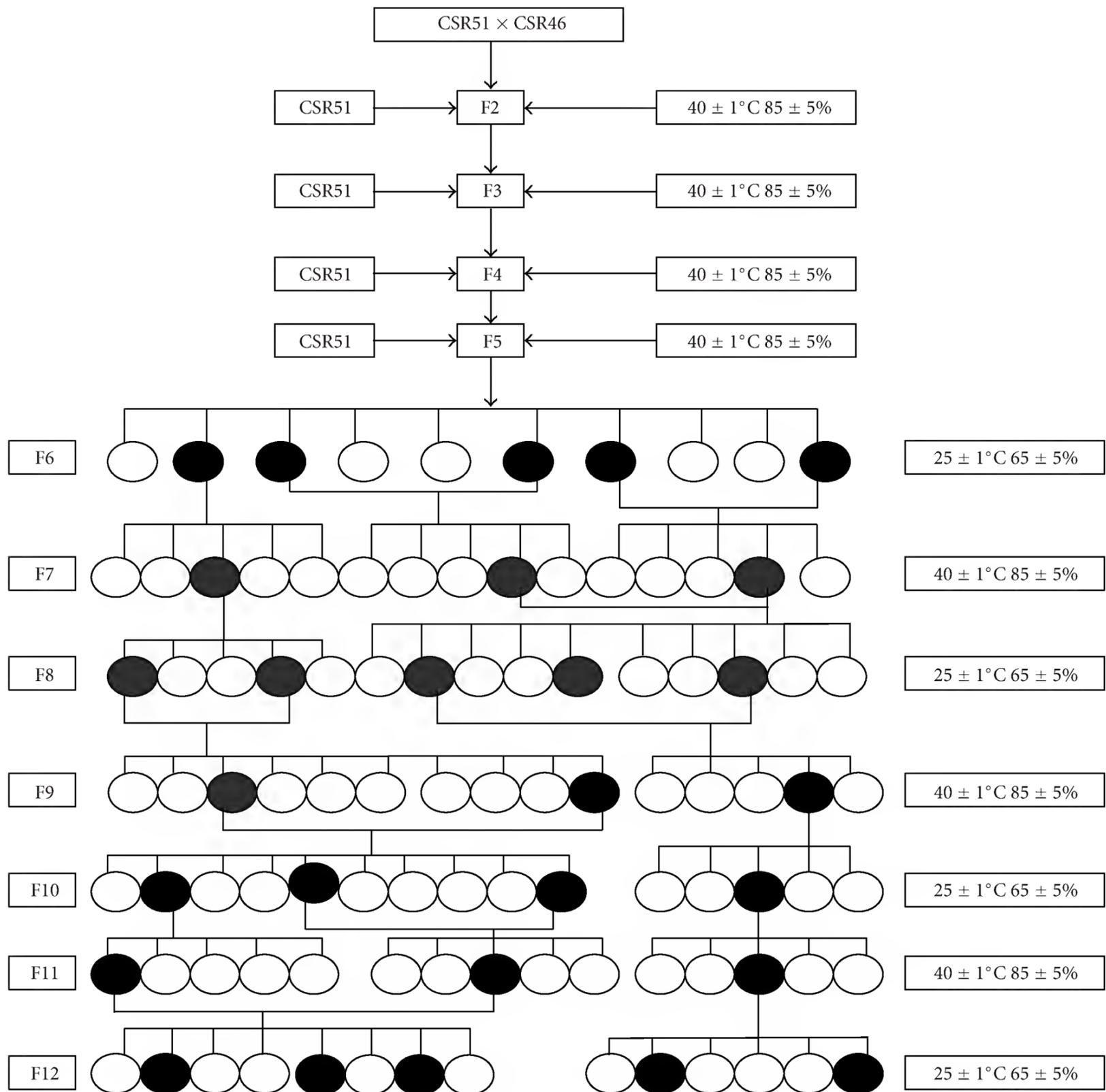


FIGURE 3: Breeding plan for HH12.

lowest (89.33 p) in (F5). Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest was observed in F4 and F6 (92.67 p) and the lowest (90.67 p) in F7 and F11. Analysis of variance for reeling of HH10 showed nonsignificant differences for all the traits at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. However at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, with regard to filament length (m), filament size (d) recorded highly significant difference ($P > .001$), and reelability recorded significant difference ($P > .01$) (Table 5).

3.3. Performance of HH12 at Two Temperature Conditions

3.3.1. Rearing Performance. Generation wise mean performance for rearing of HH12 is presented in Table 6. Highest fecundity (591) of HH1 was recorded at F8 and lowest (560) was recorded at F5 at $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH. At $40 \pm 1^\circ\text{C}$

and $50 \pm 5\%$ RH, the V age larval duration ranged from 132 to 138 hours with the shortest of 132 was recorded at F4, F7, and F11. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH V age larval span ranged from 144 hrs to 150 hrs (F5). The survival percentage in respect of HH12 at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH ranged from 77.3 to 85.0% with the highest of 85.0% recorded at F7 and the lowest of 77.3% at F1. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH the survival percentage ranged from 91.1 to 94.4% with the highest of 94.4% at F3 and the lowest of 91.1% recorded at F7. At $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH, the highest cocoon Yield/10000 larvae for HH12 was observed in F9 (16.42 kg) and the least (13.74 kg) at F7. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH12 was observed in F5 (19.81 kg) and the least (18.47 kg) at F8. The highest cocoon weight (1.600 g) for HH12 was recorded at $40 \pm 1^\circ\text{C}$

TABLE 2: Generation wise mean performance for rearing of HH8 at two temperature conditions.

Generation	40 ± 1°C and 85 ± 5% RH					25 ± 1°C and 65 ± 5% RH								
	Fecundity (No)	V age larval span (hrs)	Survival rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell%	Fecundity (No)	V age larval span (hrs)	Survival rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell (%)
F1	680	130	53.00 (46.74)	15.67	1.566	0.324	20.67 (27.04)	680	148	94.1 (75.9)	17.92	1.784	0.396	22.19 (28.11)
F2	446	134	54.7 (47.7)	14.65	1.562	0.316	20.26 (26.75)	446	144	92.6 (74.2)	17.23	1.726	0.393	22.78 (28.51)
F3	430	134	55.3 (48.8)	15.59	1.580	0.329	20.85 (27.16)	430	144	92.8 (74.4)	17.81	1.695	0.379	22.37 (28.25)
F4	420	134	65.7 (54.1)	14.82	1.588	0.328	20.67 (27.04)	420	144	92.0 (73.5)	17.61	1.781	0.406	22.81 (28.53)
F5	425	134	76.3 (60.8)	15.22	1.593	0.324	20.34 (26.31)	425	150	91.6 (73.1)	18.55	1.933	0.440	22.77 (28.50)
F6	428	—	—	—	—	—	—	428	144	90.9 (72.4)	18.58	1.789	0.402	22.45 (28.28)
F7	608	134	77.0 (61.3)	14.37	1.607	0.332	20.70 (27.05)	608	150	91.3 (72.8)	18.23	1.785	0.405	22.71 (28.46)
F8	434	—	—	—	—	—	—	434	144	91.8 (73.3)	18.05	1.803	0.405	22.45 (28.28)
F9	616	132	80.3 (63.6)	15.79	1.598	0.342	21.42 (27.57)	616	144	91.6 (73.1)	18.42	1.824	0.407	22.33 (28.20)
F10	438	—	—	—	—	—	—	438	144	91.6 (73.1)	18.48	1.767	0.397	22.46 (28.29)
F11	611	132	79.0 (62.7)	15.73	1.569	0.329	20.98 (27.26)	611	144	91.9 (73.4)	18.26	1.800	0.402	22.35 (28.22)
F12	448	—	—	—	—	—	—	448	144	91.4 (72.9)	18.12	1.720	0.388	22.56 (28.36)
Mean	499	133	67.66	15.239	1.584	0.328	20.737	499	145.00	92.0	18.10	1.784	0.402	22.52
F-test	*	ns	***	**	ns	ns	ns	*	ns	ns	*	**	**	ns
CD at 5%	3,10	—	15.02	0.79	—	—	—	3,10	—	—	0.74	0.09	0.02	—
CV%	19.66	1.14	17.57	4.32	2.28	3.56	3.91	19.66	1.69	1.475	2.97	4.14	4.35	1.26

*, **, *** Denote significant difference at 5%, 1% and 0.1% level, respectively. ns: Denote non significant. Values in parenthesis are angular transformed.

TABLE 3: Generation wise mean performance for reeling of HH8 at two different temperatures.

Generation	40 ± 1° C and 85 ± 5% RH						25 ± 1° C and 65 ± 5% RH					
	Reelability %	Filament length (m)	Renditta	Raw silk %	Filament size (d)	Neatness (p)	Reelability %	Filament length (m)	Renditta	Raw silk %	Filament size (d)	Neatness (p)
F1	82.00 (64.90)	757	6.59	15.1 (22.94)	2.42	85.00 (67.21)	86.00 (68.03)	981	5.72	17.48 (24.71)	2.83	92.33 (73.93)
F2	82.00 (64.90)	796	6.79	14.76 (22.59)	2.44	87.00 (68.87)	84.67 (66.95)	980	5.56	17.98 (25.09)	2.51	91.00 (72.60)
F3	80.00 (63.44)	825	6.68	15.01 (22.79)	2.29	86.00 (68.03)	86.67 (68.58)	1023	5.63	17.78 (24.94)	2.86	92.00 (73.57)
F4	81.00 (64.16)	808	6.66	15.02 (22.80)	2.50	88.67 (70.34)	85.33 (67.49)	1016	5.47	18.27 (25.31)	3.00	92.67 (74.30)
F5	80.67 (63.92)	879	6.76	14.79 (22.62)	2.42	87.00 (68.87)	85.00 (67.21)	1013	5.58	17.94 (25.06)	2.98	90.67 (72.23)
F6	—	—	—	—	—	—	86.33 (68.31)	1021	5.64	17.73 (24.90)	2.91	92.33 (73.93)
F7	80.67 (63.92)	898	6.63	15.11 (22.87)	2.46	90.67 (72.23)	86.00 (68.04)	1030	5.63	17.77 (24.93)	2.88	91.67 (73.26)
F8	—	—	—	—	—	—	86.33 (68.31)	993	5.58	17.93 (25.05)	2.89	91.33 (72.90)
F9	81.00 (64.16)	878	6.38	15.69 (23.33)	2.36	90.00 (71.57)	85.33 (67.49)	1033	5.59	17.88 (25.01)	2.97	92.33 (73.93)
F10	—	—	—	—	—	—	86.33 (68.31)	1106	5.68	17.61 (24.81)	2.87	91.00 (72.60)
F11	80.33 (63.68)	919	6.50	15.41 (23.11)	2.38	90.00 (71.57)	86.33 (68.31)	1007	5.66	17.66 (24.85)	2.87	92.33 (73.93)
F12	—	—	—	—	—	—	86.67 (68.59)	1021	5.57	17.95 (24.07)	2.89	92.00 (73.59)
Mean	80.96	845	6.62	15.12	2.41	88.04	85.92	1019	5.61	17.83	2.87	91.81
F-test	**	***	ns	ns	***	ns	**	***	ns	ns	***	ns
Cd at 5%	0.87	33.33	—	—	0.09	—	0.97	43.90	—	—	0.08	—
CV%	1.00	6.70	4.12	4.08	3.11	2.37	0.94	3.77	1.78	1.79	4.45	1.19

*, **, *** Denote significant difference at 5%, 1% and 0.1% level, respectively. ns: Denote non significant. Values in parenthesis are angular transformed.

TABLE 4: Generation wise mean performances for rearing of HH10 at two temperature conditions.

Generation	40 ± 1° C and 85 ± 5% RH										25 ± 1° C and 65 ± 5% RH									
	Fecundity (No)	V age larval span (hrs)	Survival rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell%	Fecundity (No)	V age larval span (hrs)	Survival rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell (%)						
F1	582	130	59.0 (50.7)	15.18	1.601	0.327	20.42 (26.86)	582	148	93.0 (74.6)	19.03	1.808	0.401	22.20 (28.11)						
F2	502	134	63.3 (52.8)	14.74	1.521	0.312	20.53 (26.94)	502	144	92.8 (74.4)	18.77	1.784	0.395	22.13 (28.06)						
F3	465	134	62.0 (52.1)	15.10	1.590	0.328	20.65 (27.03)	465	144	93.5 (75.2)	18.72	1.650	0.364	22.07 (28.02)						
F4	435	134	77.7 (61.8)	16.39	1.542	0.318	20.68 (27.04)	435	144	91.6 (73.1)	16.75	1.851	0.401	21.68 (27.75)						
F5	420	134	78.0 (62.0)	14.87	1.543	0.324	21.02 (27.29)	420	150	92.2 (73.7)	19.41	1.901	0.408	21.48 (27.62)						
F6	485	—	—	—	—	—	—	485	144	92.5 (74.1)	18.37	1.825	0.391	21.39 (27.54)						
F7	618	134	87.7 (70.3)	15.32	1.566	0.320	20.44 (26.88)	618	150	92.9 (74.5)	18.93	1.837	0.404	22.00 (27.97)						
F8	440	—	—	—	—	—	—	440	144	92.0 (73.5)	18.87	1.806	0.398	22.02 (27.98)						
F9	600	132	82.0 (64.9)	15.40	1.575	0.342	21.74 (27.79)	600	144	91.9 (73.4)	18.75	1.828	0.404	22.10 (28.04)						
F10	453	—	—	—	—	—	—	453	144	92.0 (73.5)	18.73	1.791	0.397	22.16 (28.08)						
F11	619	132	80.7 (63.9)	15.47	1.596	0.329	20.61 (27.00)	619	144	92.7 (74.3)	18.63	1.825	0.404	22.14 (28.07)						
F12	458	—	—	—	—	—	—	458	144	93.3 (75.0)	18.07	1.781	0.397	22.29 (28.17)						
Mean	506	133	73.80	15.487	1.567	0.324	20.762	506	145.00	92.5	18.59	1.807	0.397	21.97						
F-test	ns	ns	*	**	ns	ns	ns	ns	ns	ns	***	*	ns	ns						
Cd at 5%	—	—	19.3	1.07	—	—	—	—	—	—	0.76	0.10	—	—						
CV%	15.06	1.14	14.57	5.57	3.21	3.97	4.08	15.06	1.69	1.24	4.00	4.18	4.03	2.432						

*, **, *** Denote significant difference at 5%, 1% and 0.1% level, respectively. ns: Denote non significant. Values in parenthesis are angular transformed.

TABLE 5: Generation wise mean performance for reeling of HH10 at two different temperatures.

Generation	40 ± 1° C and 85 ± 5% RH						25 ± 1° C and 65 ± 5% RH					
	Reelability %	Filament length (m)	Renditta	Raw silk %	Filament size (d)	Neatness (p)	Reelability %	Filament length (m)	Renditta	Raw silk %	Filament size (d)	Neatness (p)
F1	82.33 (65.15)	852	6.61	15.15 (22.90)	2.38	85.00 (67.21)	86.00 (68.03)	984	5.69	17.58 (24.59)	2.86	92.33 (73.93)
F2	81.33 (64.40)	764	6.68	14.99 (22.77)	2.45	86.33 (68.31)	84.67 (68.95)	967	5.71	17.52 (24.74)	2.54	91.67 (73.26)
F3	81.67 (64.65)	782	6.69	14.96 (22.76)	2.42	88.67 (70.34)	86.67 (68.59)	1026	5.75	17.40 (24.65)	2.89	91.33 (72.90)
F4	81.67 (64.65)	868	6.70	14.98 (22.76)	2.54	87.00 (68.87)	85.33 (67.49)	1027	5.78	17.31 (24.58)	2.99	92.67 (74.30)
F5	81.00 (64.16)	857	6.53	15.34 (23.05)	2.42	89.33 (70.95)	85.00 (67.21)	983	5.84	17.12 (24.44)	3.00	91.67 (73.23)
F6	—	—	—	—	—	—	86.33 (68.31)	1000	5.86	17.08 (24.41)	2.91	92.67 (74.30)
F7	81.00 (64.16)	968	6.73	14.86 (22.67)	2.46	90.67 (72.23)	86.00 (68.04)	1049	5.76	17.36 (24.62)	2.90	90.67 (72.23)
F8	—	—	—	—	—	—	86.33 (68.31)	993	5.72	17.48 (24.71)	2.85	91.00 (72.56)
F9	81.00 (64.16)	878	6.30	15.87 (23.48)	2.36	89.33 (70.95)	85.33 (67.49)	1033	5.68	17.60 (24.80)	2.97	92.33 (73.93)
F10	—	—	—	—	—	—	86.33 (68.31)	1106	5.65	17.70 (24.88)	2.87	91.00 (72.60)
F11	80.33 (63.68)	919	6.61	15.14 (22.90)	2.38	90.67 (72.22)	86.33 (38.31)	1007	5.66	17.67 (24.85)	2.87	90.67 (72.23)
F12	—	—	—	—	—	—	86.67 (68.59)	1084	5.68	17.60 (24.80)	2.87	91.00 (72.56)
Mean	81.29	861	6.61	15.16	2.43	88.38	85.92	1022	5.73	17.45	2.88	91.58
F-test	ns	Ns	ns	ns	ns	ns	**	**	ns	ns	**	Ns
CD at 5%	—	—	—	—	—	—	0.97	50.26	—	—	0.05	—
CV%	0.99	7.72	4.28	4.21	3.82	2.34	0.94	4.65	2.51	2.47	4.03	1.24

*, **, *** Denote significant difference at 5%, 1% and 0.1% level, respectively. ns: Denote non significant. Values in parenthesis are angular transformed.

TABLE 6: Generation wise mean performances for rearing of HH112 at two temperature conditions.

Generation	40 ± 1°C and 85 ± 5% RH					25 ± 1°C and 65 ± 5% RH								
	Fecundity (No)	V th age larval span (hrs)	Survival rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell (%)	Fecundity (No)	V th age larval span (hrs)	Survival rate (%)	Yield/10,000 larvae (kg)	Cocoon Wt. (g)	Shell Wt. (g)	Cocoon Shell (%)
F1	600	130	53.8 (46.74)	16.04	1.600	0.325	20.33 (26.80)	600	148	91.6 (73.1)	18.76	1.756	0.382	21.77 (27.81)
F2	484	134	60.7 (51.2)	15.25	1.562	0.330	21.16 (27.29)	484	144	92.6 (74.2)	18.73	1.834	0.406	22.16 (28.08)
F3	470	134	53.0 (46.74)	14.77	1.521	0.334	21.96 (27.94)	470	144	94.4 (76.3)	18.88	1.821	0.407	22.32 (28.19)
F4	464	134	65.7 (54.1)	14.77	1.585	0.325	20.51 (26.92)	464	144	93.8 (75.5)	18.77	1.915	0.430	22.46 (28.29)
F5	450	134	56.3 (48.5)	15.93	1.571	0.329	20.96 (27.24)	450	150	92.7 (74.3)	19.81	1.947	0.438	22.50 (28.32)
F6	455	—	—	—	—	—	—	455	144	92.3 (73.8)	18.78	1.839	0.414	22.51 (28.32)
F7	625	134	77.7 (61.8)	13.74	1.579	0.336	21.30 (27.48)	625	150	91.1 (72.6)	18.94	1.823	0.415	22.75 (28.49)
F8	462	—	—	—	—	—	—	462	144	91.8 (73.3)	18.47	1.847	0.421	22.78 (28.51)
F9	632	132	83.7 (66.1)	16.42	1.598	0.327	20.46 (26.89)	632	144	92.5 (74.1)	19.27	1.863	0.424	22.74 (28.48)
F10	468	—	—	—	—	—	—	468	144	92.5 (74.1)	18.92	1.775	0.396	22.34 (28.21)
F11	610	132	83.3 (65.8)	15.94	1.569	0.327	20.83 (27.15)	610	144	93.6 (75.3)	18.71	1.808	0.411	22.72 (28.47)
F12	470	—	—	—	—	—	—	470	144	92.6 (74.2)	18.71	1.759	0.396	22.50 (28.31)
Mean	516	133	66.78	15.357	1.574	0.33	20.938	516	145.00	92.6	18.90	1.832	0.412	22.46
F-test	ns	ns	*	** *	ns	ns	ns	ns	ns	ns	ns	*	**	ns
CD at 5%	—	—	18.7	0.92	—	—	—	—	—	—	—	0.10	0.02	—
CV%	14.61	1.14	19.48	6.26	2.29	3.22	4.16	14.61	1.69	1.90	2.57	4.10	4.28	2.02

*, **, *** Denote significant difference at 5%, 1% and 0.1% level, respectively. ns: Denote non significant. Values in parenthesis are angular transformed.

and $85 \pm 5\%$ RH in F1 and the lowest (1.521 g) at F3. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH12 was observed in F5 (1.947 g) and the lowest (1.756 g) in F1. The highest cocoon shell weight at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH for HH12 was observed in F7 (0.336 g) and the lowest (0.325 g) in F1 and F4. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH12 was observed in F5 (0.438 g) and the lowest (0.382 g) in F1. The highest cocoon shell percentage at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH for HH12 was observed in F3 (21.96%) and the lowest (20.33%) in F1. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest for HH12 was observed in F8 (22.78%) and the lowest (21.77%) in F1. Analysis of variance with regard to Yield/10000 larvae recorded highly significant difference ($P > .001$) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH between generations. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, shell weight recorded significant difference ($P > .01$) and cocoon weight recorded significant difference ($P > .05$) (Table 6).

3.3.2. Reeling Performance. Generation wise mean performance for reeling of HH12 is presented in Table 7. The reelability at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH ranged from 80.67% (F2), (F5), (F7) and (F11) to 82% (F3) and (F9). Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 83.33% at F4 to 86.67% at F12. Longest filament length of 976 m was recorded at F1 and the least of 887 m in F5 at $40 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ RH. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the longest 1077 m was recorded in F3 and least 963 m in (F5). Lowest renditta of 6.28 was observed at F3 and it ranged from 6.28 to 6.82 (F4) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. However, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 5.53 (F8) to 5.83 (F3). The highest raw silk percentage at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH was recorded in F3 (15.94%) and the lowest (14.68%) in F4. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest was observed in F8 (18.08%) and the lowest (17.16%) in F1. Thinner filament size of 2.37 was observed at F9 and it ranged from 2.37 to 2.69 d (F7) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, it ranged from 2.63 d (F2) to 3.01 d (F4). Highest neatness at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH was observed in F7 and F11 (90.33 p) and the lowest (85.33 p) in (F1). Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, the highest was observed in F1, F3, F9 and F11 (92.33 p) and the lowest (90.67 p) in F5 and F7. Analysis of variance with regard to filament length (m), filament size (d) recorded highly significant difference ($P > .001$) at $40 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH between generations. Similarly, at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH, reelability, filament size (d) recorded highly significant difference ($P > .001$) and filament length (m) recorded significant difference ($P > .05$) (Table 7).

4. Discussion

The breeding of silkworm since long has been aimed towards evolving of superior and hardy breeds either by means of selection alone or by combining outcrossing or backcrossing with selection in the subsequent generations. The final aim of the breeder is primarily to evolve a breed which can give rise to stabilized crops and secondly to improve both quantity and quality of silk [11]. The breeding of silkworm races probably dates back to the beginning of the history of silkworm

rearing, but it has made great progress rather recently [12]. Sericulturally advanced countries like Japan has achieved remarkable progress by executing systematic breeding plans for the development of productive races. In silkworms, studies carried out for various characters have shown that the characters could be changed to suit the breeders choice, since selection for one trait has correlation with genetic change of other characters. The correlation for few traits is negative and for some it is positive [13–16]. Therefore, during the course of breeding of new breeds, the breeder has to be aware of the response of certain characters in selection and its correlated changes with other economic traits. Inbreeding of hybrids to stabilize silkworm breeds which bred true is well documented [12, 16–26]. Similarly, Kovolov [27] is of the opinion that improvement of silkworm races is possible by outbreeding with exotic races and improvement of cocoon quality by repeated backcrossing [28].

According to Allard and Bradshaw [29], performance of the strain itself in a given environment indicates its superiority. During evaluation, emphasis was given on the phenotypic expression of traits of economic importance under different temperature conditions. However, as the objective of the study was for greater viability and high productivity merits, equal importance was given on these two traits during selection of parents. The significant variations observed in the phenotypic manifestation for the traits analyzed can be attributed to the genetic constitution of the breeds and their degree of expression to which they are exposed during their rearing. Such variations in the manifestation of phenotypic traits of the breeds studied can be ascribed to the influence of environmental conditions. Variable gene frequencies at different loci make them to respond differently. The results are in line with the findings of [29–39].

In the present breeding programme, which envisages evolution of new hardy bivoltines, the aim was to develop more resistant bivoltines that can give rise to stable cocoon crops with better viability, even though productivity is low compared to the existing productive bivoltine breeds that are currently used in the field. In silkworms, the correlation for some characters is positive and for some is negative [15, 16]. Such a negative correlation is observed for the traits productivity and viability and hence the attempt made was to increase the viability of the developed breeds. Moreover as suggested by [40, 41], the selection parameters were primarily aimed at improving the viability character such as yielded by number without sacrificing much of the productivity traits like cocoon weight, cocoon shell weight, and yield by weight. In addition, during later generations of inbreeding, selection was applied to select desired genotypes to improve the traits of commercial importance like viability and productivity as suggested by [42, 43] to improve the yield of bivoltines.

The imposition of exposure to high temperature levels in 5th instar and the resultant low pupation rate could be attributed to the low feeding activity of the silkworm resulting in the physiological imbalance and poor health of the larvae and an increased number of nonspinning worms in the mountages. The work in [44] demonstrated that silkworms are more sensitive to high temperature during

TABLE 7: Generation wise mean performance for reeling of HH12 at two different temperatures.

Generation	40 ± 1° C and 85 ± 5% RH						25 ± 1° C and 65 ± 5% RH					
	Reelability %	Filament length (m)	Renditta	Raw silk %	Filament size (d)	Neatness (p)	Reelability %	Filament length (m)	Renditta	Raw silk %	Filament size (d)	Neatness (p)
F1	81.00 (64.17)	794	6.71	14.93 (22.72)	2.39	85.33 (37.49)	85.67 (67.76)	986	5.83	17.16 (24.47)	2.87	92.33 (73.93)
F2	80.67 (63.92)	763	6.53	15.33 (23.05)	2.45	86.33 (68.31)	84.67 (66.95)	982	5.69	17.58 (24.79)	2.63	92.00 (72.59)
F3	82.00 (64.91)	792	6.28	15.94 (23.53)	2.54	88.67 (70.34)	85.67 (67.76)	1077	5.70	17.5 (24.75)	2.88	92.33 (73.93)
F4	81.00 (64.16)	852	6.82	14.68 (22.52)	2.53	90.00 (71.61)	83.33 (65.91)	1013	5.70	17.55 (24.77)	3.01	91.67 (73.26)
F5	80.67 (63.92)	871	6.59	15.18 (22.93)	2.43	89.33 (70.95)	84.00 (66.42)	963	5.66	17.65 (24.84)	2.85	90.67 (72.23)
F6	—	—	—	—	—	—	86.33 (68.31)	981	5.64	17.73 (24.90)	2.82	91.33 (72.89)
F7	80.67 (63.92)	965	6.40	15.64 (23.30)	2.69	90.33 (71.89)	86.00 (68.04)	1057	5.57	17.94 (25.06)	2.84	90.67 (72.23)
F8	—	—	—	—	—	—	86.33 (68.31)	1037	5.53	18.08 (25.16)	2.81	91.00 (72.56)
F9	82.00 (64.91)	878	6.64	15.08 (22.84)	2.37	90.00 (71.57)	85.33 (37.49)	1027	5.57	17.95 (25.07)	2.90	92.33 (73.93)
F10	—	—	—	—	—	—	86.33 (68.31)	1024	5.62	17.79 (24.95)	2.88	92.00 (73.59)
F11	80.67 (63.92)	976	6.57	15.27 (23.00)	2.42	90.33 (71.89)	86.33 (68.31)	1023	5.63	17.76 (24.92)	2.93	92.33 (73.93)
F12	—	—	—	—	—	—	86.67 (68.59)	1065	5.59	17.91 (25.03)	2.95	91.00 (72.60)
Mean	81.08	894	6.57	15.26	2.48	88.79	85.56	1020	5.65	17.72	2.86	91.64
F-test	ns	***	ns	ns	***	ns	***	*	ns	ns	***	ns
CD at 5%	—	29.20	—	—	0.11	—	1.01	66.06	—	—	0.08	—
CV%	1.58	5.33	4.19	4.20	4.69	2.17	1.32	4.69	2.12	2.12	3.42	1.17

*, **, *** Denote significant difference at 5%, 1% and 0.1% level, respectively. ns: Denote non significant. Values in parenthesis are angular transformed.

4th and 5th instars. The productive bivoltine breeds are reported to be susceptible to high temperature; the authors of [8, 45] noticed higher survival in the hybrids than the pure races under high temperature conditions. In the present investigation, when lines are exposed to high temperature continuously there is a drastic reduction in the pupation rate and cocoon traits. The work in [46] observed that at high temperature (35°C) and low humidity (50 ± 5%) and high humidity (85 ± 5%) conditions, pupation rate was drastically reduced in productive hybrid, CSR2 × CSR5. Such drastic change is usually obtained as it is low heritable trait in the silkworm and is prone to large variations in environment and management [47]. The work in [48] observed that the pupation rate in Indian popular bivoltine breed, NB4D2, is significantly influenced by both low and high humidity.

Silkworm breed which are reared over a series of environments exhibiting less variation are considered stable. One of the objectives of the breeder is to recommend stable breeds to the farmers for rearing under different environmental conditions. Effect of high temperature and low humidity in terms of cocoon crop depends on several factors that operate within and outside the body of the silkworm. In the present study, it was observed that apart from the temperature, humidity also influences the productivity pattern in the silkworm and is in agreement with [49, 50]. It was also observed that the cocoon Yield/10000 larvae, cocoon weight, cocoon shell weight, and cocoon shell percentage were also low in the high temperature treated batches when compared to the batches reared under optimum rearing conditions. The work in [51] reported the deleterious effect of high temperature and high humidity on quantitative traits of parents, foundation crosses, and single and double hybrids of bivoltine silkworm breeds of *Bombyx mori* L.

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Research Article

Histochemical Comparison of the Hypopharyngeal Gland in *Apis cerana* Fabricius, 1793 Workers and *Apis mellifera* Linnaeus, 1758 Workers

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Received 9 April 2010; Revised 9 July 2010; Accepted 3 August 2010

Academic Editor: Diana E. Wheeler

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Hypopharyngeal glands of honeybee are age-dependent structures that change with the size of acini and are correlated with various social behaviors. The histochemical structure of *Apis cerana* and *A. mellifera* worker hypopharyngeal glands in four different developmental stages was stained with ninhydrin Schiff's and periodic acid Schiff's reagents (PAS) for localization of proteins and carbohydrates, respectively, and examined with light microscopy. Nurse bees of both honeybee species had significantly larger glands as compared to guards and foragers, but there were no statistically significant differences between these two species after accounting for caste. Gland protein concentration increased progressively in nurse bees, and this was correlated with the appearance of enriched protein granules in the cytoplasm. In addition, the hypopharyngeal gland protein concentration of *A. mellifera* was higher than that of *A. cerana* even though gland size was not significantly different between species. However, gland size was shown to have decreased progressively in foragers and guards.

1. Introduction

The development of hypopharyngeal glands (HPGs) in dwarf honeybee workers primarily depends on age. These glands begin to differentiate at pupal stage and are largely undeveloped at emergence [1]. When workers become nurse bees, they perform brood rearing that is associated with HPGs development. The size of HPGs is correlated with glandular production and generally increases with age from 6 to 18 days in nurse bees [2, 3]. HPGs synthesize and secrete proteinaceous substances and royal jelly that are fed to the queen and brood [3]. The highest rate of protein synthesis occurs during nursing ages from 8 to 16 days [4, 5]. In bees older than 18 days (guards and foragers), the HPGs decrease considerably in size and secrete enzymes such as α -glucosidases, leucine arylamidase and invertase [6–9]. Forager gland size is reduced and correlated with the gland activity [3, 4]. We previously demonstrated the location of proteins and carbohydrates within HPGs of dwarf

honeybee workers among different ages [1]. The glands were composed of several secretory apparatus. Each opened into a secretory duct and then passed through the mouthparts. In pupae, the secretory cells were irregular in shape with low concentrations of proteins and carbohydrates while the glands of nurse bees and foragers were fully developed with numerous secretory vesicles [1]. For this study, we measured the size of glandular acini, examined protein concentration in hypopharyngeal glands, and identified the location of proteins and carbohydrates in the hypopharyngeal gland workers of *Apis cerana* and *A. mellifera*.

2. Materials and Methods

2.1. Honeybees. *Apis cerana* and *A. mellifera* workers of different ages were collected from Samut Songkarm province, Thailand. Pupae (dark brown-eyed stage) were grasped from the cells. Nurse bees were taken from their colony while they were feeding brood. Guards were collected from in front of

TABLE 1: Hypopharyngeal gland sizes (mean \pm S.E.) of *A. cerana* and *A. mellifera* workers in different stages of life: nurse, forager, and guard.

Honeybees	Workers	Hypopharyngeal gland sizes (mean \pm s.e.)	
		width (μm)	length (μm)
<i>A. cerana</i>	Nurse	101.57 \pm 4.68 ^b	128.55 \pm 4.41 ^a
	Guard	83.59 \pm 3.80 ^{cd}	108.28 \pm 6.53 ^{bc}
	Forager	91.56 \pm 3.33 ^{bc}	113.91 \pm 6.28 ^{ab}
<i>A. mellifera</i>	Nurse	116.41 \pm 4.25 ^a	122.51 \pm 4.31 ^{ab}
	Guard	68.12 \pm 2.54 ^e	94.06 \pm 4.77 ^c
	Forager	74.69 \pm 2.03 ^{ed}	97.66 \pm 2.84 ^c

Note: Means \pm S.E. followed with different letters in the same column denote significant differences (ANOVA-Duncan's multiple range test; $F = 24.98$, $df = 5$, $P < .0001$; $F = 7.32$, $df = 5$, $P < .0001$).

the hive entrance. Foragers (bees with pollen loads) were captured inside the colonies.

2.2. Glandular Size Measurement. Measurements of glandular size were made from nurse bees, guards, and foragers. Under a stereomicroscope, HPGs of each life stage were removed from the head using modified blades and then transferred into insect saline solution (NaCl 8.766 g., CaCl₂ 0.188 g., KCl 0.746 g., MgCl₂ 0.407 g., NaHCO₃ 0.336 g., sucrose 30.807 g., and trehalose 1.892 g., pH 7.6). Using a micrometer, gland diameters were measured (width and length) under light microscopy.

2.3. Preparation of Protein Sample. Ten worker bees were collected to represent each species and anesthetized on dry ice. Protein extraction from HPGs was modified from the work of Li et al. [9]. Glands were transferred to 50 μL 0.1 M phosphate buffer (PB) pH 7.8 in a 1.5 mL microcentrifuge tube, homogenized for 10 min on ice, sonicated for 2 min, then centrifuged at 1500 rpm for 10 min at 4°C. The supernatant was transferred to another tube. The pellet was resuspended in 10 μL PB and then centrifuged at 1500 rpm for 10 min at 4°C. The supernatant from this resuspension was removed and added to the previous supernatant and stored at 4°C.

2.4. Protein Assay. Glands were homogenized and then centrifuged at 1000 rpm for 2 min. The supernatant was analyzed using the Bradford protein assay [10]. Standard curves were prepared using bovine serum albumin (BSA) and absorbance measured at 595 nm against a blank reagent using a Shimadzu UV-visible spectrophotometer (UV-1610). Concentrations of protein (BSA) were plotted against the corresponding absorbance value to generate a linear regression standard curve.

2.5. Histochemical Study. Bee heads of each developmental stage were dissected in insect saline (NaCl 7.5 g/L, and Na₂HPO₄ 2.38 g/L, and KH₂PO₄ 2.72 g/L) and then fixed in Bouin's solution for 24 h. Samples were dehydrated through an ethyl alcohol series: 70%, 90%, 95%, and 100% for 10 min each. Samples were soaked in xylene for an hour and then embedded in paraffin wax. The tissues were sectioned into 6 μm thickness using a rotary microtome

(Leica, Germany), stained with hematoxylin and eosin, and submitted to periodic acid Schiff's reagent (PAS) and ninhydrin Schiff's reagent for localization of carbohydrates and proteins, respectively.

2.6. Data Analysis. Statistical differences between hypopharyngeal mean gland size and protein concentration were compared using ANOVA and Duncan's multiple-range test (DMRT).

3. Results

3.1. Glandular Size. Acini of the glands started to develop at the pupal stage and increased in size at the adult stage. The size (width and length) of the acini of both species was the largest in nurse bees and then gradually decreased from nurses to guards (Table 1). However, the acini slightly increased in size when they changed their tasks to become foragers. There was no significant difference in the width of acini between *A. cerana* and *A. mellifera* ($F_{1,3} = 2.63$, $P < .1120$). However, there were significant width differences among different life stages ($F_{1,2} = 31.24$, $P < .0001$, and $n = 48$). In contrast, the length of the acinus of HPGs showed statistically significant differences between these two species and developmental stages ($F_{1,2} = 8.48$, 12.92; $P < .0048$, .0001; $n = 48$).

3.2. Protein Content. The mean total protein content of the hypopharyngeal glands taken from all three developmental stages of *A. mellifera* was significantly higher than those of *A. cerana* ($F_{1,2} = 38.88$, $P < .0001$) (Figure 2). The highest protein concentration was found in nurse bees of *A. mellifera* which were $1389.6 \pm 158.9 \mu\text{g}/\mu\text{L}$ ($69.5 \pm 8.0 \text{ mg/bee}$). Proteins (revealed with ninhydrin Schiff's reagent) decreased in concentration in later developmental stages. In contrast, the lowest protein concentrations were found in guards of *A. cerana* which were $413.8 \pm 5.1 \mu\text{g}/\mu\text{L}$ ($20.7 \pm 0.3 \text{ mg/bee}$, Figure 2). There was a significant difference in protein concentration among the developmental stages of *A. mellifera* and *A. cerana* ($F_{2,5} = 217.82$, $P < .001$, and $n = 49$, Figure 1).

3.3. Histochemical Structure for Localization of Protein. The histochemical structure of proteins from *A. cerana* and *A.*

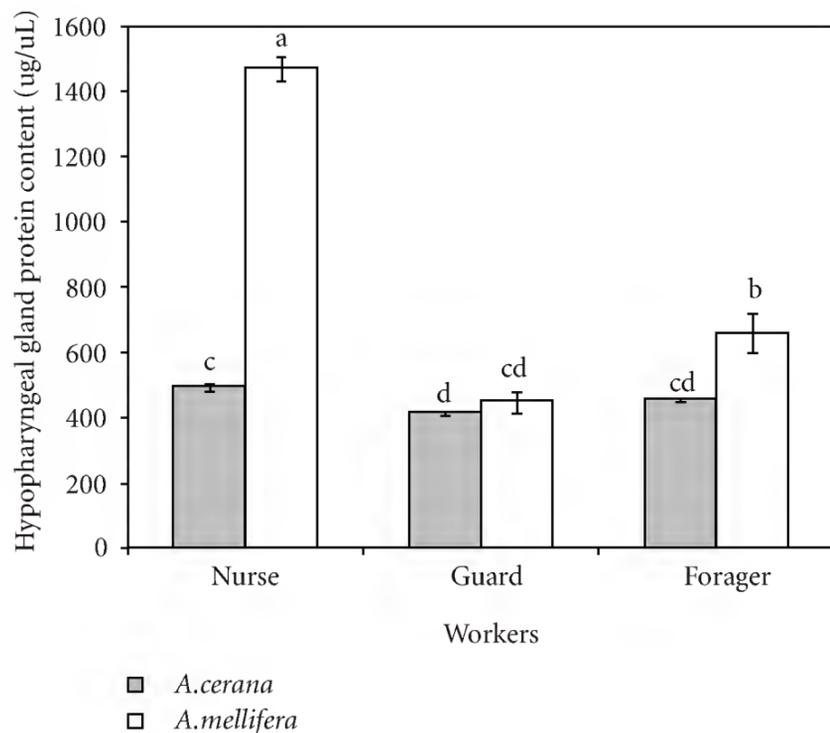


FIGURE 1: Mean \pm S.E. protein concentration of *A. cerana* and *A. mellifera* hypopharyngeal glands in different developmental stages: nurse, guard, and forager. Vertical bars with different letters represent significant differences (ANOVA-Duncan's Multiple Range Test; $F = 217.82$, $df = 5$, $P < .0001$, $n = 49$).

mellifera was relatively similar among life stages. In both species, several clusters of acini were connected to a long slender secretory duct. Each acinus was composed of 8–10 aggregated secretory cells and the glands consisted of an incomplete irregularly shaped secretory unit with unequal secretory cells. However, there were differences among stages of either *A. cerana* or *A. mellifera*. The histochemical staining shows a glandular cell cytoplasm rich in secretory vesicles and presenting glycoprotein secretions.

3.3.1. Pupae. The hypopharyngeal glands developed and formed an acinus. The glands consisted of an incomplete structure of secretory units which were irregular in shape and composed of 8–10 secretory cells. Each cell had an oval nucleus which stained green. The slightly pink cytoplasm can be seen in this stage, but secretory vesicles do not appear (Figure 2(b)).

3.3.2. Nurse Bees. Each acinus consisted of numerous vesicles that were not stained with Schiff's reaction. These white and clear vesicles were found surrounding the nucleus of acinar cells. Strongly positive Schiff's staining was found in the periphery of secretory cell cytoplasm. Ninhydrin Schiff's staining clearly distinguished the unstained vesicles from the peripheral area of the cytoplasm that included the nucleus that was strongly positive with pink staining. Nevertheless, areas of some vesicles stained magenta (Figures 2(c) and 2(d)).

3.3.3. Guards. In the guards, some HPGs demonstrated the beginning of retrogression through the formation of irregular secretory cells. Guard cytoplasm stained weakly with Ninhydrin Schiff's reagent as compared to the stronger

staining of nurse bees and foragers. Even the secretory vesicles of guards were smaller in size than nurse bees or foragers, the vesicles were elongated and cylindrical in shape. The wider extracellular space between two adjacent secretory cells was clearly seen in this stage (Figure 2(e)).

3.3.4. Foragers. The glands were composed of several acini smaller than those of nurse bees but larger than those of guards. Each acinus consisted of 8–10 pyramidal secretory cells, with oval nuclei staining positive using Ninhydrin Schiff's reagent in the area of euchromatin. The cytoplasm of secretory cells, except vesicles, was strongly magenta positive when stained with Ninhydrin Schiff's reagent (Figure 2(f)).

3.4. Histochemical Structure for Localization of Carbohydrate. Carbohydrate levels in the HPGs of the four stages of *A. cerana* and *A. mellifera* workers were similar to each other. The glands stained strongly positive to PAS, indicating the existence of carbohydrates (Figures 3(a) and 3(b)). However, there were clear differences among developmental stages in both species.

3.4.1. Pupae. The glands began forming a cluster of paired irregularly shaped secretory units that were composed of 8–10 cell aggregations. The cells gave weak positive staining to PAS with pink in cytoplasm.

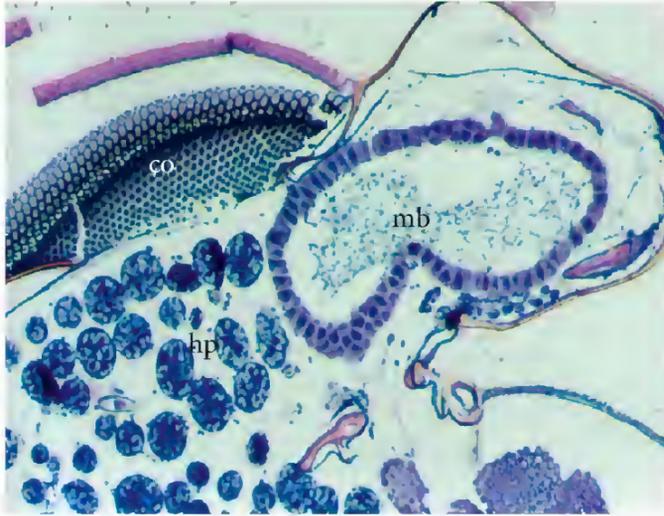
3.4.2. Nurse Bees. The HPGs were fully developed and composed of several secretory units made up of 8–10 aggregated pyramidal acinar cells. Within the acinar cells there were numerous red-pink secretory vesicles indicating a strong positive with PAS reaction and corresponding to glandular sizes (Figure 3(c)) (Table 1).

3.4.3. Guards. For guards, the glandular structure and histochemistry were slightly different to foragers. For example, each acinus was composed of 8–10 cells with small secretory vesicles and also lower number than those of foragers. However, they showed large extracellular space between adjacent cells, unlike the glands of nurse bees and foragers where there was minimal space (Figures 3(d) and 3(e)).

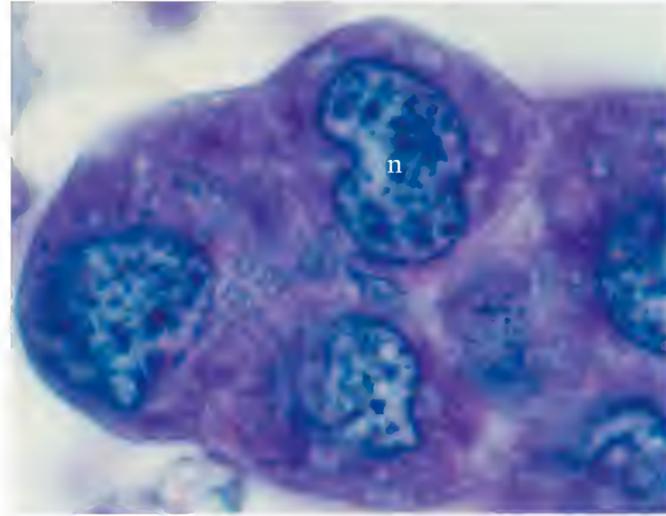
3.4.4. Foragers. The glands of foragers showed complete structure consisting of 8–10 aggregated acinar cells. The secretory vesicles of foragers were slightly larger than those of guards. The secretory vesicles of foraging bees gave strong positive staining with PAS which was similar to those of nurse bees (Figure 3(f)).

4. Discussion

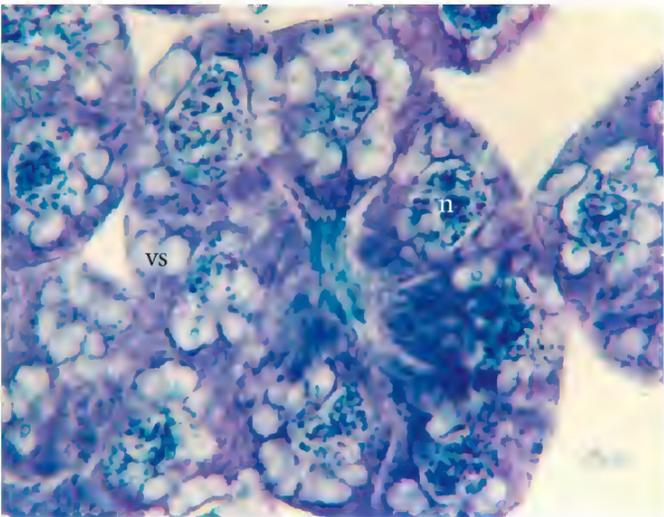
The structure of honeybee hypopharyngeal glands depends on the development and age of individuals, which corresponds with age-specific tasks and is known as age polyethism [3, 11]. The results of this study showed that the histochemical structure of carbohydrate and protein in *A. cerana* and *A. mellifera* was correlated with honeybee



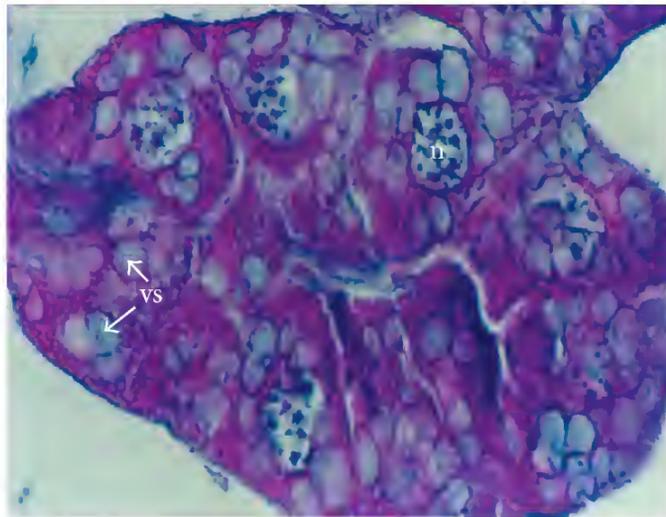
(a) A section of a nurse bee head in *Apis cerana* showing the location of the hypopharyngeal gland located beside the compound eye and close to the mandibular gland (40x)



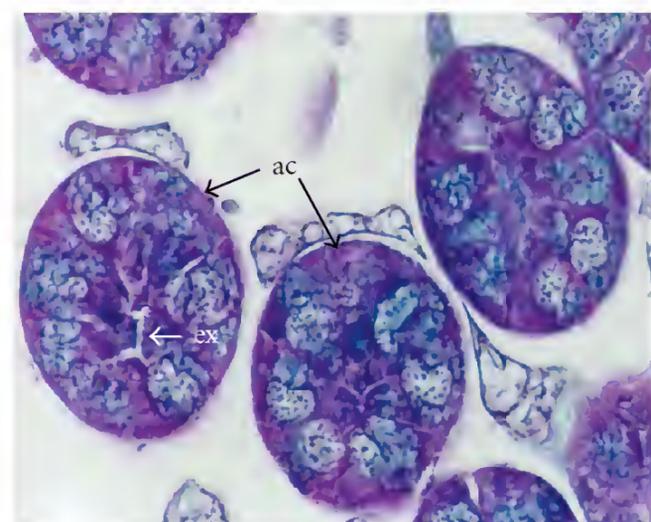
(b) A section of the hypopharyngeal gland of pupa of *Apis cerana* worker showing the incomplete irregular shaped secretory units (1000x)



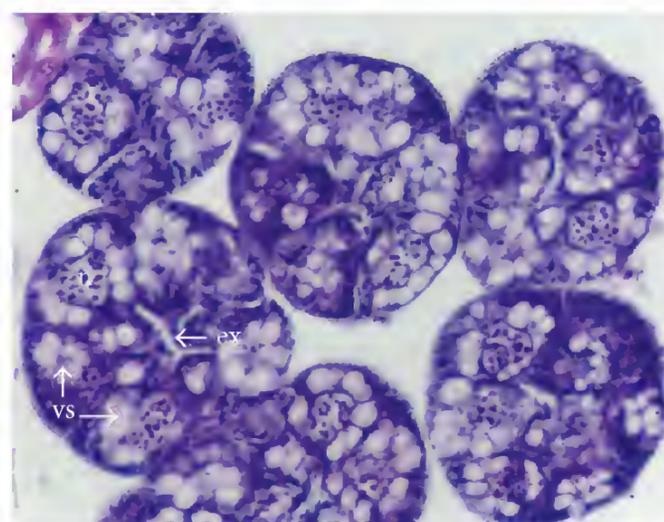
(c) The micrograph illustrates the secretory units of the complete hypopharyngeal gland of *A. cerana* nurse bee, the cytoplasm containing a large amount of proteins is characterized by staining purple-pink color with NHS (200x)



(d) The micrograph has been stained by a histochemical method NHS to demonstrate the presence of proteins which are stained magenta to purple pink in the secretory cells of the glands of *A. mellifera* nurse bee (200x)

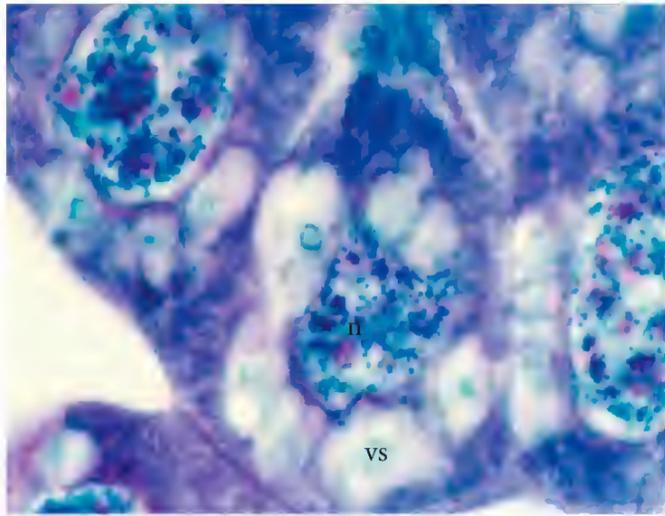


(e) A micrograph showing the histochemical appearance of the secretory units of the complete developed gland of an *A. cerana* guard; the cytoplasm is stained pink with NHS technique showing the narrow extracellular space between adjacent acinar cells seen by white color separating them from each other (100x)

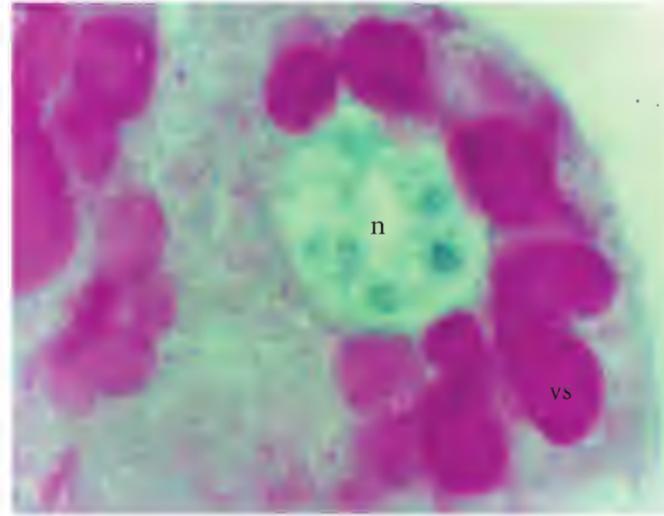


(f) A histochemical micrograph of an *A. mellifera* forager showing the cytoplasm of the secretory cell seen to contain a variable number of secretory vesicles that are almost unstained with NHS (100x)

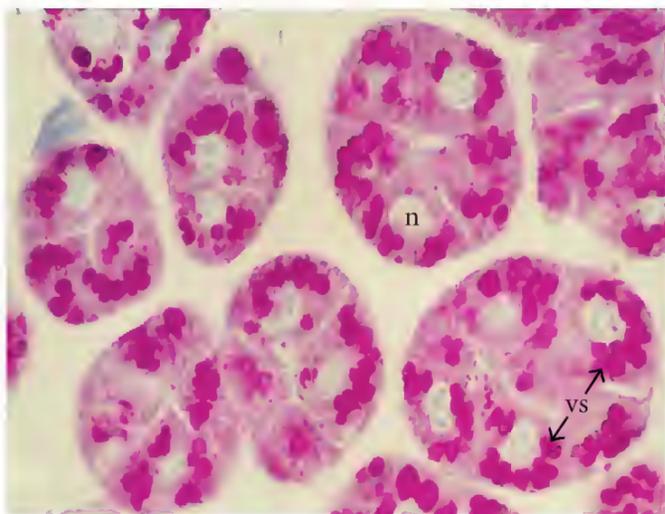
FIGURE 2: Light microscope micrographs of hypopharyngeal gland acini stained with ninhydrin Schiff's reagent (NHS). *Abbreviations:* ac: acinus; co: compound eye; ex: extracellular space; hp: hypopharyngeal gland; mb: mandibular gland; n: nucleus; vs: vesicle.



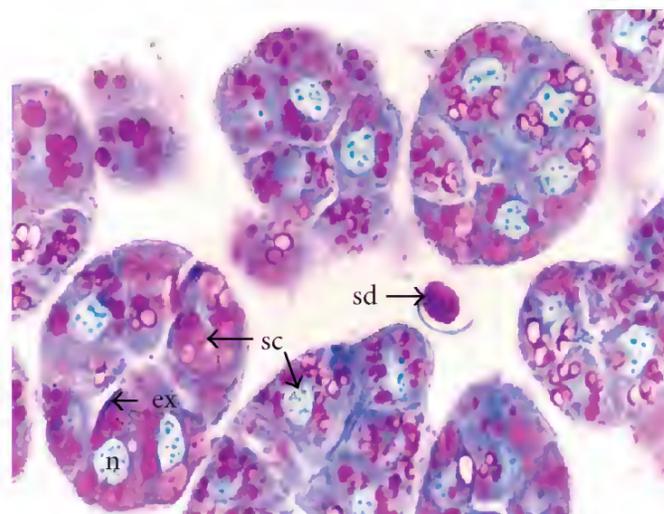
(a) High-resolution micrograph of the *A. cerana* nurse bee hypopharyngeal gland stained pink-purple surrounding the secretory vesicles (1000x)



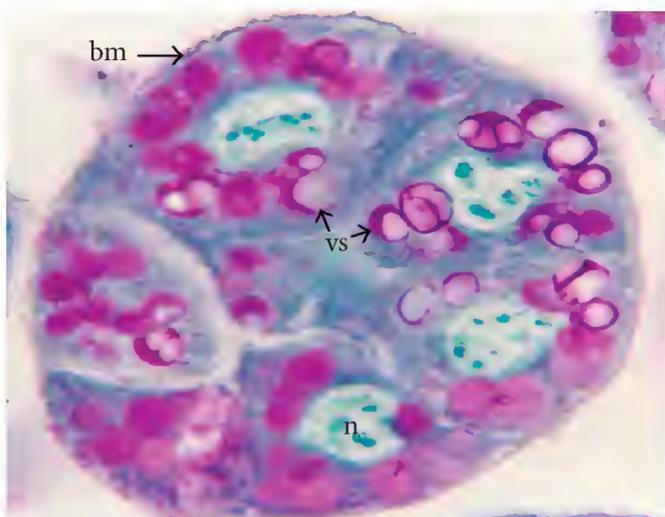
(b) A section of the hypopharyngeal gland of an *A. mellifera* worker nurse bee showing the cytoplasm of the secretory cell containing a variable number of secretory vesicles which is stained red-pink with PAS. The oval nucleus stains pale greenish with light green (1000x)



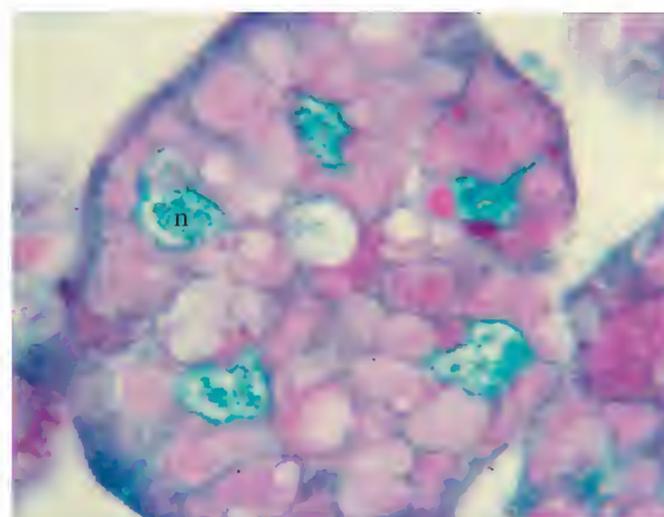
(c) A medial section of the completely developed acinar gland of *A. cerana* nurse bee showing a cluster of several secretory units. The secretory cell cytoplasm is stained pink with PAS and the cell has a large nucleus. (100x)



(d) The secretory units of the hypopharyngeal gland of *A. mellifera* forager showing the smaller size of secretory vesicles containing less carbohydrate. These are characterized by staining slightly red-pink with PAS and showing the wide extracellular space between adjacent acinar cells which is seen by white color separating it from the other secretory cells (100x)



(e) With a higher magnification of *A. mellifera* guard, the secretory cell contains various sizes of secretory vesicles which are less stained to PAS than that of nurse bee (200x)



(f) A medial section of the hypopharyngeal gland of an *A. cerana* guard showing the shrinkage and damaged plasma membrane (400x)

FIGURE 3: Light microscope micrographs of hypopharyngeal gland acini stained with PAS. *Abbreviations:* bm: basement membrane; ex: extracellular space; n: nucleus; sc: secretory unit; sd: secretory duct; vs: vesicle.

age-specific tasks of the colony. Young worker nurse bees care for and feed their brood with royal jelly that is synthesized and secreted from the hypopharyngeal glands. These hypopharyngeal glands were strongly positive to PAS and Ninhydrin Schiff's reagent reactions in this study. However older workers, when they became guards, had less positive staining to PAS as compared to nurse bees. This may be related to the development of the hypopharyngeal glands which are fully developed when young workers take care of their brood by synthesizing and secreting royal jelly. Older workers no longer feed the brood, and thus gland atrophy is expected [3, 4, 8]. However, when bees become foragers, the hypopharyngeal gland contains significant amounts of carbohydrate and protein. This finding is consistent with the finding that the hypopharyngeal glands are the site of conversion of nectar to simple sugars by enzymes [6–9].

The hypopharyngeal gland has been studied in several ways, including its ultrastructure, protein complement, and histochemical structure in *Apis andreniformis* and *A. florea*. [1, 3, 5, 8, 9, 12]. In this study, we found similarities in glandular structure between species, and the glands were fully developed in nurses, guards, and foragers. Moreover, the secretory units of the HPGs were filled with numerous vesicles that gave a strong positive staining with PAS and Ninhydrin Schiff's reagent [1]. This indicates that the glands of nurses, guards, and foragers in four species of honeybees (i.e., *A. andreniformis*, *A. florea* [1], *A. cerana*, and *A. mellifera*.) play an important role not only in secretion of carbohydrate rich substance but also in the secretion of enzymes for converting nectar to honey [1, 13, 14]. However, there were also differences found in structure of the glands between the hive cavity nest honeybees, *A. cerana* and *A. mellifera*, and the single open nest honeybees, *A. andreniformis* and *A. florea*. The structure of the extracellular space between adjacent cells of *A. andreniformis* and *A. florea* was wider than that of *A. cerana* and *A. mellifera*. In addition, the secretory units of hypopharyngeal glands of *A. mellifera* from this study were different from results in the study of Deseyn and Billen [3] who showed that the volume of acini decreased in foragers or displayed degenerative structure while that was not found in this study.

In the present study, the development and histochemical aspects of the hypopharyngeal glands were evaluated in *A. cerana* and *A. mellifera*. The findings are in agreement with our previous study [1]. Additionally, glandular sizes were the greatest in nurse bees. Hypopharyngeal glandular size is known to be positively correlated with gland activity and is influenced by larval feeding [2, 4, 15, 16]. These glands gradually decrease in size when honeybees become guards, cease feeding, and begin defending the colony [3]. However, the hypopharyngeal gland size of foragers was significantly larger than that of guards. The results in this study indicate that glandular development corresponded well with total protein synthesis in the hypopharyngeal glands at different adult life stages. A number of reports indicate that the HPGs produce enzymes that are used to hydrolyze nectar into honey, including amylase, α -glucosidases, glucosidase oxidase, galactosidase, esterase, leucine arylamidase, and invertase [4, 6–9]. It can be inferred that the HPGs perform

two functions: first producing protein rich royal jelly for the nursing brood (by nurse bees) and then enzyme production (by foragers). However, the function of hypopharyngeal glands has flexibility depending on the colony condition and the need for feeding brood [4, 16]. The protein concentration of the hypopharyngeal glands peaked in nurse bees and declined in older workers. This corresponds to the degree of protein synthesis and is correlated with the abundance of rough endoplasmic reticulum (RER) found in adult workers. RER of acinar cells of worker glands developed and increased significantly a few days after emergence. Most of the cytosol space was filled with RER stacks a few days after emergence, while in foragers the RER decreased [5]. However, the total mean protein content of the hypopharyngeal glands of *A. mellifera* workers was significantly higher than that of *A. cerana* workers ($F_{1,2} = 38.88$, $P < .0001$). This is related to glandular sizes. In *A. mellifera*, the hypopharyngeal glands and average body sizes are larger than those of *A. cerana*. In summary, the hypopharyngeal glands of *A. cerana* and *A. mellifera* workers are fairly similar in terms of the histochemical structure. Each gland begins development as pupae and had a fully forming acinus in nurse bees. Gland regression occurred when the bees developed into foragers and guards. Protein concentration peaked in nurse bees and declined in guards. In the hypopharyngeal glands, the histochemical staining of carbohydrates and protein corresponded well with the glandular size and protein content.

Acknowledgments

The authors would like to thank James Nieh, Division of Biological Sciences, Section of Ecology, Behavior, and Evolution, University of California San Diego and anonymous reviewers for their comments on this paper. They sincerely thank Department of Biology, Faculty of Science, Burapha University, Chon Buri province, Thailand for providing the research facilities.

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Research Article

Citizen Science Observations of Monarch Butterfly Overwintering in the Southern United States

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Received 3 May 2010; Revised 14 July 2010; Accepted 26 August 2010

Academic Editor: Michael Rust

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Members of the public have long had a fascination with the monarch butterfly, *Danaus plexippus*, because of its amazing long-distance migration to overwintering sites in central Mexico, and many participate in online citizen-science programs where they report observations of its life history in North America. Here, we examine a little-studied aspect of monarch biology, the degree of overwintering in the southern United States. We compiled 9 years of sightings of overwintering monarchs in the southern United States that were reported to Journey North, a web-based citizen science program, to map the distribution of areas where monarchs are capable of surviving during the winter (i.e., in January and February), differentiating between adult sightings and sightings of breeding activity. We also statistically compared the latitudes of adult and breeding sightings, examined differences across years in latitude of sightings, and quantified the number of monarchs reported with each sighting. Of all 254 sightings, 80% came from Florida and Texas, with the remainder coming from South Carolina, Louisiana, Georgia, Alabama, Mississippi, North Carolina, and even one in Virginia. This distribution was generally consistent with the winter range predicted by prior investigators based on climatic conditions of this region. Sightings of adults were on average from higher latitudes than reports of breeding activity and there was significant variation across years in the average latitude of all sightings. The majority of sightings (94.2%) were of fewer than 10 adult monarchs per location, and there were no reports of clustering behavior that is typical of monarch overwintering in California and Mexico. The results of this investigation broaden our collective understanding of this stage of the monarch life cycle and, more generally, highlight the value of citizen science programs in advancing science.

1. Introduction

The life cycle of the monarch butterfly, *Danaus plexippus*, in eastern North America is unique among insects, as every fall the late-summer population undergoes a famous 3000 km⁺ southward migration to overwintering sites in central Mexico [1]. There, they spend the winter clustered in high-altitude fir forests before flying back northward in March to recolonize their breeding range [2]. This fascinating life cycle has not only garnered the monarch a high degree of scientific attention [3, 4], but it has made it the focus of many “citizen-science” programs, whereby school children, public citizens, and naturalists document and collect observational data on various aspects of its biology. One of these programs is

Journey North, a nonprofit education-focused organization with a website that allows users to input records of a variety of monarch-related observations. Several of these Journey North data sets have already contributed to the collective scientific knowledge of this insect’s biology; sightings of northward-migrating monarchs were used previously to map the progression northward [2] and to estimate the rate of northward travel [5]. More recently, the sightings of fall roosts were mapped to elucidate the southward migration flyways [6]. Here, we use other data from this program to examine a little-studied aspect of monarch biology, the degree of overwintering in the southern United States.

Scientists have known for many years that not all monarchs in the population east of the Rocky Mountains reach

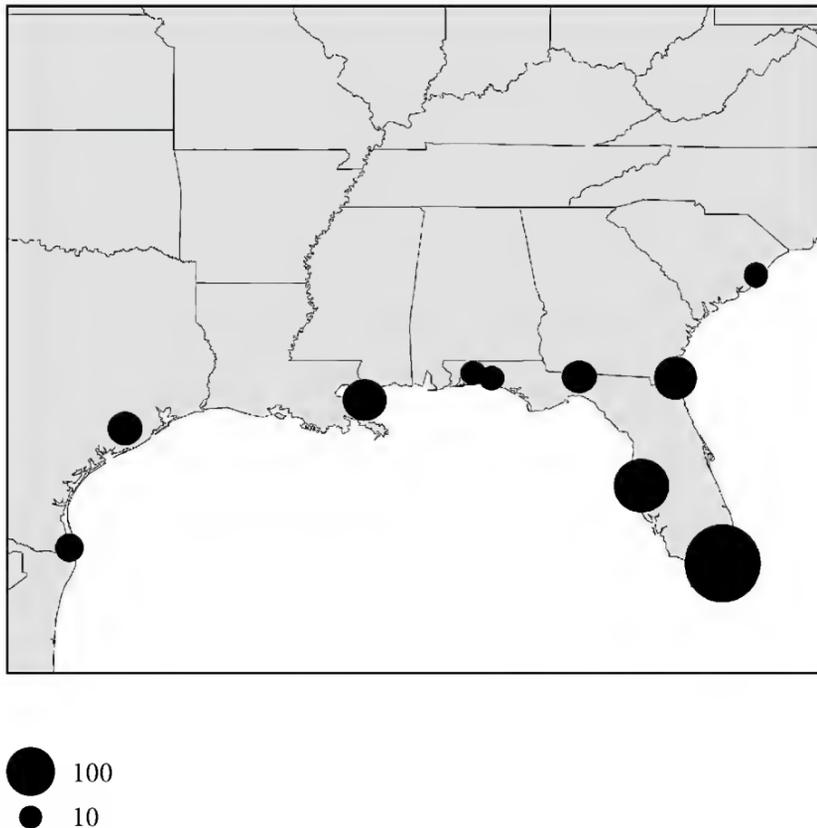


FIGURE 1: Predicted distribution of adult monarch butterflies in the southern United States in winter, based on a CLIMEX model that calculates suitable conditions for adult survival from environmental data (redrawn with permission from [12]). Size of black circles refers to an index score of abundance given by the CLIMEX model (not number of monarchs).

central Mexico each winter. Some have provided evidence that a small number ends up in Cuba [7]. Hilburn [8] reports monarchs occasionally arriving to Bermuda in the fall. In the United States, there is a well-known resident population in southern Florida [9, 10] that receives annual influxes of migrants from the larger eastern population [11]. Brower [1] compiled many early reports of monarchs wintering in peninsular Florida and other Gulf coast locations. And finally, based on regional climatic data and knowledge of adult survival thresholds, Zalucki and Rochester [12] modeled the predicted winter range of monarchs in the United States, which pointed to multiple *potential* wintering areas along the Gulf coast and Florida (Figure 1). These were areas where monarchs would be theoretically capable of surviving, given the typical temperature and moisture levels of the region in the winter. Thus, the collective evidence indicates that there are multiple areas in the southern United States where monarchs are capable of surviving during the winter.

Since 2002, the Journey North program has compiled winter observations of monarchs made by citizens and interested persons in the southern United States. In most cases, these sightings are made by people who were surprised to find a monarch in their area during the winter, given the well-known Mexican wintering colonies. As each winter sighting is reported, (and verified by Journey North staff), it is displayed on an online map for that year (<http://www.learner.org/jnorth/>). While these sightings, which are made by homeowners, amateur naturalists, and

interested citizens, were not necessarily obtained with scientific rigor, over the course of 9 years, these sightings nevertheless collectively represent an important source of information on this phenomenon, which would otherwise be nearly impossible to study scientifically because of the spatial scope involved.

In the current study, we compiled and examined 9 years of reports of monarchs overwintering in the southern United States from the Journey North program in an effort to further scientific understanding of this phenomenon. Our objectives were to (1) map the distribution of overwintering monarchs using all available records (using sightings from 2002 to 2010), distinguishing between sightings of adults or of winter breeding activity, (2) compare the latitudes of the sightings in both categories and across years, (3) estimate the number of monarchs observed with each overwintering sighting, and (4) report on other observations of biological importance made by certain Journey North participants who are located in key points within the distribution of wintering sites. The results of this investigation will help fill a large gap in the collective knowledge of this aspect of the monarch butterfly life cycle in North America.

2. Methods

2.1. Journey North Sightings. We compiled sightings of monarchs from the “monarch overwintering” sightings database which is accessible within the archived sightings section of Journey North’s website (<http://www.learner.org/jnorth/maps/archives.html>). These sightings represent observations of adults, eggs, and larvae of monarchs that are made and submitted online by Journey North participants during the wintering season. There is no specific format or requirement for the sightings, only that they be, to the best of the observer’s knowledge, of wild monarch butterflies (i.e., not reared). Each report contains a date, location (i.e., town, state, and latitude and longitude), and a summary of the observation, which is usually a statement such as “We saw three adult monarchs flying around our garden today”, or “We were surprised to find third-instar caterpillars on our milkweed plants in January”. Some participants also include photographs of the sighting. While people are free to enter overwintering sightings from January through March of each year, for the current study we selected only those sightings from January and February. This was to eliminate the possibility that the monarchs sighted were individuals returning from Mexico, which occurs in March in the southern United States [2, 5]. We categorized each sighting into one of two groups: sightings of adults only, or sightings of breeding activity, which we considered as any observation of monarch eggs, larvae, pupae or of females ovipositing. If we could not discern which category the sighting fit into based on the information given, we did not include that record. The sightings were then plotted onto a map of the southern United States using ArcView GIS software, based on the latitude and longitude coordinates with each record. Finally, we further categorized the sightings into one of three groups according to how many (adult) monarchs were seen, based on the notes provided by the observers:

TABLE 1: Summary of all sightings of “overwintering” monarchs (sightings of adults and breeding activity) made during the months of January and February from 2002 to 2010, broken down by state.

State	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
FL	9	13	6	7	7	8	12	24	13	99
TX	20	9	12	8	9	3	11	19	3	94
SC	0	0	0	2	6	2	7	1	1	19
LA	5	3	0	2	1	0	0	5	0	16
NC	1	0	0	0	4	1	0	0	0	6
GA	0	1	0	0	1	0	0	1	1	4
AL	0	0	0	0	1	1	0	0	0	2
VA	0	0	0	0	1	0	0	0	0	1
MS	0	0	0	0	0	0	1	0	0	1
All States	35	26	18	19	30	15	31	50	18	242

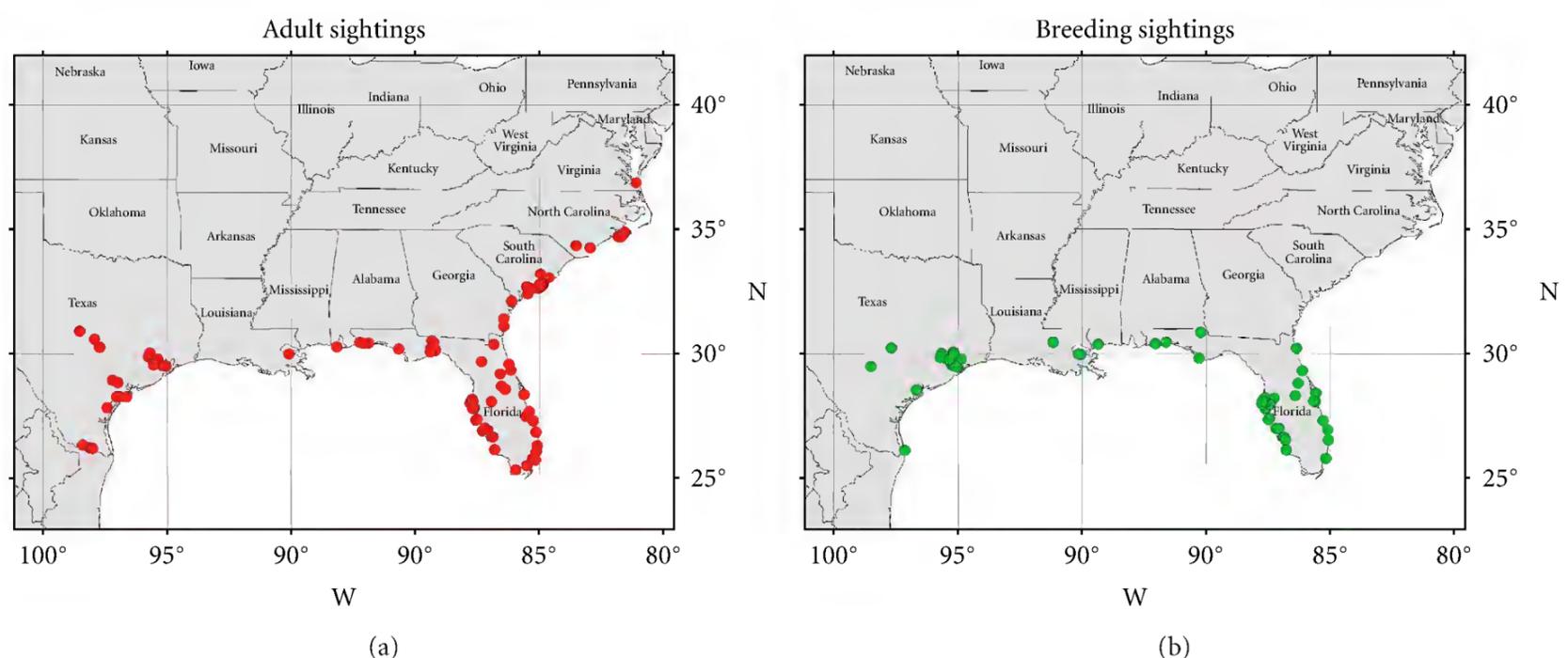


FIGURE 2: Locations of winter sightings of (a) adult and (b) breeding monarch butterflies (i.e., larvae, pupae, or ovipositing females) in the southern United States, as reported to Journey North in January and February from 2002 to 2010.

one adult; a small number of adults (2–9 individuals); or 10 or more adults. In some cases, observers reported seeing “lots of larvae” or “milkweeds covered with larvae”, and in these cases we assumed that there were less than 10 adult monarchs present (since a single female can produce many larvae).

2.2. Data Analyses. We were primarily interested in knowing if the latitude of sighting (response variable) differed between breeding and adult categories, which necessitated the use of circular statistics [13]. Therefore, using the data from all sightings, we used a Watson-Williams test to compare latitudes between categories. We used a similar test to compare latitudes across years. Analyses were conducted using MATLAB software with the CircStat toolbox installed [14].

3. Results

3.1. General. A total of 242 sightings of overwintering monarchs were made over the 9-year time frame of this study

(Table 1). Of these, 193 (80%) were of sightings in Texas and Florida. The other sightings came from South Carolina, Louisiana, Georgia, Alabama, Mississippi, North Carolina, and even one in Virginia during the winter of 2006 (see verification of this sighting below). We note that all of these are coastal states and in fact most sightings occurred along the coastlines of these states (below).

3.2. Spatial Distribution of Sightings. The distribution of sightings differed visibly between the two categories. Sightings of adult monarchs from all years are mapped in Figure 2(a), which shows that sightings occurred in each of the 9 states listed above, with locations appearing to generally fall close to the coastline. However, sightings of breeding monarchs, which also appeared to fall along coastlines, were only made at locations below 31°N latitude (Figure 2(b)). The adult monarch sightings in Virginia in February 2006 were extremely unusual because of the high latitude, however, the observers who made this report (David and Joyce Williams) provided detailed evidence to support the observation. During the prior fall (of 2005),

TABLE 2: Summary of average latitudes of monarch winter sightings across all years and sighting categories (adults only or confirmed breeding). Numbers in parentheses indicate 95% confidence intervals. Means and confidence intervals calculated using circular statistics [13].

Year	Adult(s) only		Breeding		Combined	
2002	29.7°	(0.7)	29.0°	(0.9)	29.4°	(0.5)
2003	29.2°	(0.8)	29.0°	(0.6)	29.1°	(0.5)
2004	29.0°	(1.5)	29.8°	(0.3)	29.5°	(0.5)
2005	30.7°	(1.0)	28.7°	(0.9)	29.5°	(0.8)
2006	31.7°	(1.3)	28.6°	(1.0)	30.7°	(1.0)
2007	29.7°	(1.8)	28.9°	(1.1)	29.5°	(1.3)
2008	30.4°	(1.1)	28.9°	(0.7)	29.7°	(0.7)
2009	28.8°	(0.7)	29.3°	(0.4)	29.0°	(0.4)
2010	28.2°	(1.6)	28.2°	(1.0)	28.2°	(0.9)
All years	29.8°	(0.4)	29.0°	(0.2)	29.4°	(0.2)

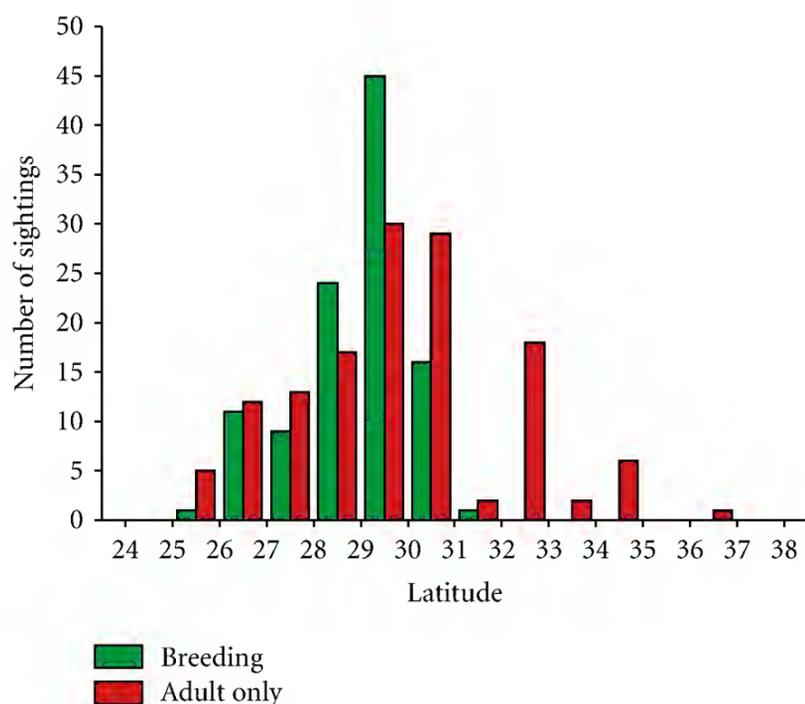


FIGURE 3: Distribution of the sighting latitude of adult and breeding monarch butterflies in January and February from 2002 to 2010.

these participants had been capturing and tagging adult monarchs with numbered MonarchWatch stickers [15], and in February of 2006 they observed a tagged monarch in their yard in Virginia Beach. They subsequently captured two monarchs two weeks later and discovered that both had been tagged by them in the fall on two successive days (September 25 and 26). Thus, they are confident that these monarchs stayed in the vicinity of their yard in Virginia from September 05 through March 06.

3.3. Statistical Analyses of Sighting Latitudes. Consistent with the spatial pattern shown in Figure 2, the initial Watson-Williams test indicated that the average latitudes of all adult sightings (mean = $29.8^\circ \pm 0.4$) differed significantly from that of the breeding sightings (mean = $29.0^\circ \pm 0.2$; $F_{1,240} = 10.11$, $P = .0017$; Table 2, Figure 3). Given that 1° latitude is equivalent to 111 km, this equates to an average difference of 89 km between categories. This difference is also apparent when viewing the annual averages in Table 2, where in 6 of

the 9 years, the average latitude of adult sightings was greater than that of the breeding sightings. The second Watson-Williams test revealed significant variation across years in the average latitudes of sightings ($F_{8,233} = 3.32$, $P = .0013$). This variation can also be seen in Table 2, where a noticeable decline in latitude occurred in the last year of records (2010); in this year, the average latitude of all sightings was approximately 1° or 111 km lower than most prior years.

The drop in latitude in 2010 can be attributed to the unusually cold winter in that year in the southern United States, when many observers reported below-freezing January temperatures in regions where it normally does not freeze. In Port Lavaca, TX (a gulf-coast town in southern Texas), one of us (H. Aschen) observed 3-4 monarchs per day in December and January until the first week of January when Texas had one of the hardest freezes in almost twenty years, down to 24°F . After that, H. Aschen saw no butterflies. Moreover, after the freeze, H. Aschen contacted a number of other regular monarch observers in southern Texas and asked if they had seen monarchs, and all reported none. Later, another observer from League City, Texas reported to Journey North: “I’ve spotted only one monarch since our big freeze, most of my milkweed was lost.” There was also a noticeable difference in the location of sightings during this season, which is evident in Table 1. In most years, the proportion of sightings that come from the state of Florida ranged between 23% and 53% of the total. However, in the 2010 overwintering season, 72% of all sightings came from Florida, and of these, all were between 25° and 28° north latitude.

3.4. Numbers of Monarchs Seen. Of all 242 sightings, 89 (36.8%) were of single adult monarchs, 139 (57.4%) were of a small number of adults (i.e., less than 10), and 14 sightings (5.8%) were of 10 or more adults. Collectively then, 94.2% of the sightings were of fewer than 10 monarchs per location. Of the reports of 10 or more adults, all were of monarchs seen flying or nectaring in gardens and areas with flowing plants. None of the reports (in any category) made mention of clustering or roosting behaviors that are typical of monarch overwintering in Mexico or California (e.g., [16, 17]).

4. Discussion

This study adds to the collective knowledge of monarch butterfly biology in a number of ways. First, the map of all adult monarch sightings over the 9-year period considered here (Figure 2(a)) effectively elucidates the current range in the southern United States where monarchs are capable of surviving during the winter, which is not dissimilar from the predicted range based on suitable climate conditions for adult survival (Figure 1, [12]). In the same way, the map drawn from the sightings of breeding monarchs (Figure 2(b)) shows where monarchs are able to form continuously-breeding populations. These maps, plus the statistical analyses of the latitudes associated with the sightings, make it clear that the breeding locations are at lower latitudes in general than the sightings of adults. In fact, the breeding locations appear to be restricted to areas below the 31° N parallel. It should be pointed out, however, that larval forms could have been present (but not seen) in the sites where only adults were observed, so this latitudinal threshold is likely not absolute.

The suitability of wintering sites in the southern United States for overwinter survival, especially in the northernmost locations, appears to vary among years. This point is made especially clear by the reports from the 2010 wintering season, which indicated that monarchs wintering in most areas except southern Florida were either killed by freezing temperatures, or they suffered high mortality due to reductions in nectar or hostplant availability. This same conclusion was reached by Brower [1], who reviewed much of the early literature and anecdotal reports on monarch wintering in the southern United States and also determined the frequency of lethal winter temperatures (to adult monarchs) in this region. Thus, Brower concluded that “about once each decade weather conditions in northern Florida would result in 50% mortality if the butterflies remained dry, or 100% mortality if they were previously wetted by rain.” Given that one of the 9 years examined here appeared to meet this scenario, Brower’s conclusion appears to be supported by these data.

While the number of documented wintering locations in Figure 2 appears large, it is important to consider that the number of monarchs reported for most locations tended to be fewer than 10 adults. Thus, in comparison to the millions of adults that overwinter in the Mexican colonies each year, the number of monarchs wintering in the southern United States is likely only a tiny fraction of the eastern population. Furthermore, these monarchs that are present in winter months on the Gulf coast appear not to display the typical overwintering behavior seen in Mexico or California (e.g., [16, 17]), since there were no reports of clustering behavior or even aggregations on vegetation. As such, rather than calling these “overwintering sites” which calls into mind massive clusters of monarchs hanging from trees, they may be more appropriately termed “winter sightings of monarchs”. To be fair, we must point out that the majority of the sightings in our data set were from homeowners viewing monarchs in their backyards (which are usually in urban areas), and not necessarily where monarchs might indeed

form winter clusters, such as on Florida barrier islands. Plus, the nature of the sightings (made by amateurs and public citizens) might also account for the lack of clustering reports since clusters of immobile monarchs can be hard to observe. Thus, it may be that clustering monarchs are indeed present in some areas along the Gulf coast, and this citizen-science approach simply fails to detect them.

The apparent lack of clustering behavior observed here may also help to explain why the data from this study appear to contradict the conclusion reached by Batalden et al. [18], who demonstrated that wintering monarchs require different ecological niches than do breeding monarchs. In that study, the environmental conditions required by overwintering monarch colonies in central Mexico (from [19]) were compared to those of breeding monarchs in the United States. The wintering locations presented here (Figure 2) appear inconsistent with this conclusion until one considers that the “overwintering” conditions examined by Oberhauser and Peterson [19] reflected the conditions required by entire monarch *colonies*, which may be different from the winter conditions needed by small groups of (nonclustering) adult monarchs, which is what the majority of reports to Journey North entail.

Since the overwintering sightings program in Journey North has only been operating since 2002, we have no way of knowing if the patterns observed in the 9 years here are a new development in the biology of monarchs east of the Rockies, or if these locations (i.e., Figures 2(a) and 2(b)) have always hosted wintering monarchs. While the locations in southern Florida have likely been present for some time [1], we suspect that most of the locations along the northern Gulf coast are more recent, based on evidence that we do have for one Gulf coast location where we documented winter breeding activity (Baton Rouge, LA). Surveys of adult monarchs here in the mid-1980s [20] showed the earliest spring sightings of adults were always in mid to late March, which is consistent with the timing of the return of the Mexico cohort to this state [2], and there was no mention of adult presence earlier than this. Further, Riley attempted to document all milkweed species present on his survey routes and found 6 species: *A. longifolia*, *A. amplexicaulis*, *A. obovata*, *A. tuberosa*, *A. viridis*, and *A. verticillata*. Moreover, he reported that no milkweeds had emergent stems in late February. This is compared to the recent sighting from the Journey North database for this location, in which an observer reports that on January 8, 2009, monarch caterpillars were found on *A. curassavica* in its yard, and that no other milkweed species were present. At least for this location then, there seems to have been a change in the seasonal occurrence of monarchs over the last 30 years and a change in hostplant availability throughout the winter.

Aside from the changing conditions within wintering sites, the entire wintering range of monarchs in the United States is predicted to expand northward if global temperatures rise by as little as 0.1°C per latitude [12]. In fact, increasing global temperatures are already expected to shift spring and summer breeding distributions northward over the next 50 years [18], so it would also hold true that overwintering sites would progress northward as well. If this is the case, then it will be important to continue monitoring

the distribution of wintering areas in this region and watch for these predicted changes in the coming years. With the help and dedication of the many citizen scientists who follow the life of this fascinating insect, this goal should be attainable.

Acknowledgments

This paper is based on reports made by citizen scientists who participate in the Journey North program, and the authors thank all of them for their dedication and keen observations. Lincoln Brower provided expert assistance to Journey North on countless occasions. The paper benefitted from helpful discussions with Sonia Altizer, and from comments by two anonymous reviewers.

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Research Article

Western Amazonian *Ticapimpla* (Hymenoptera: Ichneumonidae: Pimplinae): Four New Species from Colombia, Ecuador, and Peru, with a Key to Species of the Genus

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Received 11 June 2010; Accepted 1 September 2010

Academic Editor: Robert Matthews

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Ticapimpla is a small genus closely related to the New World genera *Acrotaphus* and *Hymenoepimecis*. It has been previously reported from Costa Rica and Brazil. In this paper, we describe four new species: *T. amazonica* from Ecuador and Peru, *T. carinata* from Colombia and Peru, *T. matamatae* from Colombia, and *T. soinii* from Colombia, Ecuador, and Peru. All have been collected in Western Amazonia, suggesting a South American origin for the genus. A key to the known species of the genus is provided.

1. Introduction

The *Polysphincta* group of genera (tribe Polysphinctini *sensu* Townes, [1]) is of great interest as the species are koinobiont ectoparasitoids developing on active spiders (see, e.g., [2, 3]). The genus *Ticapimpla* was described by Gauld [4] as a monotypic genus closely related to *Acrotaphus* Townes and *Hymenoepimecis* Viereck. These three genera constitute a monophyletic assemblage [5] that can be separated from the remaining neotropical genera of the *Polysphincta* group by the following combination of apomorphic features: (1) occipital carina strongly raised; (2) absence of occipital notch; (3) epomia completely absent; (4) epicnemial carina absent or extremely reduced. According to Gauld [4] *Ticapimpla* can be distinguished from *Acrotaphus* + *Hymenoepimecis* by the densely hirsute mesoscutum and the complete submetapleural carina. Another distinctive character of the genus mentioned by Gauld—the auxiliary teeth on the tarsal claws—is also present in some species of *Hymenoepimecis* and, according to the material examined in the present study, at least two species of *Ticapimpla* possess a preapical lobe instead of the auxiliary tooth. Additionally, *Ticapimpla* differs from *Hymenoepimecis* by

having the pronotum simple, without a mid-dorsal, pocket-like structure.

The only described species of the genus, *T. vilmae* Gauld, is known from Costa Rica and Brazil [4, 6]. In this paper, we describe four new species of *Ticapimpla* from Colombia, Ecuador, and Peru. We also provide images of *T. vilmae* for comparison. All of the new species have been collected in the Western Amazonian basin, suggesting that *Ticapimpla* is a taxon of South American origin. Unfortunately, nothing is known about the biology of the genus.

2. Material and Methods

Morphological terminology and forms of description used in the study largely follow those of Gauld [4]. The specimens are deposited in the following collections: the entomological collection of the Instituto Alexander von Humboldt, Villa de Leyva, Colombia (IAVH), Smithsonian Institution, USA (USNM), the Natural History Museum, UK (BMNH), Departamento de Entomología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru (UNSM), and The Zoological Museum, Section of

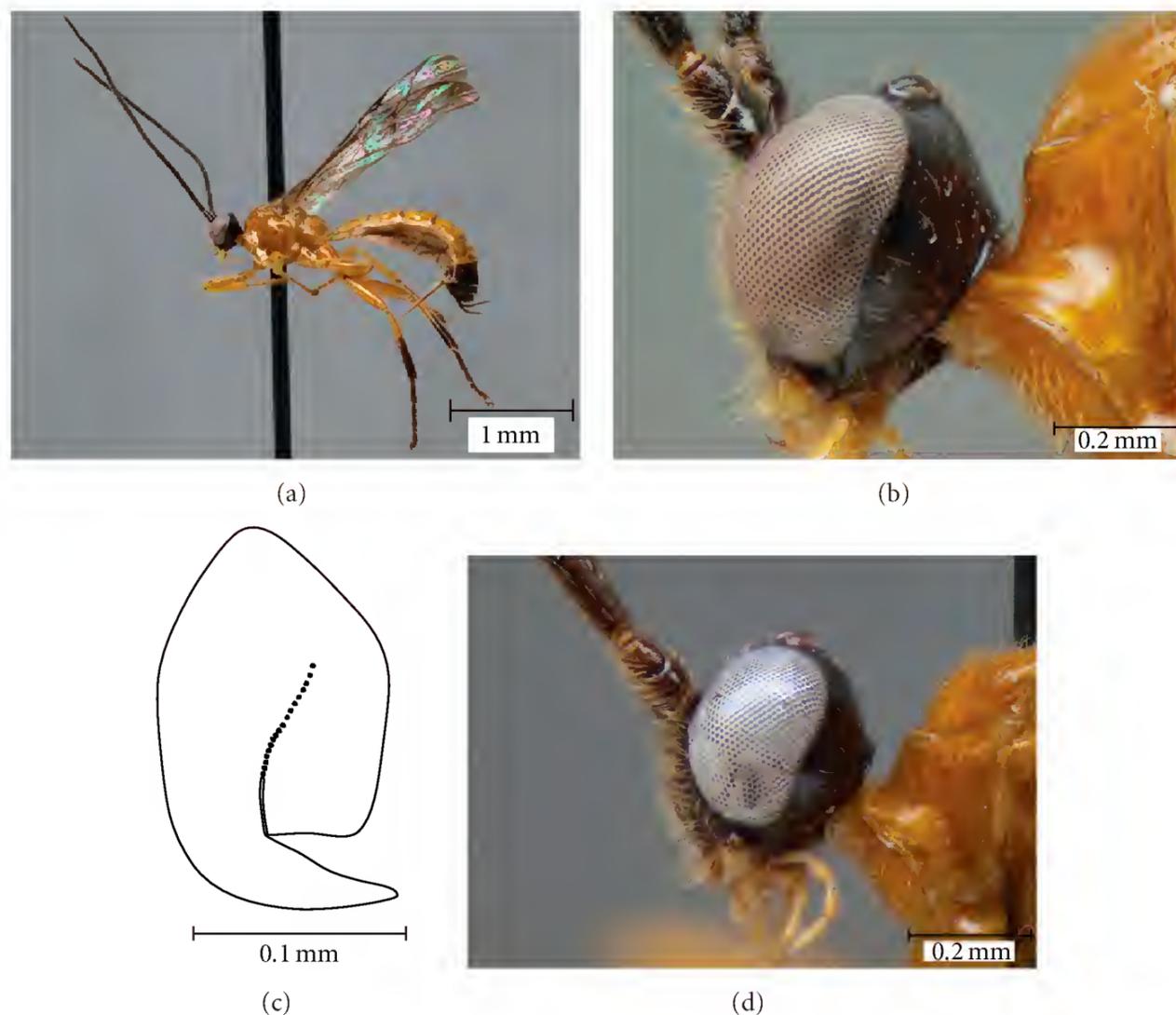


FIGURE 1: *T. amazonica* sp.n.: holotype female habitus (a), occiput detail (b), fore tarsal claw (c), and paratype male occiput detail (d).

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The Colombian specimens examined in this study were all collected during a Malaise trap inventory carried out in Colombia between 2001 and 2003 in several natural parks of the country. This inventory is part of the Diversidad de Insectos en Áreas Protegidas Project, developed jointly by the Instituto von Humboldt (Colombia), the special administrative unit of the Colombian natural park system (UAESPNN) and the University of Kentucky, with the support of the National Science Foundation (NSF). The Ecuadorian specimens of the genus were collected by canopy fogging by Dr. Terry Erwin and his research team (USNM). Peruvian specimens were collected by Malaise trapping during a large-scale Western Amazonian ichneumonid sampling programme coordinated by the third author (e.g., [7]). All Peruvian specimens were collected in the National Reserve of Allpahuayo Mishana.

Observations were made using Leica MZ12 and Olympus SZX10 stereomicroscopes. Layer photos were taken in ZMUT using an Olympus SZX16 stereomicroscope attached to an Olympus E520 digital camera. Digital photos were combined using the Deep Focus 3.1, QuickPHOTO CAMERA 2.3, and CombineZP programmes. At BMNH, images were taken using a Zeiss Stemi SV11 stereomicroscope attached to a Canon EOS 450D digital camera and partially focused images were combined using Helicon Focus v. 4.80 software.

3. Description of the New Species

3.1. *Ticapimpla amazonica* sp.n. (See Figures 1(a)–1(d))

Type Material. Holotype female (UNSM): Peru, Department of Loreto, Iquitos Area, Allpahuayo, Bosque Terraza. Malaise Trap H1(16). I. E. Sääksjärvi et al. leg. 7-21.XII.2000. Paratype females: 1 female (UNSM) Peru, Department of Loreto, Iquitos area, Mishana, 1-16.XII.1998, Clay, Malaise trap A1(8), I. E. Sääksjärvi et al. leg.; 1 female (ZMUT): Peru, Department of Loreto, Iquitos area, Allpahuayo, 23.I.-20.II.2000, Varillal, Malaise trap G1(1), I. E. Sääksjärvi et al. leg.; 1 female (ZMUT): same locality, 24.III-16.IV.2000, Varillal, Malaise trap E3(4), I. E. Sääksjärvi et al. leg.; 1 female (BMNH): same locality, 24.I.-20.II.2000, bosque terraza, Malaise trap H1(1), I. E. Sääksjärvi et al. leg.

Paratype males: 1 male (ZMUT): Ecuador, Orellana Transect Ent. 1 km S. Onkone Gare Camp Reserva Etnica Waorani Onkone Gare Camp 216 m, 3.X.1996, 00° 39' 25.7'' S 076° 27' 10.8'' W, T. L. Erwin et al. leg. Fogging terra firme forest, Lot # 1725; 1 male (USNM): same locality, 3.X.1996, 00° 39' 25.7'' S 076° 27' 10.8'' W, T. L. Erwin et al. leg., Fogging terra firme forest, Lot # 1735. 1 male (BMNH): same locality, 2.X.1996, 00° 39' 25.7'' S 076° 27' 10.8'' W, T. L. Erwin et al. leg., Fogging terra firme forest, Lot # 1708.

Female Description. Malar space at narrowest point about 0.4 times as long as basal mandibular width; lower face

elongate, about 1.0 times as broad as high (from weak supraclypeal suture to base of antenna), rather flat, with many long, conspicuous setae; head in dorsal view with genae relatively long and narrowed behind eyes; ocelli moderately large, the lateral one separated from the eye by about 0.8 times its own diameter; occipital carina not arising from a strongly raised flange of the occiput. Pronotum relatively long, so that distance from front margin of tegula to head is about $0.6 \times$ distance from hind edge of tegula to hind margin of propodeum; mesoscutum very finely punctate; scutellum, in profile, moderately convex, posteriorly abruptly declivous; mesopleuron polished, ventrally bearing long close setae; epicnemial carina represented by a short midventral section about $1.4 \times$ the width of fore coxae. Metapleuron polished, sparsely but evenly pubescent, with setae arising from fine punctures; propodeum smooth, anteriorly and laterally with close fine setiferous punctures; propodeum, in profile, more convex than in *T. carinata*, anterior margin with a pair of small median tubercles; transverse groove before propodeum deep, in section U-shaped, barely interrupted laterally by raised extensions of propodeum. Tarsal claws without auxiliary tooth, instead with a preapical, flattened lobe, lobe with inner margin convex; teeth of claw simply pointed. Fore wing length 5.0–5.8 mm; *cu-a* from opposite to distal of *Rs&M* by about $0.2 \times$ length of *cu-a*; *2rs-m* about 0.2 times as long as abscisa of *M* between *2rs-m* and *2m-cu*; hind wing with distal abscisa of all veins more or less spectral, *cu-a* joining subbasal cell clearly closer to 1A than to *M-Cu*. Metasoma moderately stout, tergite I 2.0 times as long as posteriorly broad, with lateral carina only present at extreme anterior end flanking the anterior concavity, lateral longitudinal carina present only anteriorly, reaching the spiracle; tergite II 1.1 times as long as posteriorly broad; tergite III 1.1 times as long as posteriorly broad; ovipositor 0.90–0.95 times as long as hind tibia, lower valve proximally with a rather long, weakly convex swelling.

Head and antenna black, mouthparts and distal edge of clypeus orange. Mesosoma entirely orange. Metasoma orange, with tergites VI + and ovipositor sheath black. Anterior two pairs of legs orange; hind legs orange with distal 0.6 of tibia and entire tarsus black. Wings very faintly yellowish, the fore wing with apex and area adjacent to pterostigma very faintly infumate; pterostigma black.

Male. Similar to female but fore wing length about 3.7–4.0 mm; tarsal claws simple, without auxiliary tooth or preapical lobe.

Variation. One of the males has the apex of the fore wings more clearly infumated than other specimens (females and males).

Diagnosis. This species can be distinguished from other species, except *T. soinii* sp.n., of the genus by the absence of an auxiliary tooth on female tarsal claws; instead, it has a flattened lobe resembling the common condition found in the *Sericopimpla* group of genera. It may be easily separated from *T. soinii* by the shape of the occiput; in *T. amazonica*

sp.n. occipital carina does not arise from a strongly raised flange of the occiput.

Biological Notes. Nothing is known about the hosts of this species.

Etymology. The specific name refers to the distributional area of the species, the Amazonia. It also reflects the possible South American origin of the genus.

3.2. *Ticapimpla carinata* sp.n. (See Figures 2(a)–2(c))

Type Material. Holotype female (IAVH): Colombia, Department of Amazonas, PNN Amacayacu, Mocagua, 3°41' S, 70°15' W, 150 m elev., D. Chota leg. Malaise trap, 26.II-12.III.2001. Paratypes: 1 female (IAVH): Colombia, Department of Vaupés, R. N. Mosiro-Itajura, Caparú, 1°4' S, 69°3' W, 60 m elev., J. Pinzón leg. Malaise trap, 1-8.XII.2003; 1 female (UNSM): Peru, Department of Loreto, Iquitos area, Allpahuayo, 15.X-8.XI.2000, clay, Malaise trap J2(15), I. E. Sääksjärvi et al. leg.

Female Description. Malar space at narrowest point 0.7 times as long as basal mandibular width; lower face elongate, 1.2 times as broad as high (from supraclypeal suture to base of antenna), rather flat, with many long, conspicuous setae; head in dorsal view with genae long and narrowed behind eyes; ocelli moderately large, the lateral one separated from the eye by about 0.8 times its own diameter; occipital carina arising from a strongly raised flange of the occiput. Pronotum relatively long, so that distance from front margin of tegula to head is about $0.45\text{--}0.5 \times$ distance from hind edge of tegula to hind margin of propodeum; mesoscutum very finely punctate; scutellum, in profile, moderately convex, posteriorly abruptly declivous; mesopleuron polished, ventrally bearing long close setae; epicnemial represented by a short midventral section about $1.0 \times$ the width of fore coxae. Metapleuron polished, sparsely but evenly pubescent, with setae arising from fine punctures; propodeum smooth, anteriorly and laterally with close fine setiferous punctures, anterior margin with a pair of small median tubercles; transverse groove before propodeum deep, in section U-shaped, barely interrupted laterally by raised extensions of propodeum. Tarsal claws with a preapical auxiliary tooth; teeth of claw simply pointed. Fore wing length 6.0–6.9 mm; *cu-a* distal of *Rs&M* by about $0.1\text{--}0.2 \times$ length of *cu-a*; *2rs-m* about 0.2 times as long as abscisa of *M* between *2rs-m* and *2m-cu*; hind wing with distal abscisa of all veins more or less spectral, *cu-a* joining subbasal cell distinctly closer to 1A than to *M-Cu*. Metasoma moderately stout, tergite I 1.7 times as long as posteriorly broad, with lateral carina only present at extreme anterior end flanking the anterior concavity, lateral longitudinal carina present only anteriorly, slightly surpassing the spiracle; tergite II 1.1 times as long as posteriorly broad; tergite III 0.9 times as long as posteriorly broad; ovipositor 0.95–1.0 times as long as hind tibia, lower valve proximally with a rather long, weakly convex swelling.

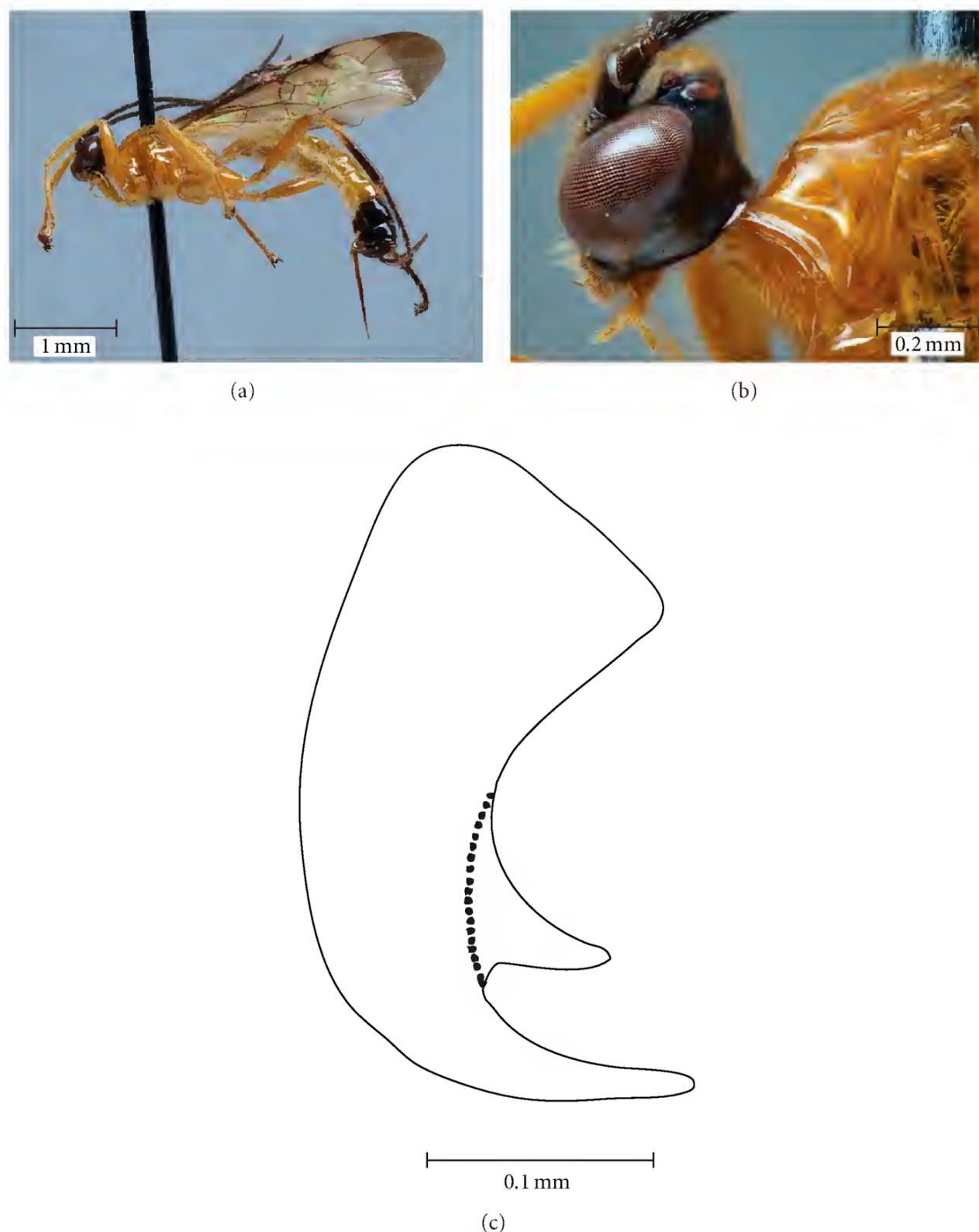


FIGURE 2: *T. carinata* sp.n.: paratype female habitus (a), occiput detail (b), and fore tarsal claw (c).

Head and antenna black, mouthparts and distal edge of clypeus orange. Mesosoma entirely orange. Metasoma orange, with tergites VI + and ovipositor sheath black. Anterior two pairs of legs orange; hind legs orange with distal 0.6 of tibia and entire tarsus black. Wings very faintly yellowish, the fore wing with apex and area adjacent to pterostigma clearly infumate; pterostigma black.

Male. Unknown.

Diagnosis. This species can be distinguished from *T. vilmae* Gauld and *T. matamatae* sp.n. by the presence of a short but discernible epicnemial carina. *T. carinata* sp.n. resembles *T. amazonica* sp.n. and *T. soinii* sp.n. in the general color pattern and by the presence of a short epicnemial carina, but has the auxiliary tooth on the female tarsal claws and a slightly

slenderer metasoma. It also differs clearly from *T. amazonica* by the shape of the head; the occipital carina arises from a strongly raised flange of the occiput.

Biological Notes. Nothing is known about the hosts of this species.

Etymology. The specific name refers to presence of a short ventral epicnemial carina.

3.3. *Ticapimpla matamatae* sp.n. (See Figures 3(a) and 3(b))

Type Material. Holotype female (IAVH): Colombia, Department of Amazonas, PNN Amacayacu, caño Matamata, 3°41' S, 70°15' W, 300 m elev., M. Kelsey leg. Malaise trap, X.1989.

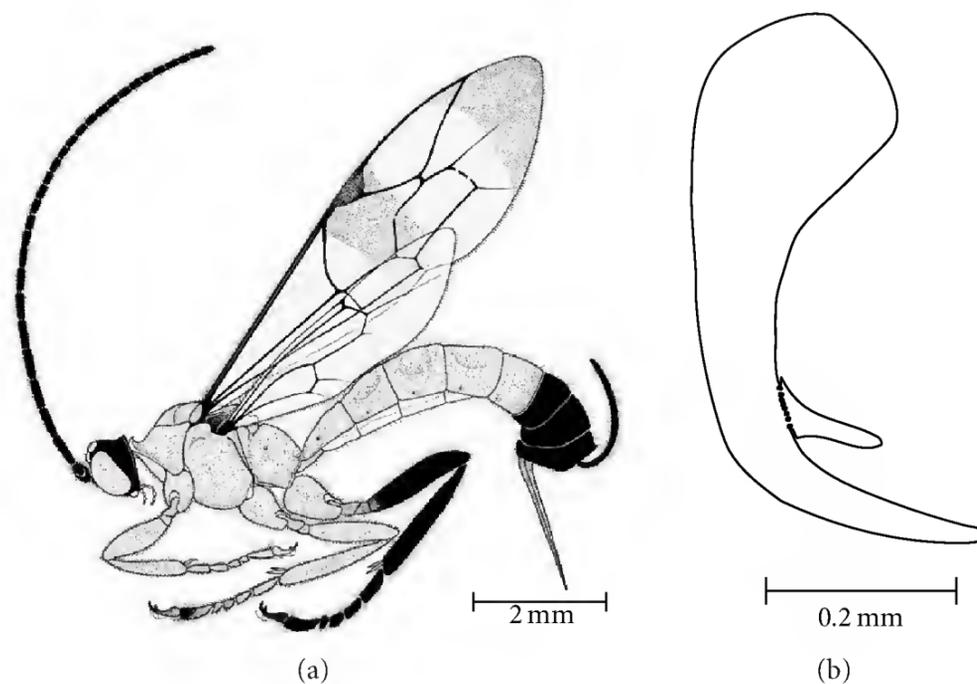


FIGURE 3: *T. matamatae* sp.n.: holotype female habitus (a) and fore tarsal claw (b).

Female Description. Malar space at narrowest point 0.6 times as long as basal mandibular width; lower face elongate, 0.9 times as broad as high (from supraclypeal suture to base of antenna), rather flat, laterally with scattered punctures which bear long conspicuous setae; head in dorsal view with genae long and narrowed behind eyes; ocelli moderately small, the lateral one separated from the eye by about 1.2 times its own diameter; occipital carina arising from a strongly raised flange of the occiput. Pronotum relatively long, so that distance from tegula to head is about $0.5 \times$ distance from tegula to hind margin of propodeum; mesoscutum smooth and impunctate; scutellum, in profile, moderately convex, posteriorly abruptly declivous; mesopleuron polished, ventrally bearing long close setae; epicnemial carina completely absent; metapleuron polished, sparsely but evenly pubescent, with setae arising from fine punctures; propodeum smooth, anteriorly and laterally with close fine setiferous punctures, anterior margin without median tubercles; transverse groove before propodeum deep, in section U-shaped, barely interrupted laterally by raised extensions of propodeum. Tarsal claws with a preapical auxiliary tooth; teeth of claw simply pointed. Fore wing length 9 mm; *cu-a* more or less opposite to base of *Rs&M*; *2rs-m* about 0.2 times as long as abscissa of *M* between *2rs-m* and *2m-cu*; hind wing with distal abscissa of all veins more or less spectral, *cu-a* joining subbasal cell clearly closer to *1A* than to *M-Cu*. Metasoma stout, tergite I 1.4 times as long as posteriorly broad, with lateral carina only present at extreme anterior end flanking the anterior concavity, lateral longitudinal carina present only anteriorly and barely reaching the spiracle; tergite II 0.8 times as long as posteriorly broad; tergite III 0.65 times as long as posteriorly broad; ovipositor 0.9 times as long as hind tibia, lower valve proximally with an inconspicuous swelling.

Head and antenna black, mouthparts orange brown. Mesosoma entirely orange. Metasoma orange, with tergites VI + and ovipositor sheath blackish. Anterior two pairs of legs orange; hind legs predominantly blackish, with coxa, trochanters and proximal ends of femur and tibia

orange. Wings very faintly yellowish, the fore wing with apex infumate and with a weak blackish preapical band; pterostigma blackish.

Male. Unknown.

Diagnosis. This species can be distinguished from all other species of the genus by the presence of a preapical band in the anterior wings, hind femur almost entirely black, a slightly stouter metasoma, and a more inconspicuous proximal swelling on the lower ovipositor valve.

Biological Notes. Nothing is known about the hosts of this species.

Etymology. The specific name refers to the Colombian Amazonian locality surrounding “caño Matamata” where the specimen was found.

3.4. *Ticapimpla soinii* sp.n. (See Figures 4(a)–4(e))

Type Material. Holotype female (UNSM): Peru, Department of Loreto, Iquitos area, Allpahuayo, 17.XII-27.XII.2000, varillal, Malaise trap G2(18), I. E. Sääksjärvi et al. leg. Paratype females: 1 female (UNSM): Peru, Department of Loreto, Iquitos area, Allpahuayo, 24.I.-20.II.2000, bosque terraza, Malaise trap H1(1), I. E. Sääksjärvi et al. leg.; 1 female (ZMUT): same locality, 8.XI-15.XII.2000, varillal, Malaise trap E3(17), I. E. Sääksjärvi et al. leg.; 1 female (ZMUT): same locality, 8.XI-1.XII.2000, varillal, Malaise trap K2(16), I. E. Sääksjärvi et al. leg.; 1 female (BMNH): same locality, 16.VII-2.VIII.2000, bosque terraza, Malaise trap H1(10), I. E. Sääksjärvi et al. leg.; 1 female (IAVH): Colombia, Department of Putumayo, PNN La Paya, Cecilio Cocha, $0^{\circ}7' S$, $74^{\circ}56' W$, 220 m elev., C. Sarmiento leg. Malaise trap, 26-29.I.2002.

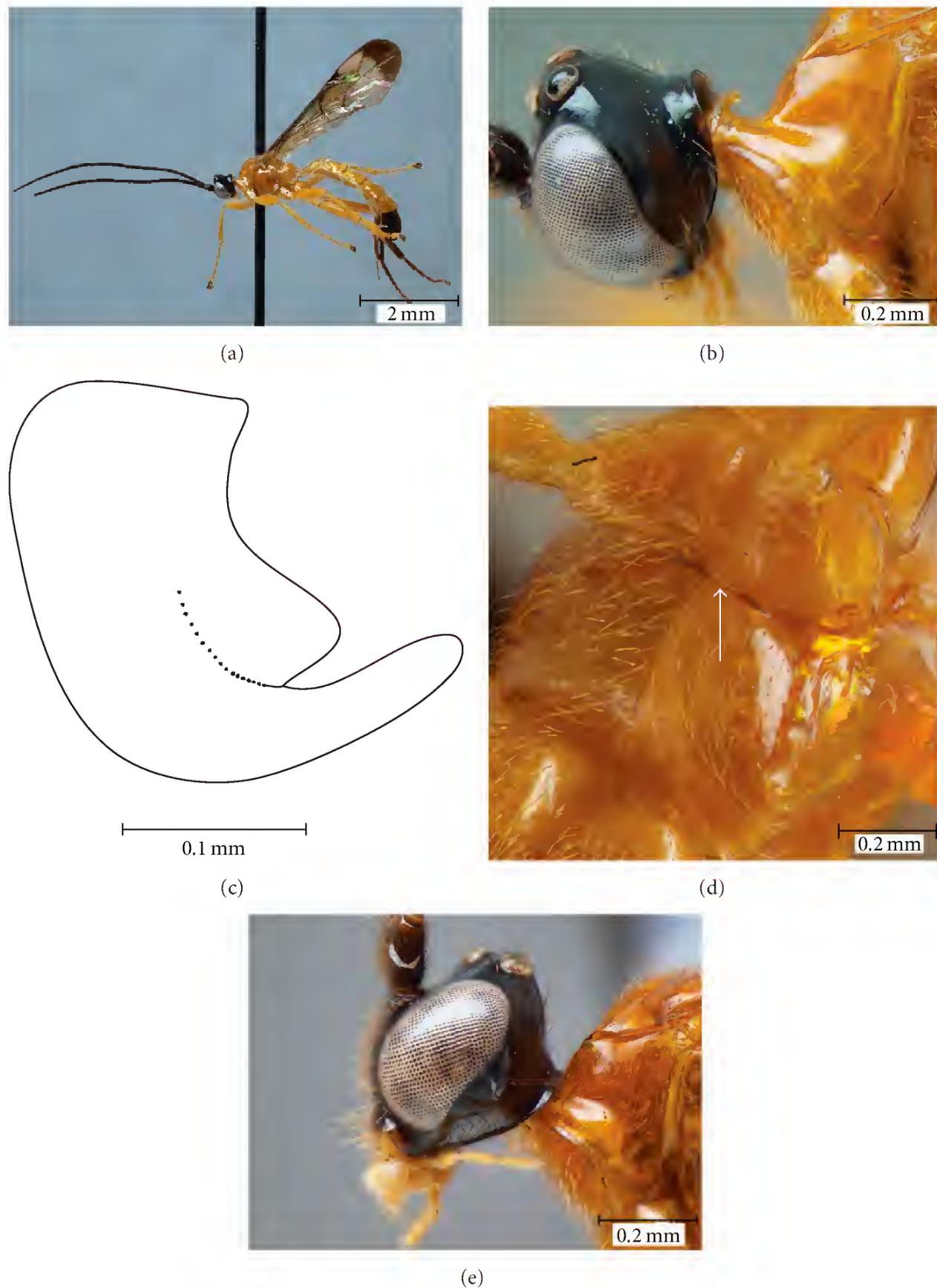


FIGURE 4: *T. soinii* sp.n.: holotype female habitus (a), occiput detail (b), fore tarsal claw (c), ventral view of epicnemium (d), and paratype male occiput detail (e).

Paratype males: 1 male (ZMUT): Ecuador, Department Orellana, Onkone Gare, 216.3 m, 00°39'25.7'' S 076°27'10.8'' W, 22.X.2005, T. L. Erwin et al. leg., Canopy fogging, Lot # 3062; 1 male (USNM): same locality, 216.3 m, 00°39'25.7'' S 076°27'10.8'' W, 7.X.1995, T. L. Erwin et al. leg., Canopy fogging, Lot # 1248.

Female Description. Malar space at narrowest point about 0.4 times as long as basal mandibular width; lower face elongate, about 1.0 times as broad as high (from weak supraclypeal suture to base of antenna), rather flat, with many long, conspicuous setae; head in dorsal view with genae

long and narrowed behind eyes; ocelli moderately large, the lateral one separated from the eye by about 0.8 times its own diameter; occipital carina arising from a strongly raised flange of the occiput. Pronotum relatively long, so that distance from front margin of tegula to head is about $0.6 \times$ distance from hind edge of tegula to hind margin of propodeum; mesoscutum very finely punctate; scutellum, in profile, moderately convex, posteriorly abruptly declivous; mesopleuron polished, ventrally bearing long close setae; epicnemial represented by a short midventral section about $1.0 \times$ the width of fore coxae. Metapleuron polished, sparsely but evenly pubescent, with setae arising from fine punctures;



FIGURE 5: Habitus of *T. vilmae*, paratype female (BMNH).

propodeum smooth, anteriorly and laterally with close fine setiferous punctures; propodeum, in profile, more convex than in *T. carinata*, anterior margin with a pair of small median tubercles; transverse groove before propodeum deep, in section U-shaped, barely interrupted laterally by raised extensions of propodeum. Tarsal claws without auxiliary tooth, instead with a preapical, flattened lobe, lobe with inner margin clearly concave; teeth of claw broad and concave internally. Fore wing length about 5.3–6.4 mm; *cu-a* from opposite to distal of *Rs&M* by about $0.2 \times$ length of *cu-a*; *2rs-m* about 0.2 times as long as abscissa of *M* between *2rs-m* and *2m-cu*; hind wing with distal abscissa of all veins more or less spectral, *cu-a* joining subbasal cell clearly closer to 1A than to *M-Cu*. Metasoma moderately stout, tergite I 2.0 times as long as posteriorly broad, with lateral carina only present at anterior end flanking the anterior concavity and reaching about 0.3 the length of tergite I, lateral longitudinal carina present only anteriorly, reaching the spiracle; tergite II about 1.0 times as long as posteriorly broad; tergite III about 0.9 times as long as posteriorly broad; ovipositor 0.90–0.95 times as long as hind tibia, lower valve proximally with a rather long, weakly convex swelling.

Head and antenna black, mouthparts and distal edge of clypeus orange. Mesosoma entirely orange. Metasoma orange, with tergites VI + and ovipositor sheath black. Anterior two pairs of legs orange; hind legs orange with distal 0.6 of tibia and entire tarsus black. Wings very faintly yellowish, the fore wing with apex and area adjacent to pterostigma clearly infumate; pterostigma black.

Male. Similar to female but fore wing length about 4.7 mm; tarsal claws simple, without auxiliary tooth or preapical lobe; tergites IV and V with dark lateral markings.

Diagnosis. This species can be distinguished from other species, except *T. amazonica* sp.n., by the absence of an auxiliary tooth on female tarsal claws; instead, *T. soinii* has a flattened and internally clearly concave lobe (in *T. amazonica* sp.n. the lobe is internally convex). It may be easily separated from *T. amazonica* sp.n. by the shape of the occiput; occipital carina arises from a strongly raised flange of the occiput.

Biological Notes. Nothing is known about the hosts of this species.

Etymology. The specific name refers to Mr. Pekka Soini (1941–2004), a Finnish tropical biologist who worked for years in the National Reserve of Allpahuayo Mishana, the type locality of *T. soinii* sp.n. His scientific contributions were among the key elements in the conservation process of this megadiverse and threatened Western Amazonian rain forest.

4. Key to the Known Species of *Ticapimpla*

(1) Females—2

Males (only the males of *T. amazonica* and *T. soinii* are known)—6

(2) Tarsal claws with flattened, preapical lobe (Figures 1(c) and 4(c))—3

Tarsal claws with auxiliary, preapical tooth (Figures 2(c) and 3(b))—4

(3) Occipital carina on strongly produced flange posteriorly (Figure 4(b)). Teeth of tarsal claws broad and concave internally, lobe of the claw concave (Figure 4(c)). Fore wing with sharply defined infumate apex (Figure 4(a))—*soinii* sp.n.

Occipital carina on weakly produced flange posteriorly (Figure 1(b)). Teeth of tarsal claws simply pointed, not concave internally, lobe of the claw convex (Figure 1(c)). Fore wing slightly infumate apically, infumate patch not sharply defined (Figure 1(a))—*amazonica* sp.n.

(4) Fore wing with distinctive preapical band. Hind femur almost entirely black (Figure 3(a)). Fore wing length >8 mm—*matamatae* sp.n.

Fore wing at most with infumate area adjacent to pterostigma. Hind femur entirely orange (Figures 2(a) and 5). Fore wing length <8 mm—5

(5) Epicnemial carina vestigial, represented midventrally by a very short section about $0.3 \times$ the width of fore coxa. Metasoma with tergite VI orange (Figure 5)—*vilmae* Gauld

Epicnemial carina very short but distinct, represented midventrally by a short section about $1.0 \times$ the width of fore coxa (as in Figure 4(d)). Metasoma with tergite VI black (Figure 2(a))—*carinata* sp.n.

(6) Occipital carina on strongly produced flange posteriorly (Figure 4(e)). Fore wing with sharply infumate apex—*soinii* sp.n.

Occipital carina on weakly produced flange posteriorly (Figure 1(d)). Fore wing with slightly infumate apex—*amazonica* sp.n.

5. Discussion

Although Pimplinae is the taxonomically best known subfamily of Ichneumonidae in the Neotropics, many species remain undescribed especially in northern South America. Moreover, it is clear that the limits of several genera are still poorly defined because the novel material found in this part of the Neotropics often represents entirely new species-groups or anomalous taxa that extend the morphological limits of genera. The latter is true of *Ticapimpla*, for which the newly described species have provided a wider concept of the genus.

Three morphological features of the genus are particularly interesting in this respect: (1) the shape of the female tarsal claws, (2) the epicnemial carina, and (3) the shape of the occiput. According to Gauld et al. [8], the preapical teeth on the female tarsal claws of pimplines represents a more derived condition than the flattened lobe. In *Ticapimpla*, the female tarsal claws show three different shapes: first, a short claw with a wide, flattened preapical lobe (that should be considered the plesiomorphic condition); second, a short claw with a narrower, preapical flattened lobe; finally, a large claw with a tooth-like, preapical process (the more derived condition). The absence of the epicnemial carina is generally considered a derived condition within Pimplinae and within the *Polysphincta* group of genera [5, 8]. The mid-ventral section of the epicnemium of *Ticapimpla* may have a short, but clearly distinct carina (the plesiomorphic condition), a much reduced, vestigial carina, or be simple, without any trace of carina (the more derived condition). Within the *Polysphincta* group of genera, the occiput is flanged in the clade *Ticapimpla* + (*Acrotaphus* + *Hymenoepimecis*) but simple in their closest sister group (*Polysphincta*) and other closely related lineages (see [5]). In *Ticapimpla*, the occiput varies from a weakly produced flange (possibly the plesiomorphic condition) to a very strongly produced one.

At one extreme of this range of variation is *T. amazonica*, possibly the less derived from within the genus, with *T. matamatae* at the other extreme—possibly the more derived one—which closely resembles some species of *Acrotaphus*. The only known non-Amazonian species of *Ticapimpla* is *T. vilmae* [4, 6], and it is possible that additional new species of *Ticapimpla* will be found in the future, further extending the limits of this genus.

Acknowledgments

The authors are indebted to Dr. Terry L. Erwin for providing them with his interesting canopy fogging samples which yielded several specimens of *Ticapimpla*, including the only known male specimens of the genus. The Colombian material in IAVH was collected thanks to the support of NSF grant DEB 0205982 to M. J. Sharkey and B. V. Brown. Claudia Alejandra Medina from IAVH kindly provided access to specimens under her care. The Peruvian material was collected with the help of José Alvarez, Mario Escobedo, Gerardo Lamas, Manuel Reategui, Jukka Salo, and Pekka Soini. They are grateful to the following Peruvian institutions for providing good facilities and research permits during

the study: Universidad Nacional de la Amazonía Peruana, UNAP, Instituto Nacional de Recursos Naturales, INRENA, Instituto de Investigaciones de la Amazonía Peruana, IIAP and Instituto Nacional de Investigación Agraria, INIA. The Peruvian material in ZMUT was collected with the support of the Ministry of Education, Finland and the Entomological Society of Finland. Salla-Riikka Vesterlund checked the language of the discussion. The comments of Dr. Mark R. Shaw and two anonymous reviewers helped to improve the manuscript.

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Research Article

Atypical Wing Venation in *Dialictus* and *Hemihalictus* and Its Implications for Subgeneric Classification of *Lasioglossum*

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Received 28 May 2010; Accepted 30 August 2010

Academic Editor: David Roubik

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The subgeneric classification of hundreds of species in *Lasioglossum* Curtis *sensu lato* is currently unstable due to differing opinions on the suitability of wing venation characters for differentiating subgenera. The subgenera *Dialictus* Robertson and *Hemihalictus* were both originally defined primarily by the forewing having two submarginal cells. I present examples of variation in submarginal cell number in the type species of these two subgenera: *L. (Dialictus) anomalum* (Robertson) and *L. (Hemihalictus) lustrans* (Cockerell). These results suggest that submarginal cell number is insufficient for recognizing subgenera in *Lasioglossum*. The variability of this character is used to refute the classification proposed by some authors that *Chloralictus* Robertson, but not *Dialictus*, be synonymised with *Evylaeus* Robertson.

1. Introduction

Lasioglossum Curtis *sensu lato* (Apoidea: Halictidae) is the largest genus of bees with over 1700 described species [1]. This cosmopolitan genus includes many commonly collected bees which can, in both temperate and tropical areas, dominate the bee fauna in terms of number of individuals (see [2] for Ontario, [3] for Louisiana, [4] for North Carolina, and [5] for Maryland, Chihuahuan desert, and Columbia plateau, Ngo et al. in prep. for Costa Rica). In addition, the behaviourally diverse *Lasioglossum s.l.* has been the focus of numerous sociobiological studies (reviewed in [6–9]) and is an ideal group for studying the evolution of social behaviour [10].

There are competing classifications currently in use within *Lasioglossum s.l.* [11–15] that result in unstable nomenclature for many species and confusion among researchers [16]. These classifications depend on whether wing venation characters are sufficient to recognise genus-group names in *Lasioglossum s.l.* The utility of these characters is examined in an attempt to provide support for a more stable classificatory system. Stable classification and nomenclature for these important bees are highly desirable to facilitate continued study and communication of results.

Lasioglossum s.l. has been subdivided into two “series” based on the strength of the distal veins of the forewing [11, 17]. The *Lasioglossum* series has the outermost veins, 2rs-m, 2m-cu, and the second abscissa of M weak; the *Hemihalictus* series has an additional weakened vein, 1rs-m. In some cases 1rs-m is absent resulting in two rather than three submarginal cells (Figure 1). The *Hemihalictus* series, at least, seems to be a monophyletic group [17, 18] and likely resulted as a transition from an ancestral strong vein state with the state seen in the *Lasioglossum* series as a possible intermediate. The *Hemihalictus* series consists of more than half (>900) of the species in the genus *Lasioglossum*, including those under consideration in this paper.

The presence or absence of vein 1rs-m has been used to recognise genus-group names for taxa included in the *Hemihalictus* series (see [11, 13, 14, 19–25], and [16] for a complete list of genus-group names). In competing classifications, several hundred species in the *Hemihalictus* series are classified as either *Dialictus* Robertson or *Evylaeus* Robertson depending on the status of the genus-group name *Chloralictus* Robertson [11, 13]. *Chloralictus* was formerly in common use for many species [23] but now is conventionally treated as a junior synonym of either *Dialictus* [11, 15, 24, 26]

or *Evyllaesus* [12–14]. *Hemihalictus* Cockerell is the oldest name in the *Hemihalictus* series but has only ever been applied to a single species, *L. lustrans* (Cockerell).

These four genus-group names were first erected based primarily on differences in wing venation and colouration (Table 1) [20, 21]. *Dialictus* and *Chloralictus*, as originally defined, both have metallic colouration but differ in their number of submarginal cells: *Dialictus* has two (vein 1rs-m absent) and *Chloralictus* has three (vein 1rs-m present). *Hemihalictus* and *Evyllaesus* have a similar relationship; both are nonmetallic but have two and three submarginal cells, respectively. The presence or absence of metallic colouration is now widely regarded as a poor character for recognising genus-group names in these bees because it can vary within species and among closely related species (see [11, 27, 28]). As a result *Dialictus s.l.* (+*Chloralictus*) and *Evyllaesus s.s.* both include species with and without metallic colouration [11, 28]. Mitchell [26] was the first to treat *Chloralictus* as a junior synonym of *Dialictus*. Individuals from many species of *Chloralictus* and *Dialictus s.s.* may have vein 1rs-m present in one wing and absent in the other [29]. At least two metallic species not closely related to the type species of *Dialictus*, *L. anomalum* (Robertson), are known to be polymorphic for the presence or absence of vein 1rs-m, *L. parvum* (Cockerell) [30] and *L. asteris* (Mitchell) [15]. *Lasioglossum parvum* belongs to the *L. tegulare* species-group of Gibbs [31] whereas *L. asteris* is a social parasite [32] only distantly related to the aforementioned groups (see [18], Gibbs unpublished data). Mitchell [26] considered the absence of vein 1rs-m to be an unreliable character for these bees. Many subsequent authors have followed his classification (e.g., [11, 15, 24, 31, 33, 34]).

In contrast, Ebmer [13, 25, 27] has argued that the presence or absence of vein 1rs-m is sufficient to classify *Dialictus* and *Chloralictus* as separate subgenera. As such, *Chloralictus* is then considered by him to be a junior synonym of *Evyllaesus* because colour is not considered a reliable character [27]. The classification espoused by Ebmer [13, 25] is followed by others [12, 14] and results in a paraphyletic *Evyllaesus* (+*Chloralictus*) [18, 35] because *Dialictus sensu* Ebmer [13], Pesenko et al. [12] and Murao and Tadauchi [14] is derived from within it (see [18]; Gibbs unpublished data). Ebmer [13] explicitly rejects a strict cladistic classification for these bees. Thus, the classification set forth by Ebmer [13, 25], and used by many Old World authors, depends solely on the reliability of the presence or absence of vein 1rs-m for separating *Dialictus* from *Chloralictus*.

The genus-group name *Hemihalictus* has only ever been applied to a single species, *L. lustrans*, a solitary oligolege on *Pyrrhopappus* DC [36] and related Asteraceae in the tribe Cichorieae (M. Arduser *in litt.*). *Hemihalictus* renders *Dialictus s.l.* paraphyletic [18] but has never been treated as a synonym because it has priority over all other names in the *Hemihalictus* series [11, 16] and would require hundreds of name changes if the synonymy was applied. *Hemihalictus* is characterized by the lack of vein 1rs-m, nonmetallic integument, serrate inner hind tibial spurs of females, and short flagellomeres in males. The flagellomere character is also seen in some *Dialictus s.l.* (e.g., *L. pectorale* (Smith))

as well as some *Evyllaesus s.s.* (e.g., *L. marginatum* (Brullé)), and the hind tibial spur character is similar to those of some *Evyllaesus s.s.* (e.g., *L. laeve* (Kirby) and *L. lineare* (Schenck); see [12] for variation in hind tibial spurs). All of the species in the preceding sentence would be considered *Evyllaesus* in some classifications [13, 24, 26, 27].

Variation in the wing venation of *L. anomalum*, the type species of *Dialictus*, and *L. lustrans*, the type species of *Hemihalictus*, is described herein based on large-scale taxonomic studies of *Dialictus s.l.* [15, 31, 37]. The implications of this variation for the subgeneric classification of *Lasioglossum s.l.* are discussed.

2. Methods

My revisionary studies of North American *Dialictus s.l.*, which include the type species of both *Dialictus* and *Chloralictus*, have involved the examination of many tens of thousands of specimens [15]. In addition to morphology, my studies have included a molecular component for aiding taxonomic study [15, 31, 37–39]. A database of over three thousand homologous DNA sequences (DNA barcodes) for *Lasioglossum s.l.* is currently stored on the Barcode of Life Data Systems [40] and GenBank.

DNA barcoding, the use of a standard gene fragment for species-level identification [41], was used to verify the identity of some of the specimens described herein. The standard fragment used for animals is 658 bp on the 5' end of cytochrome *c* oxidase subunit 1 [42]. Sequencing was performed at the Canadian Centre for DNA Barcoding at the University of Guelph (Guelph, Ontario). DNA was extracted from a single dried leg (or in some cases two legs) using automated extraction protocols for 96-well plates [43]. One of two primer pairs was used to amplify the DNA barcode region (LCO1490 and HCO2198 [44] or the variants LepF1 and LepR1; [45]). Samples that failed to amplify were then reattempted using internal primer pairs (LepF1 and C_AntMr1D-RonIdeg_R [46] and LepR1/MLepF1; [47]). PCR and sequencing reactions followed standard Canadian Centre for DNA Barcoding protocols [48]. Sequences were uploaded to the Barcode of Life Data Systems [40].

3. Results

Seven individuals of *Lasioglossum (Dialictus) anomalum* with atypical wing venation were examined (Table 2). Six of these had vein 1rs-m present in one wing but absent in the other. The final specimen, collected in Guelph, Ontario, Canada, approximately 900 km Northeast of the type locality in Carlinville, Illinois, had vein 1rs-m present in both wings (Figure 2) resulting in wing venation typical of *Chloralictus* and *Evyllaesus*. The specimens are otherwise morphologically identical to *L. anomalum*. The DNA barcode sequences of the Ontario and Michigan specimens matched DNA barcode sequences of *L. anomalum* individuals with vein 1rs-m absent in both wings sampled throughout its range, including approximately 300 km from the type locality (Figure 3). A

TABLE 1: Characteristics of *Hemihalictus*, *Dialictus*, *Evylaeus*, and *Chloralictus* type species.

Genus-group name	Type species	Date of publication	Vein 1rs-m	Integument colour	Female inner hind tibial spur	Male flagellomere length
<i>Hemihalictus</i> Robertson	<i>Panurgus lustrans</i> Cockerell	1897, p. 288	Absent	Nonmetallic	Serrate/denticulate	short
<i>Dialictus</i> Robertson	<i>Halictus anomalus</i> Robertson	1 Feb. 1902, p. 48	Absent	Metallic	Pectinate	short
<i>Evylaeus</i> Robertson	<i>Halictus arcuatus</i> Robertson*	10 Sep. 1902, p. 247	Present	Nonmetallic	Serrate/denticulate	long
<i>Chloralictus</i> Robertson	<i>Halictus cressonii</i> Robertson	10 Sep. 1902, p. 248	Present	Metallic	Pectinate	long

* Junior subjective synonym of *Halictus cinctipes* Provancher.

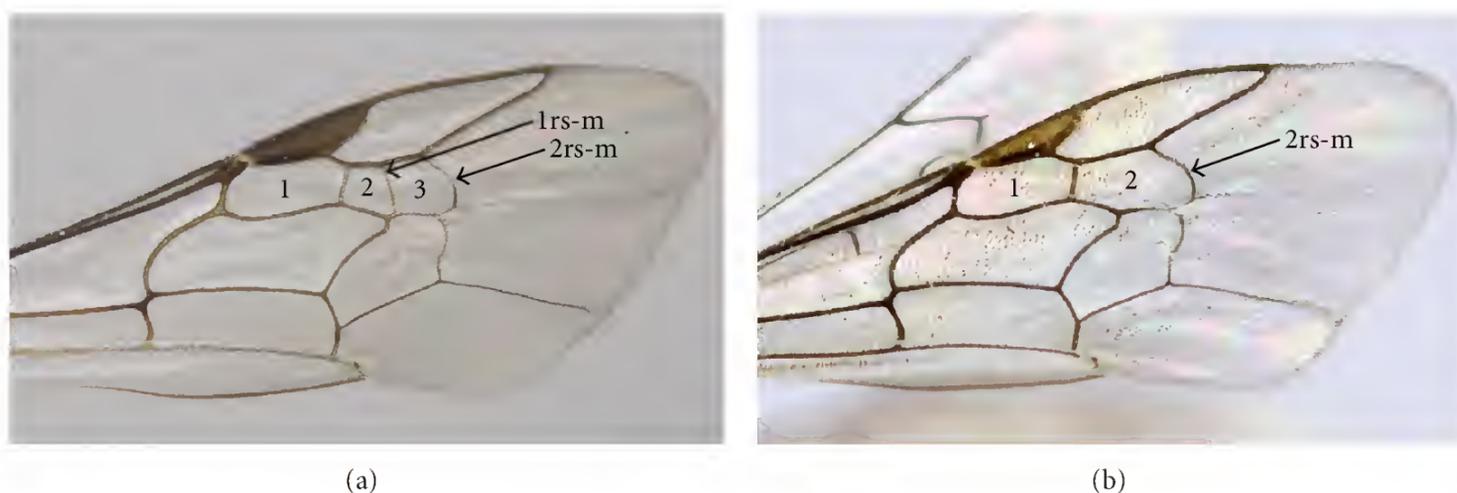


FIGURE 1: Forewing of *Lasioglossum* belonging to the *Hemihalictus* series. Numbers indicate submarginal cells. (a) Vein 1rs-m present. (b) Vein 1rs-m absent. Modified from [31].



FIGURE 2: Lateral habitus of *Lasioglossum (Dialictus) anomalum* with three submarginal cells. Bar = 1 mm.

series of *L. anomalum* collected from the same site as the Ontario specimen had vein 1rs-m absent.

A single male specimen of *L. (H.) lustrans* with vein 1rs-m present in both forewings has been examined (Figure 4). In other respects, it appears to be a normal specimen of *L. lustrans*. The identification was also verified using DNA barcodes. The locality data for this specimen is as follows: USA, Wisconsin, Marinette Co., Dunbar Barrens, N45.65149 W088.2415, 13.vii.2005 (C. Destree). A second male specimen with vein 1rs-m absent was also examined from the same locality. Both specimens are stored at the Richter Museum of Natural History, University of Wisconsin, Green Bay, Wisconsin.

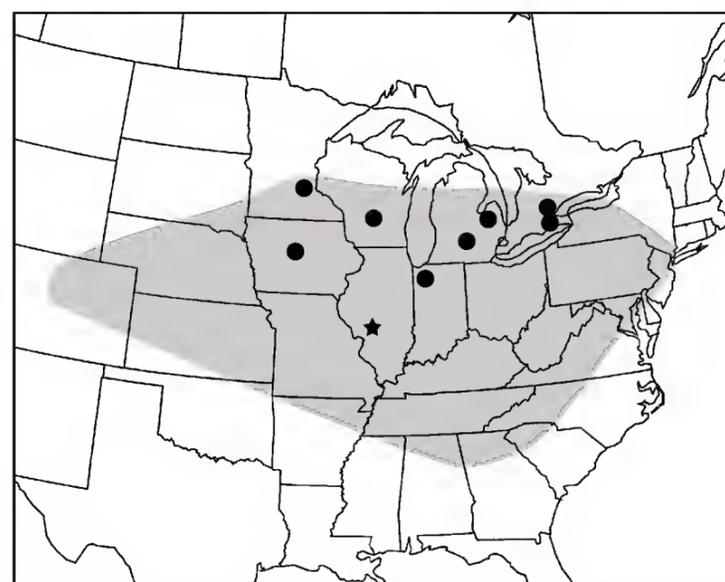


FIGURE 3: Map of *Lasioglossum anomalum* distribution in eastern North America with type locality indicated by a star. Circles indicate collection localities of DNA barcoded specimens. Modified from [31].

4. Discussion

Ebmer [27] argued that metallic colouration was not a reliable character for recognising genus-level differences between *Evylaeus* and *Chloralictus*. To support this argument, he used the examples of *L. viride* (Brullé) and *L. morio* (Fabricius). In the case of *L. viride*, both black and metallic forms

TABLE 2: *Lasioglossum anomalum* individuals with vein 1rs-m present. PCYU: Laurence Packer's Collection, York University (Toronto, Canada), AMNH: American Museum of Natural History (New York, USA), ARC: Albert J. Cook Arthropod Research Collection, Michigan State University (East Lansing, USA), and IRCW: University of Wisconsin–Entomology (Madison, USA).

Depository	Forewing (s) with 1rs-mPresent	Country	Province or state	Latitude (north)	Longitude (west)	Collection date
PCYU	2	CANADA	Ontario	43.5	80.31	16.viii.2007
PCYU	1	USA	Iowa	43.32472	91.13444	15.viii.2005
PCYU	1	USA	Michigan	43.61667	83.31739	16-20.vi.2008
ARC	1	USA	Michigan	43.69311	83.20706	30.vii.2009
ARC	1	USA	Michigan	43.69311	83.20706	30.vii.2009
AMNH	1	USA	New York	40.86806	73.42611	26.vii.1962
IRCW	1	USA	Wisconsin	43.28245	89.58043	5.vi.1995



FIGURE 4: Dorsal habitus of *Lasioglossum (Hemihalictus) lustrans* with three submarginal cells. Bar = 1 mm.

are known from the same locality. Morphologically they are indistinguishable and the colour variation in these two forms is considered to be a polymorphism. Colour aberrations also occur—*Halictus balticus* Blüthgen was the name given to a black specimen of the normally metallic *L. morio*. Other examples of metallic/nonmetallic polymorphism are known from *Lasioglossum* (J. Gibbs, unpublished data) and in the genus *Agapostemon* (L. Packer, unpublished observation).

The examples given herein for wing venation are analogous to those provided by Ebmer [27] for colouration. At least two species in the *Hemihalictus* series, *L. parvum* and *L. asteris*, show both the presence and absence of vein 1rs-m with a high frequency. *Lasioglossum parvum* belongs to the *L. parvum/tegulare* species group [18, 31] whereas *L. asteris* is a distantly related parasitic species [18]. Neither of these species is believed to be a close relative of *L. anomalum*, a view supported by preliminary phylogenetic analyses (J. Gibbs, unpublished data). In the *Lasioglossum* series, *L. (Ctenonomia) bakeri* Pauly was described from two individuals, each with a different number of submarginal cells [49]. Less frequent variation, such as that of the *L. anomalum* and *L. lustrans* individuals described here, fails to support the utility of this character for species-level identification, let alone genus-level classification.

Even disregarding the benefits of a cladistic classification (for discussion see [50–52]) these examples, strongly suggest that the presence or absence of vein 1rs-m is not sufficiently reliable to recognise *Dialictus* and *Chloralictus* as separate genera, subgenera or even to recognise species. *Chloralictus*

should be considered a junior synonym of *Dialictus* based on the principle of priority (Article 23.3, [53]) following Mitchell [26], Krombein [33], Hurd [54], Moure and Hurd [24], Michener [7, 11], and many others. *Chloralictus* cannot justifiably be considered a synonym of *Evylaeus* without the latter name in turn becoming a junior synonym of the older name *Dialictus*. The type species of *Evylaeus* belongs to the “carinate-*Evylaeus*” which is sufficiently different morphologically and phylogenetically [18] from *Dialictus* + *Chloralictus* to be recognised as distinct. The evidence presented here and previously [29, 30] does not support the classification used by Ebmer [13, 25] even if a phenetic classification was considered appropriate.

The subgenus *Hemihalictus* is also recognised primarily on the basis of the absence of vein 1rs-m. This name has priority over all other names in the *Hemihalictus* series, and its sole species is clearly nested within the *Dialictus s.l.* clade [17, 18, 35]. The existence of individuals with vein 1rs-m present provides additional support for considering *Hemihalictus* synonymous with *Dialictus s.l.* If this synonymy were made, the subgeneric placement of hundreds of species would change. *Hemihalictus* is an uncommonly collected, monotypic taxon. A petition to set aside the principle of priority in the case where *Hemihalictus* is considered a synonym of *Dialictus* or *Evylaeus* has been submitted to the International Commission of Zoological Nomenclature [16].

Some authors have chosen to elevate subgenera of *Lasioglossum* to the level of genus [24, 26, 27, 54, 55] which would seem unwise given the difficulty in distinguishing between these higher taxa and the probability that many *Lasioglossum* subgenera are paraphyletic [17, 18]. A subdivision of *Lasioglossum s.l.* into smaller genera may be desirable but should await a more complete phylogeny of the group to allow a stable classification [11].

Acknowledgments

The author would like to thank Marianna Horn for allowing him to examine her specimens of *L. anomalum*. John Ascher generously provided information on the New York specimen of *L. anomalum* and sent him the specimens of *L. lustrans* discussed here. Laurence Packer and Cory Sheffield provided useful comments on earlier drafts of this paper. This paper was supported through funding to the Canadian Barcode

of Life Network from Genome Canada, NSERC, and other sponsors listed at <http://www.bolnet.ca/>.

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Research Article

Survey of the Aphid Parasitoids (Hymenoptera: Braconidae: Aphidiinae) of Costa Rica with Information on Their Aphid (Hemiptera: Aphidoidea): Plant Associations

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Received 23 July 2010; Accepted 7 October 2010

Academic Editor: Martin H. Villet

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Species of Aphidiinae (Braconidae) have never been surveyed in Central America. Here we present the results of an initial inventory of the aphidiine species of Costa Rica and record the presence of ten species (four undetermined), in six genera. The material was obtained by rearing aphids from both crop and noncrop plants throughout the country. In total 2832 aphidiine specimens were reared from 24 species of aphids. *Aphidius colemani* and *Lysiphlebus testaceipes*, which are probably not native to Costa Rica, accounted for nearly 90% of all the specimens. Many of the other aphidiines are also probably exotic species, as are most of their host aphids.

1. Introduction

The primary parasitoids of aphids are restricted to two taxa of Hymenoptera, Aphidiinae (Braconidae) and *Aphelinus* (Aphelinidae), but the vast majority of species and rearing records pertain to the former. Research on the aphid parasitoids of the Neotropical region has been dominated by work done in Cuba [1–6] although some data exist for Mexico [7, 8], Guadeloupe [9], Venezuela [10], Brazil [11], Argentina [12], and Chile [13]. Despite their importance in biological control [14], the species of Aphidiinae (Braconidae) have never been extensively surveyed in Central America.

Knowledge of the aphid parasitoids of this region is important for at least two reasons. First, biocontrol companies are currently offering exotic species for sale in Central America, but before granting permission to these companies, governmental agencies need to know whether a particular commercial species is already present. If it is not present, an

evaluation needs to be done of the potential impact of liberating an exotic species, but this is not possible if the existing parasitoid fauna is unknown. Second, aphids are among the very few groups of insects in Central America where the vast majority of species are not native. About 90 aphid species are reported from Costa Rica and the vast majority of these are probably not native to the country [15]. About 37.5% of the Costa Rican aphid species are Nearctic, 34.1% Palearctic, 14.8% Oriental, 6.8% Neotropical, and 6.8% are of unknown affinities [16]. Thus, it might be predicted that most of their parasitoids are also exotic species, having been intentionally or inadvertently introduced in the recent past. In this respect, a possible environmental hazard following an introduction of a biocontrol agent is negligible.

The objective of this study is to provide initial survey of the primary aphid parasitoids present in Costa Rica and to evaluate the geographic affinities of these species, in particular, which ones are native and which ones are exotic. The data presented here treat only primary parasitoids;

hyperparasitoids were also reared, but these will be treated in a separate publication.

2. Materials and Methods

Aphidiine parasitoids were obtained by collecting as many aphid species as possible from their host plants throughout various locations in Costa Rica. Aphid populations sampled in the field varied in size, and approximately 25 to 200 aphids were taken per sample. A subsample of 5–70 aphids was preserved in 70% ethanol for later identification. When the identity of the plant was unknown samples were dried in a plant press for later identification. GPS Garmin Etrex was used for recording the geographic coordinates and elevation of each site where aphids were collected. Arc Map 9.2 software was used to create the parasitoid distribution map (Figure 1).

Each part of plant sampled with aphids was placed in square plastic containers of 10 cm in length and 10 cm height. The containers were maintained for 25–30 days under a temperature range of 24°–28°C with constant ventilation and light. The samples were checked daily for emerged parasitoids. After emergence, the aphidiine parasitoids were placed in 70% ethanol for identification. The specimens are deposited in the Museum of Zoology at the University of Costa Rica and in P. Starý's collection (České Budějovice). All the material was sampled by the first author who also identified the aphids, with the help of Nicolás Pérez Hidalgo, University of Leon, Spain. The parasitoids were identified by P. Starý.

3. Results

In total, 2832 aphidiine specimens, comprising ten species in six genera, were reared from 24 species of aphids, from a total of 35 localities (Figure 1). Below, the parasitoid species are listed in alphabetical order, along with their aphid and host plants (exotic plants are marked with an asterisk), collecting locality, geographic coordinates, elevation, date, number of specimens (spns.), and (in parenthesis) lot number (Museum of Zoology, University of Costa Rica). Abbreviations used for Costa Rican provinces are Al-Alajuela, Ca-Cartago, Gu-Guanacaste, He-Heredia, Pu-Puntarenas, and SJ-San José.

3.1. *Aphidius colemani* Viereck

Aphis craccivora Koch on *Vicia sativa*. Ca, Cot, 9°53.964'N, 83°52.727'W, 1895 m, 25-VIII-09, 1 spn. (S-133).

Aphis gossypii Glover on *Cyphomandra betacea*. Ca, Tierra Blanca, 9°56.120'N, 83°52.963'W, 2382 m, 4-IX-09, 373 spns., (S-138); SJ, Pérez Zeledón, 9°29.914'N, 83°36.781'W, 1706 m, 20-IX-09, 4 spns., (S-157); Ca, Taras, 9°52.944'N, 83°53.835'W, 1598 m, 30-IX-09, 6 spns., mixed with *Myzus ornatus*, (S-182).

Aphis nerii Boyer de Fonscolombe on *Asclepias curassavica*. Ca, Turrialba, 9°90.291'N, 83°68.561'W, 680 m, 5-X-08, 93 spns., (S-712); Pu, Monteverde, 10°19.150'N, 84°49.428'W, 1317 m, 10-V-09, 13 spns., (S-71); Ca, Cervantes, 9°52.730'N, 83°49.210'W, 1413 m, 23-VII-09, 4 spns., (S-90); Gu, Liberia, 10°33.301'N, 85°23.843'W, 133 m, 31-VII-09, 4 spns., (S-102); on *Gomphocarpus physocarpus**: SJ, Coronado, 9°58.556'N, 84°00.464'W, 1400 m, 15-IX-08, 40 spns., (S-7); on *Gonolobus edulis*: SJ, Montes de Oca, 9°56.380'N, 84°03.003'W, 1214 m, 20-VII-09, 24 spns., (S-125); on *Tabernaemontana alba*: He, San Miguel, 9°58.600'N, 84°04.600'W, 1165 m, 3-VIII-08, 58 spns., (S-1).

Brachycaudus helichrysi (Kaltenbach) on *Ageratum conyzoides*. Pu, Monteverde, 10°19.335'N, 84°49.468'W, 1367 m, 15-VIII-09, 25 spns., (S-115); SJ, Pérez Zeledón, 9°30.057'N, 83°36.817'W, 1721 m, 20-IX-09, 1 spn., mixed with *Myzus ornatus*, (S-156); on Asteraceae: Ca, Cerro Buena Vista, 9°44.514'N, 83°57.002'W, 2112 m, 20-V-09, 1 spn., (S-53); on *Emilia sonchifolia*: Ca, Coris, 9°51.227'N, 83°59.379'W, 1498 m, 08-VIII-09, 8 spns., (S-105); Ca, Paraíso, 9°49.103'N, 83°51.530'W, 1303 m, 08-VIII-09, 1 spn., mixed with *Aulacorthum solani*, (S-106); SJ, Desamparados, 9°54.205'N, 84°02.429'W, 1199 m, 8-VIII-09, 16 spns., (S-110); SJ, Pérez Zeledón, 9°30.008'N, 83°35.783'W, 1675 m, 20-IX-09, 39 spns., (S-155); on *Senecio grandifolius*: Ca, Oreamuno, 9°58.557'N, 83°50.580'W, 3345 m, 14-VIII-08, 83 spns., (S-3), 25-III-09, 7 spns., (S-25); on *Solanum* sp.: SJ, Cerro Buena Vista, 9°40.727'N, 83°52.755'W, 2465 m, 29-IX-09, 77 spns., mixed with *Myzus ornatus* and *Rhodobium porosum*, (S-179).

Myzus ornatus Laing on Asteraceae. SJ, Montes de Oca, 9°56.345'N, 84°02.836'W, 1184 m, 29-IV-09, 1 spn., (S-42); on *Cyphomandra betacea*: Ca, 9°52.944'N, 83°53.835'W, 1598 m, 30-IX-09, 6 spns., (S-182); on *Rubus urticifolius**: Ca, Cerro Buena Vista, 9°33.736'N, 83°44.421'W, 3161 m, 2-V-09, 4 spns., mixed with *Aphis gossypii*, (S-47); on *Rumex* sp.: SJ, Cerro Buena Vista, 9°41.281'N, 83°54.019'W, 2520 m, 25-IX-09, 2 spns., mixed with *Brachycaudus helichrysi*, (S-176); on *Solanum* sp.: SJ, Cerro Buena Vista, 9°40.727'N, 83°52.755'W, 2465 m, 29-IX-09, 77 spns., mixed with *Brachycaudus helichrysi* and *Rhodobium porosum*, (S-179).

Myzus persicae (Sulzer) on *Drymaria cordata*. SJ, Montes de Oca, 9°56.380'N, 84°03.003'W, 1214 m, 11-VII-09, 44 spns., (S-81); on *Solanum lycopersicum*: Al, Zarcero, 10°11.058'N, 84°23.472'W, 1648 m, 07-V-09, 9 spns., mixed with *Aphis gossypii* and *Aphis spiraecola*, (S-50).

Pentalonia nigronervosa Coquerel on *Costus pulverulentus*. SJ, Montes de Oca, 9°44.23'N, 83°50.101'W, 1214 m, 22-V-09, 3 spns., (S-57).

Rhopalosiphum maidis (Fitch) on *Zea mays**. SJ, Pérez Zeledón, 9°30.008'N, 83°36.783'W, 1675 m, 20-IX-09, 3 spns., (S-153).

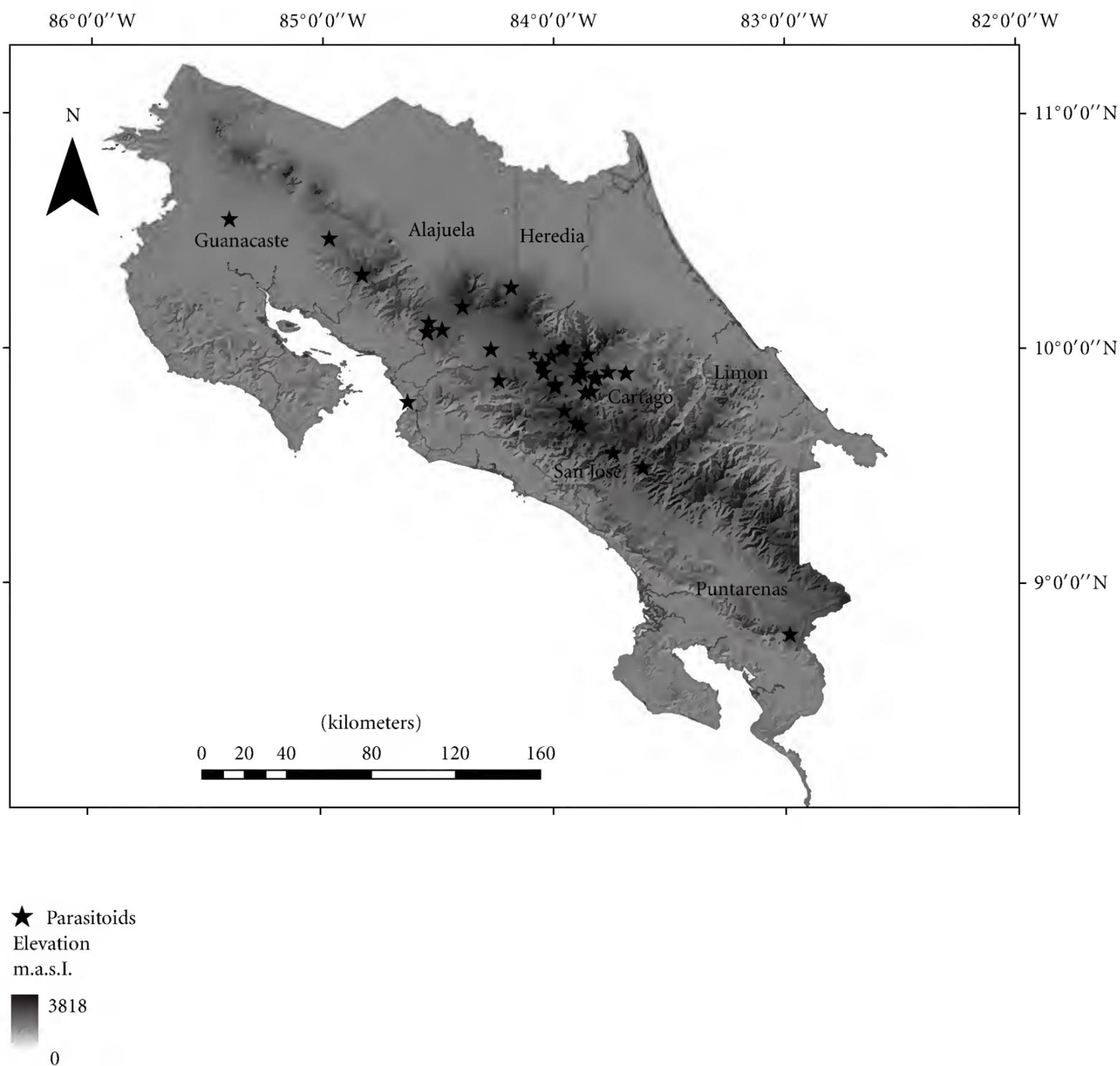


FIGURE 1: Geographic distribution of aphid-Aphidiinae associations in Costa Rica.

Toxoptera citricida (Kirkaldy) on *Citrus sinensis**. Pu, Tárcoles, 9°55.247'N, 84°03.407'W, 60 m, 21-V-09, 1 spn., (S-56).

3.2. *Aphidius* sp. near *colemani*

Brachycaudus helichrysi (Kaltenbach) on *Ageratum conyzoides*. Ca, Cot, 9°53.964'N, 83°52.727'W, 1895 m, 25-VIII-09, 19 spns., (S-132); on *Senecio grandifolius*: Ca, Oreamuno 9°58.557'N, 83°50.580'W, 3346 m, 14-VIII-08, 19 spns., (S-3).

Illinoia morrisoni Swain on *Cupressus lusitanica**. SJ, Coronado, 10°00.220'N, 83°57.563'W, 1724 m, 12-VII-09, 5 spns., (S-86).

Macrosiphum salviae Bartholomew on *Morella pubescens*. Ca, Cerro Buena Vista, 9°44.491'N, 83°57.038'W, 2123 m.s.n.m., 20-V-09, 20-V-09, 2 spns., (S-55).

Myzodius modestum (Hottes) on *Polytrichum juniperinum*. SJ, Cerro Buena Vista, 9°41.281'N, 84°54.018'W, 2519 m, 25-IX-09, 1 spn., (S-225).

Remarks. This species is characterized by a combination of 16-17 segmented antenna (19 in males), tentorial index of 0.4, labial palpi 4 segmented, maxillary palpi 3 segmented, and petiole bearing 4 costae (female). It belongs to the *A. colemani* species group and is possibly a distinct species, but its status must await a reexamination of South American material routinely grouped under *A. colemani*. It commonly occurs together with *A. colemani* in the same sample.

3.3. *Aphidius* sp. A

Microparsus (Picturaphis) pojanii (Cermeli et Smith) on *Phlebodium pseudoaureum*. Ca, Cot, 9°53.940'N, 83°52.576'W, 1893 m, 25-VIII-09, 26 spns., (S-128).

Remarks. This species is characterized by combination of 17-segmented antenna, tentorial index of 0.6, maxillary palpi 4 segmented, labial palpi 2 segmented, and petiole costulate anterolaterally.

3.4. *Aphidius* sp. *B. Uroleucon* (Lambersius) *gravicorne* (Patch) on *Conyza canadensis*: SJ, Montes de Oca, 9°55.953'N, 84°02.786'W, 1214 m, 5-IV-09, 4 spns., (S-30).

3.5. *Binodoxys solitarius* Starý

Aphis gossypii Glover on *Cyphomandra betacea*. SJ, Pérez Zeledón, 9°29.914'N, 83°56.781'W, 1706 m, 20-IX-09, 2 spns., (S-157).

Brachycaudus helichrysi (Kaltenbach) on *Asteraceae*. Ca, Cerro Buena Vista, 9°44.514'N, 83°57.002'W, 2112 m, 20-V-09, 1 spn., (S-53); on *Nasa triphylla*: SJ, Coronado, 10°00.784'N, 83°57.101'W, 1758 m, 9-VIII-09, 4 spns., mixed with *Myzus persicae* and *Lipaphis erysimi*, (S-112).

Myzus ornatus Laing on *Lamiaceae*. SJ, Cerro Buena Vista, 9°40.727'N, 83°52.755'W, 2465 m, 2 spns., (S-177).

Toxoptera aurantii (Boyer de Fonscolombe) on *Cuphea appendiculata*. SJ, Mora, 9°52.267'N, 84°1°4.029'W, 1207 m, 11-VIII-09, 2 spns., mixed with *Aulacorthum solani*, (S-113).

3.6. *Binodoxys* sp. (Male). *Aulacorthum solani* (Kaltenbach) on *Bocconia frutescens*: SJ, Coronado, 9°58.556'N, 84°00.464'W, 1402 m, 18-V-09, 1 spn., (S-76).

3.7. *Diaeretiella rapae* (M'Intosh)

Brevicoryne brassicae (L.) on *Brassica campestris*. Ca, Alvarado, 9°52.985'N, 83°48.742'W, 1420 m, 5-VI-09, 14 spns., mixed with *Myzus persicae*, (S-75); Ca, Cot, 9°53.964'N, 83°52.727'W, 1895 m, 25-VIII-09, 8 spns., mixed with *Myzus persicae* and *Aphis spiraecola*, (S-131); Ca, Coris, 9°50.677'N, 83°59.469'W, 1424 m, 8-VIII-09, 21 spns., (S-111).

Lipaphis pseudobrassicae Davis on *Brassica campestris*. SJ, Cerro Buena Vista, 9°11.281'N, 83°54.018'W, 2519 m, 25-IX-09, 38 spns., (S-175).

3.8. *Ephedrus lacertosus* (Haliday)

Uroleucon (Lambersius) *gravicorne* (Patch) on *Rubus* sp.: SJ, Cerro Buena Vista, 9°40.727'N, 83°52.755'W, 2465 m, 25-IX-09, 3 spns., (S-173).

3.9. *Lipolexis oregmae* (Gahan)

Aphis illinoisensis Shimer on *Vitis tiliifolia*. Pu, Coto Brus, 8°46.978'N, 82°58.294'W, 1311 m, 21-III-09, 2 spns., (S-20).

Toxoptera citricida (Kirkaldy) on *Citrus aurantium**. Al, San Ramón, 10°904.676'N, 84°32.447'W, 916 m, 24-V-09, 1 spn., (S-58); on *Zanthoxylum* sp., Al, San Ramón, 10°04.676'N, 84°32.447'W, 885 m, 13-VII-09, 132 spns., (S-68).

3.10. *Lysiphlebus testaceipes* (Cresson)

Aphis coreopsidis (Thomas) on *Bidens pilosa*. Al, La Garita, 10°00.242'N, 84°16.087'W, 847 m, L., 23-IX-09, 2 spns., (S-163).

Aphis gossypii (Glover) on *Bauhinia purpurea**. Ca, Taras, 9°52.944'N, 83°53.835'W, 1598 m, 30-IX-09, 4 spns., (S-181); on *Cyphomandra betacea*: Al, Zarcero, 10°11.058'N, 84°23.472'W, 1648 m, 14-V-09, 200 spns., mixed with *Aulacorthum solani*, (S-45); SJ, Pérez Zeledón, 9°29.914'N, 83°36.781'W, 1706 m, 20-IX-09, 44 spns., (S-157); on *Drymaria cordata*: SJ, Montes de Oca, 9°56.380'N, 84°03.003'W, 1214 m, 11-VII-09, 1 spn., (S-81); on *Jacaranda mimosifolia**: SJ, Montes de Oca, 9°56.380'N, 84°03.003'W, 1214 m, 20-IV-09, 38 spns., mixed with *Aphis spiraecola*, (S-72); on *Lycopersicon esculentum*: Pu, Buenos Aires, 9°90.669'N, 83°76.280'W, 350 m, 25-X-09, 65spns., (S-226); on *Piper* sp.: SJ, Montes de Oca, 9°56.380'N, 84°03.003'W, 1214 m, 11-VII-09, 312 spns., (S-79).

Aphis helianthi Monell on *Furcraea cabuya*. SJ, Montes de Oca, 9°49'423'N, 83°50.101'W, 1214 m. 5-I-09, 12 spns., (S-18); on *Yucca guatemalensis*: SJ, Montes de Oca, 9°49.423'N, 83°50.101'2, 1214 m, 26-IV-09, 31 spns., (S-40).

Aphis illinoisensis Shimer on *Vitis tiliifolia*. Pu, Coto Brus, 8°46.978'N, 82°58.294'W, 1311 m, 21-III-09, 4 spns., (S-20).

Aphis nerii Boyer de Fonscolombe on *Asclepias curassavica*. Pu, Monteverde, 10°19.150'N, 84°49.428'W, 1317 m, 10-V-09, 22 spns., (S-71); on *Gomphocarpus physocarpus**: SJ, Coronado, 9°58.556'N, 84°00.464'W, 1400 m, 15-IX-08, 14 spns., (S-7).

Aphis spiraecola Patch on *Bauhinia purpurea**. He, Cariblanco, 10°16.072'N, 84°10.858'W, 848 m, 26-II-09, 14 spns., mixed with *Aphis gossypii*, (S-21); on *Piper* sp.: SJ, Montes de Oca, 9°56.380'N, 84°03.003'W, 1214 m, 11-VII-09, 312 spns., (S-200); on *Schefflera* sp.: Al, San Ramón, 10°05.182'N, 84°28.673'W, 1098 m, 26-VII-09, 7 spns., (S-99).

Brachycaudus helichrysi (Kaltenbach) on *Gnaphalium* sp. Ca, Cerro Buena Vista, 9°44.482'N, 83°57.054'W, 2122 m, 20-V-09, 1 spn., (S-54).

Hysteroneura setariae (Thomas) on *Paspalum* sp.*. SJ, Montes de Oca, 9°56.345'N, 84°02.837'W, 2122 m, 29-IV-09, 3 spns., (S-43).

TABLE 1: Aphid-Aphidiinae associations in Costa Rica. Note that the record of *Binodoxys solitarius* from *Aulacorthum solani* is actually an undetermined species of *Binodoxys* (see text). Letters refer to provinces shown in map (Figure 1): Al = Alajuela, Ca = Cartago, Gu = Guanacaste, He = Heredia, Pu = Puntarenas, SJ = San José.

Aphid/Parasitoid	Aphidius colemani	Aphidius nr. colemani	Aphidius sp. A and B	Binodoxys solitarius	Diaritiella rapae	Ephedrus lacertosus	Lipolexis oregmae	Lysiphlebus testaceipes
<i>Aphis coreopsidis</i>								Al
<i>Aphis craccivora</i>	Ca							
<i>Aphis gossypii</i>	Ca SJ			SJ				Al Ca Pu He SJ
<i>Aphis helianthi</i>								SJ
<i>Aphis illinoisensis</i>							Pu	Pu
<i>Aphis nerii</i>	Ca Gu Pu SJ							Pu SJ
<i>Aphis spiraecola</i>								Al He SJ
<i>Aulacorthum solani</i>				SJ				
<i>Brachycaudus helichrysi</i>	Ca Pu SJ	Ca		Ca				Ca
<i>Brevicoryne brassicae</i>					Ca			
<i>Hysteroneura setariae</i>								SJ
<i>Illinoia morrisoni</i>			SJ					
<i>Lipaphis erysimi</i>				SJ				
<i>Lipaphis pseudobrassicae</i>					SJ			
<i>Macrosiphum salviae</i>		Ca						
<i>Microparsus pojanii</i>			Ca					
<i>Myzodinium modestum</i>		SJ						
<i>Mizus ornatus</i>	Ca SJ			SJ				
<i>Myzus persicae</i>	Al SJ			SJ	Ca			
<i>Pentalonia nigronervosa</i>	SJ							Gu SJ
<i>Rhopalosiphum maidis</i>	SJ							
<i>Toxoptera aurantii</i>				SJ				
<i>Toxoptera citricidus</i>	Pu						Al	Al, Pu SJ
<i>Uroleucon gravicorne</i>			SJ			SJ		

Pentalonia nigronervosa Coquerel on *Costus pulverulentus*. SJ, Montes de Oca, 9°49.423'N, 83°560.101'W, 1214 m, 22-V-09, 143 spns., (S-57); on *Xanthosoma mexicanum*: Gu, Tilarán, 10°28.413'N, 84°57.961'W, 561 m, 17-IX-09, 2 spns., mixed with *Aphis nasturtii*, (S-145).

Toxoptera citricida (Kirkaldy) on *Citrus aurantium**. Al, San Ramón, 916 m, 10°04.676'N, 84°32.447'W, 10-IV-09, 59 spns., (S-33); Al, San Ramón, 10°14.676'N, 84°32.447'W, 916 m, 24-V-09, 6 spns., (S-58); on *Citrus sinensis**: SJ, Montes de Oca, 9°49.423'N, 83°50.101'W, 1200 m, 17-IX-08, 17 spns., (S-9); Pu, Tárcoles, 9°55.247'N, 84°03.407'W, 60 m, 21-V-09, 1 spn., (S-56); Al, San Ramón, 10°4.493'N, 84°323.600'W, 916 m, 25-V-09, 59 spns., (S-59); on *Zanthoxylum* sp.: Al, San Ramón, 10°04.676'N, 84°32.447'W, 885 m, 13-VI-09, 47 spns., (S-68).

4. Discussion

The results of the present study allow us to examine the geographic affinities of the aphid parasitoids found in Costa Rica in order to determine whether they show a pattern similar to that of their aphid hosts, that is, whether a majority of aphidiines are exotic species. The results also

allow a comparison to be made between the host records of aphidiines in Costa Rica (Table 1) and those reported from elsewhere.

Aphidius colemani occurs in warmer regions around the world and is common in South America, but presumably originated in the Oriental region [17]. Based on host records from Venezuela [10], Chile [13], and Brazil [11], it is probable that this species attacks a greater range of aphids in Costa Rica than that reported here. Biocontrol companies are currently selling exotic populations of *A. colemani* in Costa Rica, but the species has probably been in the country for some time.

Binodoxys solitarius was previously known only from Mexico [7], and its occurrence in Costa Rica suggests that this species is native to Mexico and Central America, although this suggestion requires further investigation. The only previous host record (in Mexico) was from *Aphis solitaria* (Baker) but our results document a wider host range.

Diaeretiella rapae is Palearctic in origin but is now cosmopolitan, and is almost exclusive to aphids on crucifers, but it has been reared from aphids on other plant families [10]. *Ephedrus lacertosus* is known from the Palearctic region [18, 19] Thailand [20] and the Nearctic region [21]. The present record from Costa Rica (just one locality) represents

a considerable range extension for this species. It has probably been introduced in the country, but this requires confirmation.

Lipolexis oregmae is originally from the Oriental region and was introduced into the Americas, although it is unclear exactly when, where, and how it was first introduced. Specimens from Guam were released in Florida beginning in 2000 in order to control *Toxoptera citricidus*, an Asian aphid and vector of citrus tristeza virus that invaded the Caribbean basin during the 1990's [22]. However, the third author has specimens of this species that were collected in Florida in 1986 (from Sugarloaf Mountain near Clemont, by S. J. Peck). There were plans to release *L. oregmae* in Jamaica and Dominica, but before this could happen it arrived fortuitously in both countries [23, 24]. Its introduction into Costa Rica was also fortuitous, but it is not known exactly when it arrived.

Lysiphlebus testaceipes is probably originally a North American species but now occurs throughout Central America and most of South America. It has been reared from a wide diversity of aphid species both in Costa Rica (this study) and elsewhere, for example in the Pacific Northwest of the United States [21], Mexico [8], Brazil [11], and Chile [13].

The number of specimens reared of each aphidiine species is as follows: 1105 *Aphidius colemani*, 46 *Aphidius* sp. near *colemani*, 26 *Aphidius* sp. A, 4 *Aphidius* sp. B., 11 *Binodoxys solitarius*, 1 *Bionodoxys* sp., 81 *Diaeretiella rapae*, 3 *Ephedrus lacertosus*, 135 *Lipolexis oregmae*, and 1420 *Lysiphlebus testaceipes*. Nearly 90% of all the specimens reared in this study belong to just two species, *Lysiphlebus testaceipes* (50%) and *Aphidius colemani* (39%). Both of these species have probably entered the country as a result of human activity, as have the next two most common species, *Lipolexis oregmae* (5%) and *Diaeretiella rapae* (3%). It is therefore possible that four exotic species account for 97% of all the specimens.

The question remains whether there are any native aphid parasitoids in Costa Rica. It is possible that *Binodoxys solitarius* is a native species, although most of its host aphids are exotic species. Although the vast majority of aphid species in Costa Rica are exotics, there are a few native aphids, and one might expect there to be a few native parasitoids associated with these aphids. For example, *Microparsus pojanii* is a native aphid species from which we reared *Aphidius* sp. A, but further taxonomic study is required before conclusions can be made regarding the geographic affinities of the four undetermined species found in this study. Other native aphids include *Idiopterus nephrolepidis*, *Impatientinum americanum*, and undescribed species from high altitudes. More collecting, especially at high altitudes, and further taxonomic research are needed before the question of native Aphidiinae can be answered.

Acknowledgments

The authors thank the University of Costa Rica, the Agencia de Cooperación Española, and Idea Wild for financial support. Their cordial thanks are expressed to N. Pérez

Hidalgo (University of Leon, Spain) for identification of the aphids. Majorie A. Hoy (University of Florida, USA) kindly supplied information on biocontrol of the brown citrus aphids in Florida and some other areas. The contribution by P. Starý was partially supported from the Entomology Institute Project AV0Z50070508 (Academy of Sciences of the Czech Republic).

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Research Article

Taxonomic Review of the *Caudatella heterocaudata* (McDunnough) and *C. hystrix* (Traver) Complexes (Insecta: Ephemeroptera: Ephemerellidae)

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Received 20 August 2010; Accepted 27 October 2010

Academic Editor: Ai-Ping Liang

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Caudatella columbiella (McDunnough, 1935), new combination, (Insecta: Ephemeroptera: Ephemerellidae) is removed from synonymy with *Caudatella heterocaudata* (McDunnough, 1929), and a new junior synonym is recognized, based on comparative examination of type material and larval exuviae associated with adults from the type locale of *C. columbiella* (= *C. californica* (Allen and Edmunds, 1961), new status, new synonym). *Caudatella circia* (Allen and Edmunds, 1961), new status, is recognized as a strict specific synonym of *C. heterocaudata* (McDunnough, 1929) (= *C. circia* (Allen and Edmunds, 1961), new synonym). A neotype is designated for *Caudatella hystrix* (Traver, 1934), based on a specimen collected in Western Montana, USA, during June 2000. Morphological differences between the type specimen of *C. hystrix* and the type specimens of its two junior synonyms, *Ephemerella cascadia* Allen and Edmunds, 1961, and *E. spinosa* Mayo, 1952, are detailed. An identification key for larvae of the genus *Caudatella* is included.

1. Introduction

The genus *Caudatella* Edmunds (Insecta: Ephemeroptera: Ephemerellidae) is restricted to Western North America, where larvae live in mountain streams, often associated with aquatic moss [1]. Larval and winged stages of *Caudatella* are distinguished from other Ephemerellidae genera by the median caudal filament being wider at the base and longer than the cerci. *Caudatella* was described initially as a subgenus of *Ephemerella* Walsh [2], but it was subsequently elevated to genus [3]. All nominal *Caudatella* species and subspecies were described originally as *Ephemerella*.

Caudatella contains ten nominal species and subspecies, with six of them recognized as valid in the most recent revisionary synopsis of the genus [4]: *C. edmundsi* (Allen, 1959); *C. heterocaudata heterocaudata* (McDunnough, 1929) [5] (= *Ephemerella columbiella* McDunnough, 1935 [6]); *C. heterocaudata californica* (Allen and Edmunds, 1961) [7]; *C. heterocaudata circia* (Allen and Edmunds, 1961) [7]; *C. hystrix* (Traver, 1934) [8] (= *Ephemerella spinosa* Mayo, 1952; = *Ephemerella cascadia* Allen and Edmunds, 1961 [7]);

C. jacobi (McDunnough, 1939) (= *Ephemerella orestes* (Allen and Edmunds, 1961) [7]).

Aspects of *Caudatella* species taxonomy have remained questionable despite recent revisionary work [4], especially subspecies designations and species that are polytypic. The subspecies classifications, in particular, are questionable because they were established at a time when mayfly taxonomists tended to define any distinct morphological variant as a nominal subspecies; currently, subspecies designations usually are reserved for geographically isolated variants. This paper addresses issues associated with the *C. heterocaudata* and *C. hystrix* species complexes.

2. Materials and Methods

Specimens were examined with dissecting microscopes and are deposited at the following institutional collections: California Academy of Sciences, San Francisco California, United States of America (CAS); Canadian National Collection of Insects, Ottawa, Ontario, Canada (CNC); Cornell University Insect Collection, Ithaca, New York, United States of

America (CUIC); Purdue University Entomological Research Collection, West Lafayette, Indiana, United States of America (PERC).

2.1. Primary Type Material Examined

2.1.1. *Ephemerella californica*. HOLOTYPE: United States of America, California, Madera County, Sierra National Forest, Chilacoot Creek, 1 mile above Bass Lake, 19-VI-1959, R. K. Allen, larva (PERC).

2.1.2. *Ephemerella cascadia*. HOLOTYPE: United States of America, Oregon, Clackamas County, Branch of Still Creek on road to Timberline Lodge, Mt. Hood, 30-VIII-1958, G. F. Edmunds, Jr., & R. K. Allen, larva (PERC).

2.1.3. *Ephemerella circia*. HOLOTYPE: United States of America, Oregon, Douglas County, Umpqua River, Sawyer Rapids, 26.5 mi E of Reedsport, 17-VI-1958, Mike Johnson, larva (PERC).

2.1.4. *Ephemerella columbiella*. HOLOTYPE: Canada, British Columbia, Peachland, Trepanier Creek, 4-VII-1934, A. N. Gartrell, male adult (reared from larva) (CNC).

2.1.5. *Ephemerella heterocaudata*. HOLOTYPE: United States of America, Wyoming, Yellowstone National Park, Upper Geyser Basin, 29-VII-1928, J. McDunnough, male adult (CNC).

2.1.6. *Ephemerella hystrix*. NEOTYPE (new designation), United States of America, Montana, Sweet Grass County, small tributary of Big Timber Creek, at canyon road crossing, 1 mile south of Big Moon Campground, 46°1'30"N, 110°8'48"W, 10-VI-2000, W. P. McCafferty et al., larva (CUIC).

2.1.7. *Ephemerella spinosa*. HOLOTYPE, United States of America, California, Inyo County, 4-VII-1938, larva (specimen number 8645) (CAS).

2.2. Other Material Examined

2.2.1. *Ephemerella californica*. United States of America, California, Los Angeles County, San Gabriel River at Camp Bonita (62°), 16-VI-1959, R. K. Allen, one larva, paratype (PERC).

2.2.2. *Ephemerella cascadia*. United States of America, Oregon, Clackamas County, Branch of Still Creek on road to Timberline Lodge, Mt. Hood, 30-VIII-1958, G. F. Edmunds, Jr., & R. K. Allen, two larvae (same collection data as holotype) (CNC); United States of America, Washington, Pierce County, Mt. Rainier National Park, spring-fed stream, Westside Road, 1.2 miles north of Highway 706, 16-VI-2004, emerged 21-VI, Kondratieff, Schmidt, three male adults, one female adult, associated larval exuviae (PERC).

2.2.3. *Ephemerella columbiella*. Canada, British Columbia, Peachland, Trepanier Creek (same collection locale as holotype), A. N. Gartrell, sub 15-VII-1934, adult 17-VII-1934, one set larval exuviae (CNC); same locale and collector, sub 6-VII-1934, adult 10-VII-1934, one set larval exuviae (CNC); same locale and collector, sub 5-VII-1934, adult 6-VII-1934, one set larval exuviae (CNC).

2.2.4. *Ephemerella heterocaudata*. United States of America, Wyoming, Yellowstone National Park, Firehole River, above Chimney Bridge, 22-VII-1928, J. McDunnough, nine larvae (CNC).

2.2.5. *Ephemerella hystrix*. Canada, British Columbia, Keremos, Shingle Creek Road, sub 26-VII-1935, adult 28-VII-1935, A. N. Gartrell (699-1192) (CNC); United States of America, Montana, Sweet Grass County, small tributary of Big Timber Creek, at canyon road crossing, 1 mile south of Big Moon Campground, 46°1'30"N, 110°8'48"W, 10-VI-2000, W. P. McCafferty, et al., (same collection data as neotype), five larvae (PERC).

3. Systematic Accounts

3.1. *Caudatella heterocaudata* Species Complex. This species complex is defined herein to contain two nominal species. Adults and larvae of these two related species are distinguished from congeners by abdominal sterna that have three longitudinal stripes and by cerci that are much shorter than the median filament (usually less than half as long).

Caudatella heterocaudata has been divided into three nominal subspecies: *C. h. heterocaudata*, *C. h. californica*, and *C. h. circia* [7]. Allen and Edmunds [7] listed *Ephemerella columbiella* as a junior synonym of *C. heterocaudata* based on its falling within their concept of variability for the latter species. Remarkably, they listed *E. columbiella* with the *C. h. heterocaudata* subspecies [7]. The former is removed from synonymy with the latter, below.

3.1.1. *Caudatella columbiella*: New Combination

Larval Diagnosis. In contrast to *C. heterocaudata*, *C. columbiella* has paired medial spines on abdominal tergum 1, the other paired medial spines sharp at the tips, and cerci that are approximately one-sixth the length of the median filament, or about one-half the length of the abdomen.

Adult Diagnosis. Male adults of *C. columbiella* (forewing length ca. 6 mm) are smaller than those of *C. heterocaudata* (forewing length at least 7 mm), and *C. columbiella* male adults usually have abdominal maculation that is not as highly contrasted from the base coloration [5, 6].

Remarks. *Caudatella columbiella* (newly revised concept) is known from Southern British Columbia and Central California [6, 7], which is within the geographic distribution of the more widespread *C. heterocaudata* [7]. Based on the consistent differences detailed above, *C. columbiella*

should be a valid species and not a synonym of *C. heterocaudata*.

Caudatella columbiella must have been considered synonymous with the *C. h. heterocaudata* subspecies [7] independent from the recognition of the other two nominal subspecies, because larval exuviae associated with adult specimens of *C. columbiella* from the type locale (listed as having been examined by Allen and Edmunds [7]) are indistinguishable from the holotype of *C. h. californica*. *Caudatella californica*, new status, therefore, should be recognized as a species separate from *C. heterocaudata* and as a subjective junior synonym of *C. columbiella* (= *C. californica*, new status, new synonym).

3.1.2. *Caudatella heterocaudata*

Larval Diagnosis. In contrast to *C. columbiella*, *C. heterocaudata* does not have paired medial spines on abdominal tergum 1, and the other paired medial spines are blunt at the tips. The cerci are longer than those of *C. columbiella*, being approximately one-third the length of the median filament, or about the same length as the abdomen.

Adult Diagnosis. Male adults of *C. heterocaudata* (forewing length at least 7 mm) are much larger than those of *C. columbiella* (forewing length ca. 6 mm), and *C. heterocaudata* male adults usually have more contrast between the dark maculation and base coloration of the abdomen [5, 6].

Remarks. Allen and Edmunds' [7, Figure 3] is actually a ventral view of the *C. heterocaudata* penes that was labeled incorrectly as a dorsal view; all of their other penes figures are correctly labeled as dorsal views.

Caudatella heterocaudata circia was described originally as a subspecies of *C. heterocaudata* that has prominent, characteristic setose, wartlike protuberances on the pro- and mesonota of the thorax; the adults are not known [7]. Its status as more than merely a morphological variant must be regarded as questionable, however, due to observed variation in the development of the characteristic dorsal protuberances on the thorax (the defining character of the subspecies) within the type locale population (Willamette River, Lane County, Oregon, United States of America) [7]. Furthermore, this nominal variant and typical *C. heterocaudata* "probably meet and intergrade" elsewhere near the type locale [7]. Based on current views of subspecies in Ephemeroptera and of morphological variation among species of Ephemerellidae, *C. h. circia* should be considered a strict species synonym of *C. heterocaudata* (= *C. circia*, new status, new synonym).

3.2. Caudatella hystrix Complex. This species complex consists of one polytypic species. Larvae are distinguished from congeners by having long, almost hook-like, paired medial spines on the middle abdominal terga. Adults are distinguished from congeners by having abdominal sterna with broad medial maculation that occupies most of the segment, usually taking the form of a chevron shape

[7], and male penes with the gonopores in a subparallel or convergent orientation and with dorsally or medially directed projections on the dorsal aspects of the penes.

The name *Ephemerella hystrix* was established based on a single larva collected from western Montana, United States of America [8] that had been described prior to its formal naming [9]. The concept of the species later was expanded to be polytypic, encompassing the nominal species *Ephemerella spinosa* [10] and *Ephemerella cascadia* [11], based on variation of the defining characteristics of the nominal species from throughout their geographic ranges.

In order to emphasize the polytypic nature of *C. hystrix* and to provide a base for future study of *Caudatella* diversity, the type specimens and some additional material of the three nominal species included in the polytypic concept of *C. hystrix* are discussed under the heading of their original generic combinations. In each case, the type specimen is a larva.

3.2.1. Ephemerella hystrix. The *E. hystrix* holotype was collected 29 June 1906 from Big Blackfoot River, Potomoc, Montana, United States of America [8]. The last direct report of the *E. hystrix* holotype was made in 1954 [10]. The specimen's deposition location was listed in 1961 [7], but no evidence indicates that it was examined directly by those authors at that time.

Unfortunately, the type specimen (no. 1287.1) of *E. hystrix* is missing from the Cornell University Insect Collection (E. R. Hoebeke, pers. comm.). Correspondence with personnel from insect collections at the California Academy of Science, Florida A & M University (Tallahassee, Florida, USA) and Purdue University also failed to locate the specimen. Almost all existing mayfly specimens examined by R. K. Allen, W. C. Day, and G. F. Edmunds, Jr., are housed in these three collections, and thus these were selected as the locations most likely to house the specimen in question, if it were still in existence.

In order to maintain the identity of *E. hystrix*, *sensu stricto*, and to provide a basis for future study of *Caudatella* species diversity, a neotype is designated for *E. hystrix* (see Primary Type Material Examined), which was selected from a series of specimens collected from western Montana in June 2000. The lost holotype of *E. hystrix* also was collected from western Montana in June [8]. The new specimens match the lost *E. hystrix* holotype descriptions [8, 9, 12] and Traver's [12, Figure 53.b]. The neotype designation herein satisfies the conditions and recommendations of Article 75 of the *International Code of Zoological Nomenclature* [13].

The neotype larva of *E. hystrix* does not have spicules on the dorsal surfaces of the large, paired medial spines of abdominal terga 5–7, and these medial spines have tips that curve slightly outwards. The abdominal terga have dark medial coloration, and the legs have numerous long, hairlike setae along the outer margins. Most of the abdominal sterna have distinct chevron-shaped maculae [7].

3.2.2. Junior Synonym: Ephemerella cascadia. The somewhat discolored *E. cascadia* holotype has the medial spines of the

abdominal terga with long spicules dorsally, as shown in Allen and Edmunds' [7, Figure 25], and these paired spines have a subparallel orientation of the apical portions. The legs have relatively few long, hairlike setae on the outer margins. The abdominal sterna have a solid dark brown color pattern [7].

Previous study indicated that the maculation of larval sterna can be variable [11], but sometimes the extent of maculation is dependent upon which instar is examined. The medial sternal maculation of recently reared adults appears to be consistent with the type concept of *E. cascadia* (solid color). Reexamination of this material from Washington (previously listed as *C. hystrix* [4]) revealed that the gonopores of the penes are not strongly oriented medially, as historically indicated for *C. hystrix* [7]. More specimens of both nominal variants should be studied to determine whether this is a consistent difference because the orientation of the penes lobes of preserved specimens can vary dramatically.

3.2.3. Junior Synonym: *Ephemerella spinosa*. *Ephemerella spinosa* has abdominal terga that lack medial coloration, and the paired medial spines on the abdominal terga have dorsal spicules. The medial spines on abdominal terga 5–7 are distinctly more divergent than the others and have tips that curve inwards. The legs have numerous long, hairlike setae on the outer margins. Most of the abdominal sterna have distinct chevron-shaped maculae. No adults have been associated with this name.

Ephemerella spinosa Mayo, 1952, should not be confused with *Ephemerella spinosa* Morgan, 1911, a nomen nudum [4], nor should it be confused with *Ephemerella spinosa* Ikononov, 1961, a preoccupied name, which correctly was renamed *E. ikononovi* Puthz, 1971 [14]. *Ephemerella ikononovi* is the type species of the genus *Quatica* Jacobus and McCafferty [4].

4. Updated Taxonomic Synopsis of the Genus *Caudatella*

Caudatella edmundsi (Allen, 1959) [15]

Caudatella columbiella (McDunnough, 1935) [6],
comb. n.

=*Ephemerella californica* Allen and Edmunds, 1961
[7], stat. n., syn. n.

Caudatella heterocaudata (McDunnough, 1929) [5]

=*Ephemerella circia* Allen and Edmunds, 1961 [7],
stat. n., syn. n.

Caudatella hystrix (Traver, 1934) [8]

=*Ephemerella spinosa* Mayo, 1952 [10]

=*Ephemerella cascadia* Allen and Edmunds, 1961
[7, 11]

Caudatella jacobi (McDunnough, 1939)

=*Ephemerella orestes* Allen and Edmunds, 1961
[7, 16].

5. Key to Larvae of the Genus *Caudatella* Edmunds

- (1) Maxillary palp vestigial; tarsal claw with two prominent rows of denticles—(*edmundsi*).
- (1') Maxillary palp with three distinct segments; tarsal claw with only one distinct row of denticles—(2).
- (2) Paired medial spines on abdominal terga long and curved (at least on middle segments), some almost hook like—(*hystrix*).
- (2') Paired medial spines on abdominal terga relatively straight, none longer than respective segment—(3).
- (3) Cerci approximately two thirds length of median filament; abdominal sterna with solid color, never with longitudinal stripes or other such markings—(*jacobi*).
- (3') Cerci less than one-half length of median filament; abdominal sterna almost always with three dark, longitudinal markings—(4).
- (4) Cerci approximately one-third length of median filament (about the length of the abdomen); distinct pair of medial spines present only on abdominal terga 2–9, with the spine tips blunt—(*heterocaudata*).
- (4') Cerci approximately one-sixth length of median filament (about half the length of the abdomen); distinct pair of medial spines present on abdominal terga 1–9, with the spine tips sharp—(*columbiella*).

Acknowledgments

Richard Hoebeke (Cornell University, Ithaca, New York, USA), Vincent Lee (California Academy of Science, San Francisco, California, USA), Patrick McCafferty (Purdue University, West Lafayette, Indiana, USA), and Janice Peters and Bart Richard (Florida A&M University, Tallahassee, Florida, USA) assisted in attempting to locate the *E. hystrix* holotype. Ai-Ping Liang (Institute of Zoology, Chinese Academy of Sciences, Beijing, China), Michel Sartori (Museum of Zoology, Lausanne, Switzerland), and an anonymous reviewer provided criticisms and suggestions that led to important improvements in this study. This study was funded in part by CanaColl Grant 178.

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Research Article

Correlations of Rainfall and Forest Type with Papilionid Assemblages in Assam in Northeast India

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Received 7 June 2010; Accepted 8 October 2010

Academic Editor: David Roubik

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No comprehensive community studies have been done on the butterflies of the tropical monsoon forests of the East Himalayan region. We described the Papilionidae at one site within the continuous moist deciduous forest belt of Northeast India and their variation with season and forest type. We surveyed 20 permanent line transects, varying with respect to canopy openness and observed levels of disturbance. A total sample effort of 131 days during the dry and wet seasons of a two-year study resulted in 18,373 individuals identified from 28 Papilionidae species. Constrained canonical correspondence ordination was used to examine the effects of season, forest type, rainfall, year, altitude, and geographical position on the species assemblages. Results showed that rainfall, forest type, and season accounted for most variance in papilionid abundance. Rainfall was strongly correlated with the abundance of some species. Nine species were associated with gaps, 16 species were restricted to closed forest, and three species were encountered in both gaps and closed forest. Six species with narrow geographic range were found only in closed forest. The results confirm the strong seasonality of continental Southeast Asian butterfly assemblages.

1. Introduction

Studies focusing on insect seasonality have been carried out in many parts of the world including the equatorial tropics. Amongst the insects, butterflies form a suitable model group for ecological studies specifically pertaining to seasonality because rainfall and plant phenology are two interrelated factors that influence the life histories of these phytophagous insects [1–3]. The habitat preferences are also closely related to life strategies of different species just as much as the geographical range is related to each species' ecological strategy [4–6].

Butterfly populations are closely controlled by weather and many species are constrained by climate [7–10], reproduce quickly, have high dispersal ability and an annual or more frequent life cycle [11, 12]. Seasonal environments (tropical and temperate regions) and climate gradients like temperature and precipitation play an important role in defining the differences in habitat preferences, biology, adult

activity, reproductive strategy, and adaptive polyphenisms of butterflies [13–15].

The IUCN had identified the “Indo-Burma” hotspot as a “Papilionidae-rich zone” and had also drawn up the “Swallowtail Conservation Action Plan” [16]. India occupies the 6th rank in the list of priority countries for “Swallowtail Conservation”. From the Indian subcontinent, 84 species had been documented, of which six are endemic [17]. Evans [18] and Talbot [19] described 90 species from the Indian subcontinent; 15 species reported from Ceylon, 19 in South India, 6 in Baluchistan, 11 in Chitral, 50 in southern Burma, 13 in the Andaman and Nicobar islands, 31 in the Western Himalayas, and 69 in North-east India, which is a part of the Eastern Himalayas biodiversity hotspot. In Assam, of the Eastern Himalayas, five species of Papilionidae were listed as endemic [18]. Evans [18] also reported six species as endemic in the entire Sikkim-Assam region. In recent times, records on the papilionids from the Indian Himalayan region were documented by Haribal [20] and Kunte et al. [21]. As few

studies have focused on butterflies of the Northeast Indian region, and in keeping with the conservation value of this ecoregion, with respect to the Papilionidae, we therefore proposed to investigate the papilionid assemblage within a protected forest reserve in Assam.

The primary vegetation in Northeast India has been disturbed by human activities. The forest ecouality has also been deteriorating with the dense forests (canopy closure >60%) becoming degraded into open-forests or scrub. In Assam the percentage of “Very Dense” forest cover is only 2.1% (1,684 km²), “Moderately Dense” forest cover is 14.5% (11,358 km²) “Degraded/Open forests” is 18.9% (14,784 km²) and “Nonforest” area is 64.5% of the total area of forest and tree cover [22]. Under the directives of the National Forest Policy (1988) [23] and the Joint Forest Management (JFM) resolution (1990) [16] involving the local peoples’ participation, regeneration and maintenance of degraded forests as well as the protection and conservation of forest resources has assumed priority and significantly contributed towards the management strategies for sustainable forestry in India, including Assam.

This paper aims at documenting the effects of rainfall and habitat on a forest papilionid community in Assam, while also evaluating its conservation status. Thus, we focused on two main ecouestions: (1) is there any difference in the composition and distribution of species assemblages between open-forest or gaps and closed-forest? and (2) is the species abundance and distribution trend within the study area correlated with environmental variables such as season, forest type, year, rainfall, altitude and geographical position (latitude and longitude)?

We hypothesized that (1) papilionid assemblages have a higher species richness in closed-forest as compared to gaps, although in an Amazonian forest fragment, Ramos (2000) found that forest edges and areas of intermediate to high disturbances presented higher species richness and diversity of nymphalid butterfly communities; (2) closed-forest supports more species with restricted ranges, as, for example, a high proportion of Papilionidae are primarily forest-dwelling species, but some species are also known to be associated with open habitats [17]; (3) Season, rainfall and forest type may be important determinants for the distribution and abundance of the papilionid assemblage in the study area.

2. Methods

2.1. Study Area. The study area is a 23,231 ha protected forest reserve, “Rani-Garbhangha Reserve Forest” located on the south bank of the river Brahmaputra in Assam (26°55′ to 26° 0.5′ N and 91°35′ E to 91°49′ E [24], for management purposes, the reserve is divided into two forest ranges—the Garbhanga range (18,861 ha) and the Rani range (4,370 ha). The difference in management is due to the variation in the vegetation structure, regeneration of Sal (*Shorea robusta*) and Bamboo (*Dendrocalamus hamiltonii*), replacement of Teak (*Tectona grandis*) plantations by indigenous species and involvement of local communities in the sustainable management of land-use methods for converting degraded

areas into productive ones. Five villages are in the immediate transition zone of this reserve. These fringe villages do have a strong effect on the protected reserve in the form of disturbances like small wood collection, stone ecouarrying, occasional illegal logging, grazing and man-animal conflict. A wetland with a core area of 4.14 km² (Deepor Beel) lies at the northern boundary of the reserve and is the only Ramsar site in Assam and a representative wetland type found within the biogeographic province “Burma Monsoon Forest” [25]. The forest reserve is also contiguous with the Jarasal-Kwasing Reserve, Nakhalliyang Wildlife Sanctuary and Jirang Unclassed State Forest of the neighbouring state of Meghalaya. It serves as a natural elephant corridor [24] by linking about 70 km of the reserve from the hills of the Meghalaya plateau into the Deepor Beel in the northern boundary of the reserve (Figure 1).

The forest type in this reserve corresponds to Champion and Seth’s [26] “Assam valley tropical mixed moist deciduous forest”. This reserve was the original habitat of the “Sal”, *Shorea robusta* with *Shorea assamica* and *Schima wallichii* as the principal associate species. However, following excessive logging due to its high commercial viability as a timber yielding species, much of the Sal vegetation was gradually replaced by “moist deciduous secondary bamboo brakes”. *Shorea robusta*, *S. assamica*, *Dipterocarpus macrocarpus*, *Schima wallichii*, *Lannea coromandelica*, *Gmelina arborea*, *Tetrameles nudiflora*, *Lagerstoemia parviflora*, *Bridelia retusa*, *Albizia lebeck* and *Ficus hispida* were observed to be some of the dominant species forming the upper canopy, while the middle storey was composed of *Holarrhena antidysenterica*, *Tricalysia singularis*, *Oroxylum indicum*, *Salix tertrasperma*, *Malletus albus*, *Careya arborea*, *Semicarpus anacardium*. *Dendrocalamus hamiltonii* (locally known as Kako Bamboo) was the dominant bamboo species within the forest reserve [27].

We used satellite imagery to select five study sites, covering a total area of 50 ha within the forest reserve. All these sites were partially to heavily disturbed, although the disturbance levels were not ecouantified but evaluated on a visual scale (Table 1). Three of the study sites were located in the Garbhanga range and two sites in the Rani range.

2.2. Sampling Design. Based on the visual levels of disturbance and degree of canopy closure, we demarcated two zones within each study site for butterfly sampling—scattered or open-forest (SCF) and Closed forest (CF). We followed the line transect method [12, 28] where four transects were selected in each study site—transects T1 and T2 in SCF and transects T3 and T4 in CF. Thus we had 12 fixed transects in three study sites of the Garbhanga range (SCF = 6, CF = 6) and eight transects in two study sites of the Rani range (SCF = 4, CF = 4). Each transect was one kilometer in length and five meters wide and took two observers approximately 30 minutes to walk and record butterflies on both sides of the transect. Species were confirmed by both observers before recording to avoid observer bias [29]. Each transect was sampled usually twice a day, in the morning between 07.00 to 13.00 h and in the afternoon 14.00 to 17.00 h. The sampling time during

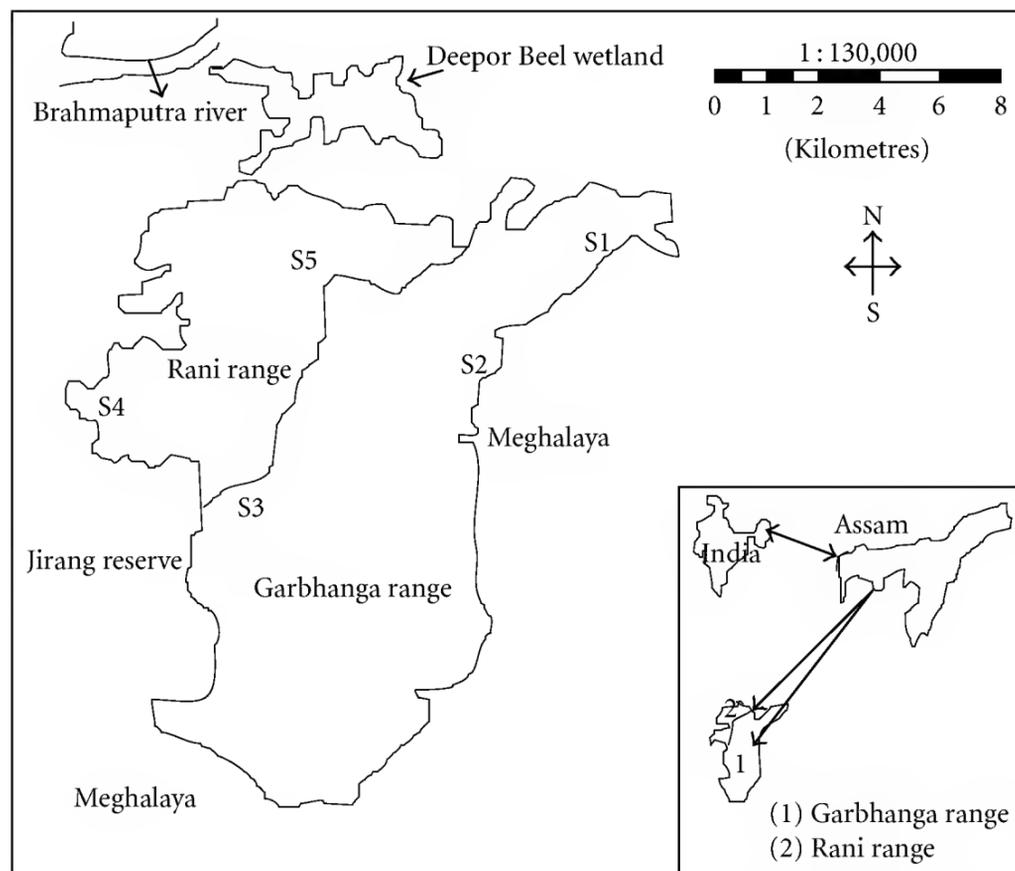


FIGURE 1: Map of Rani-Garbhangha Reserve forest in Assam, Northeast India.

TABLE 1: Characteristics of the five study sites selected for butterfly sampling.

Study sites	Geographical position	Area in hectares	Habitat type and landscape element	Altitude (m) above MSL	Level and type of human disturbance
S1	26°05'26.71"– 91°46'39.01"	15	Mixed moist deciduous secondary Sal forest with good cover of grasses	102	Partially disturbed human settlement, earth cutting, small wood collection
S2	26°03'46.49'– 91°43'41.56"	5	Mixed-moist deciduous secondary forest with intermittent tracts of Bamboo brakes. Closed canopy with trees > 20 m in height. Abundance of climbers	130	Partially disturbed stone ecouarrying, earth cutting, selective logging
S3	26°01'39.18"– 91°39'03.91"	5	Secondary euphorbiaceous scrub with grasses growing up to 10–15 cm on the rocky slopes and hills in the areas near to the abandoned patches of shifting cultivation	170	Heavily disturbed (shifting cultivation, illegal logging)
S4	26°01'52.20"– 91°35'51.32"	10	Degraded secondary deciduous forest edge with teak plantation, Sal regeneration, cropland, household plantation, shrubs and grasses, and scrubland	100	Heavily disturbed (teak plantation, selective logging, grazing, road construction, human settlements)
S5	26°04'49.77"– 91°40'03.07"	15	Secondary mixed deciduous forest near human settlement	60	Heavily disturbed (forest village, selective logging, intensive grazing)

the afternoon counts was reduced due to less observed activities of the butterflies. The two-year sampling period was divided into two seasons—Dry season (January–March) and Wet season (August–October) (Figure 2). Our sampling effort was 35 days during the dry season and 42 days during the wet season for study sites S1, S2, and S3 in the Garbhanga range. Studies at S4 and S5 in the Rani range were 27 days each during the dry and wet seasons. The sampling days were different for the study sites of the Garbhanga and Rani ranges. This amounted to nine hours of sampling per day for

the three study sites in the Garbhanga range and six hours per day in the two study sites of the Rani range. All sampling activities were carried out on days with sunny weather and a mean daily maximum temperature of 30°C during the wet season and 22°C during the dry season.

2.3. Collection, Identification, and Geographic Range Classification. The papilionid individuals were collected using butterfly nets whenever identification on the wing was not possible. We used a camera for recording and later counting

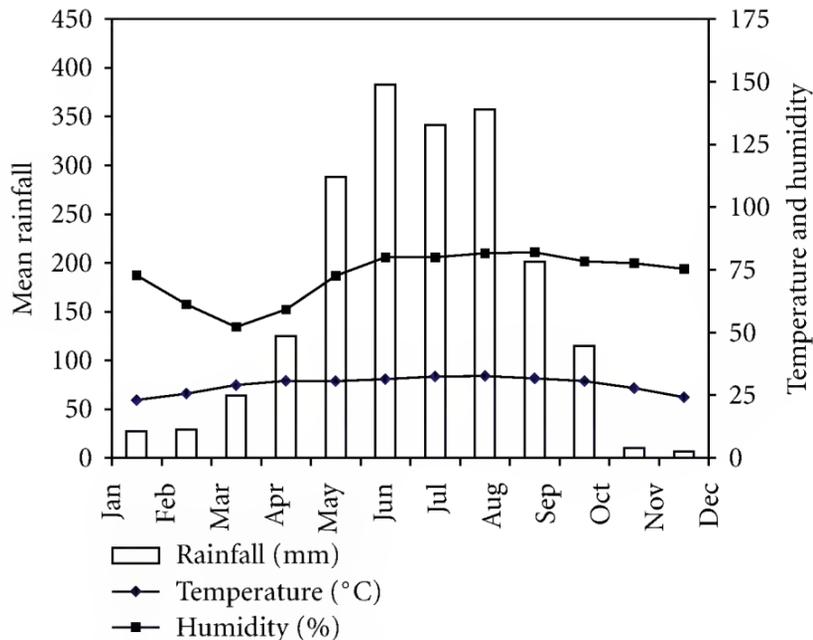


FIGURE 2: Ombrothermic diagram (Precipitation scale = $2 \times$ Temperature scale) for the study area (mean of years 2001–2005). The relative humidity is also plotted on the same axis as temperature.

and identifying individuals detected on transects along mud-puddling spots. As within the East-Himalayan Papilionidae, there are polymorphic species [30]; therefore the individual counts for such species were restricted to the species level only. Similarly for the East-Himalayan endemic subspecies, we restricted our sample count to species level only. For species with polymorphic forms as well as for those species that exhibited mimicry, wherever identification on the wing was not confirmed, we excluded such counts in order to avoid sampling bias [29]. We also handled the case of sexual dimorphism and distinct seasonal forms amongst some species in a similar manner.

Identification followed Evans [18], Talbot [19] and Haribal [30] while nomenclature followed Evans [18] and D’Abrera [31]. The geographic distribution ranges had been categorized on a scale of 1–6 (smallest to largest) as used by Spitzer et al. [32]: (1) Eastern Himalayas, Yunnan and Northern Indo-China; (2) North India and all Indo-China; (3) Oriental (Indo-Malayan) region; (4) Indo-Australian region (Australasian tropics); (5) Palaeotropics; (6) Larger than Palaeotropics—Cosmopolitan.

Within the Indian Himalayan Region (IHR), the Indian extent of the Eastern Himalayas includes all of Northeast India. Therefore in our study, the lowest ranked species with score 1 was endemic to the Eastern Himalayas, excluding Yunnan and Northern Indo-China and the highest ranked species with a score 4 was the most widely distributed species. No species with geographic range scores 5 and 6 were recorded in our study area.

2.4. Data Analysis. Canonical Correspondence analysis (CCA) was used for the ordination of the sites, composition and distribution of the species assemblages within the study area. The CCA ordination was run using the axis scores centred and standardized to compartment variance and compartments were plotted on diagrams

using linear combination scores in the program R 2.3.1 (<http://cran.r-project.org>) [33]. Analyses were done separately for the study sites of the Garbhanga and Rani ranges—butterfly abundance data from the 12 fixed transects in the Garbhanga range and similarly abundance data from eight fixed transects in the Rani range were pooled by season, year and forest type. This amounted to 16 sites scores during the multivariate ordination where each site score in the ordination plots indicated the changing butterfly abundances in the transects by season and year. Site 1 in the ordination plots represented the abundance data from transect T1 in scattered/open-forest sampled during the dry season of year 1 and site 5 represented the abundance data from the same transect T1 sampled during the wet season of year 1. Similarly, site 9 represented the abundance data from the same transect T1 sampled during the dry season of year 2 and site 13 represented the abundance data from transect T1 sampled during the wet season of year 2 (Appendix 1, see Supplementary Materials available at doi:10.1155/2010/560396).

In order to evaluate environmental effects on mean butterfly abundance, we selected five independent variables within the environment matrix—altitude, geographical position (latitude and longitude), year and rainfall, while season (that subsumes mean maximum temperature, rainfall and humidity) and forest type were used as categorical variables to explain the community structure of the Papilionidae. The first two axes of CCA matrix were plotted as standard plots to compare changes as a result of the environmental variables. The significance of species-environment relationships were tested by running the permutation tests using the “ANOVA” function in the “Vegan” package of the program “R” (Appendices 2 and 3).

Variation in abundance of papilionid individuals between seasons and year were computed by One-Way ANOVA, using the program STATISTICA 7.1 [34].

3. Results

3.1. General Aspects. A total of 18,373 individuals representing 28 species (eight genera) were recorded during the study period (Table 2 and Appendix 4). The overall abundance of papilionid individuals recorded in the study sites of the Garbhanga range was higher than in the study sites of the Rani range throughout the study period (Figure 3). Two species, *Atrophaneura sycorax* and *Papilio paris*, were not recorded in the Rani range. All species present in the Rani range were also recorded in Garbhanga.

The differences in mean annual transect abundance of butterfly individuals between the dry and wet seasons of the two-year study period were not significant for the study sites of the Garbhanga range (one-way ANOVA, $F_{1,14} = 3.9452$, $p = 0.0669$), but significant for the study sites of the Rani range (One-way ANOVA, $F_{1,14} = 38.626$, $p = 0.00002$) (Figure 3). Differences in total abundance between the genera were also observed—*Graphium* and *Papilio* had highest mean abundances (>1000 individuals) while *Chilasa* and *Lamproptera* had the lowest mean abundance (<100 individuals) (Table 2). Four endemic species from the genera

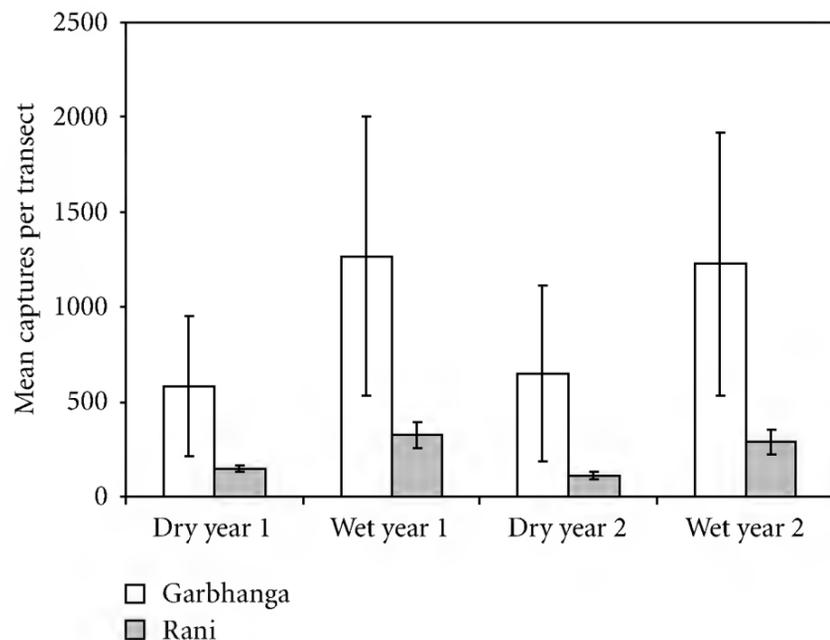


FIGURE 3: Differences in seasonal abundance of papilionids sampled as individuals during the dry and wet seasons of the two years study period from the 20 fixed transects of study sites S1, S2, S3 in Garbhanga (open bars) and S4 and S5 in Rani (dotted bars).

Atrophaneura, *Papilio* and *Pathysa* with geographic range score 1 were recorded in our study. Six species from three genera (*Graphium*, *Papilio* and *Pathysa*) had the widest distribution (geographic range 4) while the highest number of species (16) from seven genera was represented by the geographic range score 3 (Appendix 4).

3.2. Ordination of Species Assemblages. The ordination of the species assemblages in the five study sites as shown in the CCA-biplots (Figures 4(a) and 4(b)) clearly indicated the separating effect of forest type as a variable on the papilionid community. *Graphium* (3 spp.), *Pathysa* (4 spp.), *Pachliopta* (1 sp.), and *Papilio* (1 sp.) were characteristic of the open/scattered forests while *Atrophaneura* (5 spp.), *Troides* (2 spp.), *Pachliopta* (1 sp.), *Papilio* (7 spp.), and *Chilasa* (1 sp.) were confined to the closed-forests. Three species (*Papilio polytes*, *Lamproptera meges*, *L. curius*) could be classified as “Intermediate” as they were encountered in both gaps and closed-forests. Six species from the closed-forest habitats and two gap species had restricted geographic range score (1 and 2) (Appendix 4).

The site scores in the ordination plots (numbers in black) overlaid on the ordination matrix showed the succession of butterfly abundance with respect to the effects of season and year.

Endemic species from the closed-forest like *Atrophaneura dasarada*, *Atrophaneura varuna*, *Troides aeacus*, *Papilio castor* and *Papilio krishna* with restricted geographic ranges 1-2 were deviated from the center and could be classified as “locally rare” while open-forest species with wider geographic ranges 3-4 like *Papilio demoleus*, *Pathysa antipathies*, *Pathysa macareus*, *Papilio memnon* were relatively close to the origin and could be classified as “locally common” (Figure 4(a)). Similarly endemic species from the open-forest like *Pathysa aristeus* and *Pathysa xenocles* were closer to the origin and could be classified as “locally common”. Although

Graphium species recorded the highest total abundance (Table 2), in the constrained ordination matrix, this group assemblage is seen to show a large deviation from the center (Figure 4(a)), which could be implicated either to the fact that they were almost exclusively encountered in the open-forest transects or to the significant effect of rainfall on their abundances during the wet season. The ordination of the species assemblages in the study sites of the Rani range showed that fourteen species (belonging to five genera) were confined to the closed-forests while nine species belonging to four genera were associated with the open-forests or gaps. Three species belonging to two genera could be classified as “Intermediate” as they were encountered in both gaps and closed-forest transects (Figure 4(b)). Forest-dependant species like *Troides aeacus*, *T. helena*, *Papilio polycter*, *P. nephelus*, *Atrophaneura aidoneus*, *A. polyceuctes* and gap species like *Graphium agammemnon*, *G. doson*, *Pathysa antipathies*, *P. aristeus* and *P. macareus* could be classified as “locally common” species as their positions in the ordination matrix were relatively closer to the origin. *Papilio helenus*, *P. castor* and *Atrophaneura varuna* characteristic of the closed-forest and *Graphium sarpedon*, *Pathysa xenocles*, *Pachliopta aristolochiae* and *Papilio polytes* from the open-forests were fairly abundant. There were exceptions where *Atrophaneura varuna* which is strongly deviated from the centre could be classified as “locally rare” while in case of *Papilio demoleus*, as this species was mostly encountered in the gaps while the counts from the closed-forest transects were almost negligible, therefore its position in the ordination matrix could be deviated from the centre.

3.3. Variables and Species Abundance in the Study Sites of the Garbhanga Range. The variable “year” was linked with papilionid abundance (Figure 4(a)). Closed forest restricted species like *Atrophaneura* spp., *Troides aeacus*, *Papilio paris* and gap species like *Graphium* spp. and *Pathysa xenocles* recorded higher abundances during the wet seasons of the 2-year study period. The second variable “rainfall” was strongly correlated with abundances of closed-forest species like *Atrophaneura dasarada*, *A. polyceuctes*, *Papilio paris* and gap species like *Graphium doson* and *G. agammemnon*. Gap species like *Papilio demoleus*, *Graphium sarpedon*, *Pathysa* sp. and those from intermediate areas like *Papilio polytes* and *Pachliopta aristolochiae* showed declining abundances with decreasing rainfall. Altitude as a variable influenced the abundances of the papilionids; closed-forest species recorded greater abundance at higher elevations while gap species recorded higher abundances at lower elevations. The effect of geographical position (latitude and longitude) on papilionid abundance could not be meaningfully explained probably due to the restricted geographical size of the study area.

3.4. Variables and Species Abundance in the Study Sites of the Rani Range. Closed forest species like *Papilio helenus*, *P. castor*, *Atrophaneura varuna* and gap species like *Graphium agammemnon*, *Papilio demoleus*, *Pachliopta aristolochiae* and *Lamproptera curius* recorded higher abundances during the wet season of the second year of study (Figure 4(b)).

TABLE 2: Total number of individuals of Papilionid (per genus) sampled during the dry and wet seasons of 2003-2004 within the study area.

Genus	DS 1	WS 1	DS 2	WS 2	Total abundance
<i>Graphium</i>	1212	2771	1886	2825	8694
<i>Papilio</i>	1092	1784	921	1653	5450
<i>Pathysa</i>	271	456	194	376	1297
<i>Chilasa</i>	59	106	46	129	340
<i>Atrophaneura</i>	90	491	87	627	1295
<i>Troides</i>	96	273	100	287	756
<i>Pachliopta</i>	65	186	39	143	433
<i>Lamproptera</i>	26	39	19	24	108

Notes: DS 1: dry season year 1, WS 1: wet season year 1, DS 2: dry season year 2, WS 2: wet season year 2.

Rainfall strongly affected the abundances of *Papilio demoleus*, *Graphium agammemnon*, *Pachliopta aristolochiae*, *Papilio castor*, *P. helenus*, *Lamproptera curius* and *Atrophaneura varuna*. The effect of altitude on papilionid species abundance was found to be significant as the gap species recorded higher abundances at lower elevations and the closed-forest species were found to occur in greater abundance at higher elevations. Geographical position did not have a significant influence on the species abundance.

4. Discussion

4.1. Species Composition and Association with Forest Type. As we hypothesised, the species composition of our papilionid community was different between the closed and open-forest habitats, with a higher number of restricted range species found in the closed-forest [5, 32, 35, 36].

The choice of forest types by the papilionid assemblage in our study area could have been influenced by several biological factors for the adults—availability of suitable oviposition sites by the gravid females, floral phenology, predators and mimics [35, 37]. Ecological factors like suitable mud-puddling sites and more sheltered conditions as well as structural variables like size of the area, topography, temperature, humidity, light, gaps and ground pattern could have also accounted for the preference shown by the Papilionidae for different habitats or forest types [5, 32, 35, 38–42].

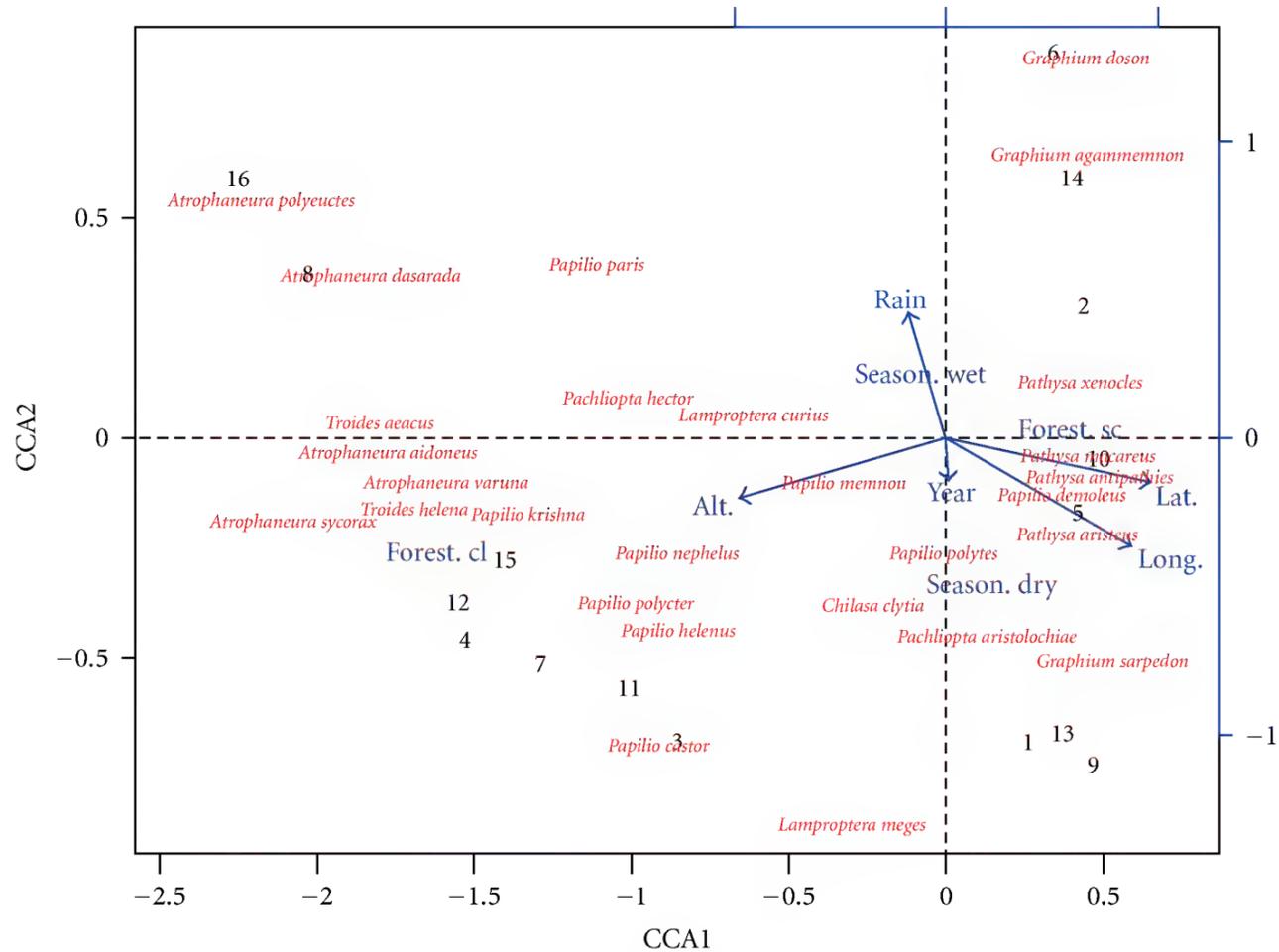
In our present study, the *Atrophaneura*, *Troides* and the *Papilio* spp. were found to prefer the closed-forest habitats. *Troides aeacus* and *Atrophaneura* spp. are known to occur [41] in forest gaps and along riparian corridors in tropical forests. In our study area, *T. aeacus* and *A. dasarada* recorded higher abundances in closed-forests but sightings were also made in gaps. This could be attributed to the fact that the increased canopy openness and light penetration caused by disturbance increases the abundance of herbaceous growth and vines and favours species freecounting tree fall gaps and streams for mud-puddling and sun basking [43]. Similarly, the habitat preferences of the seven *Papilio* spp. could have been affected by structural variables like temperature, light and humidity [44, 45].

The geographically wide-ranging *Papilio* spp. are also migratory species flying over forested areas, thereby giving

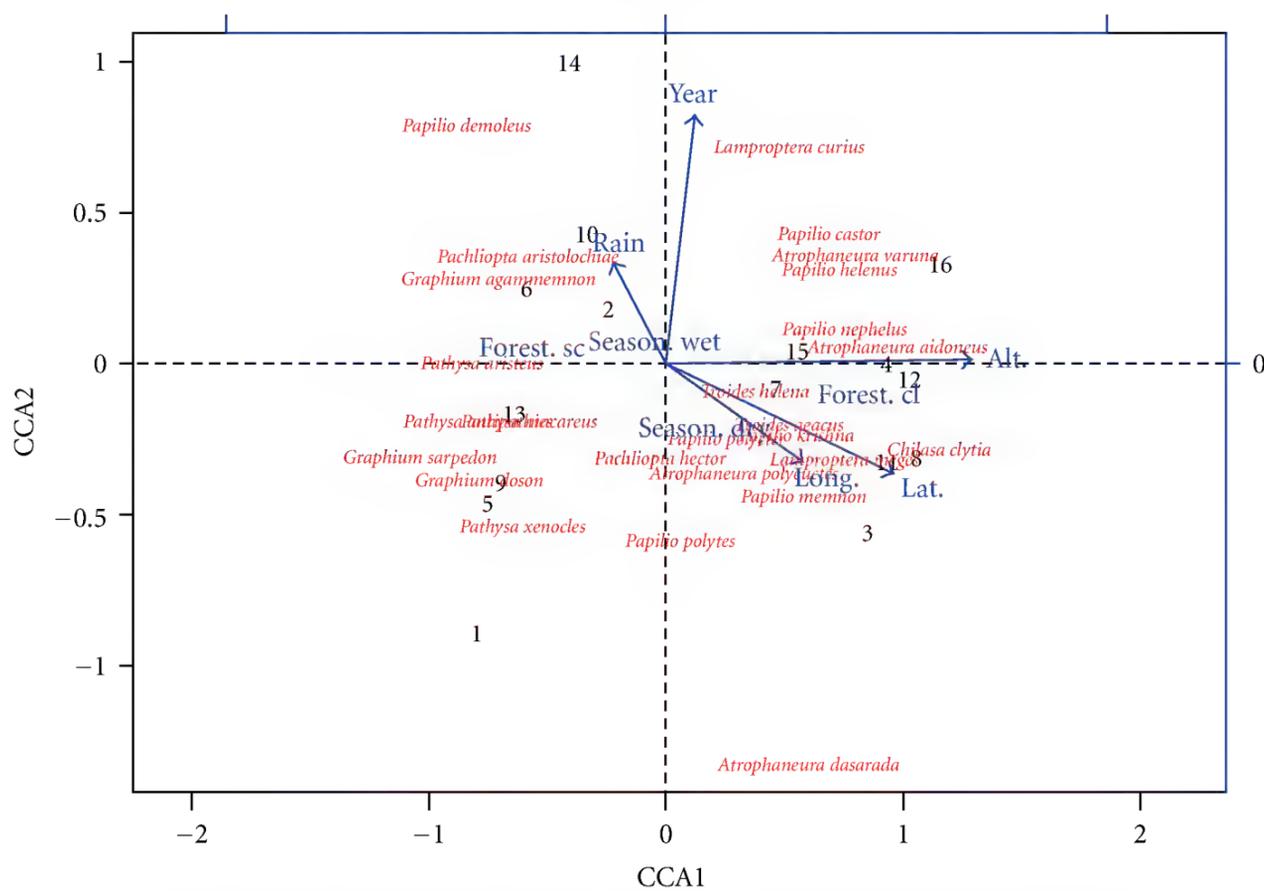
them an opportunity for visiting flowers in both open habitats and forest canopy (e.g., *Papilio paris* and *P. helenus*) [32]. The genera *Graphium* (3 spp.), *Pathysa* (4 spp.), *Pachliopta* (1 sp.), and *Papilio* (2 sp.) had preferences for the open-forest habitats [32]. They were mostly observed at forest edges near human settlements and gaps created by human disturbances. These species were frequently observed mud-puddling on wet soil in open sunny patches near human habitations, excreta of domestic animals and foraging on plants like *Ixora coccinea*, *Hibiscus rosa sinensis*, *Lantana camara* and *Vitex nugundo* that occurred in higher abundances in areas near human settlements. Such opportunists are able to persist in the disturbed landscape, including human settlements, as they are more adaptable and better able to exploit a wide range of ecological niches [46, 47]. They are known to use a wide range of host plants and therefore can fly over the forest canopy to gaps. This accounts for their wide distribution as opportunist species and may explain our results on higher abundance of the wide-ranging *Graphium* and *Papilio* species in the gaps [48, 49]. The *Graphium* spp. are known to have expanded their ranges to *Cinnamomum* tree plantations throughout Southeast Asia [20]. Monoculture of *Polyalthia longifolia*, an important host-plant of *Graphium* spp. in human settlements close to the forest edges and gaps as observed in our study could have resulted in high abundances of *Graphium* spp. This is contradictory to the reports of monoculture—like conditions in plantations of economic trees that are normally known to cause a decline in both floral and butterfly diversity [21, 50].

The habitat preferences of the two *Lamproptera* spp. was categorised as “intermediate” although they were observed to have stronger preferences for the gaps within the study area. *Lamproptera curius* is a typical gap species [32] and it had showed the lowest abundance amongst all the 28 species recorded in the study area. The status of *Lamproptera* spp. is “Vulnerable” and there is lack of information on its host plant availability in Assam.

4.2. Effect of Rainfall. In the tropical monsoon climate, the temperature fluctuations between the dry and wet seasons are very little whereas the differences in rainfall are very high. The combined effects of temperature and moisture gradients are known to influence the biology and ecology of butterflies, particularly the variation in adult abundance



(a) CCA ordination plots of papilionid species matrix on sampling locations of Garbhanga



(b) Ordination plots of CCA of Papilionidae species matrix on sampling locations of Rani

FIGURE 4: Ordination plots of CCA of papilionid species matrix on sampling locations of Garbhanga (a) and Rani (b) ranges during the wet and dry seasons of 2003-2004. Note: Lat for latitude, Long for longitude, Alt for altitude, Forest CL for closed forest, Forest SC for scattered/open Forest.

and activity. Arguably, variation in rainfall patterns is the most important factor affecting the seasonality of tropical insects [51, 52]. Corresponding to our hypothesis, the ordination results showed a strong correlation between rainfall and papilionid abundances, thereby predicting the

influence of the monsoon climate on butterfly seasonality. The abundances of species like *Papilio demoleus*, *Pachliopta aristolochiae* and *Graphium* spp., showed a strong correlation with rainfall and were abundant all throughout the monsoon period. *Troides aeacus*, *Troides helena*, *Papilio helenus* and

Atrophaneura spp. from the closed-forest and *Graphium sarpedon*, *Papilio* spp. and *Pathysa* spp. from the open-forests showed moderate seasonal trends with rainfall. While the flight periods of open-forest species reached a peak in the summer months, that of *Troides* and *Atrophaneura* spp. peaked during the last part of the wet season, and the flight period of *Papilio helenus* was found to peak during the late monsoon until the early part of the dry season. Previous studies on the effects of season and habitat on butterfly communities in the northern Western Ghats within the Indian subcontinent reported fluctuations in seasonal abundance with peak populations during late monsoon and early winter [53]. Some species like *Pachliopta hector* and *Papilio castor* were strictly seasonal and were found to be on the wing during the monsoons alone. Such a seasonal trend could be attributed to synchrony with the phenology of food plants [41].

4.3. Effect of Altitude and Geographical Position (Latitude and Longitude). An effect of altitude on the abundance and distribution of the Papilionidae was indicated in our study area. The forest species preferred the higher elevations while the open-forest species preferred the gaps at lower elevations. Although the altitudinal variation in the sampled transects were not large, differences in abundances between open and closed-forests were observed. Narrow elevational gradients could sometimes influence some of the biological activities of the butterflies, like fecundity and opportunities to lay eggs [54]. The sampling design might have also affected the results as gaps were mostly located at lower elevations. The sampling scale was also small which might explain why we did not find a pronounced effect of the geographical position on papilionid abundance.

4.4. Conclusion and Recommendations for Conservation. Our overall study results showed that papilionid abundance was influenced by the seasonal monsoon climate and this was in agreement with the findings of Spitzer et al. [32] and Leps and Spitzer [5]. Our results were also in conformity with our hypothesis on higher species richness in the closed-forest habitats. Sixteen out of 28 species were found to be associated with the closed-forest, and six species with restricted geographic range scores (1-2).

Species like the Bhutan Glory (*Bhutanitis lidderdalei*) and Kaiser-I-Hind (*Teinopalpus imperialis*), which were already listed as rare in the Assam region in the early part of the 20th century, have not been sighted in several years. They were subjected to extensive commercial trade. But there are many more species, which have not been recorded since their early documentation, and it is not known whether they still exist. The Yellow-Crested Spangle (*Papilio elephenor*), an IUCN Red-listed species endemic to the Eastern Himalayas, which was last reported from Assam in 1907 [55] was spotted in a protected reserve in western Assam in 2009 (Eastern Himalayas Bulletin 2009). The area where this species has been sighted is still a pristine habitat although reports of human pressure on the reserve clearly indicate the existing threats to the habitat. The conversion of primary forest cover into secondary forests and open scrublands will destroy the

pristine habitats and the papilionids, which are primarily a forest group, will be most affected, because their host-plants will disappear. Based on previous records of high Papilionidae diversity in the Eastern Himalayas, we therefore considered it logical to investigate a protected secondary forest reserve within the moist deciduous forest zone of Assam. Large scale deforestation and habitat fragmentation has led to a decline in the butterfly population in Assam during the last decade and the status of many species which were listed as common during the early part of the 20th century is presently not clear. Most of the protected reserves do not even have inventories of butterflies. In India, butterflies have been recently included in biodiversity conservation programmes [56]. Research and documentation of butterflies needs to be re-initiated in the Assam region, with conservation priorities to be focused on stenotopic closed canopy species that are also the most endangered groups [42]. Monitoring of butterfly populations and ecological studies focusing on the possible causes of decline and developing conservation strategies in protected areas have to be initiated in order to save the remaining populations from extinction. It is also essential to bring more forests under a protected area network. A wildlife sanctuary for butterfly conservation under the Indian Wildlife (Protection) Act, 1972 [57] would serve an important purpose.

Northeast India was largely closed to the outside world for the last fifty years primarily due to its remote location, sharing the international boundary with several countries and an uniecoue cultural identity. Apart from being a biodiversity hotspot, this region is also a cultural hotspot with over 240 distinct ethnolinguistic groups [27]. In recent decades, deforestation and watershed deterioration have progressed rapidly due to land clearing by both the local and migrant population and heavy demand for timber from neighbouring Bangladesh and other urban centers of India. Shifting cultivation largely practiced by the hill tribes is another major issue. Although the indigeneous communities are recognized as the rightful stakeholders of much of the forest land in Northeast India, there is still a lack of external support within the legal policy framework due to the larger economic interests of the private sectors and government agencies. However there is a great scope for community based forest management due to a very rich heritage of Traditional Ecological Knowledge system (TEK) and the need for actions that promote the conservation and sustainable use of the region's endangered forests and the changing land-use practices in the last fifty years. Promotion of eco-tourism that will primarily focus on "butterfly watching" can also help in generating an alternative source of livelihood for the forest fringe villagers. We also call for the development of a resource database on butterfly biology in the Assam region as it has been shown to be critical for butterfly conservation [39, 40].

Acknowledgments

The authors would like to thank the Department of Forests, Government of Assam, India for the permission granted to conduct the research study in Rani-Garbhanga Reserve Forest. They would like to thank the Ministry of Environment

and Forests, Government of India for the financial grant and the research team in the Department of Zoology, Gauhati University, Assam for the fieldwork. The authors acknowledge the special contribution of Dr. Matthias Waltert, Centre for Nature Conservation, University of Göttingen, who has given the scientific inputs and helped in the drafting of the paper. Special thanks are due to N. K. Bhagobaty, Senior Instructor, Institute of Advanced Study in Science and Technology, Guwahati, Assam, India for assistance with the statistical analyses. In authorship they followed the “FLAE” norm.

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Research Article

Phylogeographic Investigations of the Widespread, Arid-Adapted Antlion *Brachynemurus sackeni* Hagen (Neuroptera: Myrmeleontidae)

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Received 10 June 2010; Accepted 16 November 2010

Academic Editor: Coby Schal

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Several recent studies investigating patterns of diversification in widespread desert-adapted vertebrates have associated major periods of genetic differentiation to late Neogene mountain-building events; yet few projects have addressed these patterns in widespread invertebrates. We examine phylogeographic patterns in the widespread antlion species *Brachynemurus sackeni* Hagen (Neuroptera: Myrmeleontidae) using a region of the mitochondrial gene cytochrome oxidase I (COI). We then use a molecular clock to estimate divergence dates for the major lineages. Our analyses resulted in a phylogeny that shows two distinct lineages, both of which are likely distinct species. This reveals the first cryptic species-complex in Myrmeleontidae. The genetic split between lineages dates to about 3.8–4.7 million years ago and may be associated with Neogene mountain building. The phylogeographic pattern does not match patterns found in other taxa. Future analyses within this species-complex may uncover a unique evolutionary history in this group.

1. Introduction

Phylogeographic analyses investigate the relationship between genealogies and their geographic distribution [1]. Many recent studies have investigated the historical biogeography of the Nearctic arid lands through the phylogeographic analyses of wide-ranging, desert-adapted taxa [2–7]. These studies often associated major genetic divergences with mountain-building events that took place in the late Neogene. As a result of these late Neogene events, deeply divergent clades were found to be restricted to the eastern (Chihuahuan) and western (Mojave and Sonoran) deserts. While the various hypotheses detailing the causes of the diversification of the Nearctic's arid-adapted biota approach a generalized model [8], little work has been done on wide-ranging, arid-adapted arthropods [7]. Phylogeographic analyses of these organisms will aid in the development of a generalized model detailing diversification in the deserts.

In addition to the importance of phylogeographic analyses to historical biogeography, these analyses often uncover

the existence of cryptic species [9–13]. Recognition of these complexes is an essential aspect of documenting biodiversity and can be beneficial in the development of conservation strategies [14, 15].

While some phylogeographic investigations have been done on arid-adapted arthropods, like beetles [16, 17], velvet ants [7, 18], and spiders [2, 19], several diverse arthropod groups remain unexplored. One such group are the antlions (Neuroptera: Myrmeleontidae). Antlions in the tribe Brachynemurini are ideal candidates for phylogeographic analyses investigating the history of the Nearctic deserts because they are most abundant in the arid or semiarid regions of the southwestern United States and northern Mexico [20]. Furthermore, because antlions are economically important as predators, adults commonly feed on caterpillars and aphids [21], and as potential pollinators—several species are associated with flowers and potentially transfer pollen [20]—understanding the genetic diversity and species limits of antlions may be helpful to biologists and land managers.

TABLE 1: Descriptive information for all of the taxa used in this study.

Species	Voucher ID	Lineage	Collection location	COI accession no.
<i>Brachynemurus sackeni</i>	MY02	1	NV: White Pine Co., near Cherry Creek	HQ386913
<i>Brachynemurus sackeni</i>	MY14	1	UT: Washington Co., near St. George	HQ386914
<i>Brachynemurus sackeni</i>	MY13	1	NV: Clark Co., Toquop Wash, W. Mesquite	HQ386915
<i>Brachynemurus sackeni</i>	MY15	1	TX: Dimmit Co., Chaparral Wildlife Management Area	HQ386916
<i>Brachynemurus sackeni</i>	MY03	1	CA: San Diego Co., Ocotillo Wells	HQ386917
<i>Brachynemurus sackeni</i>	MY19	1	CA: Imperial Co., Algodones Sand Dunes	HQ386918
<i>Brachynemurus sackeni</i>	MY09	1	CA: San Diego Co., Anza Borrego State Park	HQ386919
<i>Brachynemurus sackeni</i>	MY18	1	UT: San Juan Co., Valley of the Gods	HQ386920
<i>Brachynemurus sackeni</i>	MY16	1	UT: Emery Co., Green River	HQ386921
<i>Brachynemurus sackeni</i>	MY21	2	CA: San Bernardino Co., 8 mi N Big Bear City	HQ386922
<i>Brachynemurus sackeni</i>	MY08	2	CA: San Bernardino Co., 5 mi S Barstow	HQ386923
<i>Brachynemurus sackeni</i>	MY06	2	UT: Washington Co., Beaver Dam Slope	HQ386924
<i>Brachynemurus sackeni</i>	MY11	2	NV: Nye Co., Pahrump	HQ386925
<i>Brachynemurus sackeni</i>	MY12	2	CA: Riverside Co., Deep Canyon Reserve	HQ386926
<i>Brachynemurus sackeni</i>	MY05	2	NM: Harding Co., Kiowa National Grasslands	HQ386927
<i>Brachynemurus sackeni</i>	MY17	2	TX: Jeff Davis Co., Davis Mtns. State Park	HQ386928
<i>Brachynemurus sackeni</i>	MY04	2	TX: Brewster Co., Big Bend Ranch State Park	HQ386929
<i>Brachynemurus hubbardi</i>	MY23	2	AZ: Yavapai Co., near Camp Verde	HQ386930
<i>Scotoleon yavapai</i>	MY22	2	AZ: Cochise Co., happy camp road	HQ386931

FIGURE 1: Male *Brachynemurus sackeni* from Deep Canyon, California.

In this study, we investigate the phylogeographic patterns among populations of a widespread antlion, *Brachynemurus sackeni* Hagen (Figure 1), in order to gain insight into the diversification of the Nearctic desert biota. We apply a molecular clock, in order to estimate the dates associated with the major divergences within this species and compare those dates to published records of diversification in

other desert-adapted animals. This study represents the first phylogeography and one of the first molecular phylogenetic analyses conducted on antlions.

2. Materials and Methods

2.1. Taxon Sampling. Specimens were collected from sites across western North America (Figure 2) from 2002 to 2009 using black light traps and fluorescent lantern traps. All specimens were placed directly into 95% ethanol, and those used for molecular examination have been labeled as voucher specimens and deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS). Desert boundaries to discuss species distributions and historical biogeography are altered from Omernik [22].

2.2. Molecular Methods. DNA was extracted from a middle and hind leg of each specimen using the High Pure PCR Template Preparation Kit (Roche Pharmaceuticals, Indianapolis, IN). A portion of the mitochondrial gene cytochrome oxidase I (COI) was amplified using the primer pair LepF1 (ATTCAACCAATCATAAAGATATTGG) and LepR1 (TAAACTTCTGGATGTCCAAAAAATCA) [23], which amplified an approximately 700 bp DNA fragment of the mitochondrial COI gene. PCR took place in a 20 μ L volume with the following conditions: 3 mM MgCl₂, 200 pM dNTPs, 2 units of *Taq* polymerase, 1 mM of each primer, and standard PCR buffer concentration. For each PCR, approximately 20 ng of template DNA was added to the reaction. The PCR program included an initial step of 94°C for 150 sec, followed by 35 cycles of 94°C for 30 sec, 47°C for 60 sec, and 72°C for 60 sec, with a final step of 72°C for 10 min. Amplified products were visualized on



FIGURE 2: Map of Western North America showing the major deserts and arid lands altered from Omernik [22]. Closed circles (●) indicate collection locations of specimens from lineage 1, and open circles (○) indicate collection locations of specimens from lineage 2.

agarose gels, stained with ethidium bromide. Successful PCR products were cleaned using isopropanol purification. Sequences were analyzed with an ABI Prism 3730 Genetic Analyzer. PCR products were sequenced in both directions and sequence contigs assembled using Sequencher 4.0 (Gene Code Corp., Ann Arbor, MI). DNA sequences were aligned using Clustal W [24] and alignments were visually inspected and corrected in MacClade 4.07 [25]. All COI sequences were deposited in GenBank (Accession numbers HQ386913-HQ386931; Table 1). Genetic distances between major clades were calculated as pairwise percentages by determining the number of differences (point mutations and insertions or deletions) divided by the number of base pairs of the longer of the two sequences.

Two additional DNA regions, the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2), were amplified for a limited number of specimens to investigate the possibility of these intergenic regions being useful for phylogeographic analysis. The primers 5'-GATTACGTCCCTGCCCTT-TG-3' (forward-18S) and 5'-CGATGATCAAGTGTCCTG-CA-3' (reverse-5.8S) [26] were used for the ITS1 locus and

5'-GGCTCGTGGAATCGATGAAGAACG-3' (forward 5.8S) modified from Weekers et al. [27] and 5'-GCTTATTAATAT-GCTTAAATTCAGCGG-3' [27] were used for ITS2. The PCR programs included an initial step of 94°C for 150 sec, followed by 35 cycles of 94°C for 30 sec, 52°C (ITS1) or 56°C (ITS2) for 60 sec, and 72°C for 60 sec, with a final step of 72°C for 10 min.

2.3. Phylogenetic and Network Analyses. The genetic locus COI was subjected to Bayesian analysis using MrBayes v3.1.2 [28]. Sequences were analyzed according to the general time-reversible model of sequence evolution [29] with invariant sites and gamma-distributed rate variation across sites (GTR + I + Γ) and with all parameters unlinked across loci. Bayesian analyses included four independent runs with three heated chains and one cold chain in each run. The MCMC (Markov Chain Monte Carlo) chains were set for 3,000,000 generations and sampled every 100 generations; chains were run until the average standard deviation of the split frequencies dropped below 0.01. The burn-in period

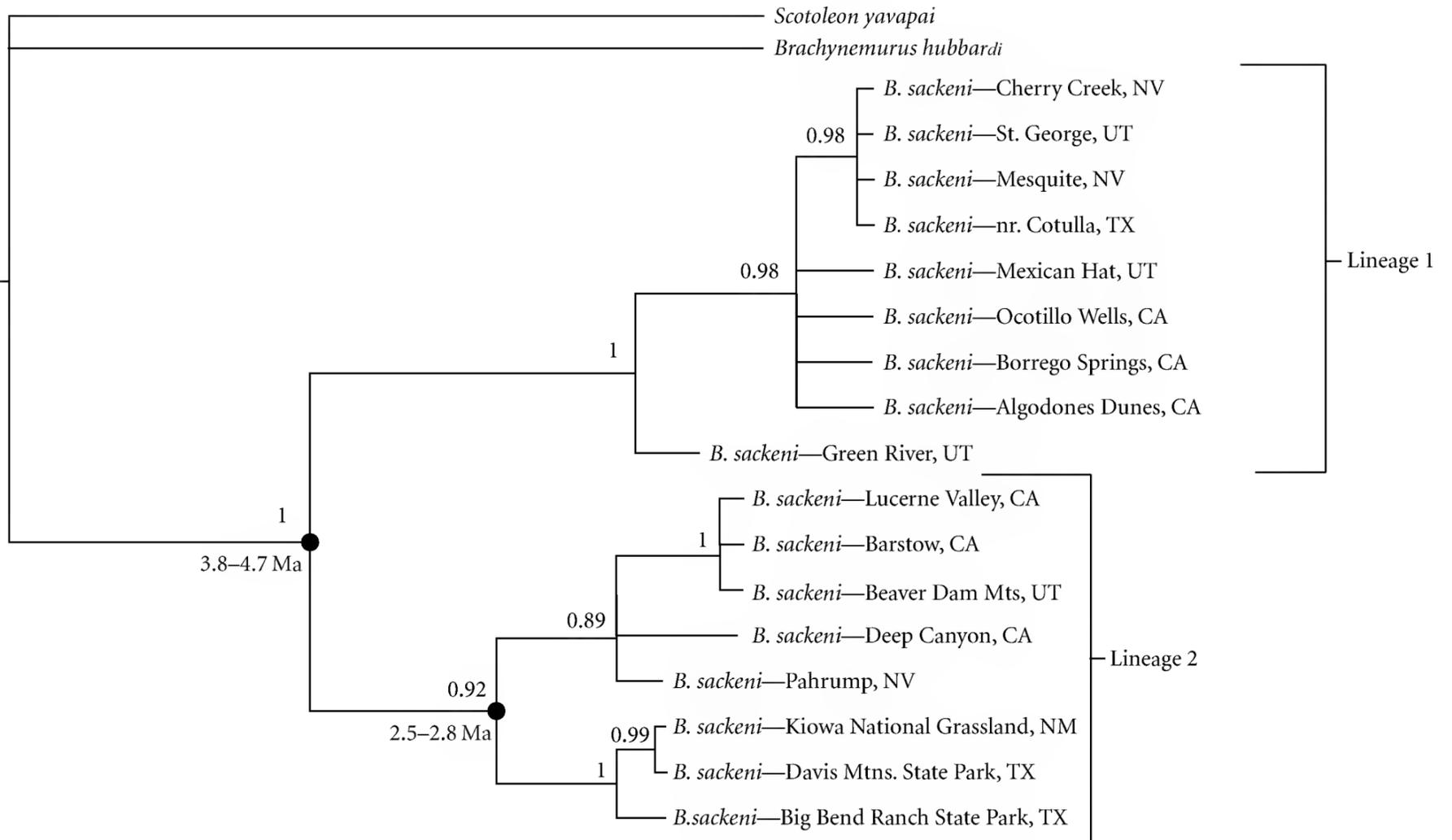


FIGURE 3: Consensus tree of the Bayesian analysis done on the aligned COI data set. Numbers at each node represent posterior probabilities. Collection locations for each *B. sackeni* specimen are given. Estimated divergence dates are given for two nodes marked with black circles.

of 3,000 samples was removed for each analysis after graphical determination of stationarity. *Brachynemurus hubbardi* Currie and *Scotoleon yavapai* (Currie) were included as outgroups, because they are closely related to *B. sackeni* [20].

We constructed a parsimony-based haplotype network using the aligned COI sequences for all *B. sackeni* specimens using TCS version 1.21 [30]. The program estimated the 95% reconnection limit between haplotypes with gaps treated as missing data.

3. Results

3.1. Phylogenetic, Haplotype Network Results. Sequences from the two intergenic regions (ITS1 and ITS2) were A/T rich (ITS1: A = 42.7%, T = 42%, C = 6%, G = 9.3%. ITS2: A = 42.1%, T = 42%, C = 8.1%, G = 7.8%). Because both of these regions were A/T rich, which can be problematic phylogenetic analyses, we did not use these sequences in the subsequent analyses.

Usable COI sequences were obtained from a total of 17 *B. sackeni* specimens that were collected from sites across the Nearctic deserts (Figure 2). A total of 679 bp of COI were sequenced. Bayesian analysis of the molecular data produced a tree that clearly shows two divergent *B. sackeni* lineages (Figure 3). The genetic distances between these two lineages are relatively high (8.7–10.9%). There is no clear biogeographic pattern between the two major lineages, but there is some geographic structuring within Lineage 2

(Figure 3). Lineage 2 is split into two subclades, one made up of populations from the Mojave and western Sonoran desert and the other from the Chihuahuan Desert and nearby areas. While some phylogenetic structuring is found within Lineage 1, there is no clear biogeographic pattern to these clades.

Haplotype Network analysis also shows that a large amount of genetic variation exists among populations of *B. sackeni*. A total of seven networks were formed based on the aligned COI dataset (Figure 4). Four of these networks are composed of single individuals. The populations associated with Lineage 1 formed a single network except for one individual from Green River, Utah, and one individual from Mexican Hat, UT, which were each placed in their own network. Lineage 2 was split into two monotypic networks and two networks composed of three populations each (Figure 4).

4. Discussion

Stange [20] suggested that *B. sackeni* is highly variable morphologically, and more detailed studies may lead to division of the species. Our analysis shows that this species is also highly variable genetically. Genetic distances, phylogenetic estimation, and haplotype networks suggest that *B. sackeni* should be split into two lineages (our Lineage 1 and Lineage 2; Figure 3) and is likely two different species. *Brachynemurus sackeni* has two synonyms, which were originally distinguished by color and size. Based on subsequent

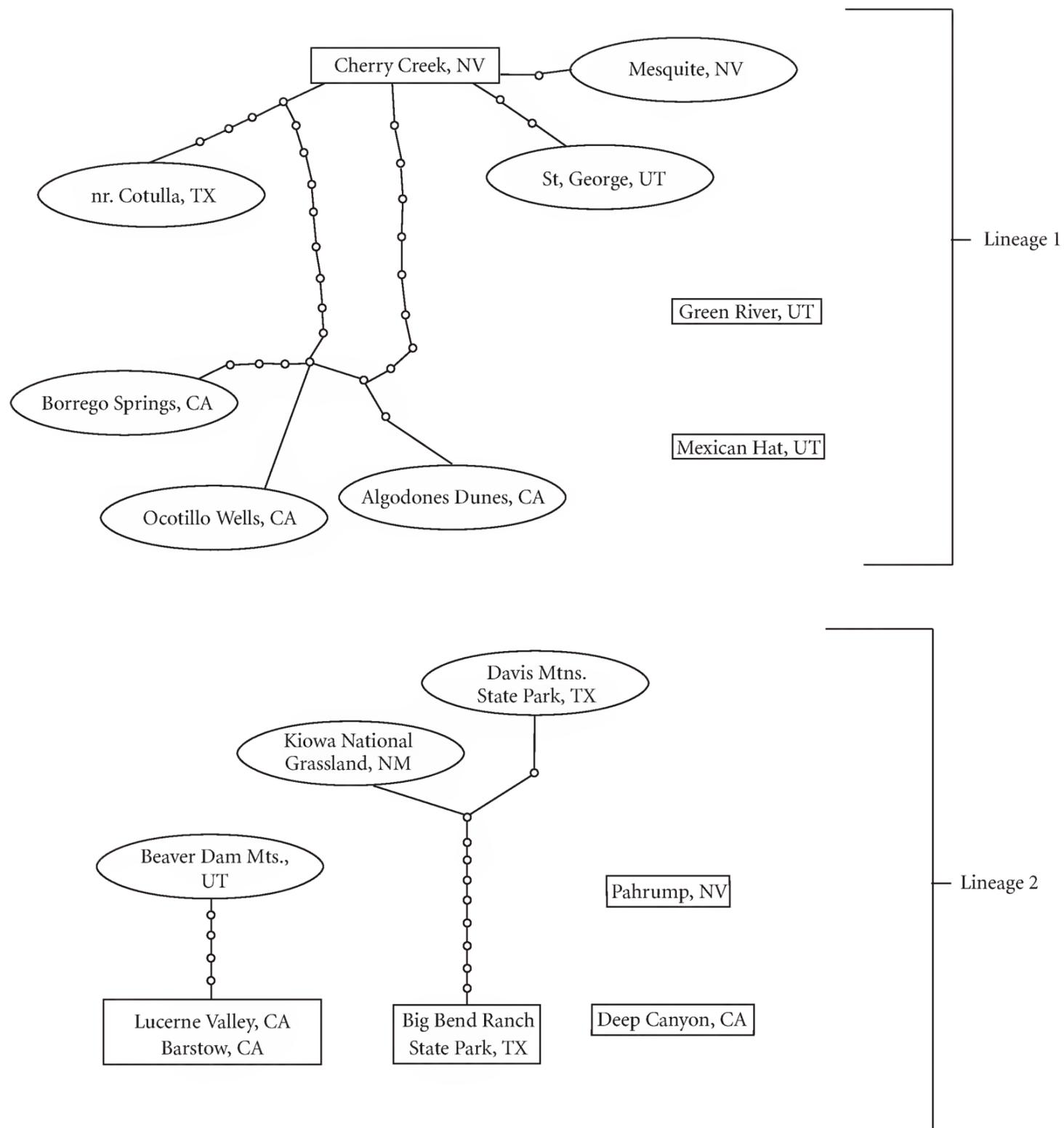


FIGURE 4: Haplotype networks based on the aligned COI data set. Populations are named based on their collection locality, and populations corresponding to Lineage 1 and Lineage 2 from Figure 3 are marked. Haplotypes surrounded by a rectangle were estimated to be the ancestral haplotype for network.

analysis of the holotypes, Stange [20] suggested that the coloration and size differences fit within the variation seen in *B. sackeni*. While our analyses suggest that *B. sackeni* is composed of multiple species, we agree with Stange [20] that color and size differences are variable and do not represent distinct species in this case because multiple color forms and sizes are present throughout both major lineages of our phylogeny. Detailed analysis of morphological characters, especially genitalia, may uncover morphological differences between the two *B. sackeni* species. Because these species are genetically distinct, yet cannot easily be distinguished at present based on the morphological characters presented in the literature, *B. sackeni* represents the first known cryptic species-complex in Myrmeleontidae.

While several of the analyses investigating the historical biogeography of the Nearctic deserts have described an east/west split in closely related species with sister species often being restricted to eastern (Chihuahuan) and western (Mojave and Sonoran) deserts [2–5, 7], our analysis did not uncover this pattern between the species in the *B. sackeni* cryptic species-complex. Instead we collected species 1 in the western Sonoran, Mojave, and Great Basin deserts as well as the Colorado Plateau and in southern Texas near the Chihuahuan Desert (Figure 2). Similarly, we collected species 2 in the western Sonoran, Mojave, and Chihuahuan deserts, as well as the high plains of northern New Mexico (Figure 2). While we do not see any biogeographical pattern among species in the *B. sackeni* cryptic species-complex, we do see

an east/west split among populations in species 2, with one lineage being found in the Mojave and western Sonoran Desert, and the other lineage being found in the Chihuahuan Desert and nearby areas (Figure 3). Patterns like this have been linked to both late Neogene mountain-building events [3–5, 7] and Pleistocene climate change [2, 18]. Divergence dates are needed in order to understand what processes led to diversification within the *B. sackeni* cryptic species-complex.

Using our COI sequence data and a global arthropod molecular clock estimate of 2.3% sequence divergence between lineages per million years [2, 31], we roughly dated the divergence time between species, and between major lineages in species 2. Divergence time estimates for the split between species suggest that the speciation event occurred around 3.8–4.7 million years ago. The split between major lineages in species 2 was estimated to be from 2.5–2.8 million years ago. Because of the wide range of dates that have been proposed for the uplift of the mountain ranges in western North America, divergence dates ranging from 2–15 million years could likely be associated with mountain-building events [32]. Therefore, even though no clear biogeographic pattern exists between species, the major divergences within this species-complex, and those divergences within species 2, may be linked to mountain building events in the late Neogene that caused the formation of many of the western deserts and drove diversification in numerous arid-adapted species.

Because no biogeographic pattern can be seen between species in the *B. sackeni* cryptic species-complex, it is likely that both vicariant events and dispersal events shaped the history of this group. Given that this analysis is based on a limited number of specimens, it must be viewed as preliminary. Future analyses examining more detailed phylogeographic patterns within this species-complex may uncover additional patterns of diversity that could aid in the understanding of the processes that led to diversification in the Nearctic deserts and arid lands.

Acknowledgments

The authors thank Carol von Dohlen at Utah State University for the use of laboratory space and equipment. Funding for this research was provided in part through the AMNH Theodore Roosevelt Memorial Fund grant and the Southwestern Research Station. Additional funding was provided through the California Desert Research Fund at The Community Foundation. This research was also supported by the Utah Agricultural Experiment Station, Utah State University, Logan, UT and was approved as journal paper no. 568.

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Research Article

Two New Species of *Chaetopteryx* Stephens, 1837 from Turkey with a Description of the Unknown Female of *C. bektasensis* Sipahiler, 2008 (Trichoptera, Limnephilidae: Limnephilinae: Chaetopterygini)

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Received 31 August 2010; Accepted 23 November 2010

Academic Editor: John Heraty

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Two new species of Trichoptera from Turkey are described and illustrated: *Chaetopteryx akgolensis* sp.n. and *Chaetopteryx sinopica* sp.n. (Limnephilidae). Both belong to the tribe Chaetopterygini in the Limnephilinae. *C. akgolensis* sp.n. is close to *C. bektasensis* Sipahiler, 2008, whereas *C. sinopica* sp.n. is closely related to *C. nalanae* Sipahiler, 1996. The previously unknown female of *Chaetopteryx bektasensis* Sipahiler, 2008 is described and figured.

1. Introduction

The genus *Chaetopteryx* Stephens was previously thought to be represented in Turkey by four species. Two of them, *C. bosniaca* Marinkovic, 1955 (found in the Carpathians, the Balkans, and Turkey) and *C. abchazica* (Martynov, 1916) (found in the Caucasus, Iran, and northeastern Turkey), are widely distributed species; the others, *C. bektasensis* Sipahiler, 2008 and *C. nalanae* Sipahiler, 1996 have restricted distributions, being found in northeastern and northwestern Turkey, respectively [1–3]. In the present paper, two new species of this genus are described: *C. akgolensis* sp.n., which is closely related to *C. bektasensis* and *C. abchazica*, and *C. sinopica* sp.n., which is close to *C. nalanae*. Both are found in northwestern Anatolia.

2. Materials and Methods

Specimens were collected in autumn during the day using a hand net. The material was preserved in 75% ethyl alcohol and deposited in my collection in Hacettepe University Department of Biology Education. For the code of depository the abbreviation, CD is used.

3. Taxonomy

3.1. *Chaetopteryx akgolensis* Sp.n. (Figures 1 and 2)

Material. Holotype male and paratype female: Turkey, Sinop, Hanönü, Ayanick direction, Çangal Mountain (CD: R-1251), 1130 m, a small spring near Akgöl, 41°41' N, 34°34' E, 3.x.2009; same place (CD: R-1262), 26.x.2009, 1 female, leg. and coll. Sipahiler.

Antennae, palps, legs, and wings pale brown; forewing with a white spot on the medial vein, and a larger one on anal vein 1 located near anastomosis and near the margin, respectively. Spur formula of male is 0.3.3, of female 1.3.3. Length of anterior wing of male 14.5 mm, of female 15–15.2 mm.

Male Genitalia (Figure 1). Spinulose zone of tergite VIII large; in dorsal view, the posterior and anterior edges almost straight and the sides rounded. In lateral view, the sides of segment IX are dilated on the anterior margin; the ventral part of segment IX is narrow. The preanal appendages are more or less rounded; in caudal view, the inner side of the apical edge with a large, almost rounded sclerotized

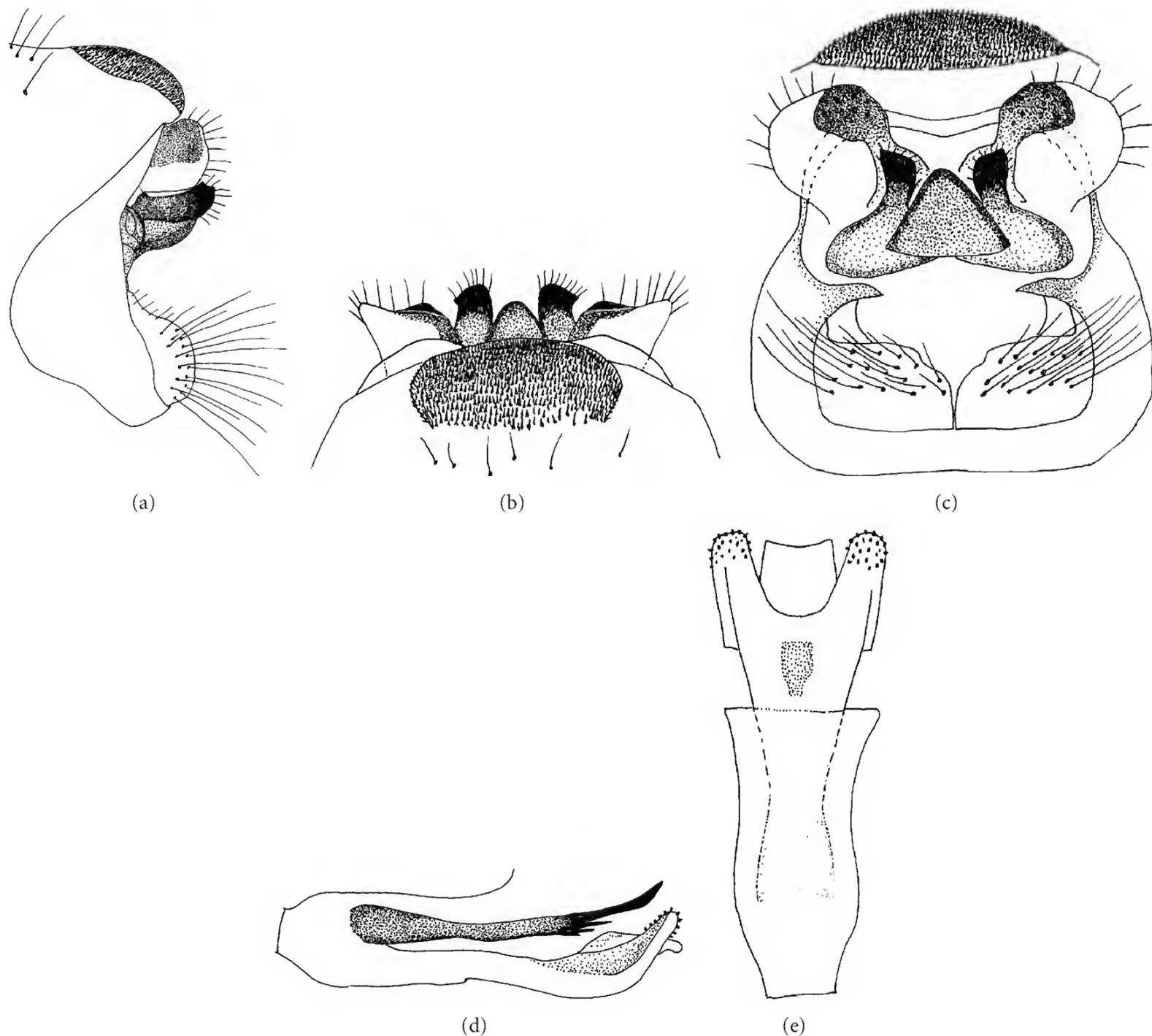


FIGURE 1: *Chaetopteryx akgolensis* sp.n., male genitalia: (a) lateral; (b) dorsal; (c) caudal; (d) phallic apparatus, lateral; (e) phallic apparatus, ventral.

zone. The intermediate appendages are nearly quadrangular and covered with white-yellowish short hairs; in lateral view, the ventral edge is somewhat dilated subdistally, the apex with two small pointed projections. The supra-anal plate is very large and long, almost triangular, strongly sclerotized; in dorsal view, it is seen as a large lobe between the intermediate appendages. The inferior appendages are short; in lateral view, the posterior edge broadly rounded. In caudal view, the inner edges are sinuate, and the dorsal edges are straight. The phallic apparatus is large on the apical portion; in ventral view, the sides are almost smooth, with the apical edge deeply and roundly excised, forming rounded lobes on each side, which are sclerotized and covered with tubercles; the median part with a weakly sclerotized almost quadrangular plate; parameres long, strongly sclerotized, and each possesses a long and rather broad spine, which is curved subdistally inside, and has three small spines, located subdistally.

Female Genitalia (Figure 2). In dorsal view, the dorsal part of segment IX is broadly and roundly excised, forming finger-shaped lobes on each side; the median part of this excision is broadly dilated; segment X is tube shaped, strongly sclerotized and located between the side lobes of segment IX; its dorsal part is deeply and roundly excised in the middle, and the sides of the excision are straight; the ventral part is longer than the dorsal part, and the apical margin is more or less straight, bearing short spines on the dorsal surface; in lateral view, the tube-like part of segment X is longer than the side lobes of segment IX, and the cavity is large. In caudal view, the median lobe of the vulvar scale is moderately large, apex rounded.

Remarks. *Chaetopteryx akgolensis* sp.n. is closely related to *C. bektasensis* Sipahiler, 2008 [3], but differs from this species by the following features: in *C. bektasensis*, the spinulose zone of tergite VIII is roundly dilated in the middle of the

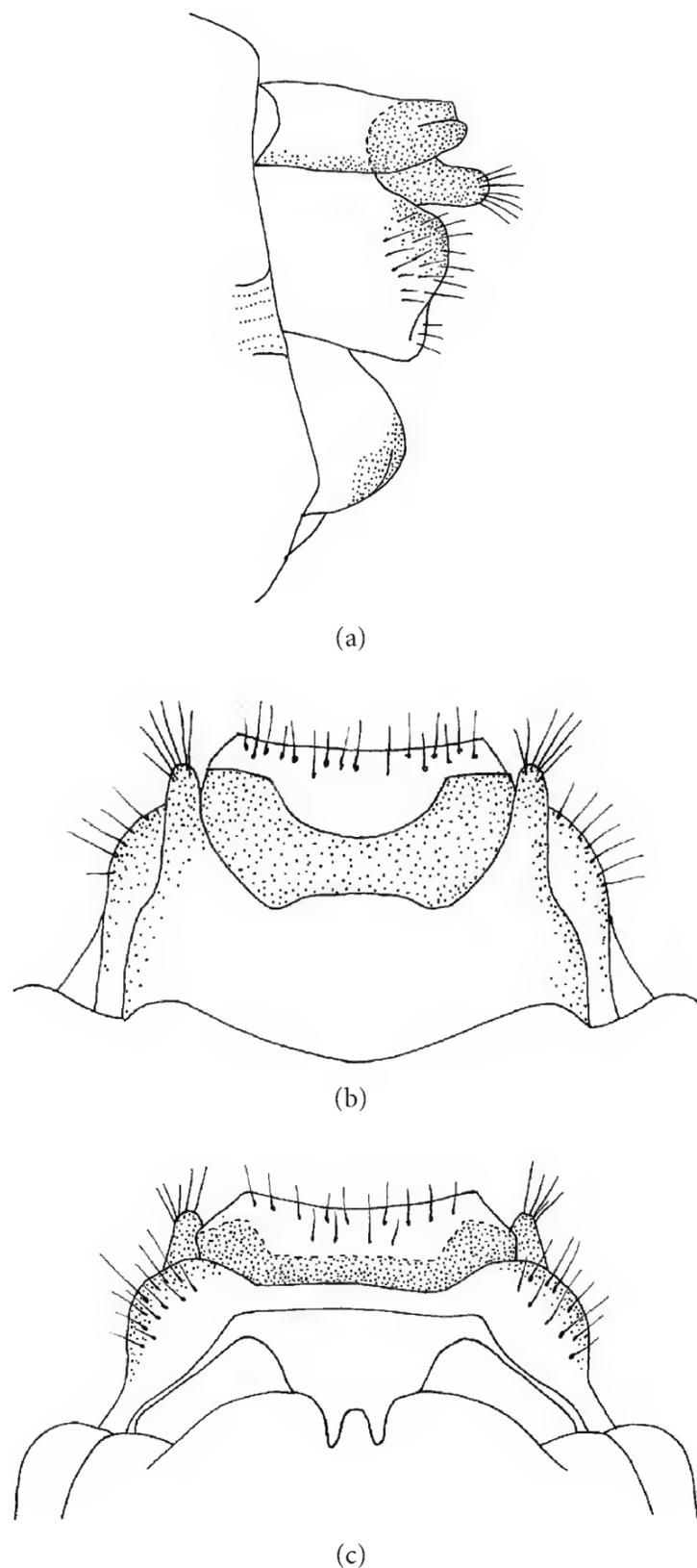


FIGURE 2: *Chaetopteryx akgolensis* sp.n., female genitalia: (a) lateral; (b) dorsal; (c) ventral.

apical edge; segment IX is broad on the ventral portion; in lateral view, the preanal appendage is elongate and dilated on the ventral edge, with the sclerite small and located on the inner corner, while in *C. akgolensis* sp.n., the apical edge of the spinulose zone of tergite VIII is broadly rounded. Segment IX is narrower ventrally, with the preanal appendage in lateral view shorter and almost rounded; the sclerite on the inner surface continues as a large band towards the base; the supra-anal plate of the related species is short and small but in *C. akgolensis* it is very large and long. Laterally, the inferior appendage of *C. bektasensis* is long, broad at the base, and narrowing towards the apical edge. In the new species, it is short and broad. The phallic apparatus of *C. bektasensis*, in ventral

view, with a bilobed median portion, with the basal parts of the paramere not totally sclerotized. Laterally, there is a short finger-shaped lobe, bearing long spines, of which the inner one is longer than the others and the phallic apparatus. In *C. akgolensis* sp.n., the median part of the phallic apparatus is quadrangular, and the parameres are strongly and completely sclerotized, having no basal lobe. The longer sclerotized spine, located on the inner side, is as long as the phallic apparatus. Differences in the female genitalia are also evident. In *C. bektasensis*, the dorsal and ventral edges of the tube-like part of segment X are narrower, and the ventral edge is roundly excised in the middle, forming rounded lobes on each side. The cavity of the tube-like part is small; in the new species, the tube-like part of segment X is broad, the cavity is large, the dorsal edge roundly excised medially, the sides are straight, and the ventral edge is smooth.

Etymology. Named after the place where the type specimens were collected.

3.2. *Chaetopteryx sinopica* Sp.n. (Figures 3 and 4)

Material. Holotype male and paratypes (4 males, 2 females): Turkey, Sinop, Küre Mountains, Boyabat, Bürnük (CD: R-1248), 41°39' N, 34°51' E, 1146 m, 2.x.2009; other paratypes: Sinop, Hanönü, Akgöl, Çangal Mountain (CD: R-1250), 3.x.2009, 3 males, 2 females; same places, 41°41' N, 34°34' E, 1130 m (CD: R-1261), 26.x.2009, 1 male, 1 female; Sinop, Dikmen, Durağan direction, Küre Mountains, 41°31' N, 35°09' E, 917 m, (CD: R-1265), 25.x.2009, 2 males, 2 females; leg and coll. Sipahiler.

Scapus pale brown, other segments of the antennae dark brown; palps and legs pale brown, wings brown; spur formula of male is 0.3.3, of female 1.3.3. Length of the anterior wing of males 10-11 mm, of females 12-13 mm.

Male Genitalia (Figure 3). In dorsal view, spinulose zone of tergite VIII is large, almost oval, with sides dilated; in lateral view, anterior edge and dorsolateral part of segment IX strongly sclerotized; dorsal cavity rather large, with preanal appendage small; in caudal view, preanal appendages rounded, ventral parts becoming narrower, forming a petiole. In lateral view, intermediate appendage is broad and rather short, dorsal edge slightly dilated, and ventral edge roundly dilated; apical portion becoming narrower and quadrangular. In dorsal view, triangular with apex curved on sides. In lateral view, inferior appendage is long, curving inside; outer portion with long hairs. In caudal view, apical part is short, strongly sclerotized, almost quadrangular. Supra-anal plate is narrow, weakly sclerotized; apical part slightly rounded. In lateral view, the phallic apparatus is curved dorsally. In ventral view, apical part as broad as basal portion; apical edge roundly excised in the middle, with a small membranous part, sides are straight. Apex with sclerotized thick spine on each side, the shaft with long and thin sclerotized bands. Parameres dilated subdistally and each bears six spines, of which the outer one is thicker than the others.

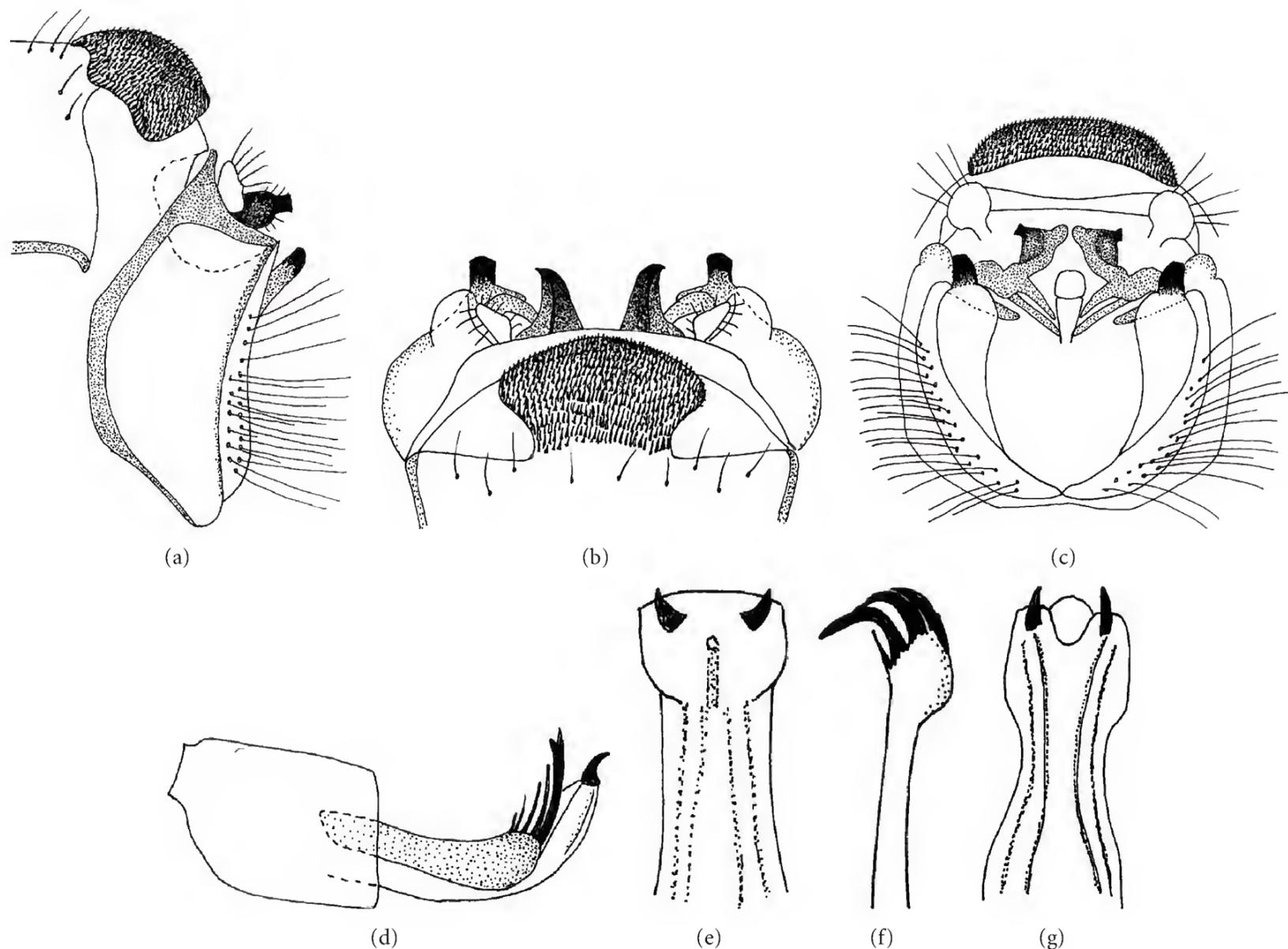


FIGURE 3: *Chaetopteryx sinopica* sp.n., male genitalia: (a) lateral; (b) dorsal; (c) caudal; (d) phallic apparatus, lateral; (e) phallic apparatus, dorsal, (f) left paramere, dorsal; (g) phallic apparatus, ventral.

Female Genitalia (Figure 4). In dorsal view, dorsal part of segment IX broadly and roundly excised, forming two triangular lobes on each side; covering the cavity of segment X. The ventral part is trapezoidal, with apical margin very short and smooth, bearing two long spines on each corner. In lateral view, ventral margin of tube-like part of segment X is pointed at the tip and as long as side lobes of segment IX. In caudal view, median lobe of the vulvar scale is large, with apex rounded.

Remarks. *Chaetopteryx sinopica* sp.n is closely related to *C. nalanae* Sipahiler, 1996, described from Bolu province in northwestern Turkey [1]. The following differences exist in the male genitalia: in *C. nalanae*, in dorsal view, the spinulose zone of tergite VIII is nearly trapezoidal, the apical and lateral edges are straight, the preanal appendages are broadly rounded and without petioles on the ventral parts, the intermediate appendages are long and narrow, the phallic apparatus very large on the apical portion, of which the sides are rounded, and the parameres are broad on the distal portion. In the new species, the spinulose zone is almost elliptical; at the preanal appendages, petioles are present ventrally, and the intermediate appendages are short and broad. Quadrangular projections are protruding apically, apical part of the phallic apparatus narrow, and the sides

straight and the parameres distally narrow. The differences in the female genitalia are as follows: in *C. nalanae*, in dorsal view, the side lobes of segment IX are rounded, the ventral part of segment X is long, almost quadrangular, and the apical margin slightly excised. In the new species the side lobes of segment IX are triangular, the ventral part of segment X is as long as the side lobes and trapezoidal. The apical edge is very short, bearing two long setae, and, in lateral view, it is pointed at the tip.

Etymology. Named after the place where the type specimens were collected.

3.3. *Chaetopteryx bektasensis* Sipahiler, 2008 (Figure 5)

Material. Turkey, Giresun, direction to Bektaş Yaylası (CD: R-1102), 2000 m, 12.x.2007 1 female; Giresun, Kümbet Yaylası, 40°33' N, 38°23' E (CD: R-1173), 1600 m, 2.x.2008, 1 male, 1 female; same place, Çıkırıktepe, 1871 m, (CD: R-1164), 2.x.2008, 1 male, 1 female; Giresun, Karagöl Yaylası direction, 1825 m (CD: R-1159), 1.x.2008, 40°33' N, 38°12' E, 2 females; same place, 1.x.2008 (CD: R-1180), 1 male, 2 females; Trabzon, Macka, Sumela, Camiboğazı Yaylası, 2077 m (CD: R-1179), 3.x.2008, 1 female; Sivas, Koyulhisar, Eğriçimen Yaylası, 4.x.2008 (CD: R-1161), 1600 m, 40°21' N,

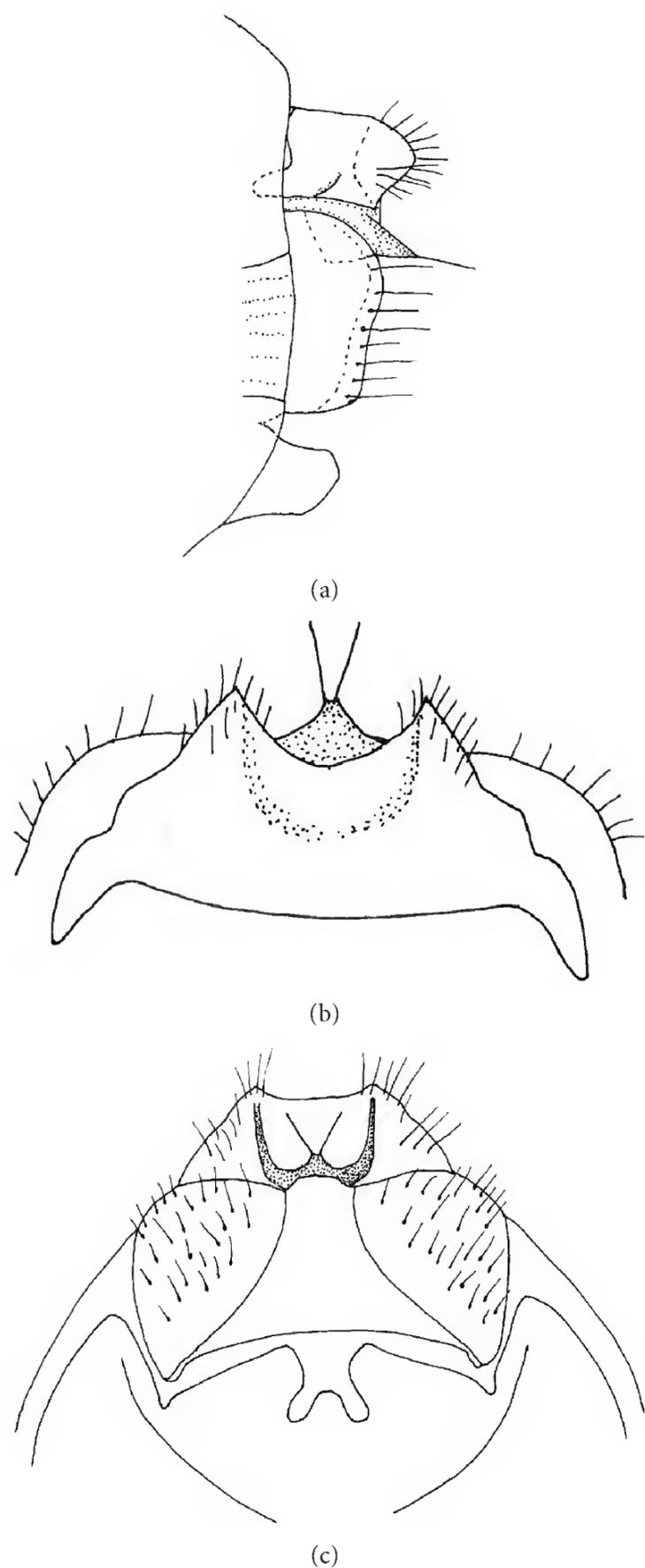


FIGURE 4: *Chaetopteryx sinopica* sp.n., female genitalia: (a) lateral; (b) dorsal; (c) ventral.

37°47' E, 1 female; same place (CD: R-1110), 11.x.2007, 1 female; Giresun, Kumbet Yaylası, Köprü, 2.x.2008 (CD: R-1162), 1 male, 3 females; Ordu, Çambaşı Yaylası, Yeşilce-Mesudiye direction, 1960 m (CD: R-1207), 40°35' N, 37°53' E, 19.8.2008, 1 female; leg. and coll. Sipahiler.

Antennae, palps and legs pale brown; wings brown, both membrane and the veins with upright hairs; spurs 1.3.3. Length of anterior wing 15-16 mm.

Female Genitalia (Figure 5). Segment IX dorsally broad at base, narrower in middle; apical edge roundly excised,

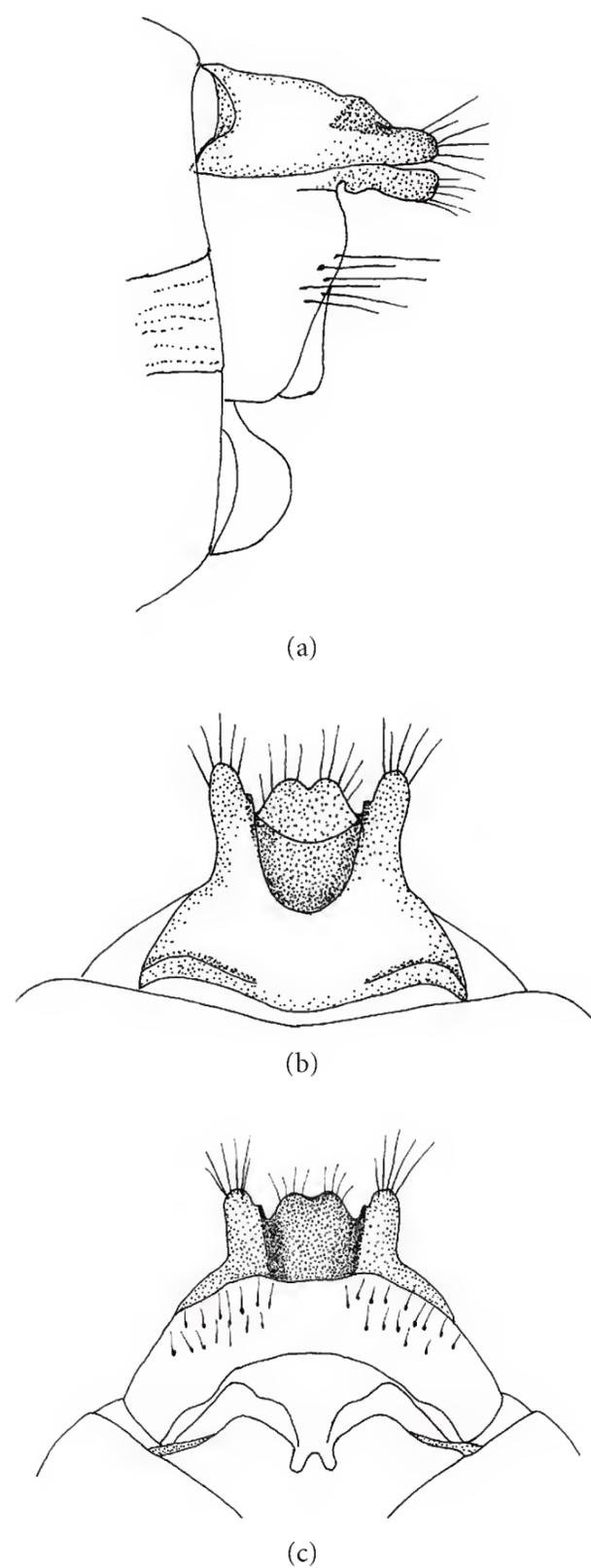


FIGURE 5: *Chaetopteryx bektasensis* Sipahiler, female genitalia: (a) lateral; (b) dorsal; (c) ventral.

forming narrow lateral lobes, which are rounded at the tips. Ventral plate of segment X as long as side lobes of segment IX; in dorsal and ventral view, somewhat narrower towards apex. Apical margin with a small excision and sides rounded. Median lobe of the vulvar scale small and obtuse at tip.

Remarks. The female genitalia of *C. bektasensis* differs from the female of *C. abchazica* by the following features: in *C. abchazica*, lateral lobes of segment IX shorter than ventral plate of segment X, somewhat divergent and the median lobe of the vulvar scale is lacking [4]. In *C. bektasensis*, lateral lobes of segment IX as long as the ventral plate, and the median lobe of the vulvar scale is small.

Acknowledgment

This study was supported by Grant no. 09D05704001 (4884) from Hacettepe University Scientific Research Centre.

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Review Article

A Review of the Biology of Cerataphidini (Hemiptera, Aphididae, Hormaphidinae), Focusing Mainly on Their Life Cycles, Gall Formation, and Soldiers

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Received 22 September 2010; Accepted 8 December 2010

Academic Editor: Ai-Ping Liang

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Species of the aphid tribe Cerataphidini (Hormaphidinae) form galls of various shapes on *Styrax* trees, their primary host, throughout East and Southeast Asia including tropical rainforests. All known species of the tribe produce second-instar sterile soldiers on the primary host and some also produce first-instar sterile soldiers on the secondary host. Here, we review their complicated life cycles with or without host alternation, the formation process of their remarkable galls (flower-like multiple-cavity galls in particular), and all morphs including soldiers. The life cycles of cerataphidines are basically the same as those of the subfamily Eriosomatinae, but in tropical and subtropical regions their life cycles are not very rigidly tuned to seasonal changes in the climate if any. In addition, cerataphidine galls in these regions last at least several months, or at times even for over one year; thus it often takes longer than one year to complete their life cycles.

1. Introduction

The tribe Cerataphidini is an aphid group of approximately 90 species [1], whose members produce sterile soldiers [2, 3]. So far as is known, all species produce sterile second-instar soldiers in their galls on the primary host, and some species also produce sterile first-instar soldiers in their open colonies on the secondary host [4–7]. They induce remarkable galls on trees of the genus *Styrax* (Styracaceae), which become huge (up to 35 cm across) in some species [8–11]. For these reasons, the group has recently attracted much attention from researchers both inside and outside aphidology [12–14]. However, although some good reviews of aphid soldiers in general have been published [6, 7, 15], no extensive review of the life cycles of Cerataphidini (nor of their gall formation) has been available to date. Because their life cycles and the process of gall formation are complicated, it can be difficult for non-aphidologists (and even aphidologists, too) to gain a thorough understanding of their biology. In this review, we first focus on the life cycles and illustrate their various

kinds in temperate, subtropical, and tropical regions. Second, we focus on how their galls, their flower-like multiple-cavity galls in particular, are formed by the aphids and explain the hypothesis that the aphids may exploit the mechanism of flower formation in the host plant for their gall formation. Third, we present a review of aphid morphs that appear in the life cycles. In this section, behavioral aspects of soldiers are reviewed and discussed.

2. General Features of the Tribe Cerataphidini

2.1. Taxonomic Position. The tribe Cerataphidini belongs to the gall-forming subfamily Hormaphidinae. The Hormaphidinae is a sister group of another gall-forming subfamily, the Eriosomatinae (formerly called “Pemphiginae”) [50] (but see also [51]), whose life cycles have been studied better [52–54] because many species are distributed in Europe and North America. The Hormaphidinae consists of three tribes, Cerataphidini, Hormaphidini, and Nipponaphidini,

TABLE 1: Primary hosts of Cerataphidini.

Cerataphidine genus	<i>Styrax</i> species	<i>Styrax</i> series
<i>Astegopteryx</i>	<i>S. suberifolius</i> [16], <i>S. benzoides</i> [17], <i>S. benzoin</i> [18, 19]	<i>Benzoin</i>
<i>Ceratovacuna</i>	<i>S. japonicus</i> [20, 21], <i>S. formosanus</i> [22], <i>S. obassia</i> [23, 24], <i>S. tonkinensis</i> ¹ , <i>S. serrulatus</i> ² , <i>S. paralleloneurus</i> [25]	<i>Cyrta</i> , <i>Benzoin</i>
<i>Pseudoregma</i>	<i>S. suberifolius</i> [26, 27], <i>S. benzoides</i> [28], <i>S. paralleloneurus</i> [29]	<i>Benzoin</i>
<i>Ceratoglyphina</i>	<i>S. suberifolius</i> [30], <i>S. ?benzoin</i> [31], <i>S. fraserensis</i> [32], <i>S. paralleloneurus</i> [31]	<i>Benzoin</i>
<i>Chaitoregma</i>	Unknown	
<i>Cerataphis</i> (with <i>Buchnera</i>)	<i>S. suberifolius</i> [33], <i>S. subpaniculatus</i> [10]	<i>Benzoin</i> , <i>Cyrta</i>
<i>Cerataphis</i> (without <i>Buchnera</i>)	<i>S. benzoides</i> ³ , <i>S. benzoin</i> [34], <i>S. suberifolius</i> [35]	<i>Benzoin</i>
<i>Tuberaphis</i>	<i>S. japonicus</i> [36], <i>S. formosanus</i> [37, 38], <i>S. obassia</i> [39], <i>S. tonkinensis</i> [11], <i>S. subpaniculatus</i> [40, 41]	<i>Cyrta</i>
<i>Glyphinaphis</i>	Unknown	

¹The junior author found many galls of *Ceratovacuna* sp. formed on *Styrax tonkinensis* at Nangoa, northern Vietnam, on 11 May 1997.

²We examined a gall of *Ceratovacuna* sp. collected from *Styrax serrulatus* by P. W. Fritsch at Ithum Khola River (5000 ft alt.), eastern Nepal, in September 1994.

³We found some galls of *Cerataphis* sp. near *brasiliensis* on *Styrax benzoides* in Chiang Mai, northern Thailand, on 3 November 2002.

TABLE 2: Secondary hosts of Cerataphidini.

Cerataphidine genus	Plants
<i>Astegopteryx</i>	Gramineae (Bambusoidea, grass) [42], Zingiberaceae [42], Palmae [42], Pandanaceae [42], Musaceae [43, 44]
<i>Ceratovacuna</i>	Gramineae (Bambusoidea, grass) [42]
<i>Pseudoregma</i>	Gramineae (Bambusoidea, grass) [42], Zingiberaceae [42]
<i>Ceratoglyphina</i>	Gramineae (Bambusoidea) [42]
<i>Chaitoregma</i>	Gramineae (Bambusoidea) [45]
<i>Cerataphis</i>	Gramineae (Bambusoidea) [46], Palmae [42], Pandanaceae [42], Orchidaceae [42], Araceae [42], Strelitziaceae [47], Smilacaceae [48], Moraceae [43], Zingiberaceae ¹
<i>Tuberaphis</i>	Loranthaceae [42], Santalaceae [49]
<i>Glyphinaphis</i>	Gramineae (Bambusoidea) [42]

¹We examined specimens (apterous adults and nymphs) of *Cerataphis* sp. collected from a plant of Zingiberaceae by T. Fukatsu at Genting Highland, West Malaysia, on 17 November 1995.

in addition to a few genera (*Aleurodaphis*, *Doraphis*, *Protohormaphis*, and *Tsugaphis*) whose taxonomic positions are unclear within the subfamily and which have been tentatively assigned to some of the three tribes. The members within each tribe are well unified in the sense that the primary hosts of Hormaphidini, Nipponaphidini, and Cerataphidini are confined to a single plant genus, *Hamamelis* (Hamamelidaceae), *Distylium* (Hamamelidaceae), and *Styrax* (Styracaceae), respectively. (Galls of some nipponaphidines have recently been found on trees of the genera *Distyliopsis* and *Sycopsis* (Hamamelidaceae) in Taiwan [55].)

2.2. *Genera within the Cerataphidini.* The Cerataphidini consists of the following eight genera, *Astegopteryx*, *Ceratovacuna*, *Pseudoregma*, *Ceratoglyphina*, *Chaitoregma*, *Cerataphis*,

Tuberaphis, and *Glyphinaphis*. The genus *Aleurodaphis* was once placed in this tribe [36, 42], but molecular data did not support this placement [6, 56]. In addition, one species of *Aleurodaphis* was found to induce galls on *Stewartia monadelphica* (Theaceae) [57], not on *Styrax*. The monotypic genus *Doraphis* has also been placed in the Cerataphidini [1, 54], perhaps because the second generation on *Populus* has a pair of frontal horns. However, its life cycle [52] is similar to those of *Hamamelistes* species (Hormaphidini) [58, 59] in that coccidiform first-instar nymphs hibernate on twigs of the host tree, and the genus is unlikely to belong to the Cerataphidini.

Within the Cerataphidini, the first four genera (*Astegopteryx*, *Ceratovacuna*, *Pseudoregma*, and *Ceratoglyphina*) are known to induce peculiar, multiple-cavity galls and, together with *Chaitoregma* whose galls are unknown, constitute a monophyletic clade [20, 60]. This has been confirmed by molecular phylogenetic analyses [18, 31, 56, 61]. The genera *Cerataphis* and *Tuberaphis* form single-cavity galls as many other gall aphids do. Galls of *Glyphinaphis* are yet unknown. So far as is known, all species of *Tuberaphis* and *Glyphinaphis* and most (but not all) species of *Cerataphis* harbor extracellular, eukaryotic symbionts instead of the prokaryote *Buchnera* [60]. If the acquisition of the extracellular symbionts happened only once in the lineage, the species group with the extracellular symbionts (*Tuberaphis*, *Glyphinaphis*, and some *Cerataphis*) constitutes a monophyletic clade, but the genus *Cerataphis*, which includes species with extracellular symbionts (e.g., *C. brasiliensis*, *C. jamuritsu*) and those without them (*C. vandermeermohri*, *C. bambusifoliae*), is not [33]. Molecular phylogenetic analyses have not yet definitely settled the issue [18, 31, 56, 61, 62].

2.3. *Geographic Distribution.* Cerataphidines are mainly distributed in East and Southeast Asia. They induce galls on trees of the genus *Styrax*, their primary host. All known

primary-host generations have been found there. The northernmost record is of *Tuberaphis styraci* on *Styrax obassia* in Sapporo (43°N), Hokkaido, northern Japan [63]. The southernmost record is in Java [42, 64]. The westernmost record is in eastern India or Nepal (see footnote 2 of Table 1), and the eastern border is approximately the line from Japan through Sulawesi [65] to Java. Trees of the genus *Styrax* are also distributed in the New World, and the species occurring in eastern North America (e.g., *S. americanus*, *S. grandifolius*) belong to the series *Cyrta* (Section 2.4). The series includes Asian *Styrax japonicus* and *S. obassia* [66, 67], which are used as the primary hosts of several cerataphidines. Fritsch [66] suggests that a single vicariance event between eastern Asia and eastern North America accounts for the intercontinental disjuncts in the series *Cyrta*. The absence of cerataphidine galls in the New World therefore suggests that cerataphidines went into temperate regions of Asia in a relatively recent era, after the series *Cyrta* was divided into the two continental groups.

Like many other aphids, most cerataphidines can live on the secondary host throughout the year. Some cerataphidines are thereby distributed beyond the range of the primary host. *Cerataphis brasiliensis* (on palms) and *Pseudoregma panicola* (on grass) have a pan-tropical distribution [68] and probably have recently invaded the New World, Africa and Australia. *Cerataphis brasiliensis*, *C. lataniae* (on palms), and *C. orchidearum* (on orchids) often occur in the greenhouse in temperate regions [69–71].

2.4. Primary Hosts. The primary hosts of cerataphidines are trees of the genus *Styrax*. According to Fritsch [66], the genus is divided into two sections, *Styrax* and *Valvatae*. Species of the section *Styrax* are temperate (or tropical) deciduous trees, while those of *Valvatae* are tropical evergreen. The section *Styrax* is further divided into two series, *Styrax* and *Cyrta*. Both have disjunct distributions between the Old and New worlds. Species of the series *Styrax* are known from western North America and western Eurasia, and those of the series *Cyrta* from eastern Asia and eastern North America. The section *Valvatae* is also divided into two series, *Valvatae* and *Benzoin*. The former is a neotropical clade, while the latter is a paleotropical clade, occurring in Southeast Asia. Cerataphidines are associated with the series *Cyrta* and *Benzoin*. So far, the aphid genera *Pseudoregma*, *Astegopteryx*, and *Ceratoglyphina* have been known from *Benzoin*, *Tuberaphis* from *Cyrta*, and *Cerataphis* and *Ceratovacuna* from both series (Table 1). Some cerataphidine species form galls on more than one *Styrax* species within a single series (e.g., *Ceratovacuna nekoashi* on *Styrax japonicus* [21], *S. formosanus* [22], and *S. obassia* [23, 24]), but no cerataphidine species has been known from *Styrax* species across series.

2.5. Secondary Hosts. The secondary hosts of the Cerataphidini are wider in range than their primary hosts and include several unrelated plant families (Table 2). All the genera but *Tuberaphis* contain at least one species that uses Bambusoidea (*Bambusa*, *Dendrocalamus*, *Gigantochloa*, *Pleioblastus*,

Sasa, etc.) as the secondary host. Palmae (palms) and/or Zingiberaceae (gingers) are also often used by tropical species of the genera *Pseudoregma*, *Astegopteryx*, and *Cerataphis*. The genus *Tuberaphis* is peculiar in that its secondary hosts are confined to mistletoes of the families Santalaceae (*Viscum*) [49] and Loranthaceae (*Loranthus*, *Scurrula*, *Dendrophthoe*, and *Macrosolen*) [42].

3. Life Cycles

Life cycles of the Cerataphidini can be classified into (1) host-alternating (heteroecious), (2) non-host-alternating (monoecious) life cycles (on the primary host), and (3) anholocycly (on the secondary host) (Figure 1). Host alternation can further be classified into (Figure 1(a)) obligate host-alternation and (Figure 1(b)) facultative host alternation. In obligate host alternation, aphids on the secondary host perish after alates (sexuparae) have flown back to the primary host. In facultative host alternation, some aphids survive on the secondary host after sexuparae fly away. From facultative host-alternating life cycles, anholocycly on the secondary host can be easily derived. In an anholocyclic species or population, aphids can persist on the secondary host over many years without sex; alate sexuparae may or may not be produced, but they are functionless and do not pass their genes to subsequent generations. Non-host-alternating life cycles on the primary host are known to occur sporadically within the Cerataphidini: one species in *Astegopteryx* [17], one in *Ceratoglyphina* [72], and three in *Tuberaphis* [11, 39, 40]. In some species with host-alternating or non-host-alternating life cycles, it takes longer than one year to complete their life cycles; their galls grow slowly and do not produce any alates within the first year ([30, 33, 73], see also [74]). In the following, we describe examples of such life cycles in temperate, subtropical, and tropical Asia.

3.1. Obligate Host Alternation in Temperate Asia. The life cycle of a temperate species, *Ceratovacuna nekoashi*, is easy to understand if readers have some knowledge about the life cycles of Eriosomatinae such as *Pemphigus bursarius* [75, 76] or *Tetraneura ulmi* [77]. In Tokyo and its vicinity, fundatrices of *C. nekoashi* hatch in spring and transform a lateral bud of a developing shoot of the deciduous snowbell *Styrax japonicus* into a characteristic “cat’s-paw” gall [21] (Figure 2(a)). How such a gall is formed is explained in Section 4.2.4. The fundatrix is followed by one or two generations of apterous adults in the gall. Some of the offspring of these apterous adults grow into second-instar sterile soldiers that play a defensive role. From July onward, alates called “emigrants” develop in the gall, and they fly to the grass *Microstegium vimineum*, their secondary host, and give birth to first-instar offspring on the undersides of its leaves. The nymphs and adults of the secondary-host generation (called “exules”; see Section 5.4) (Figure 2(b)) are characterized by a pair of frontal horns. In October, alates called “sexuparae” (Section 5.5) appear on *M. vimineum* and fly back to *S. japonicus*. They deposit tiny sexuals (males and oviparous females) on the undersides of snowbell leaves.

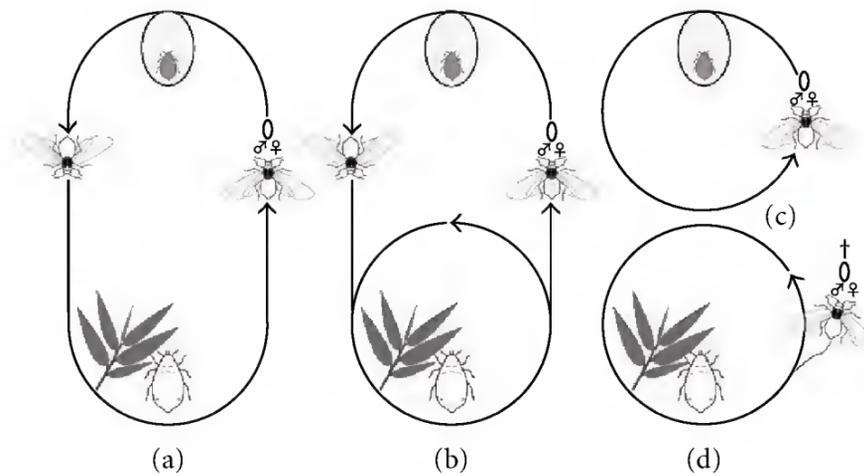


FIGURE 1: Life cycles of cerataphidines. Gall generation (upper) and secondary-host generation (lower), together with alates (emigrant and sexupara), are schematically shown. (a,b) Host-alternating (heteroecious) life cycle: in obligate host alternation (a) aphids cannot persist without returning to the primary host, while in facultative host alternation (b) aphids can survive on the secondary host throughout the year. (c) Non-host-alternating (monoecious) life cycle on the primary host; alate sexuparae produced in the gall fly to the primary host. (d) Anholocycle on the secondary host; alate sexuparae (if any) are functionless.

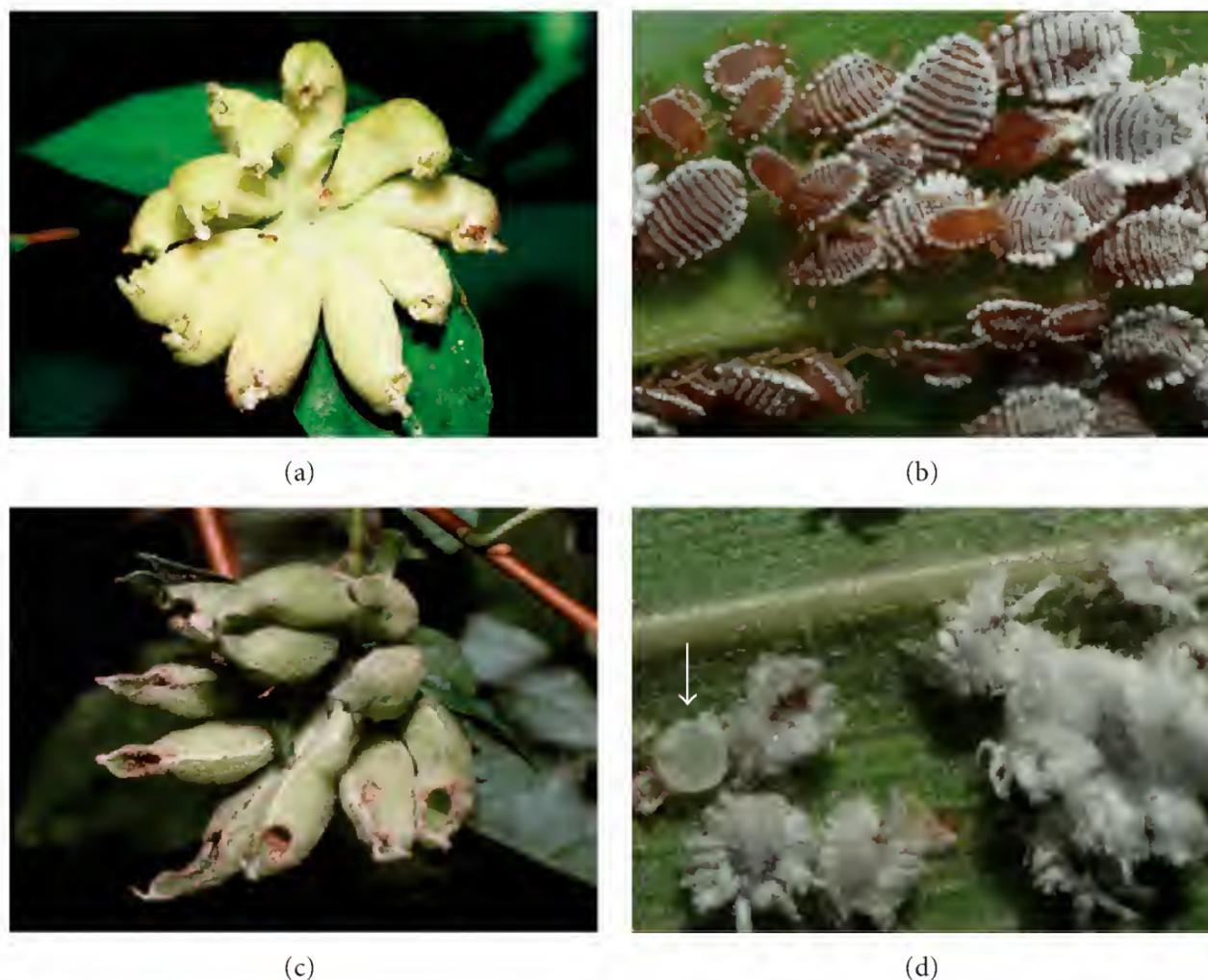


FIGURE 2: (a) A gall of *Ceratovacuna nekoashi* on *Styrax japonicus* (Seoul, South Korea; 3 August 1998). (b) A colony of *C. nekoashi* on the grass *Microstegium vimineum* (Niiza, Japan; 12 August 2009). (c) A gall of *Ceratovacuna japonica* on *S. japonicus* (Niiza; September 1990; photo by D. L. Stern). (d) A colony of *C. japonica* on the bambusoid *Pleioblastus chino*; an egg of the predaceous lycaenid *Taraka hamada* (indicated by an arrow) is laid in the colony (Kumagaya, Japan; 24 October 2009).

The sexuals move to branches and mature in the fissures of bark without feeding [78]. There, they copulate and females each lay a single egg that overwinters [79, 80].

Because *Microstegium vimineum* is an annual grass, no aphids persist on the secondary host during winter. In this sense, its host alternation is obligate (Figure 1(a)).

3.2. *Facultative Host Alternation in Temperate Asia.* The life cycle of another temperate species, *Ceratovacuna japonica*, is

almost the same as that of *C. nekoashi*. Fundatrices induce similar banana-bunch-shaped galls (Figure 2(c)) on *Styrax japonicus* in a similar manner [20]. In central Japan, alates appear in the gall from July onward and fly to secondary hosts, small bambusoids such as *Pleioblastus chino* [81]. Alate sexuparae appear in October and fly back to *S. japonicus*.

Exules, or secondary-host generations, have also been recorded from *Pleioblastus simonii* (as “*Arundinaria*” *simonii* [82]) and *Sasa senanensis* [83]. These plants belong to

the Bambusoidea and are perennials. Exules of *Ceratovacuna japonica* (Figure 2(d)) propagate themselves by parthenogenesis throughout the year on these perennial secondary hosts. In this sense, host alternation of this species is facultative (Figure 1(b)). In some populations, colonies on the secondary host produce few alate sexuparae in autumn, and galls of this species are much rarer than those of *C. nekoashi* in and around Tokyo [20] despite the fact that colonies on *Pleioblastus chino* are rather commonly found there [84]. The exules are cold-tolerant. They reproduce even during winter in the Kanto District, and a colony containing apterous adults was found on leaves of *Sasa senanensis* above snow cover by Hitoshi Hasegawa (personal communication) at Hida Osaka, Gifu Prefecture, in February (recorded as *Ceratovacuna* “sp. A” [85]).

In colonies on the secondary host, *Ceratovacuna japonica* produces sterile, first-instar “pseudoscorpion-like” soldiers [83, 84]. The presence of soldiers may contribute to the persistence of colonies on the secondary host. The species does not produce alates that disperse between the secondary hosts (called “secondary migrants”; see Section 5.6). Instead of alates, first-instar nymphs disperse on the wind [84].

3.3. Anholocycly in Temperate Asia. Anholocycly is a life cycle without sexual reproduction. In Hormaphidinae, anholocycly occurs only on the secondary host. *Ceratovacuna cerbera*, which is known from southern Korea and central Japan, lives parthenogenetically throughout the year on *Sasa* spp. including *S. borealis* and *S. veichii* [86]. At the Shomaru Pass (36°N), central Japan, alates appear in October/November. However, they are not sexuparae that would fly to the primary host but secondary migrants that fly to another plant of the secondary host. Because no sexuparae are produced, the life cycle is anholocyclic there. This does not exclude the possibility that sexual reproduction might occur elsewhere. Like *C. japonica* (Section 3.2), *C. cerbera* produces sterile first-instar soldiers [86].

3.4. Facultative Host Alternation in Subtropical Asia. In subtropical regions such as Taiwan, seasonal changes are still pronounced, but the climate has a mild winter, during which aphids can reproduce. The life cycles of cerataphidines show seasonality there. Here in after we describe three facultatively host-alternating species native to this region, *Astegopteryx bambucifoliae*, *Pseudoregma bambucicola*, and *Ceratoglyphina styracicola*, all of which form galls on the evergreen *Styrax suberifolius* and migrate to Bambusoidea.

3.4.1. *Astegopteryx bambucifoliae*. Secondary-host generations, or exules, of *Astegopteryx bambucifoliae* are yellowish aphids with green patches (Figure 3(b)) and known as pests of economically important bamboos such as *Dendrocalamus latiflorus* and *Bambusa oldami* [45]. Their colonies are formed on the undersides of bamboo leaves and individual aphids are more or less spaced out from each other [87]. Unlike the temperate species mentioned above, colonies of *A. bambucifoliae* produce many alate sexuparae in spring (from February to May) instead of autumn. They fly to leaves of

Styrax suberifolius and deposit sexuals on the undersides. These first-instar sexuals soon leave the leaves and hide in narrow spaces such as in unfolding buds of the host tree, in old dead galls of cerataphidines, or even in unfolding buds of a non-host vine coiling the host tree [16]. They mature and copulate, and females each lay a single egg. First-instar fundatrices soon hatch from the eggs and induce galls (Figure 3(a)) on stems of developing shoots of *S. suberifolius*. Kurosu and Aoki [16] guessed that gall formation takes place in June in Puli (23°N), central Taiwan. However, incipient galls are seen from May to July or sometimes even in September in Taipei (25°N) [88]; thus the gall-forming period is longer in this species than in temperate species. Some galls mature as early as late July and, after one or two generations of apterous adults, produce alates (emigrants) that migrate to bamboos [16, 89]. Live galls with alates are commonly found until December, and some until February [88]. Migration to bamboos therefore lasts over several months.

3.4.2. *Pseudoregma bambucicola*. Secondary-host generations of *Pseudoregma bambucicola* form dense colonies on bamboo shoots and twigs of *Bambusa* spp. The aphids are grayish brown in color, and the colony produces many first-instar, pseudoscorpion-like soldiers. In southern Japan (the Ryukyus, Kyushu and Shikoku), where the aphids on *Bambusa multiplex* have been studied by many researchers [90–99], the life cycle is anholocyclic (Figure 1(d))—even though the colonies produce many sexuparae in autumn—because its primary host, *Styrax suberifolius*, is absent.

In Taiwan, colonies of *Pseudoregma bambucicola* on bamboo produce alate sexuparae from November to February [88]. They fly to *Styrax suberifolius*, and their grandoffspring, fundatrices, transform flower buds into galls [100]. Incipient or very young galls (Figure 8(c)) have been found in May in both Taipei [88] and Puli [100]. Mature galls containing alates (emigrants) are found from early July to September in Puli [26]. Recent researches in Taipei revealed that some galls of *P. bambucicola* even last until May of the next year, or for almost one year [88]. The production of (second-instar) soldiers in their galls [26] certainly contributes to the longevity of the galls. In the next section, we discuss a species whose galls last far longer than galls of *P. bambucicola*.

3.4.3. *Ceratoglyphina styracicola*. This aphid species has been recorded from Taiwan under the name of “*Ceratoglyphina bambusae*” [45, 89, 101–107]. However, the true *C. bambusae* is a tropical species, forming galls on *Styrax fraserensis* in the Malay Peninsula [31, 32]; its secondary-host generations (on bamboo) have been recorded from Sulawesi, Java, Sumatra, and the Malay Peninsula [32, 42, 54], but not in Taiwan. Mature galls (Figure 3(c)) of *Ceratoglyphina styracicola* are coated with wax and look entirely white, and the structure is very peculiar among aphid galls. Each gall consists of from one to a few subgalls that each look like a cauliflower’s head. Ramified coral-like projections develop from the inner wall of each subgall, and they outgrow the original subgall’s cavity to form the “head” outward [30]. Numerous aphids reside



FIGURE 3: (a) A gall of *Astegopteryx bambucifoliae* (with many subgalls) on *Styrax suberifolius* (Sun Moon Lake, Taiwan; 25 September 1990). (b) A colony of *A. bambucifoliae* on a leaf of *Bambusa* sp.; a dueling pair is indicated by an arrow (Fushan, Taiwan; 22 April 2005). (c) A full grown gall of *Ceratoglyphina styracicola* (with two subgalls) on *S. suberifolius* (Sun Moon Lake; 13 December 1990). (d) A colony of *C. styracicola* on the bambusoid *Pleioblastus* sp. (Sun Moon Lake; 24 April 2005).

among these ramified projections. A large gall may contain 100,000 aphids, approximately half of which are soldiers [108]. Many soldiers reside on the outer surface of the gall, which is coated completely with wax, probably due to the soldiers' activity. These soldiers are highly aggressive, and pierce human skin with their stylets to cause troublesome irritation. When the colony size becomes huge, they even readily fall off the gall in response to disturbance [2, 108, 109].

The gall of *Ceratoglyphina styracicola* is initially formed by a single fundatrix on the stem of a developing shoot (Figure 8(b)) in July (or perhaps also in August) around Sun Moon Lake, central Taiwan. The gall grows very slowly and produces no alates within the first year. From the end of November of the next year, 16 months after the gall formation, the gall begins to produce alates that migrate to the bambusoid *Pleioblastus* sp. [89]. Once the production of alates begins, the gall continues to produce alates until its death, until the end of May at the latest. Thus, galls of *C. styracicola* can last for up to 23 months, although many die before. One young gall marked on 9 September 1992 was found alive on 25 May 1994, thus actually having lasted for 623 days, or about 20.5 months [30].

Migration from the *Styrax* galls to the secondary host occurs from the end of November to the end of May. On the other hand, colonies of *C. styracicola* on the secondary host (Figure 3(d)) produce many alate sexuparae around

Sun Moon Lake in the end of May and in June, which fly back to *Styrax suberifolius*. Some colonies on *Pleioblastus* sp. are found in September; so they probably also persist on the secondary host throughout the year. A few alate sexuparae have been found sporadically on the secondary host in December, February, and July [30]. It is unknown whether eggs produced in these months remain unhatched until July, or whether these alates are functionless.

3.5. Anholocycly in Subtropical and Tropical Asia. The sugarcane woolly aphid, *Ceratovacuna lanigera*, is a pest of sugarcane (*Saccharum* spp.) and is widely distributed in tropical and subtropical regions of East Asia [70, 110]. The aphids also form colonies on leaves of the Chinese silver grass, *Miscanthus sinensis* [111, 112]. (In Taiwan, Takahashi [111] transferred apterae of *C. lanigera* from sugarcane to *Miscanthus* grass and vice versa “with very successful results.”) Its distribution is extended to such a temperate region as Ichihara (36°N), central Japan, where the aphids overwinter on *M. sinensis* outdoors [113]. *Ceratovacuna lanigera* is also famous for its defensive behavior, which will be treated in Section 5.4.6. Apterous adults (exules) of *Ceratovacuna lanigera* reproduce parthenogenetically throughout the year, and many alates are produced in various months [112, 114]. Most of these alates are secondary migrants (i.e., migrants between sugarcane or *Miscanthus* grasses). Alate sexuparae have been recorded in central Japan in

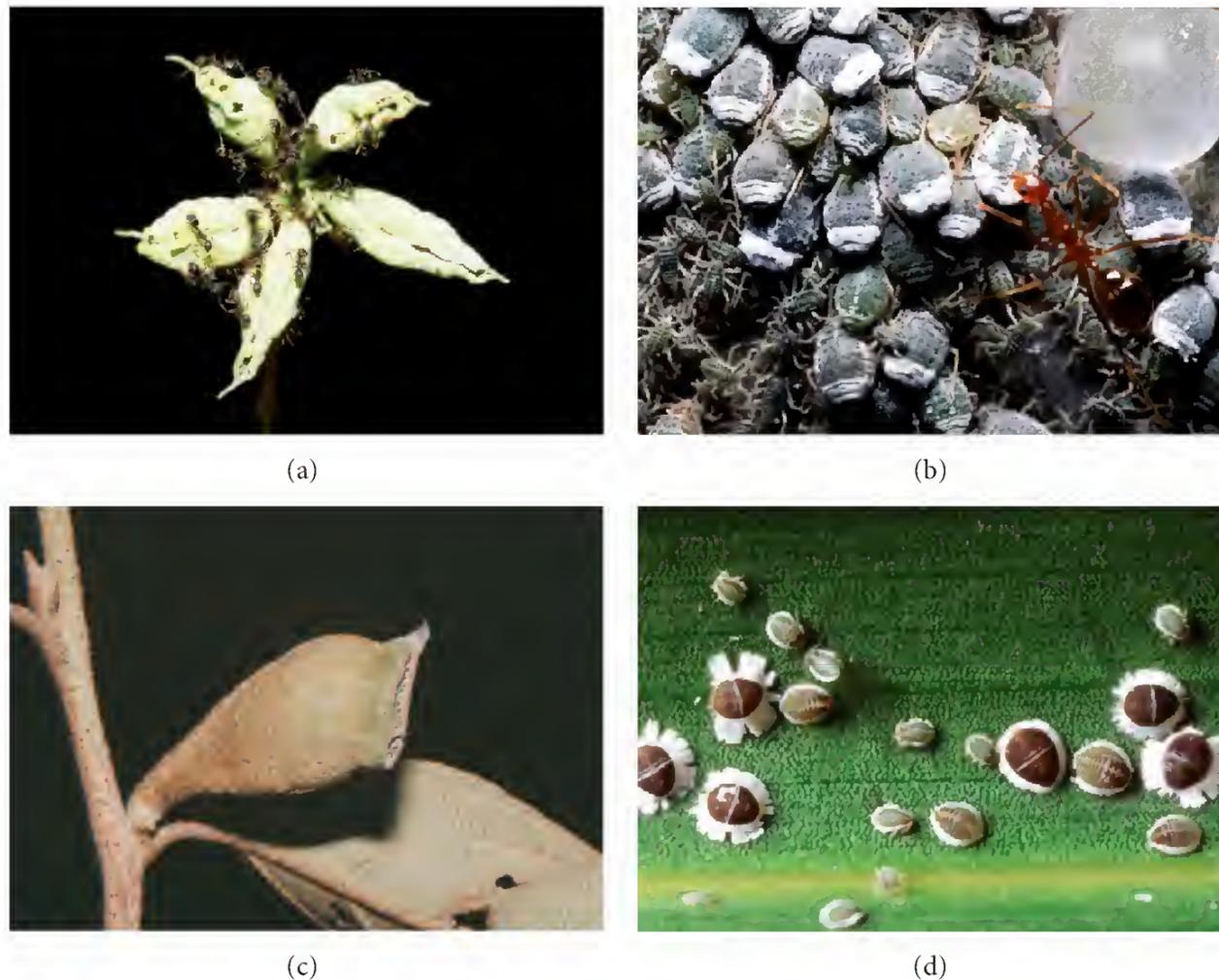


FIGURE 4: (a) A gall of *Pseudoregma carolinensis* on *Styrax benzoides*, with ants of *Dolichoderus* sp. (Chiang Mai, Thailand; 3 August 2000). (b) A colony of *P. carolinensis* on a bamboo shoot (Mae Sa, Chiang Mai Province, Thailand; 7 August 2000). (c) A gall of *Cerataphis brasiliensis* on *Styrax benzoin* (Tarutung, Sumatra; 3 March 1994). (d) A colony of *C. brasiliensis* on the palm *Areca catechu* (Bandar Baru, Sumatra; 28 August 1997).

autumn [115], and in Okinawa, southern Japan, from October to January and from April to June [116]. Although galls and associated generations are unknown, the species might retain its primary-host generations somewhere.

3.6. Facultative Host Alternation in Tropical Monsoon Asia.

Until recently the tropical bamboo aphid *Pseudoregma carolinensis* has been confused with the subtropical aphid *P. bambucicola* [117, 118]. *Pseudoregma carolinensis* is widely distributed in tropical Asia from Micronesia to India (recorded as “*Oregma bambusae*” [119] or “*P. bucktoni*” [120, 121]) and forms dense colonies on twigs and shoots of bamboo (Figure 4(b)). Its primary-host generation was found on the evergreen *Styrax benzoides* in and around Chiang Mai (19°N), northern Thailand, where the rainy season (from the end of May to November) and the dry season (from December to the beginning of May) regularly alternate every year. Mature galls of *P. carolinensis* (Figure 4(a)), containing alate emigrants, were found in August [28]. Because the gall of *P. carolinensis* is made of a flower bud, the fundatrices initiate galls when flower buds appear, probably near the end of the dry season or early in the rainy season. Galls may not last for long after August, for no galls were found in November. It is very likely that its life cycle shows a clear seasonal pattern in northern Thailand.

In contrast to the gall generations, colonies on bamboo are found throughout the year, and alate sexuparae have been

recorded in Chiang Mai Province in November and December. We also found some alate sexuparae in March in Chiang Mai (our unpublished observations). These sexuparae fly back to *Styrax benzoides*. It is not yet known whether early-produced eggs enter diapause or not.

As mentioned above, *P. carolinensis* is widely distributed in tropical regions of East Asia, beyond the range of *Styrax benzoides*. In Java, where the life cycle is probably anholocyclic, secondary migrants that disperse between bamboos occur [42].

In addition to *P. carolinensis*, two other species that form galls on *Styrax benzoides*, *Astegopteryx bambusae* and *A. singaporensis*, have facultatively host-alternating life cycles in tropical monsoon Asia (our observations in Chiang Mai). Their galls probably do not last beyond the severely dry months of March and April in northern Thailand.

3.7. Facultative Host Alternation in Equatorial Asia. In tropical rainforests of the Malay Peninsula and Sumatra, many species of cerataphidines form galls of various shapes on *Styrax* species, *S. benzoin* (Figures 4(c), 9, and 11(b)), *S. paralleloneurus* (Figures 6(c), 6(d), 12(b)), *S. subpaniculatus* (Figures 7(a)–7(c)), and so forth. [8, 122, 123]. Also, their secondary-host generations are commonly seen on bamboos, palms, gingers, pothos, climbing pandanus, and mistletoes in and around the forest (our observations in Bandar Baru, Sumatra; see also [43]). The diversity and abundance of

cerataphidines in these regions belie the widely held dogma of a paucity of aphids in the tropics (cf. [124, 125]). Most of these gall-forming cerataphidines have host-alternating life cycles, retaining a sexual generation [10, 19, 29, 31, 34]. However, it is not clear whether their galls are formed all the year round. Both mature and incipient galls of a single species are often found at the same time, but this may be merely because galls of the species last over a long period (cf. Section 3.4.3). Because trees in tropical rainforests are tall in general, no researchers have yet settled the issue by marking galls on *Styrax* trees there.

On its secondary host, *Cerataphis brasiliensis* produces scale-like aphids (Figure 4(d)), which are notorious as a pest of palms [126–128] and are widely distributed in tropical and subtropical regions of the world. The species has been recorded under the names of “*C. palmae*,” “*C. variabilis*,” and “*C. fransseni*,” and, despite the valid species epithet *brasiliensis*, is of Southeast Asian origin. Its galls (Figure 4(c)) have been recorded from the evergreen *Styrax benzoin* in Java, Sumatra, and the Malay Peninsula [34, 42, 43, 129, 130]. The fundatrix of *C. brasiliensis* transforms an axillary bud into its sac-like gall. It is therefore possible for fundatrices of the species to form galls whenever new shoots are developing. In the Malay Peninsula, galls have been recorded from June to August and from October to February [34]; in the other months no census has been made. In Ulu Gombak, Malaysia, incipient galls were found from October to January, together with mature galls [34]. These collection data suggest that galls of *C. brasiliensis* are formed irrespective of the season there. Unfortunately we do not know when alate sexuparae are produced on the secondary host in Malaysia. In Java, sexuparae were collected in September and October. Outside the range of *Styrax benzoin*, where the life cycle should be anholocyclic, sexuparae are still produced and have been collected in March (Sri Lanka), from November to January (Surinam) and in July (Taiwan) [34]. These data are, however, not very informative for inferring the life cycle in the Malay Peninsula. Secondary migrants are commonly produced on palms both inside and outside the range of the primary host.

3.8. Non-Host Alternation on the Primary Host. Non-host alternation in aphids is often referred to as “monoecy.” (The word has a completely different meaning in botany.) In the Hormaphidinae, these “monoecious” or non-host-alternating cycles have probably been derived from “heteroecious” or host-alternating cycles by discarding secondary-host generations in some way. In the tribe Cerataphidini, five species that form galls on *Styrax* complete their life cycles without migrating to the secondary host, while retaining a sexual generation (Figure 1(c)). They are *Tuberaphis styraci* on *Styrax obassia* in Japan [39], *T. leeuweni* on *S. subpaniculatus* in Sumatra [40], *T. owadai* on *S. tonkinensis* in northern Vietnam [11], *Ceratoglyphina roepkei* on *S. paralleloneurus* in Sumatra [72], and *Astegopteryx spinocephala* on *S. benzoides* in northern Thailand [17]. Here we review the biology of three species.

3.8.1. *Tuberaphis styraci*. *Tuberaphis styraci* forms coral-shaped single-cavity galls (Figure 5(a)) on the temperate deciduous *Styrax obassia* in Japan. The monoecious life cycle of *T. styraci* is peculiar in that it is a biennial cycle [73].

Aoki and Kurosu [39, 73] studied the life cycle of this aphid at the Shomaru Pass (36°N, ca. 600 m alt.), the Kanto District, Japan. The first-instar fundatrix hatches in May and forms a small, completely closed gall (Figure 5(b)) on the stem of a developing shoot of *Styrax obassia*. The gall grows slowly, and the fundatrix produces nymphs that become apterous adults. The colony size increases to approximately 30–100 individuals and the gall becomes globular and 4–10 mm in diameter at the end of the year. By that time the fundatrix dies, and the colony contains a number of soldiers but no alates are produced. Some galls have small openings, while others remain closed. After leaves of the host tree fall off, the gall, which looks somewhat like a winter bud of the host tree (Figure 5(c)), survives and overwinters in this stage. The mean temperature in Chichibu City near the Shomaru Pass is 1.6°C during January, the coldest month. (As mentioned in Section 2.3, galls of *T. styraci* were also found in Sapporo, where the mean temperature during January is –4.1°C.) At the Shomaru Pass, leaves of *S. obassia* begin unfolding in April, at which time the overwintered gall of *T. styraci* resumes growing (Figure 5(d)). The gall soon becomes large and coral shaped and has many small, exit holes (Figure 5(a)). The colony size reaches up to 15,000 [39], or 20,000, at times more than half of which are soldiers [131]. From late in July or August onward, alate sexuparae are produced. They fly to the undersides of leaves of *S. obassia* and produce sexuals there. Oviparous females lay eggs that overwinter, probably in fissures in the bark. The aphid colony in the gall can last until mid-October of the second year. The gall withers by the second winter and the dead, lignified gall remains for years on the tree.

We previously presented a hypothesis that this biennial, monoecious life cycle of *Tuberaphis styraci* may have been derived from an annual monoecious cycle [73]. However, it is now known that some host-alternating life cycles of Cerataphidini are accompanied by their long-lasting galls (Section 3.4.3). In the genus *Tuberaphis*, galls of *T. taiwana*, *T. takenouchii*, and *T. coreana*, all of which are host-alternating species, probably last for over one year (our unpublished observations). It is likely that an ancestor of *T. styraci* had such a host-alternating life cycle, and that the present life cycle of *T. styraci* has evolved by discarding the secondary-host generation in some way.

3.8.2. *Astegopteryx spinocephala*. *Astegopteryx spinocephala* forms galls (Figure 6(a)) on the evergreen *Styrax benzoides* in Chiang Mai, northern Thailand, under the tropical monsoon climate. The fundatrix hatches around June, early in the rainy season, and initiates a gall on the stem of a developing shoot [17]. The gall grows slowly and reaches nearly its full size and becomes banana-bunch shaped by November but its subgalls are still closed at that time. In March/April, near the end of the dry season, a small ostiole appears near the tip of each subgall, and several (5 or 6) soldiers cooperate to plug

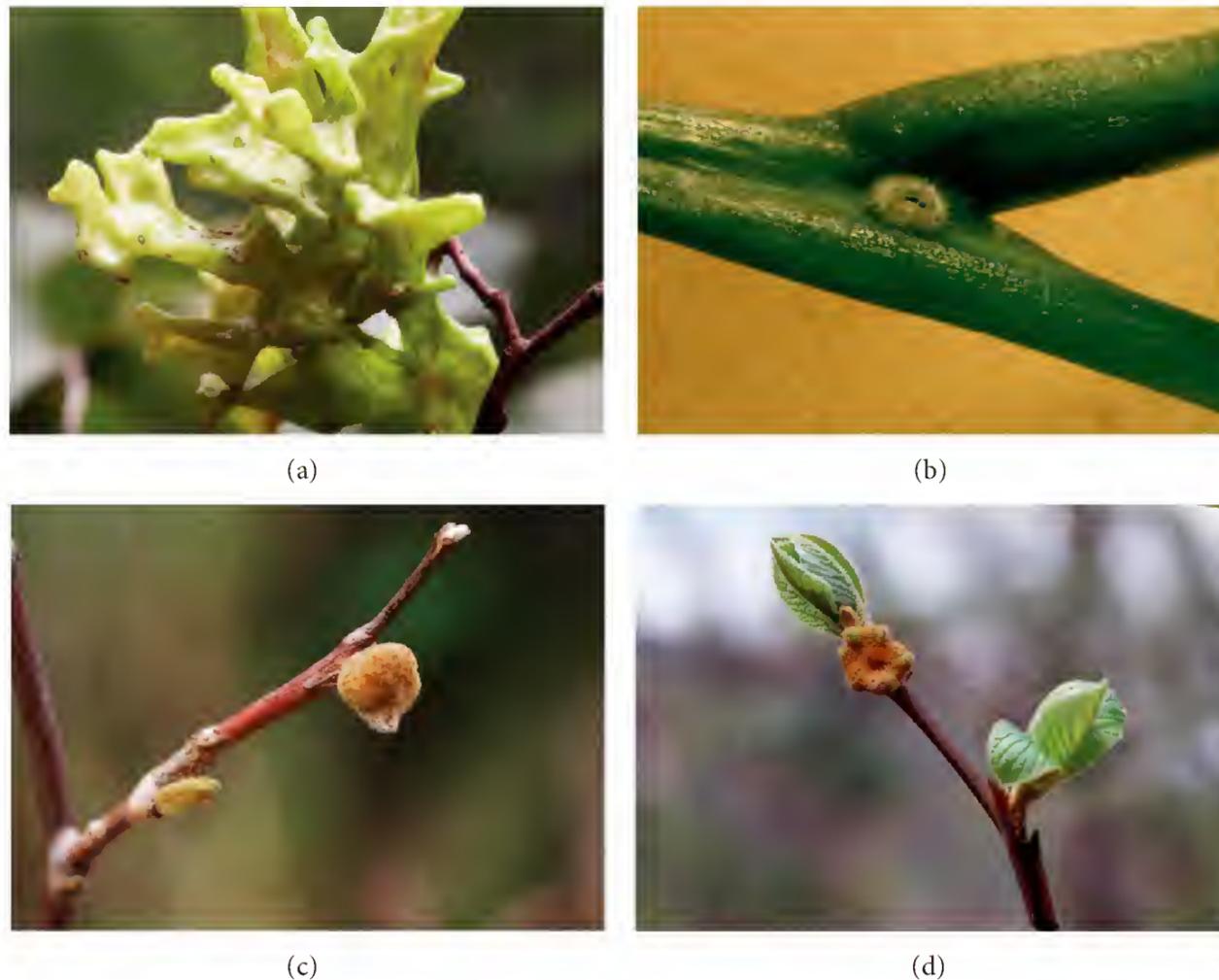


FIGURE 5: Galls of *Tuberaphis styraci* on *Styrax obassia* (Shomaru Pass, Japan). (a) A mature gall (15 July 1996). (b) An incipient (yet unclosed) gall on the stem of a developing shoot (23 May 1986). (c) A hibernating gall and a winter bud which has not yet burst (10 April 1989). (d) A hibernated gall that has resumed growing at the time of bud burst (17 April 1989).

the ostiole with their spiny heads from inside [132]. Alate sexuparae are produced in these months, and they crawl out of the subgall through the ostiole. Also in this season, many eggs (ca. 150–350 eggs per subgall [132], or ca. 2,500 eggs per gall [17]) are laid within subgalls (Figure 6(b)) and soldiers guard the eggs until they hatch in at least some galls. The mating system of *A. spinocephala* is peculiar among gall-forming aphids. Newly emerged sexuparae contain both female and male embryos. They deposit almost all female embryos (and some male embryos) within the natal subgall before flying to leaves of *S. benzoides*. After flying, they deposit the remaining embryos, almost all of which are males, on the undersides of the leaves. These first-instar males intrude into subgalls for mating despite that guarding soldiers try to prevent them from entering [133]. Such a mating system may have evolved because their galls remain viable until the next gall-forming season and can serve as safe concealments for eggs.

3.8.3. *Ceratoglyphina roepkei*. *Ceratoglyphina roepkei* forms one of the most remarkable galls (Figures 6(c) and 6(d)), which looks like a peruke with long curly frizzy hair, on the tropical evergreen *Styrax paralleloneurus* in Sumatra. The gall consists of several long, slender, tubular subgalls; each subgall is spirally twisted and has a longitudinal suture running from the base to the apex [8, 122, 123, 134]. In Bandar Baru near Medan (3°N), mature galls (Figure 6(c)) containing alate sexuparae were found in February [72]. As

in *Astegopteryx spinocephala*, (at least some) eggs are laid within live subgalls (our unpublished observations). Many incipient galls of *C. roepkei* were found on the stems of developing shoots in April. From July to September, we found more-developed, yet immature galls (Figure 6(d)) but neither mature nor incipient galls at the same locality (our unpublished observations). Hence the life cycle of *C. roepkei* is seasonal there. On the other hand, Hille Ris Lambers [134] describes alates of *C. roepkei* based on specimens collected near Adian Koting (2°N), midway from Sibolga to Tarutung, on 17 September 1931. The species therefore might produce alate sexuparae in different seasons at other localities.

4. Gall Formation

As mentioned before (Section 2.2), some groups (*Cerataphis* and *Tuberaphis*) of the Cerataphidini form single-cavity galls [34, 73], which are similar to those formed by Nipponaphidini, Hormaphidini, and other groups of aphids (e.g., [135]) in the basic structure and the process of formation. Others (*Astegopteryx*, *Ceratovacuna*, *Ceratoglyphina*, and *Pseudoregma*) form peculiar, multiple-cavity galls. The process of gall formation in the latter group is unique among aphids and will be explained in detail.

A gall is initiated by a single, first-instar fundatrix. When a fundatrix stimulates an appropriate part of the host plant by its stylets (and possibly also by its legs), plant tissues begin growing to cover the fundatrix and the fundatrix eventually



FIGURE 6: (a) Three galls of *Astegopteryx spinocephala* on *Styrax benzoides* (Chiang Mai, Thailand; 6 April 2000). (b) Cut subgalls of *A. spinocephala* (Chiang Mai; 6 April 2000): a live subgall (left) showing the inside, and another subgall after being submerged in alcohol (right) showing many eggs in its basal part (in the left photo, eggs are hard to see because they are covered with wax). (c) A mature gall of *Ceratoglyphina roepkei* on *Styrax paralleloneurus* (Bandar Baru, Sumatra; 24 February 1994). (d) A young gall of *C. roepkei* on *S. paralleloneurus* (Bandar Baru; 24 August 1997).

is confined in a closed cavity. The fundatrix becomes an adult and produces nymphs by parthenogenesis in the cavity. The colony in a *Styrax* gall therefore is a clone unless nymphs from other galls intrude into it (see Section 5.3.4).

4.1. Formation of Single-Cavity Galls. In the process of forming a single-cavity gall, the fundatrix is enclosed within the cavity until her death. The gall may later be ramified and look like a coral (Figures 5(a) and Figure 12(a)) or a bird nest (Figure 7(a)), but the single cavity is never partitioned into closed cavities (see Figure 7(b)). The gall may be made on the stem of a developing shoot, or of an axillary bud or a flower bud, or a latent bud.

4.1.1. Gall Formed on the Stem of a Developing Shoot. Galls of *Tuberaphis styraci* are formed on stems (Section 3.8.1). The fundatrix of *T. styraci* settles on the stem of a developing shoot of *Styrax obassia*. Soon the fundatrix is enclosed by tissues growing around it (Figure 5(b)). The gall swells later (Figure 5(c)) and in the next year becomes coral shaped (Figures 5(d) and 5(a)).

4.1.2. Gall Formed from a Flower Bud. Galls of *Tuberaphis leeuweni* are slender and tube-like (Figure 7(c)) and formed on inflorescences of *Styrax subpaniculatus* [40]. An illustration of its young galls by Docters van Leeuwen-Reijnvaan and

Docters van Leeuwen [8] clearly indicates that each gall is made of a flower bud. Aoki et al. [40] once mentioned that a calyx-like structure remains at the base of the gall, but we are now inclined to the opinion that the original sepals are abnormally stretched to form the entire gall. This possibility requires further investigation.

4.1.3. Gall Formed from an Axillary Bud. Galls of *Cerataphis brasiliensis* (Figure 4(c)) are initially formed on or from axillary buds of *Styrax benzoin* [34]. These incipient galls are simply enlarged to form sac-like galls. Galls of *Cerataphis bambusifoliae* are formed from axillary buds of *Styrax suberifolius*, probably in a similar way [33].

4.1.4. Gall Formed from a Latent Bud. Galls of *Tuberaphis takenouchii* (Figure 7(d)), which look like heads of broccoli, have exclusively been found on trunks or thick branches of *Styrax japonicus* [36, 136, 137] and *S. formosanus* [31, 37]. Young, small, sac-like galls have also been found on trunks and thick branches of *S. formosanus* in central Taiwan (our unpublished observations), indicating that the galls are formed from latent buds (i.e., those axillary buds whose development has been inhibited for years). Galls of *Cerataphis jamuritsu* are also found on thick branches of *Styrax suberifolius* (Figure 11(c)), suggesting that they are initiated on latent buds of the host tree [35].

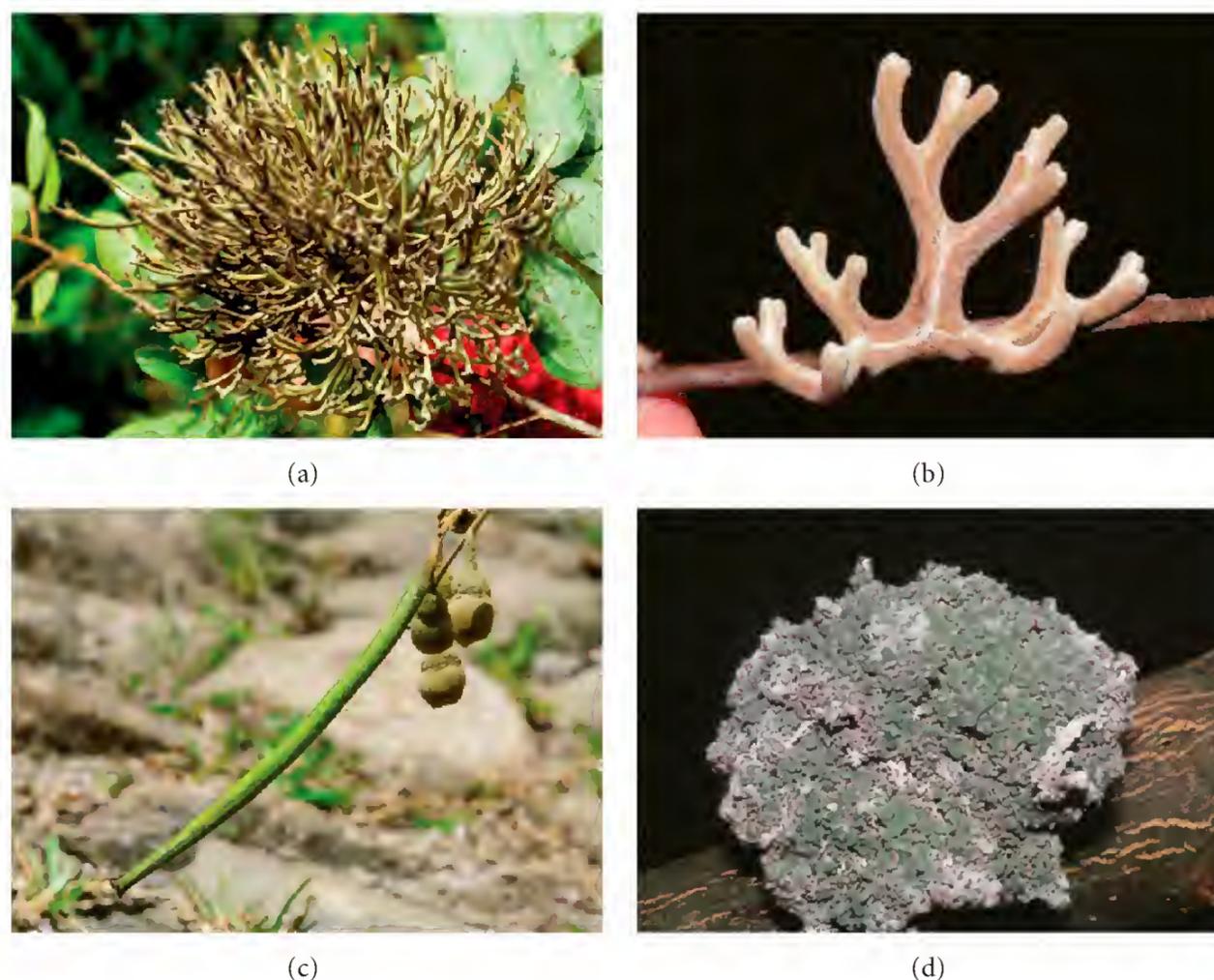


FIGURE 7: (a) A mature, well-ramified gall of *Cerataphis vandermeermohri* on *Styrox subpaniculatus* (Urung Tama, Sumatra; 12 September 1993). (b) A young gall of *C. vandermeermohri* on *S. subpaniculatus*, indicating that the ramified gall has a single cavity (Bandar Baru, Sumatra; 4 August 1996). (c) A tubular gall of *Tuberaphis leeuweni* on *S. subpaniculatus* and fruits of the host tree (Mt Sibayak, Sumatra; 19 September 1993). (d) A gall of *Tuberaphis takenouchii* on *Styrox formosanus* (upside down under natural conditions; Habon, Taiwan; 22 July 1994).

At times, galls of those species that usually form galls from axillary buds (e.g., *Cerataphis brasiliensis*) are found directly connected to thick twigs or branches [34]. This suggests that such species also utilize latent buds if available.

4.2. Formation of Multiple-Cavity Galls. Similar to the initiation of single-cavity galls, in the process of forming a multiple-cavity gall, the fundatrix is initially confined in a single cavity surrounded by lobes (Figures 8(a) and Figure 10(b)). Some of the lobes that enclose the fundatrix, however, are later differentiated into subgalls. First, inside the cavity, a niche appears on each of the lobes destined to be subgalls. At that time, the fundatrix has become an adult and begins to produce first-instar nymphs of the second generation. One or two first-instar nymphs enter each niche and begin feeding. Soon the nymphs in the niche are surrounded by plant tissues and confined in the newly formed cavity. Several subgalls are formed in this way, and the fundatrix is surrounded by the subgalls and a number of slender, solid projections which will not grow further (Figures 8(b) and 8(c)). The entire gall looks like a miniature “xiaolongbao” at this stage (Figure 8(c)). The number of subgalls varies both between and within species, but it is common to all known species that the fundatrix never enters any subgall. In some species (e.g., *Ceratoglyphina*

styracicola), a small pocket is formed at the bottom of the cavity of the entire gall and the fundatrix hides there [104]. As the subgalls, which are closed at this stage, grow outward, the entire gall opens (just as a flower opens from a bud) (Figure 8(d)) and the fundatrix is exposed again and left outside the closed subgalls. The fundatrix may still produce her offspring after all subgalls are closed. These first-instar nymphs cannot enter any subgall and cannot grow to reproduce. They defend the closed subgalls from outside, probably against lepidopteran larvae that could bore into the subgalls [104, 138], and are called “outsiders” (outside defenders).

4.2.1. Gall Formed on the Stem of a Developing Shoot. Multiple-cavity galls of *Astegopteryx bambucifoliae* [16] (Figure 3(a)), *A. spinocephala* [17] (Figure 6(a)), *A. malacensis* [8] (Figure 9(b)), *Ceratoglyphina styracicola* [104] (Figures 3(c) and Figure 8(b)) and *C. roepkei* (Section 3.8.3) (Figures 6(c) and 6(d)) are initially formed on stems of developing shoots of the host trees. Galls of *Astegopteryx basalis* (Figure 9(a)) are formed on the stem under the base of a leaf petiole [8], and those of *A. spinocephala* are sometimes formed under the base of a leaf petiole, too [17]. When these galls mature, they are firmly connected to the twig of the host tree.

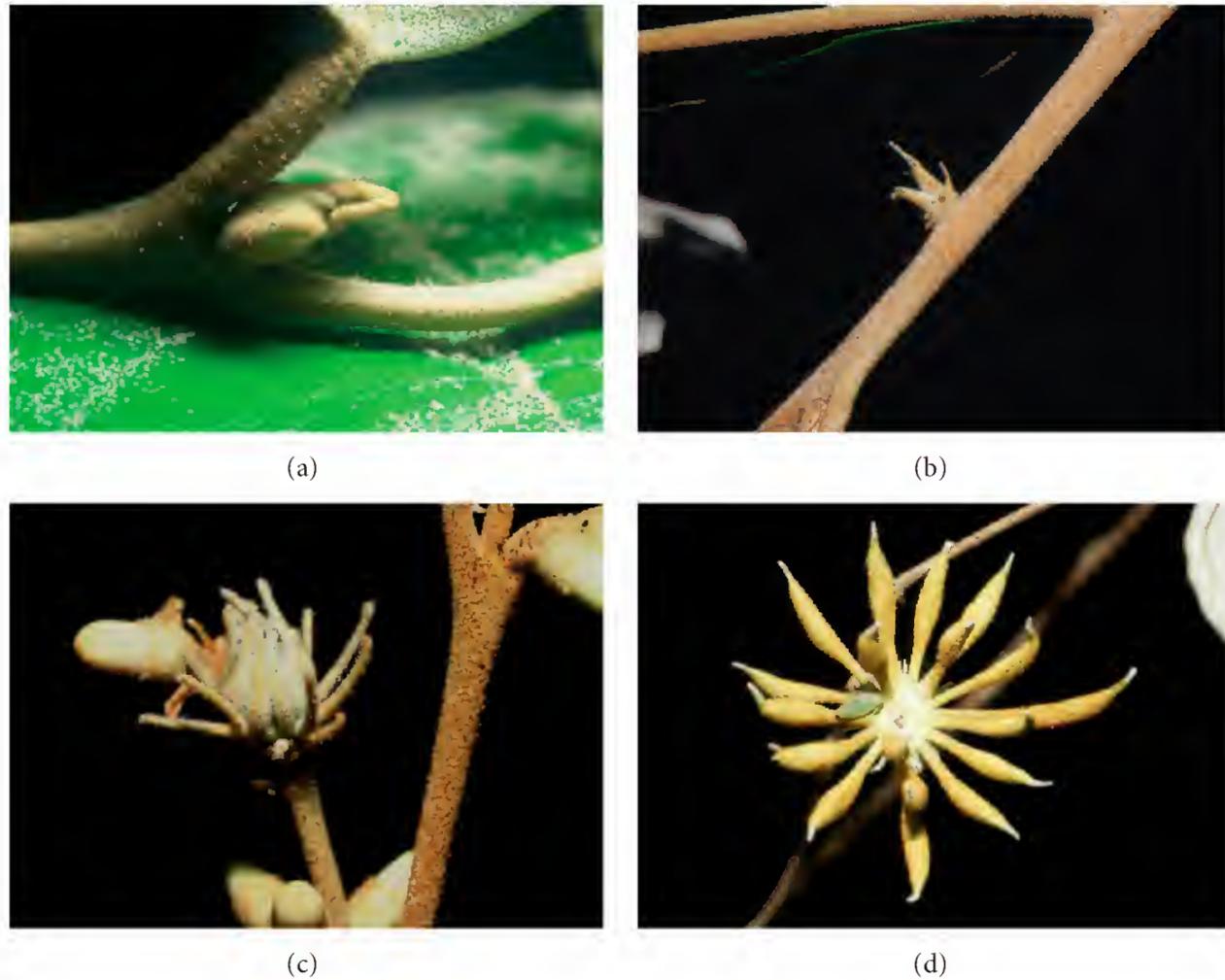


FIGURE 8: (a) An incipient gall of *Pseudoregma koshunensis* formed on an axillary bud of *Styrax suberifolius* (Taipei, Taiwan; 26 April 2005). (b) A very young gall of *Ceratoglyphina styracicola* formed on the stem of *S. suberifolius* (Sun Moon Lake, Taiwan; 18 July 1994). (c) A young gall of *Pseudoregma bambucicola* formed on an inflorescence of *Styrax suberifolius* (Fushan, Taiwan; 7 June 2004). (d) A young gall of *Astegopteryx* sp. on *Styrax benzoides* (Chiang Mai, Thailand; 1 August 2000).

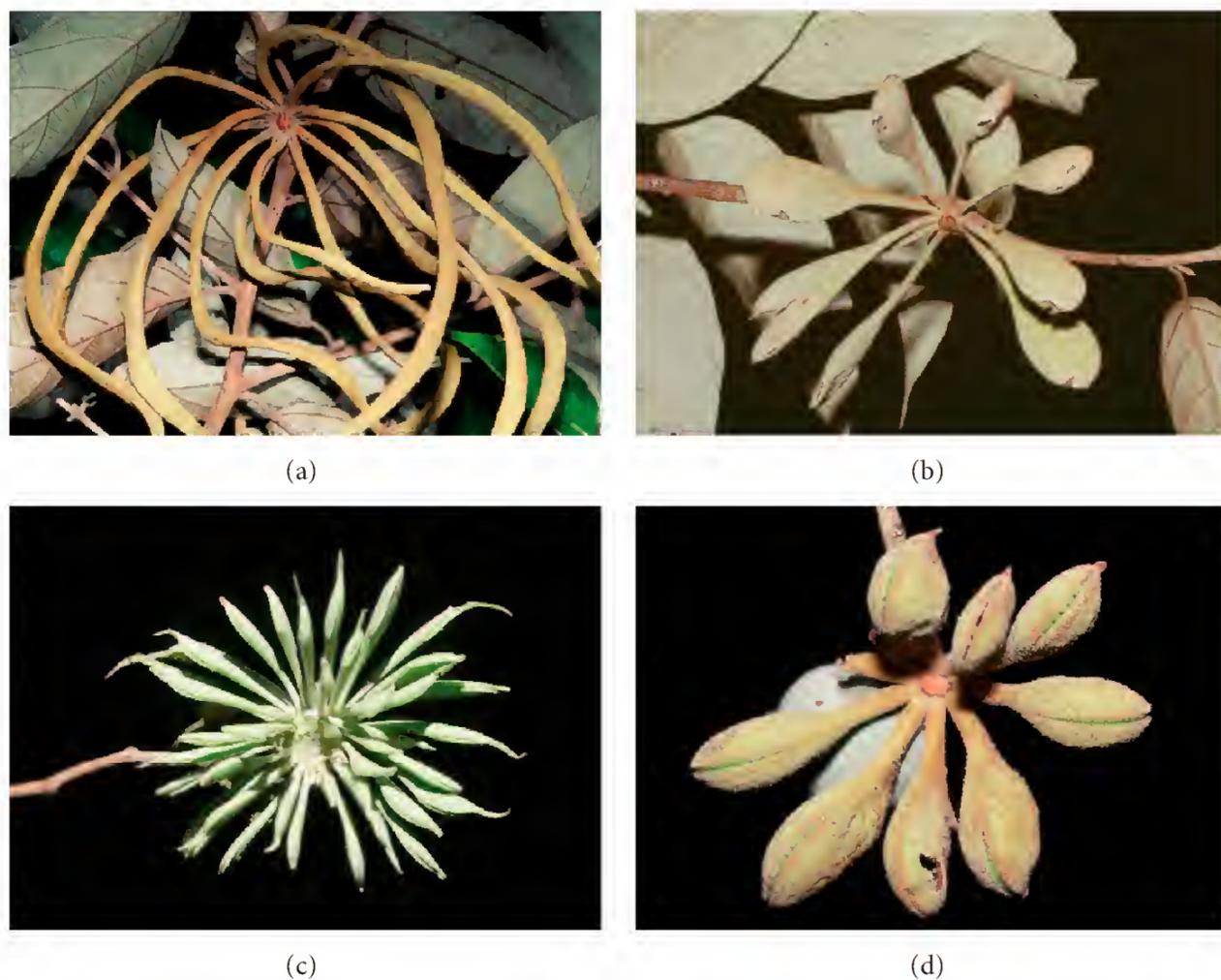


FIGURE 9: (a) A gall of *Astegopteryx basalis* on *Styrax benzoin* (Urung Tama, Sumatra; 21 August 1997). (b) A gall of *Astegopteryx malaccensis* on *S. benzoin* (Bukit Tinggi, Malay Peninsula; 31 March 1996). (c) A gall of *Astegopteryx pallida* on *S. benzoin* (Urung Tama; 1 August 1996). (d) A gall of *Astegopteryx nipae* on *S. benzoin* and a fruit of the host tree (Urung Tama; 1 August 1996).

4.2.2. *Gall Formed from a Flower Bud.* Multiple-cavity galls of *Astegopteryx styracophila* [19] (Figure 11(b)), *A. pallida* [19] (Figure 9(c)), *A. nipae* (our unpublished observation) (Figure 9(d)), *Pseudoregma bambucicola* [100] (Figure 8(c)), *P. carolinensis* [28] (Figure 4(a)), and *P. sundanica* [29] (Figure 12(b)) are formed on inflorescences of the host trees. Because these galls each have a calyx-like structure at the base (see, e.g., Figure 1(a) in [100]), it is certain that each gall is made of a flower bud. In Section 4.2.5, we will argue that the use of flower buds for gall formation is an ancestral state in the group forming multiple-cavity galls. Although the use of flower buds has not been known from the genus *Ceratoglyphina*, one species of *Ceratovacuna* is known to form galls from flower buds on *Styrax paralleloneurus* in Sumatra (a gall shown as Figure 105 by Docters van Leeuwen-Reijnvaan and Docters van Leeuwen [25] turned out to be formed by *Ceratovacuna* sp. near *keduensis*; our unpublished observation).

4.2.3. *Gall Formation from an Axillary Bud, without the Bud Shooting.* Galls of *Pseudoregma koshunensis* (Figure 11(a)) are found at the positions of axillary buds of *Styrax suberifolius*, and we found some incipient galls (Figure 8(a)) formed on axillary buds. There is therefore no doubt that, as in the case of *Cerataphis brasiliensis*, the fundatrix transforms an axillary bud into the gall without causing the bud to develop into a shoot. (A few abnormal leaves sprout in the process of gall formation, but they may later be atrophied.) Latent buds may also be utilized for gall formation. Perhaps, some other species may form their galls in this way, but up to now, among multiple-gall formers, only *P. koshunensis* is known to do this.

4.2.4. *Gall Formation from an Axillary Bud, with the Bud Shooting.* The multiple-cavity gall of *Ceratovacuna nekoashi* (Figure 2(a)) is known as “Nekoashi” (cat’s paw) among people living in Japan. The process of the gall formation is somewhat complicated. The host tree *Styrax japonicus* is deciduous and sheds all its leaves by winter in and around Tokyo. In spring, new shoots sprout from overwintered buds on the twigs. There are two kinds of new shoots. Shoots of one kind have inflorescences, and blossom in late May (Figure 10(a)). The shoots also have some (1–5) leaves (bracts), but there are no axillary buds at the bases of these leaves. Fundatrices of *C. nekoashi* cannot form their galls on these shoots. Shoots of the other kind have no inflorescences and continue to develop during the growing season, from spring to summer. As the shoots develop, they add one new leaf after another behind the terminal bud. Each leaf has an axillary bud at the base. Except when the shoot is damaged in some way, this axillary bud usually does not develop into a shoot until the next spring. (Axillary buds of *Styrax formosanus* in Taiwan rather commonly develop into shoots during the growing season.) The first-instar fundatrix of *C. nekoashi* transforms an axillary bud of a newly unfolded leaf into a small, incipient gall and conceals itself in it. The bud later becomes a multiple-cavity gall as explained before (Section 4.2), but at the same time the fundatrix causes

the bud to develop into a shoot, with the gall being located at the terminal position of the shoot [21] (Figures 10(b) and 10(d)). The fundatrix of *C. japonica* forms its gall in basically the same way [20]. Sometimes a fundatrix of *C. nekoashi* succeeds in causing an axillary bud to develop into a shoot but dies before producing its offspring in the incipient gall. In this case, the incipient gall is not developed into a normal multiple-cavity gall but often transformed into a single flower [21] (Figures 10(c) and 10(d)). What this fact implies is discussed in the next section. These single flowers originating from failed galls are noticeable because they bloom after all normal blossoms fall off the tree. According to Deguchi [139], who suggested for the first time that such single flowers of *Styrax japonicus* may be caused by *C. nekoashi*, the number of petals and the number of stamens in these flowers are often different from those in normal flowers (five petals and ten stamens). Some single flowers even have double petals [21]. At times, single flowers bore a fruit [21], which, however, may be abnormal in structure [140].

Such abnormal flowers, or “gall flowers,” have also been recorded from *Styrax formosanus*. We found a gall flower on *S. formosanus* near a gall of *Ceratovacuna nekoashi* (our observation around Sun Moon Lake, Taiwan, on 24 April 2005). In other two cases, both an abnormal flower and a live gall of *Ceratovacuna* sp. appeared at the tip of the same petiole originating from a single axillary bud of *S. formosanus* (our observations around Sun Moon Lake on 21 April 1990).

4.2.5. *Genetic Hacking Hypothesis.* A multiple-cavity gall of the Cerataphidini resembles a *Styrax* flower in its radially symmetric structure. The fact that a failed gall of *Ceratovacuna nekoashi* is often transformed into a single flower suggests that the fundatrix may exploit the mechanism of flower formation in the host plant for its gall formation. That is, the fundatrix may induce differentiation of floral meristems that would otherwise become petals and stamens (and possibly also a pistil) into subgalls. According to this hypothesis, the ancestor of the multiple-gall formers used to directly utilize a flower bud for gall formation, as the fundatrices of some extant species such as *Astegopteryx styracophila* and *Pseudoregma bambucicola* do (Section 4.2.2). Later, fundatrices of some species may have acquired the ability to form galls from other than flower buds, that is, axillary buds or stems of developing shoots, by causing differentiation of undifferentiated meristematic tissues into floral meristems. It is tempting to speculate that the fundatrices might inject a substance that activates the expression of floral meristem identity genes including *LEAFY* (see [141, 142]) into the plant to do this, thus hacking an epigenetic regulation system of flowering.

In this connection, it will be interesting to know whether gall flowers are caused by those multiple-gall formers that induce their galls on stems (*Ceratoglyphina* and some species of *Astegopteryx*) or axillary buds without the bud shooting (*Pseudoregma koshunensis*).

4.3. *Hardening of Gall Walls.* The walls of cerataphidine galls are generally not very hard. However, in *Cerataphis*

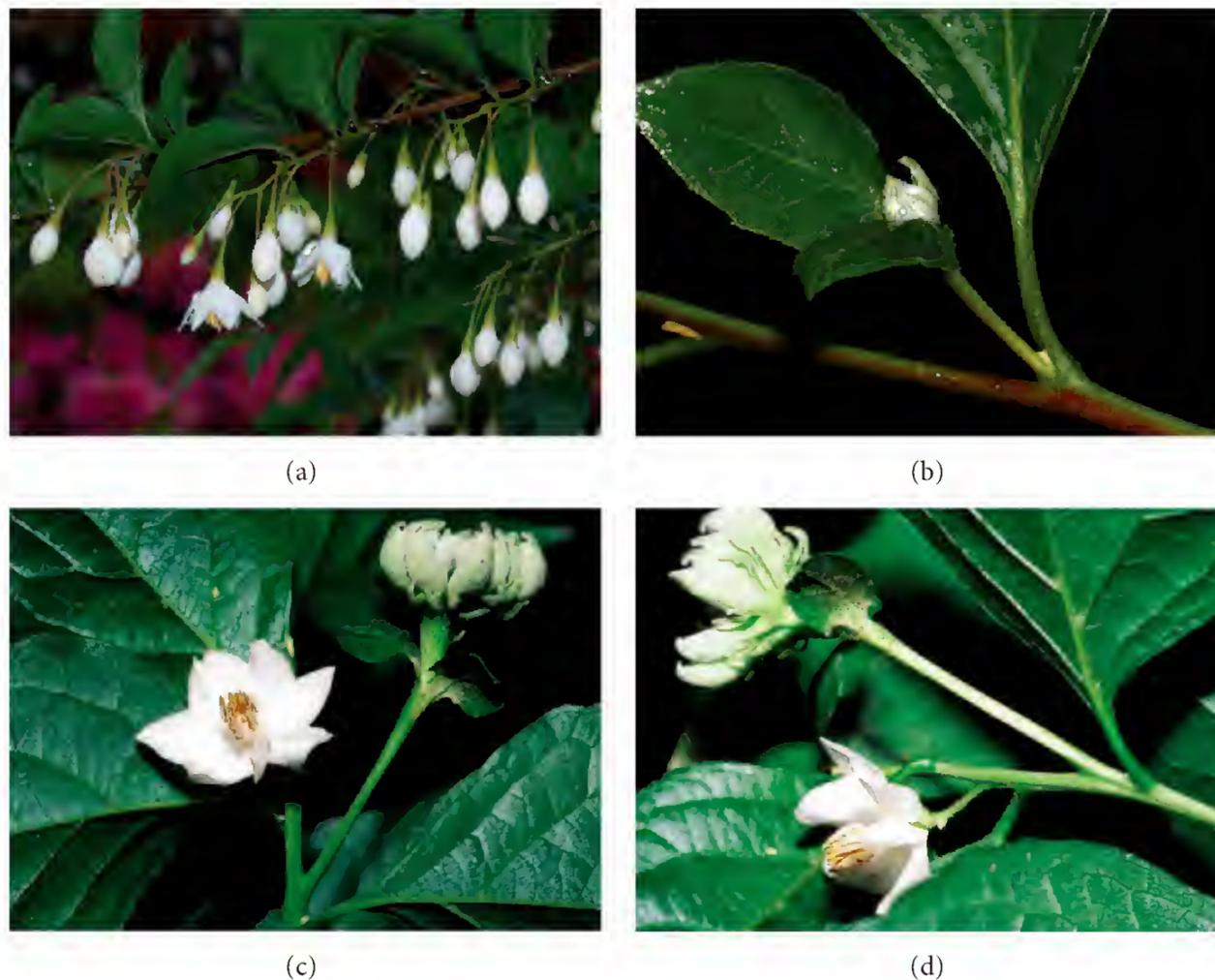


FIGURE 10: (a) Normal flowers (with five petals) of *Styrax japonicus* (Niiza, Japan; 6 May 2009). (b) A “gall shoot” that has sprouted from an axillary bud of *S. japonicus*, with a young gall of *Ceratovacuna nekoashi* at the apex, two bract-like leaves, and a new axillary bud at the base (Niiza; 6 May 2009). (c) A gall of *C. nekoashi* and an abnormal flower (with more than five petals) of *S. japonicus* (Niiza; June 1999). (d) The same abnormal flower shown from a different angle, indicating that it has sprouted from an axillary bud; petioles of abnormal flowers are variable in length and at times also with bracts (which are absent on this gall-flower shoot).

brasiliensis [34, 130] and *Pseudoregma koshunensis* [143] (Figure 11(a)), the walls of their galls become lignified and hard. In *Astegopteryx styracophila*, the apical wall of each subgall is thick and hard like a cork stopper [19] (Figure 11(b)). The hardest galls are made by *Cerataphis jamuritsu* on *Styrax suberifolius* [35, 136] (Figure 11(c)). One large gall of this species had a wall that was strongly lignified and approximately 5.8–12.1 mm thick (Figure 11(d)), and Aoki et al. [35] had to saw the gall to open it.

4.4. Coating of Gall Surface with Wax. Gall-living generations of cerataphidines, including soldiers, produce wax. Honeydew excreted by the aphids is coated with the wax to form droplets (see Figures 6(b) and 12(f)), or “aphid marbles” [144], and soldiers push them out of the gall without being trapped in the honeydew [28, 39, 131, 132, 145]. In species whose soldiers reside on the outer surface of their gall, the surface is coated with wax due to the activity of the soldiers. The outer surfaces of the galls of *Tuberaphis owadai* [11] (Figure 12(a)), *T. sumatrana* (our unpublished observation), and *Cerataphis bambusifoliae* [33] look silvery grey, and those of *Ceratoglyphina styracicola* [102] (Figure 3(c)), *Cerataphis jamuritsu* [35] (Figure 11(c)), and *Pseudoregma sundanica* [29] (Figure 12(b)) look snow white. The wax coating no doubt functions to be water repellent. The outer surfaces of the galls (e.g., of *Tuberaphis owadai* and *Ceratoglyphina*

styracicola) are densely covered with minute hairs which are likely to accumulate wax powder.

4.5. Utilization of Projections from the Inner Gall Wall. Among cerataphidine galls, the galls of *Ceratoglyphina styracicola* [2] and *Tuberaphis takenouchii* [36, 137] are peculiar in structure. The former is a multiple-cavity gall, while the latter a single-cavity gall. In both species, most aphids reside among twiggy projections which constitute the head(s) of their gall or subgalls (Figure 12(c)), rather than inside the original cavity or cavities, after the gall reaches some size. How the head of a (sub)gall is formed is similar between the two species. Here we describe the case of *C. styracicola* [30].

A young gall of *Ceratoglyphina styracicola* consists of a few spindle-shaped subgalls, which are hollow. The inner wall of each subgall is initially smooth but, as the subgall grows, small solid projections are developed from the inner wall. These projections become ramified and soon fill the cavity (Figure 12(e)). They grow further, outward through the apical slit to form the subgall’s head, which may be up to 7–10 cm in diameter [2, 101, 108] when fully developed (Figure 3(c)). The outermost sides of these projections bear minute hairs (but do not in *Tuberaphis takenouchii*) and catch wax, and the entire gall looks white, as mentioned in the previous section.

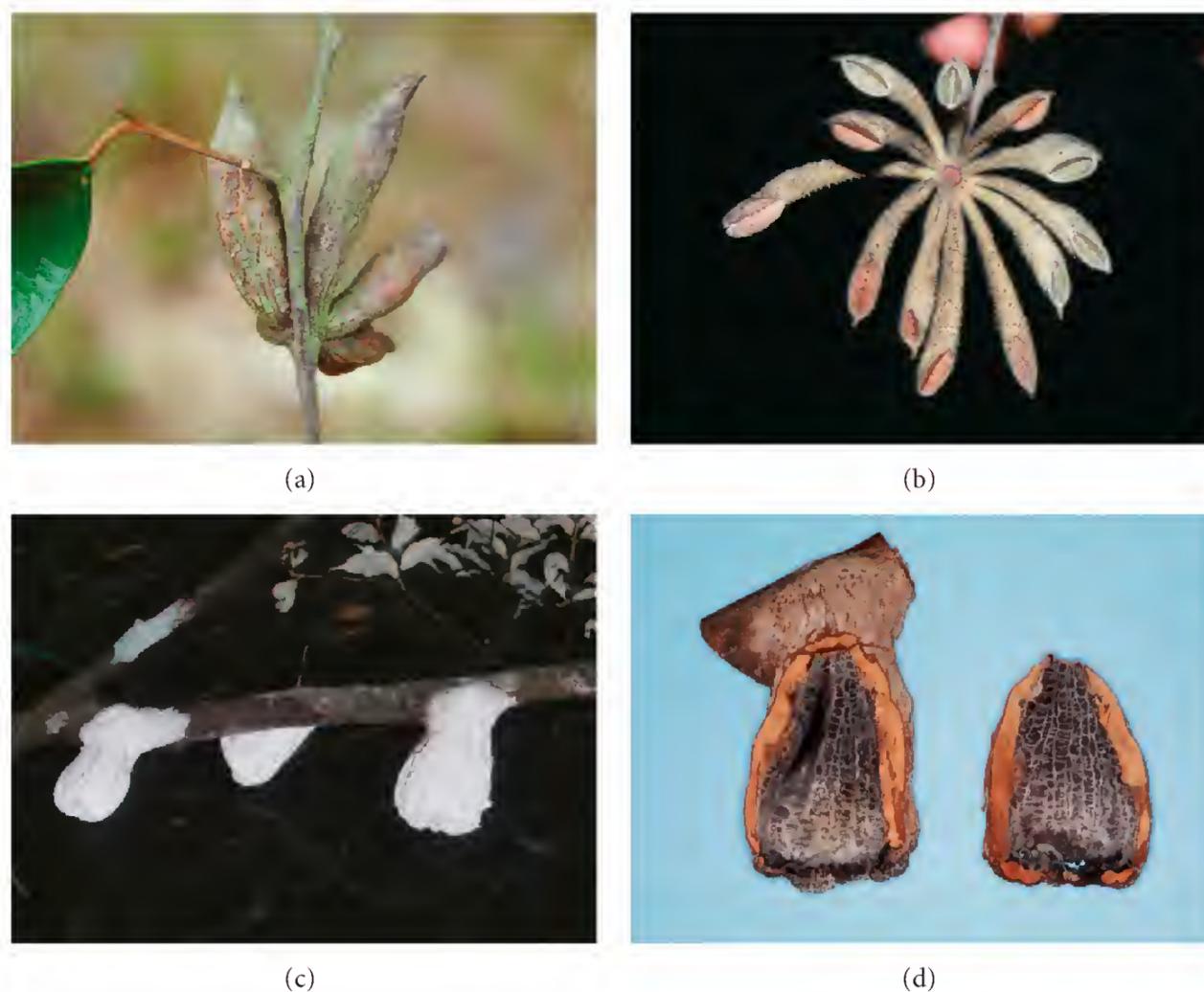


FIGURE 11: (a) A lignified (live) gall of *Pseudoregma koshunensis* on *Styrax suberifolius* (Sun Moon Lake, Taiwan; 3 June 1992). (b) A gall of *Astegopteryx styracophila* on *Styrax benzoin* (Urung Tama, Sumatra; 28 February 1994). (c) Three galls of *Cerataphis jamuritsu* on a thick branch of *S. suberifolius* (Hsinhua, Taiwan; 3 November 1994). (d) A cut gall of *C. jamuritsu*, indicating its thick wall (the rightmost gall in (c); dried after being kept in ethanol).

When part of the head is broken, the broken part may be nearly completely or partially repaired through the regrowth of projections [101]. Live, deformed galls of *C. styracicola*, which seem to have been broken by vertebrates, are often observed in the field [101]. In the gall of *Pseudoregma sundanica*, projections developed from the inner wall usually do not grow out of the subgall (see Figure 12(b) and below). However, we once found a subgall (Figure 12(d)) that looked just like a gall of *T. takenouchii*. The original gall wall was largely broken, and hyper-growth of projections formed the head. Gall repair is now known in two other aphid species belonging to the Nipponaphidini [146, 147] and Pemphigini [148].

The inner walls of subgalls of *Ceratovacuna nekoashi* (our unpublished observations), *C. japonica* [20], *Astegopteryx bambucifoliae* [16], *Pseudoregma bambucicola* [26], and *P. carolinensis* [28] are almost smooth with no or only a few short projections. On the other hand, there are many short projections on the inner walls of subgalls of *Astegopteryx styracophila* [19], *A. pallida* [19], *A. spinocephala* [17] (Figure 6(b)), and *A. nipae* (Figure 12(f)), and several, somewhat developed projections on those of *Pseudoregma sundanica* [29] (Figure 12(b)). These projections not only provide mechanical support for the structure but also increase the area of feeding sites for aphids. Thus, mature galls of *Ceratoglyphina styracicola*, consisting of well-developed ramified projections, can often sustain more than 100,000

aphids [2, 108]. Among cerataphidines that form single-cavity galls, only *Tuberaphis takenouchii* has yet been known to form galls with such projections.

5. Morphs

Aphids basically undergo cyclic parthenogenesis, in which a number of asexual generations are interrupted by a single sexual generation. As is well known, the phenotypes of aphids vary between generations of a single species. In Cerataphidini, aphids produced on the primary host are so different in appearance from those produced on the secondary host that they were once placed in different genera. The former had been grouped under the genus name “*Astegopteryx*,” while the latter under the names of “*Cerataphis*,” “*Oregma*,” and “*Ceratovacuna*” until Shibata [149, 150] and Hille Ris Lambers [151] showed that they are alternative morphs of the same group of species. In addition, nymphs are also polyphenic between and even within generations.

5.1. Fundatrix. The fundatrix is the sexually produced generation. First-instar fundatrices of cerataphidines are gall-initiators. We have examined those of *Ceratovacuna nekoashi* [21], *C. japonica* [20], *Pseudoregma bambucicola* [100], *P. koshunensis* (our unpublished observation), *Astegopteryx*



FIGURE 12: (a) A gall of *Tuberaphis owadai* on *Styrax tonkinensis* (Pha Din, Vietnam; 12 September 1995). (b) A gall of *Pseudoregma sundanica* on *Styrax paralleloneurus*; many soldiers (dark gray in color) reside on the outer surface of the gall, mainly on the basal parts of the subgalls (Mt Sibayak, Sumatra; 2 August 1996). (c) Outer surface of a gall of *Tuberaphis takenouchii* on *Styrax formosanus*; aphids live among ramified solid projections (Habon, Taiwan; 4 November 2003). (d) A once-broken gall of *P. sundanica* on *S. paralleloneurus* and a fruit of the host tree (Mt Sibayak; 2 August 1996). (e) A cut subgall of *Ceratoglyphina styracicola* on *Styrax suberifolius*, showing ramified solid projections developing inside (Sun Moon Lake, Taiwan; July 1986; photo by N. E. Pierce). (f) A cut subgall of *Astegopteryx nipae* on *S. benzoin*, showing short solid projections (Gombak Rd., Malay Peninsula; 2 December 1992; photo by D. L. Stern).

bambucifoliae [16], *A. spinocephala* [17], *Ceratoglyphina styracicola* [104], *Tuberaphis styraci* [73], and *Cerataphis brasiliensis* [34]. These first-instar nymphs are characterized by well-sclerotized tergites with long setae, well-developed setae on the tarsi, and the long, slender ultimate rostral segment. In at least one species (*C. nekoashi*), first-instar fundatrices are known to fight each other for a gall-forming site [21].

Upon feeding on the host plant, the first-instar fundatrix is soon enclosed by plant tissues (Section 4). After the first molt, its tergites become membranous and its

rostrum becomes shortened. The adult fundatrix (which is apterous) also has membranous tergites and no cornicles. In species forming single-cavity galls, the fundatrix lives with its progeny in one and the same cavity. In species forming multiple-cavity galls, the fundatrix does not enter any subgall (Section 4.2). Unlike many aphids, cerataphidine fundatrices are not very fecund. For instance, fundatrices of *Ceratovacuna nekoashi* give birth to only eight to 21 offspring [138]. In contrast, a single fundatrix of *Grylloprociphilus imbricator* (Eriosomatinae, Pemphigini) may produce more than 7,000 nymphs [152].

5.2. *Fundatrigeniae*. The parthenogenetic generations following the fundatrix on the primary host are called “fundatrigeniae.” In the gall, a number of (fundatrigenia) generations follow in succession, depending on how long the gall lasts. Adult fundatrigeniae can be winged (alate) or wingless (apterous), but alates are produced only after the gall matures, when the colony size is from 10^2 to 10^5 (Table 3). Alates that fly to the secondary host are called “emigrants,” while those that deposit sexuals on the primary host are “sexuparae” (Section 5.5).

5.2.1. *Second Generation*. The second generation, or the direct offspring of the fundatrix, plays a special role in the species forming multiple-cavity galls. They become founders of subcolonies in subgalls (Section 4.2). The number of founders per subgall is different between species, one in *Astegopteryx spinocephala* [17], one to three (mean 1.2) in *Ceratovacuna nekoashi* [21, 138], and one or two (mean 1.1) in *Ceratoglyphina styracicola* [104]. Late-born first-instar nymphs of the second generation often cannot enter subgalls because all subgalls may have been closed by that time. They defend their young gall outside the subgalls and are functionally sterile. This is the case for *Ceratovacuna nekoashi* [138] and *Ceratoglyphina styracicola* [104]. In *Astegopteryx bambucifoliae*, however, new subgalls are continuously formed over a relatively long period. It is therefore likely that late-born nymphs still can enter subgalls and may not be destined to die without growing. In fact, the number of subgalls is from five to 12 in *Ceratovacuna nekoashi* [138] and only three to six in *Ceratoglyphina styracicola* [30, 104], but up to 32 in *A. bambucifoliae* [16]. At least in *Ceratovacuna nekoashi* [138] and *C. japonica* [20], the outsiders are not different in morphology from the first-instar nymphs that have entered subgalls. Not only outsiders but also nymphs that have entered subgalls attack potential predators when the subgall is broken [20].

5.2.2. *Third and Later Generations*. Aphids of the third or later generations are not very different from those of the second generation in morphology but are generally larger than the latter [104]. While apterous adults of the second generation may not have cornicles (siphunculi) in some species forming multiple-cavity galls, those of the third and later generations always have a pair of ring-like cornicles on the sixth abdominal tergite. (Cornicles of cerataphidines are mere pores and not horn shaped, but still are referred to as “cornicles” in aphidology.) In the fundatrigenia generations, sterile soldiers appear in the second instar. The first-instar nymphs of these generations are monomorphic and, past the first molt, they develop into either “normal” second-instar nymphs or sterile soldiers. The second-instar soldiers are described in Section 5.3. The normal second-instar nymphs develop into apterous or alate adults after three more molts. (Adults are in general fifth-instar aphids.) Except for alate adults, these non-soldier fundatrigeniae may attack potential predators and/or pierce human skin with their stylets (e.g., [108]). They probably function as auxiliary defenders. Apterous adults, of course, reproduce in the gall

or subgall. Alates fly to the secondary host (if the life cycle is host-alternating) or to *Styrax* trees (if the life cycle is non-host-alternating) and give birth to their offspring on the undersides of the leaves [27, 89].

5.3. *Soldiers on the Primary Host*. All cerataphidine species whose gall-generations are known produce second-instar soldiers in their galls on the primary host. Although some authors [6, 7] use the word “soldiers” to include any defensive individuals, in the present review we use it to denote a morphological caste whose primary function is colony defense. Non-soldier individuals that play a defensive role are referred to as “defenders” or “defensive nymphs.”

5.3.1. *Morphology*. The soldiers are morphologically different from the normal second-instar nymphs (reproductives-to-be) in having sclerotized tergites with longer setae, longer claws, protruded cornicles, and one or more pairs of spine-like setae on the frons. In the genus *Pseudoregma*, the forelegs are thickened [26, 28, 29, 153] as in the first-instar soldiers produced on the secondary host (see Section 5.4.2). In *Astegopteryx pallida* (reported as *A. “setigera”* [42]) and *A. spinocephala* [132], the soldiers are armored with many spines on their heads. The number of these frontal spine-like setae is usually one pair in *Cerataphis* and *Tuberaphis* (e.g., [35, 39]), while more than one pair in *Astegopteryx*, *Ceratovacuna*, *Pseudoregma*, and *Ceratoglyphina* (e.g., [2, 16, 20, 28]). In *Ceratovacuna nekoashi*, however, these multiple pairs of frontal setae are not distinctly spine-like (our unpublished observations).

5.3.2. *Defensive Behavior*. The second-instar soldiers clasp an insect predator such as a moth larva and pierce it with their stylets. Such attacking behavior has been confirmed by us for almost all aforementioned species through introducing an insect larva into (or onto) the gall (e.g., [108]). The only exception was *Tuberaphis leeuweni*. Aoki et al. [40] failed to induce attacking behavior from its soldiers. The second-instar soldiers of all cerataphidine species we have examined so far (including *T. leeuweni*) pierce human skin, which causes irritation of various degrees. The irritation caused by soldiers of *Ceratoglyphina styracicola* is fairly severe [2, 108, 109]. We were not able to continue field observations without brushing off the soldiers that were piercing the skin on our hands. The irritation caused by soldiers of *Tuberaphis sumatrana* (U. Kurosu, her experience) and *Cerataphis vandermeermohri* [10] is as severe as by soldiers of *C. styracicola*. In contrast, soldiers of *Tuberaphis styraci* [39], *T. taiwana* [38], and *T. leeuweni* [40] cause irritation in only a slight degree. The irritation caused by soldiers of other species, including *Ceratovacuna japonica* [20], *Cerataphis jamuritsu* [35], *Pseudoregma bambucicola* [26], *Tuberaphis owadai* [11], and *Astegopteryx spinocephala* [132], falls between these two extremes. Soldiers of two species, *Ceratoglyphina styracicola* and *Cerataphis vandermeermohri*, readily fall off their gall when the gall is lightly shaken manually [2, 10, 108]. (This happens only when the gall and the colony size are large enough.) This is interpreted

TABLE 3: Colony size and the percentage of soldiers in cerataphidine galls.

Species	Colony size ¹	%Soldiers	References
<i>Ceratoglyphina styracicola</i>	100,000~200,000 ($n = 2$)	43~55%	[2, 108]
<i>Tuberaphis owadai</i>	60,000~180,000 ($n = 2$)	41~52%	[11]
<i>Cerataphis vandermeermohri</i>	8,000~94,000 ($n = 3$)	44~46%	[10]
<i>Cerataphis jamuritsu</i>	18,000~57,000 ($n = 2$)	46~48%	[35]
<i>Tuberaphis taiwana</i>	5,000~18,000 ($n = 3$)	27~42%	[38]
<i>Tuberaphis styraci</i>	8,000~15,000 ($n = 3$)	29% ($n = 1$)	[39]
<i>Pseudoregma sundanica</i>	2,000~15,000 ($n = 7$)	45~60%	[29]
<i>Astegopteryx styracophila</i>	1,400~12,000 ($n = 3$)	24~38%	[19]
<i>Cerataphis bambusifoliae</i>	2,900~9,800 ($n = 5$)	21~48%	[33]
<i>Astegopteryx pallida</i>	1,500~9,000 ($n = 3$)	30~46%	[19]
<i>Cerataphis brasiliensis</i>	6,400 ($n = 1$)	25%	[34]
<i>Astegopteryx spinocephala</i>	2,300~6,200 ($n = 2$)	59~63%	[17]
<i>Astegopteryx bambucifoliae</i>	100~5,300 ($n = 4$)	21~64%	[16]
<i>Ceratovacuna japonica</i>	400~1,700 ($n = 5$)	15~45%	[20]
<i>Pseudoregma bambucicola</i>	300~1,600 ($n = 8$)	23~41%	[26]
<i>Pseudoregma carolinensis</i>	500~1,500 ($n = 6$)	45~66%	[28]
<i>Tuberaphis leeuweni</i>	500~1,300 ($n = 5$)	21~52%	[40]

¹Colony sizes of mature galls (containing alates and/or fourth-instar wingpadded nymphs). Undeveloped or damaged galls (due to predation or for an unknown reason) are omitted from the data.

as a defensive behavior against mammals [2, 101]. Perhaps soldiers that can cause troublesome irritation in humans, including those that do not readily fall off the gall, may repel some vertebrate predators.

In *Tuberaphis styraci*, one of the main ingredients of the venom injected into natural enemies by the soldiers was identified by Kutsukake et al. [154] as a cysteine protease of the family cathepsin B. The gene encoding this protease (called “S-type cathepsin B gene”) expresses specifically in the soldier morph and leads to the production of the protease in the intestine [155]. In other *Tuberaphis* species (*T. coreana*, *T. taiwana*, *T. sumatrana*, and *T. takenouchii*), too, the same S-type gene specifically and strongly expresses in the soldier morph. However, in *Astegopteryx styracophila*, *A. spinocephala*, and *Cerataphis jamuritsu*, the S-type gene rather weakly and non-specifically expresses in the soldier morph. Although the S-type gene of *C. jamuritsu* is still transcribed, it contains a stop codon and is likely to be a pseudogene [155]. In these non-*Tuberaphis* cerataphidines, therefore, the main ingredient of the venom is something other than the cathepsin B protease.

In species whose colonies become large (e.g., *Ceratoglyphina styracicola* [102], *Pseudoregma sundanica* [29], *Tuberaphis owadai* [11]), many soldiers reside on the outer surface of their gall (Figure 12(b)). In others (e.g., *Cerataphis brasiliensis*, *Astegopteryx styracophila*), all inhabitants including soldiers reside within the gall or subgalls, and a number of soldiers guard at the small ostiole(s) and face outward from the inside of the (sub)gall [19, 88, 130]. Among them, soldiers of *Astegopteryx spinocephala* cooperate to plug the ostiole with their sclerotized spiny heads from inside [132]. When their gall is disturbed, soldiers rush out of the (sub)gall and excitedly walk around on the outer surface.

These soldiers soon begin to retreat into the (sub)gall if they encounter no enemies [19, 132].

Soldiers may emit an alarm pheromone from their cornicles. When their gall is disturbed, soldiers of *Ceratoglyphina styracicola* excitedly walk around on the outer surface of the gall while raising the tip of the abdomen upward [101, 108], perhaps emitting an alarm pheromone. Shibao et al. [156] mention that soldiers of *Tuberaphis styraci* discharge, from their cornicles, yellowish droplets containing (*E*)- β -farnesene, which is used as an alarm pheromone in many aphid species [157, 158].

5.3.3. Predators of Gall-Living Generations. Here we briefly review predators of gall-living cerataphidines. Larvae of the pyralid genus *Assara* (Lepidoptera) are one of the commonest predators. The larvae bore into cerataphidine galls. Within the gall they live in a silken net and thereby escape attack from soldiers and prey on aphids by protruding the head from the net. Three species have hitherto been identified: *Assara formosana* from galls of several cerataphidine species in Taiwan [107] and Thailand [17], *A. holophragma* from galls of *Astegopteryx styracophila* in Sumatra [19], and *Assara seminivalis* from galls of *Tuberaphis owadai* in northern Vietnam [11]. The reason why soldiers of some species reside on the outer surface of their gall (Section 5.3.2) is probably that they defend the gall against such lepidopteran larvae. Larvae of the polyphagous vine moth *Eupoecilia ambiguella* (Tortricidae) often bore into subgalls of *Ceratovacuna nekoashi* one after another and eat both the inner walls and the aphids [138]. Other *Styrax*-feeding moth larvae are also likely to be potential enemies of cerataphidine galls.

Larvae, pupae, and adults of the coccinellid genus *Sasajiscymnus* (formerly known as “*Pseudoscymnus*,” which turned out to be a junior homonym of a fish genus name [159]) are found from within some cerataphidine galls: *S. sylvaticus* from subgalls of *Ceratovacuna nekoashi* [160, 161] and *C. japonica* [20], and *S. sp.* from those of *Astegopteryx spinocephala* [17]. Unidentified larvae of other coccinellid groups have also been found from galls of *Cerataphis vandermeermohri* [9, 10] and subgalls of *Astegopteryx pallida* [19]. Predaceous beetle larvae (sometimes together with adults) of *Mimemodes* sp. (Rizophagidae, Monotominae) have been found from galls of *Cerataphis brasiliensis* [34] and subgalls of *Astegopteryx styracophila* [19], and those of *Aethina* sp. (Nitidulidae, Nitidulinae) from galls of *Tuberaphis taiwana* [38] and subgalls of *Pseudoregma bambucicola* [26].

Syrphid larvae are one of the commonest predators of aphids in general, and some (e.g., *Heringia senilis*, *Pipiza* spp.) are specialist predators of aphids within galls [162]. However, syrphid larvae have rarely been found in cerataphidine galls. Up to now we have found only one cerataphidine gall (of *Tuberaphis sumatrana* on *Styrax subpaniculatus* in Sumatra) containing several larvae of a syrphid species (our unpublished observation). For an unknown reason, no parasitoid wasps are known from gall-living cerataphidines.

As mentioned in the previous section, vertebrates such as mammals may be potential predators of cerataphidine galls. However, it is difficult to observe an incident of predation directly. Chou et al. [163] observed that the squirrel *Callosciurus erythraeus* ate a total of four galls of *Astegopteryx bambucifoliae* (reported as “*Eulachnus*” sp.; see [101]) on *Styrax suberifolius* in Taipei, Taiwan. So far, this is the only direct observation on predation of cerataphidine galls by vertebrates. Outside the Cerataphidini, Japanese monkeys (*Macaca fuscata yakui*) are known to break hard, lignified galls of *Nipponaphis monzeni* (Hormaphidinae, Nipponaphidini) with their teeth and eat the aphids inside [164]. Sunose [165] observed that galls of *Paracolopha morrisoni* (Eriosomatinae, Eriosomatini) were pecked by tree-sparrows (*Passer montanus*) and the aphids were consumed. Great tits (*Parus major*) exploit galls of *Paracletus cimiciformis* and *Forda formicaria* (Eriosomatinae, Fordini) as a source of food [166].

5.3.4. Defense against Aphids of the Same or Different Species.

Gall-living aphids send off some of their clonemates, usually nymphs, into other conspecific galls. These intruders exploit resources of foreign galls rather than of their natal gall, thus indirectly helping their clonemates remaining in the natal gall [5, 167]. This phenomenon, called “intergall migration,” is known in non-cerataphidine species including *Pachypappa marsupialis* [168], *Pemphigus* spp. [169–171], and *Adelges japonicus* [172]. Such migration of nymphs between conspecific galls is likely to occur also in cerataphidine species, because it is known that some cerataphidines, *Ceratoglyphina styracicola* [103], *Astegopteryx bambucifoliae* [26, 88], *Pseudoregma bambucicola* [88], and *Cerataphis brasiliensis* [5] intrude into galls of different species on the same host tree. In fact, soldiers of some species attack aphids

of the same or different species. Soldiers of *C. styracicola*, on the outer surface of their gall, attack conspecific aphids except conspecific soldiers, whether they are clonemates or not, in at least some seasons [102, 106]. This strongly suggests that soldiers cannot discriminate between kin and non-kin (or between clonemates and non-clonemates), that they do discriminate between soldiers and non-soldiers, and that their galls are under threat from invasion and exploitation by nearby conspecific colonies [5]. Soldiers of *P. bambucicola* and *A. bambucifoliae* also attack aphids of the same or other species at the ostiole and prevent at least some of them from intruding into the subgall [88].

5.3.5. *Cleaning Behavior.* The second-instar soldiers push globules of honeydew, cast-off skins, and dead aphids out of the (sub)gall with their heads [28, 39, 131, 132, 145]. Usually there remain few cast-off skins and few dead aphids in healthy cerataphidine galls. A possible exception may be *Ceratovacuna nekoashi*, whose soldiers do not actively push garbage out of their subgall. As mentioned in Section 5.3.1, soldiers have one or a few pairs of spine-like setae on the frons, which probably function as a brush for gall cleaning [39].

Age polyethism may occur in some species. Young soldiers of *Tuberaphis styraci* preferentially perform gall cleaning tasks, whereas aged soldiers exclusively exhibit attacking behavior [173]. In this connection, it will be interesting to know whether those soldiers of *Ceratoglyphina styracicola* that fall off their gall (Section 5.3.2) are aged ones or not.

Gall cleaning behavior has been found also in aphids belonging to other taxa, including the hormaphidines *Hormaphis betulae* and *Hamamelistes miyabei* [174, 175], and the pemphigines *Pemphigus dorocola* [176] and *P. spyrothecae* [177].

5.3.6. Ants and Aphid Soldiers on the Primary Host.

No intimate symbiosis has been recorded between gall-living cerataphidines and ants. Usually ants are not seen on cerataphidine galls (e.g., [34]), but sometimes ants collect honeydew directly from openings of a gall [28, 145] (Figure 4(a)). Kurosu et al. [145] observed that a soldier of *Ceratovacuna japonica* grasped a gall-attending ant of *Pristomyrmex pungens*, and that some ants crushed aphid soldiers with their mandibles on the same gall. Although not yet confirmed, gall-attending ants may widen openings of the gall, which may have a negative effect on the aphid colony.

5.3.7. Percentage of Soldiers in Galls.

The number and percentage of soldiers vary between gall stages and between species. In some species that form large galls (e.g., *Ceratoglyphina styracicola*, *Tuberaphis owadai*), a single colony may produce several tens of thousands of soldiers (Table 3). In those species that form small galls (e.g., *Pseudoregma carolinensis*), a single colony may contain only hundreds of soldiers. The percentage of soldiers at times reaches 40–60% when the gall matures (Table 3).

5.3.8. *Sterility of Soldiers in Galls.* Cerataphidine soldiers produced in *Styrax* galls are sterile and do not molt past the second instar. To confirm this, we have examined, for each species, approximately one hundred slide-mounted specimens of soldiers under a light microscope to determine whether they have the next (third) instar cuticle developing inside. A sample of non-soldier nymphs (i.e., reproductives-to-be) is likely to include some individuals with the next instar cuticle. For instance, of 94 first-instar non-soldiers of *Pemphigus spyrothecae* (Eriosomatinae, Pemphigini) we examined, 18 (19.1%) had the next instar cuticle developing inside [178]. If no individuals with such a cuticle are found among about one hundred soldiers of a species, it will be reasonable to conclude that soldiers of the species are sterile. Of course, if the molting rate is much smaller, say one percent, the examination of one hundred individuals will be insufficient (the probability of finding no such individual will be near e^{-1} , or 0.37). In Table 4, the results for 19 species are summarized. The data support the soldiers' sterility in general, but there have been reported two exceptional cases in which some soldiers may molt. (1) In a mature gall of *Astegopteryx bambucifoliae* with 750 aphids, four out of 476 soldiers had the next instar cuticle developing inside [16]. (2) In a very young gall of *Ceratoglyphina styracicola* with 13 live aphids, five out of six soldiers had the next instar cuticle [105]. No such soldiers were found in other galls of either species. Because soldiers are likely to be accepted by guarding soldiers of other conspecific galls [88, 106], and because soldiers are shown to intrude into galls of other species [26, 88], we suggest that the molting soldiers mentioned above were intruders from other galls. This possibility requires confirmation.

In *Tuberaphis styraci*, soldiers survive over 20 days (after the first molt) on an artificial diet, while the second stadium of normal nymphs is around 10 days [173].

5.3.9. *Proximate Factors for Soldier Production.* Proximate factors for the production of soldiers have been studied with *Tuberaphis styraci*, because the species is one of few social aphids that can be maintained on an artificial diet for over two months [179]. Shibao et al. [180] showed that high aphid density induces soldier production. When mother apterae and/or their first-instar nymphs are reared under crowded conditions, more soldiers (which are second instar) are produced. The combination of prenatal high density and postnatal high density enhances soldier differentiation in a synergistic manner [181]. Direct contact with other aphids is a cue for soldier induction [182]. Soldier production is enhanced by coexisting non-soldiers, but suppressed by coexisting soldiers [183]. Thus, the percentage of soldiers in *T. styraci* is controlled by positive and negative feedbacks consisting of density-dependent induction and suppression of soldier differentiation [183, 184].

5.4. *Exules.* "Exules" are the aphids that are produced on the secondary host but usually do not include sexuparae. Colonies on the secondary host are founded by alates (emigrants) coming from the primary host, or by alates

(secondary migrants) or first-instar nymphs from other secondary hostplants. First-instar exules are active walkers and often dispersed on the wind [84, 93, 185, 186]. Except for *Tuberaphis takenouchii* [37], *T. macrosoleni*, and *T. cerina* [42], which form and live in leaf galls or rolled leaves, most cerataphidines form an exposed colony that sprawls over a plant or a clump of plants. The demarcation of such a colony is sometimes not clear. Colonies of *Pseudoregma alexanderi* and *P. baenzigeri* that are formed on such tall bamboos as *Dendrocalamus* spp. often become huge [118, 185] and the number of aphids may exceed one million [118]. Many species such as *Pseudoregma* species form dense colonies (Figure 4(b)) on their host plants. Species of the genus *Astegopteryx* (except *A. basalis* [42]), on the other hand, form "spaced-out" colonies (Figure 3(b)) on leaves of the host plants; that is, aphids as a whole are aggregated to form a colony, but individual aphids are more or less spaced out from each other [87, 187].

Because more than one alate may come to a single leaf [86] or a single plant, and because alates or wind-dispersing nymphs may join already established colonies [27], colonies on the secondary host are not always pure clones [185].

The morphology of exules (nymphs and apterous adults) is rather uniform among genera (*Astegopteryx*, *Ceratovacuna*, *Pseudoregma*, *Ceratoglyphina*, and *Chaitoregma*) that form (or are supposed to form) multiple-cavity galls on the primary host. They all have a pair of frontal horns with minute setae. On the other hand, the morphology of exules differs fairly among genera (*Cerataphis*, *Tuberaphis*, and *Glyphinaphis*) that form (or are supposed to form) single-cavity galls. Exules of *Glyphinaphis* have no frontal horns, while those of *Cerataphis*, as its name suggests, have a pair of frontal horns which bear no setae. (The frontal horns of *Cerataphis* therefore might not be homologous with those of *Pseudoregma* and its four allied genera.) Exules of *Tuberaphis* do or do not have horn-like projections [42, 49].

5.4.1. *Butting Behavior.* Exules of many cerataphidines use their pair of frontal horns to butt conspecific aphids, or colony-mates, to gain occupation of a good feeding site on the host plant. In this interaction, a non-feeding aphid walks over to a feeding aphid, fixes all its legs on the plant, and repeatedly thrusts its body forward; the horns usually hit the aphid body being attacked. All instars but alates show this behavior. Though not frequently, soldiers (see Section 5.4.2) do, too [185]. Large instars (apterous adults or wingpadded fourth-instar nymphs) more frequently defeat small instars (early-instar nymphs) than vice versa. Note that this butting behavior is different from attacking behavior against a predator or an enemy; in the latter, the attacker clasps the predator with its forelegs or all legs, while in the former the attacker butts a conspecific aphid while keeping all its legs on the plant. The butted aphid shows some defensive behavior, which varies between species.

In *Pseudoregma alexanderi* and *Ceratovacuna nek-oashi*, which form compact colonies on the host plant (Figure 2(b)), an attacked aphid raises its abdomen when butted from behind. The attacker aphid then creeps under

TABLE 4: Molting rate of cerataphidine soldiers in *Styrax* galls.

Species	No. of soldiers examined	No. (%) of molting soldiers	References
<i>Astegopteryx bambucifoliae</i>	821	4 (0.5%)	[16]
<i>Astegopteryx styracophila</i>	109	0	[19]
<i>Astegopteryx pallida</i>	100	0	[19]
<i>Astegopteryx spinocephala</i>	100	0	[17]
<i>Ceratovacuna japonica</i>	215	0	[20]
<i>Pseudoregma bambucicola</i>	268	0	[26]
<i>ditto</i> (young gall)	94	0	[100]
<i>Pseudoregma koshunensis</i>	133	0	[153]
<i>Pseudoregma sundanica</i>	100	0	[29]
<i>Pseudoregma carolinensis</i>	100	0	[28]
<i>Ceratoglyphina styracicola</i>	126	0	[2]
<i>ditto</i>	530	0	[103]
<i>ditto</i> (young gall)	63	0	[104]
<i>ditto</i> (young gall)	37	5 (13.5%)	[105]
<i>Tuberaphis styraci</i>	139	0	[39]
<i>Tuberaphis taiwana</i>	250	0	[38]
<i>Tuberaphis leeuweni</i>	121	0	[40]
<i>Tuberaphis takenouchii</i>	8	0	[36]
<i>Tuberaphis owadai</i>	238	0	[11]
<i>Cerataphis brasiliensis</i>	100	0	[34]
<i>ditto</i> (young gall)	200	0	[34]
<i>Cerataphis jamuritsu</i>	130	0	[35]
<i>Cerataphis vandermeermohri</i>	118	0	[10]
<i>Cerataphis bambusifoliae</i>	127	0	[33]

the abdomen and begins feeding there. This leads to the formation of a compact colony. The attacker, however, sometimes does not cease butting after creeping under the abdomen. In this case, especially when the aphid being butted is solitary, the aphid is forced to withdraw its stylets from the plant tissue and driven away [4, 185].

In *Astegopteryx bambucifoliae* and *A. minuta*, which form sparse colonies on bamboo leaves, the reaction of a butted aphid is more elaborate. When butted from other than the front, the butted aphid turns itself to face toward the attacker, without withdrawing its stylets from the plant, and raises its abdomen and lowers its head to shield itself against the butt. The attacker may escalate the fight: it clasps the opponent's body with its forelegs (see a dueling pair in the lower right corner of Figure 3(b)) and repeatedly thrusts its body back and forth. In this attack, the attacker's horns no longer hit the opponent's body. The aphid being attacked then raises its abdomen further, to such an extent that its hind legs are detached from the plant and that its abdomen is bent forward to lean on the attacker, performing a headstand [187]. The attacker may or may not force the opponent away. When the attacker succeeds in forcing the aphid away, it begins feeding at the spot where the attacked aphid was feeding [188]. Hence this fighting is for a good feeding spot. By applying electrical penetration graph techniques to *Astegopteryx pallida*, which forms similar sparse colonies on bamboo leaves, Morris and Foster [189] showed that horned aphids use the exact feeding site vacated by another

individual and that the benefit they gain is rapid access to the phloem.

Apterous adults of the palm aphid *Cerataphis brasiliensis* are armored well. The head with two sharp horns and the three thoracic tergites are united to form one large sclerotic plate, and the first to seventh abdominal tergites are also united to form another large sclerite. At the butting, an attacker bends its body like a roof and snaps its anterior sclerite upward to move off a feeding aphid. In this species, the attacked aphid often counterbutts the attacker aphid. Howard et al. [190] describe the fight as follows.

“To butt another aphid, an aphid lowers its head, places its horns beneath the head of the other aphid, then snaps its head upward while simultaneously thrusting forward with the legs. The motion often lifts the other aphid at its margin. Each of the dueling pair responds to being butted within a few seconds by butting its opponent. The altercation may last up to 19 minutes, the aphids often exchanging blows about 40 times per minute and alternately resting for intervals of several minutes.”

Although Howard et al. [190] mention that “neither one (of the dueling pair) seemed to be injured by the other,” we [4] once observed under a dissecting microscope in a room that an apterous adult was snapped by another and turned upside down (at Iriomote, southern Japan, between 14 and

16 March 1986). Because these aphids were on the underside of a palm leaflet, the snapped aphid would have fallen off the leaf under natural conditions. Such an apparent cost of the duel suggests that the opponent is a non-clonemate at some probability.

In all horned cerataphidines investigated so far, butting behavior was observed ([4] and our later unpublished observations). In some species such as *Chaitoregma tattakana* and *Tuberaphis coreana*, however, the behavior is sluggish and difficult to interpret.

5.4.2. Soldiers on the Secondary Host. All but one species of the genus *Pseudoregma* [118] and several species of *Ceratovacuna* [42, 83, 84, 86, 185] produce sterile first-instar soldiers on the secondary host. The soldiers are larger than the “normal” first-instar nymphs, their forelegs are greatly thickened, and their horns are long and sharp [3, 191, 192]. They clasp such a predator as a syrphid larva with the thickened forelegs and pierce it with the sharp frontal horns (not with their stylets) [185]. Although frontal horns of cerataphidines evolved originally for intracolony butting (Section 5.4.1), they were later converted into a piercing weapon in *Pseudoregma* and *Ceratovacuna* [4, 185]. An attacking soldier repeatedly thrusts its body forward until the horns are embedded deep into the predator’s body, which may rapidly exhaust the energy reserves of the soldier and lead to its death in a few hours [193]. The soldiers also crush eggs of predators with the horns [91] and at times pierce aphids of other species on the same host plant [185]. A soldier may also clasp a conspecific aphid. In such a case, however, the attack is not escalated, and the soldier soon detaches itself from the aphid, without injuring it [185]. Schütze and Maschwitz [194] suggest haemolymph from the predator, its prey aphid, or other insects as a cue that escalates the soldier’s attacking behavior in *Pseudoregma sundanica*. It is unknown what is the first cue that causes a soldier to clasp a predator. Unlike soldiers on the primary host (Section 5.3.1) and first-instar defensive nymphs of *Ceratovacuna lanigera* (Section 5.4.6), soldiers of all species of *Pseudoregma* and most species of *Ceratovacuna* produced on the secondary host lack cornicles and therefore do not discharge droplets of cornicle secretion. Second and later instar nymphs and adults have a pair of ring-like cornicles and discharge dark, sticky cornicle secretion when attacked by a predator. It is unknown whether an alarm pheromone is involved in the defensive system. The dark secretion is often attached to the predator’s body (see photos in [195]); it may have some defensive effect, as is reported in the nipponaphidine *Quadrartus yoshinomiya* [196, 197]. Like soldiers on the primary host, soldiers produced on the secondary host cannot discriminate between kin and non-kin, or between colony-mates and non-colony-mates [84, 93, 186].

Soldiers on the secondary host are completely sterile. No soldiers with the next instar cuticle developing inside have been found (Table 5). Sakata and Itô [93] reared soldiers of *Pseudoregma bambucicola* in cages on bamboo leaves or shoots in the laboratory and found that they survived for 55 days on average, 116 days at longest ($n = 6$). Soldiers ingest

TABLE 5: Molting rate of cerataphidine soldiers on the secondary host.

Species	No. of soldiers examined	No. of molting soldiers	References
<i>Pseudoregma alexanderi</i>	123	0	[3]
<i>Pseudoregma bambucicola</i>	233	0	[185]
<i>Pseudoregma panicola</i>	13	0	[185]
<i>Pseudoregma carolinensis</i>	35	0	[117]
<i>Pseudoregma baenzigeri</i>	77	0	[118]
<i>Ceratovacuna japonica</i>	100	0	[185]
<i>Ceratovacuna cerbera</i>	78	0	[86]

plant sap and excrete honeydew [198] but less frequently than normal first-instar nymphs do [199]. The sugar content in a droplet of honeydew, on average, is $2.9 \mu\text{g}$ for the soldier and $7.6 \mu\text{g}$ for the normal first-instar nymph in *P. koshunensis*, and the amino acid composition in honeydew is also different between the two morphs [199]. These suggest a difference in nutritional requirements between them.

Soldiers and normal first-instar nymphs are differentiated already in the embryonic stage [192]. A small number of intermediate individuals between the two morphs may appear [192]. The normal first-instar nymphs also have sharp frontal horns. In at least some species, they join attack on predators and/or competitors [86, 185, 200].

Soldiers are variable in size within a single species. The armatures of soldiers of *Ceratovacuna japonica* vary in size and shape between seasons and populations [83]. In *Pseudoregma alexanderi*, soldiers in a single colony at times fall into two size groups, “majors” and “minors” [3, 191]. It is unknown whether soldiers of these two size groups play different roles.

So far as is known, these horned soldiers occur in only two closely related genera, *Pseudoregma* and *Ceratovacuna*, while all cerataphidine species produce soldiers on the primary host. This fact indicates that the evolution of soldiers on the primary host preceded the evolution of soldiers on the secondary host.

5.4.3. Percentage of Soldiers on the Secondary Host. Percentage of soldiers on the secondary host has been intensively studied for *Pseudoregma bambucicola* on *Bambusa multiplex* by a number of researchers in Kagoshima, southern Japan [90, 94, 95, 97]. Itô et al. [95] and Shibao [97], in particular, counted the number of soldiers for many colonies of *P. bambucicola*. The proportion of soldiers varies considerably across seasons (from zero to ca. 40% [90, 95]) and colonies (from zero to 35% [97]). The consensus of the researchers is that many soldiers are produced in large colonies, and few or no soldiers in small colonies [95, 97], although the correlation is not strong (Figure 13). The same tendency was also detected in *Pseudoregma sundanica* on ginger [194, 200] and perhaps holds true for soldier-producing aphids in general. The tendency can be explained if we assume logistic growth of the aphid colony and the optimal proportion

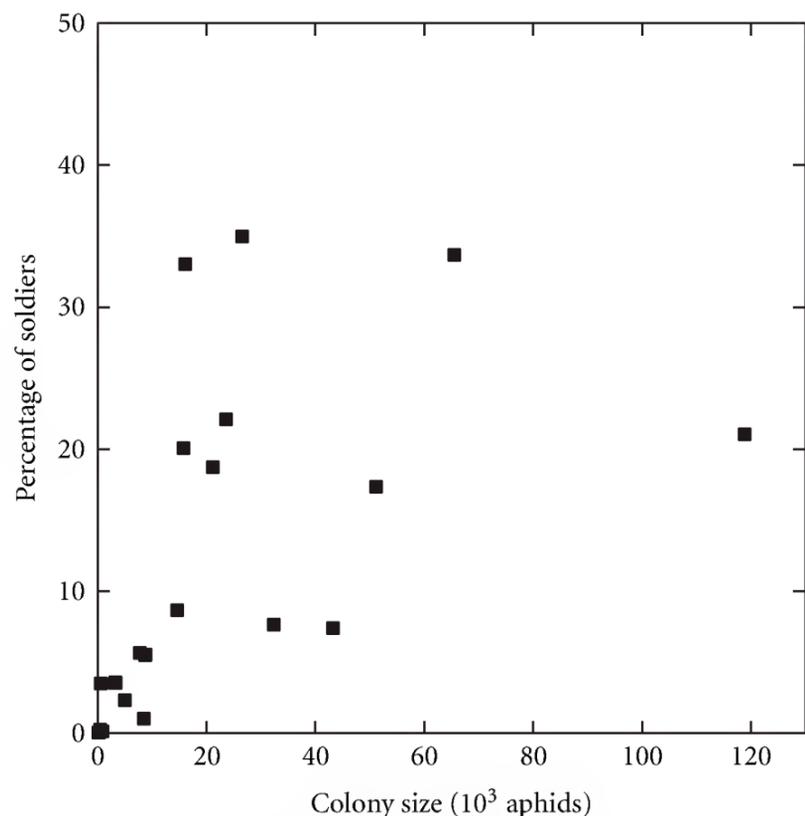


FIGURE 13: Relationship between colony size and the percentage of soldiers in *Pseudoregma bambucicola* on *Bambusa multiplex*. Data for 20 colonies sampled in Kagoshima and Miyazaki Prefectures, Japan, from October to December are shown (drawn from data in [97]). The correlation coefficient is 0.63 after arcsin square-root transformation of the percentage values.

of soldiers that maximizes the total productivity. Aoki and Kurosu [201] showed that a soldier of any given ordinal number (e.g., a first soldier, or a twelfth soldier) can be more readily produced in a large colony than in a small colony. This is because (1) a soldier benefits all non-soldiers in the colony by killing or repelling predators, and because (2) the value of a non-soldier (i.e., a reproductive) decreases as the colony size increases. Under these and some auxiliary assumptions, Aoki and Imai [202] carried out simulations, which indicated that the proportion of soldiers increases as the colony size increases. Although our model [202, 203] does not take into account the replacing cost of soldiers (i.e., assuming immortal soldiers), it indicates how effective a soldier must be if it is produced: a soldier must increase each non-soldier's productivity by larger than r_m/K . In other words, the marginal defensive efficacy of a soldier must be larger than r_m/K , where r_m and K are the maximum intrinsic rate of increase and the carrying capacity of the aphid colony without soldier, respectively. The ratio appeals to our intuition because r_m and K are well-known parameters in the logistic equation.

5.4.4. Leg-Waving Behavior. Many apterous adults and nymphs (including soldiers) of some *Pseudoregma* species in a colony, when they are disturbed (e.g., when the host plant is lightly touched), respond by waving their legs all together. This leg-waving (or leg-shaking) behavior is noticeable in *P. bambucicola* [93, 99, 111, 204] and *P. alexanderi* [185], which form colonies on bamboo shoots or twigs. Individual aphids lift their hind legs (and at times their abdomens,

too) and move them up and down, their fore and mid legs remaining in situ [93, 185]. Synchronized with this behavior, many droplets of honeydew fall from the colony [185]. According to Sakata and Itô [93], soldiers of *P. bambucicola* lift both their abdomens and hind legs more frequently than normal first-instar nymphs do. Shingleton and Foster [205] succeeded in inducing many soldiers but only a few non-soldiers of *Pseudoregma sundanica* (on ginger) to shake their legs by blowing gently on them. Stern et al. [206] observed leg waving of many aphids when predatory wasps passed near and walked over the surface of a colony of *Pseudoregma* sp. on bamboo. Ôhara [91] observed that a gravid female of the hoverfly *Eupeodes confrater*, which is a specialist predator of *Pseudoregma bambucicola* on bamboo and often lays her eggs on spider threads (Section 5.4.5), induced leg-waving behavior.

“ [A gravid] female [of *E. confrater*] approached [a] bamboo [shoot infested with *P. bambucicola*] in a straight line. She then hovered at various heights, and moved around the bamboo at a distance of a few centimeters. She examined the aphids at an angle of 45–60 degrees as she repeatedly flexed and contracted her legs. Both the soldiers and other morphs of *P. bambucicola* in the colony reacted to the fly's proximity by lifting and shaking their hind legs.”

Kenji Ôhara (personal communication) once suggested to us that, by so doing, the female of *E. confrater* might assess defensive activity of the aphid colony and, based on this information, decide where to lay her eggs, that is, whether she should oviposit directly onto the colony or on nearby spider threads so that soldiers could not readily kill her eggs.

While leg-waving is regarded as a defensive behavior [6, 96, 200, 207], how it works is still utterly unknown. Schütze and Maschwitz [207] mention that the behavior “must be understood as an unspecific defensive behavior.” Other researchers have suggested that it may deter syrphids from ovipositing [96, 99] or ward off flying parasitoids [99, 200]. In this connection it may be worth pointing out that few parasitoid wasps have been recorded from cerataphidines; the aphelinid *Encarsia flavoscutellum* from *Ceratovacuna lanigera* and *Astegopteryx nipae* [208] and *E. ceratiphivora* from *Cerataphis brasiliensis* [209] are rare exceptions. We add another yet-untested hypothesis that the simultaneous leg-waving seen in *Pseudoregma* species might be a kind of bluff that is effective against larger predators, possibly against birds.

Outside the Cerataphidini, leg-waving behavior is known in some species of Aphidinae and Lachninae [204, 210].

5.4.5. Predators of Soldier-Producing Species. Because soldiers on the secondary host can kill usual predators of aphids, predators often observed in their colonies are those that have developed some devices for escaping attack by the soldiers. The large coccinellid *Synonycha grandis*, the small coccinellid *Sasajiscymnus amplus*, the syrphid *Eupeodes confrater*, and the pyralid *Dipha aphidivora* are specialist predators of

the soldier-producing species *Pseudoregma alexanderi*, *P. bambucicola*, and *P. koshunensis* in southern Japan and/or Taiwan [185, 195, 211].

Larvae and adults of *S. grandis* prey on aphids in and on the outskirts of the aphid colony. Both are well armored with hard skins and soldiers are unlikely to pierce them with the horns; soldiers of *P. bambucicola* clasp or push the legs of the larvae and force them to fall off the bamboo [97]. Larvae of *Sasajiscymnus amplus* (see [212]), on the other hand, prey on aphids in the colony without being attacked by soldiers [185]. The larvae so much resemble prey aphids, those of *P. alexanderi* in particular (see a photograph on page 421 of Moffett [195]), that an observer frequently loses sight of them once he takes his eyes off the aphid colony. The reason for the close resemblance is yet unknown. Larvae of an unidentified species of Scymnini, whose bodies are slender in shape and which therefore do not resemble the prey aphids, have been obtained from colonies of *Pseudoregma baenzigeri* (our finding unreported in [118]).

Larvae of *Dipha aphidivora* weave silk tunnel nests within the aphid colony on the culm of bamboo and put their heads out of the tunnel to catch aphids, usually without being attacked by soldiers [195, 211, 213]. Early-instar larvae of *Taraka hamada* (Lycaenidae) and larvae of *Atkinsonia ignipicta* (= "*Oedematopoda semirubra*") (Stathmopodidae) also live in such silken nests and prey on aphids of *Pseudoregma* [211], but their main prey in Japan is *Ceratovacuna japonica* [214–217].

The syrphid *Eupeodes confrater* is famous for its acrobatic ovipositing behavior. Gravid females frequently lay their eggs onto fine threads of spiders' webs near the aphid colony [91, 96, 195, 211]. Soldiers of *Pseudoregma*, with their horns, easily crush eggs of *E. confrater* laid directly onto bamboo culms [91, 92, 97]. The oviposition on the threads is therefore an adaptation for avoiding attack by aphid soldiers. According to Ôhara [92], a single soldier of *P. bambucicola* can lift up a just-hatched larva of *E. confrater* and may fall off the colony together with the larva, but it can no longer lift up a first-instar larva after the larva has consumed an adult aphid and become heavier. Ôhara [92] has repeatedly observed that, when a soldier clasps the posterior part of the syrphid body, the soldier dies and is detached from it in a few minutes, without being counterattacked by the syrphid with its mouthhook. He suggests that the first-instar syrphid larva may secrete a chemical substance which is toxic to the aphid soldier. This interesting possibility has not yet been confirmed by later researchers. Shibao [97] experimentally showed that defense by soldiers of *P. bambucicola* is effective, to at least some degree, against the specialist predator *E. confrater* (as well as against the coccinellid *S. grandis*).

Colonies of *Pseudoregma* also attract generalist predators and the soldiers repel them [4, 185]. The syrphid *Episyrphus balteatus* and the hemerobiid *Micromus numerosus* recorded by Morimoto and Shibao [211] are likely to be such generalist predators. Both are known to prey on various species of aphids [219, 220].

Rodents sometimes eat aphids of *Pseudoregma* on bamboo [118].

5.4.6. *Defense by Monomorphic Nymphs: Piercing with Horns.* Some cerataphidine species have monomorphic first instars and do not produce morphologically distinct sterile soldiers; nevertheless these "monomorphic" first-instar nymphs have sharp horns and attack predators with the horns. Examples are an unnamed species of *Pseudoregma* found on *Schizostachyum zollingeri* in the Malay Peninsula [206] and the sugarcane woolly aphid, *Ceratovacuna lanigera*; the defensive behavior of the latter is well studied and treated here.

First-instar nymphs of *Ceratovacuna lanigera* are monomorphic and active walkers, and, when eggs of syrphids or hemerobiids are laid in or near the colony, they damage and crush the eggs with their sharp frontal horns [116, 218, 221]. Eggs of non-specialist predators such as the syrphids *Episyrphus balteatus*, *Ischidon scutellaris*, and *Syrphus ribesii*, and those of the hemerobiid *Eumicromus navigatorum* are quite easily crushed by the nymphs [116, 218, 222]. Some predators have evolved a counteradaptation to the attack by the nymphs. Adult hoverflies of *Eupeodes kuroiwayae* (referred to as "*Metasyrphus hakiensis*" in [218]) closely resemble those of *E. confrater*, a predator of *Pseudoregma* species (see Section 5.4.5), and the species has been recorded under the latter's name as a predator of *C. lanigera* ([223–225], see [116]). Eggs of the two species are, however, very different from each other. As mentioned before, egg shells of *E. confrater* are not very hard, and soldiers of *Pseudoregma bambucicola* easily pierce and kill them. Eggs of *E. kuroiwayae* are, on the other hand, slender, cylindrical in shape, and the eggshell is so hard that first-instar nymphs of *C. lanigera* cannot pierce it with their horns. Gravid females of *E. kuroiwayae* lay their eggs close to colonies of *C. lanigera*, so that aphid nymphs attack almost all laid eggs (Figure 14(a)) but fail to kill them [116, 218]. Eggs of *Dideoides latus*, another syrphid predator specialized for *C. lanigera* (and perhaps also for other *Ceratovacuna* species with soldiers), are not so tough as those of *E. kuroiwayae* but much tougher than those of the generalist syrphid predators mentioned above. First-instar aphid nymphs may kill eggs of *D. latus*, but only after their persistent attacks, often over a few days. To avoid attacks by soldiers, females of *D. latus* lay their eggs apart from aphid colonies, at times more than 1 m away from the nearest colony [218] (Figure 14(b)).

Attacking first-instar nymphs often discharge, from one cornicle or both on their posterior abdominal tergites, a droplet which contains an alarm pheromone and recruits other first-instar nymphs [222]. Unlike soldiers of *Tuberaphis styraci* (on the primary host), aphids of *C. lanigera* do not react to (*E*)- β -farnesene [116]. Because the greenbug, *Schizaphis graminum* (Aphidinae), which uses (*E*)- β -farnesene as an alarm pheromone [157], exhibits no response to the cornicle secretion of *C. lanigera*, *C. lanigera* is likely to use another unidentified chemical as the alarm pheromone [116].

First-instar nymphs of *Ceratovacuna lanigera* also attack larvae of predators. Arakaki [116] showed that their attack is effective against larvae of a generalist predator, *Episyrphus balteatus*.

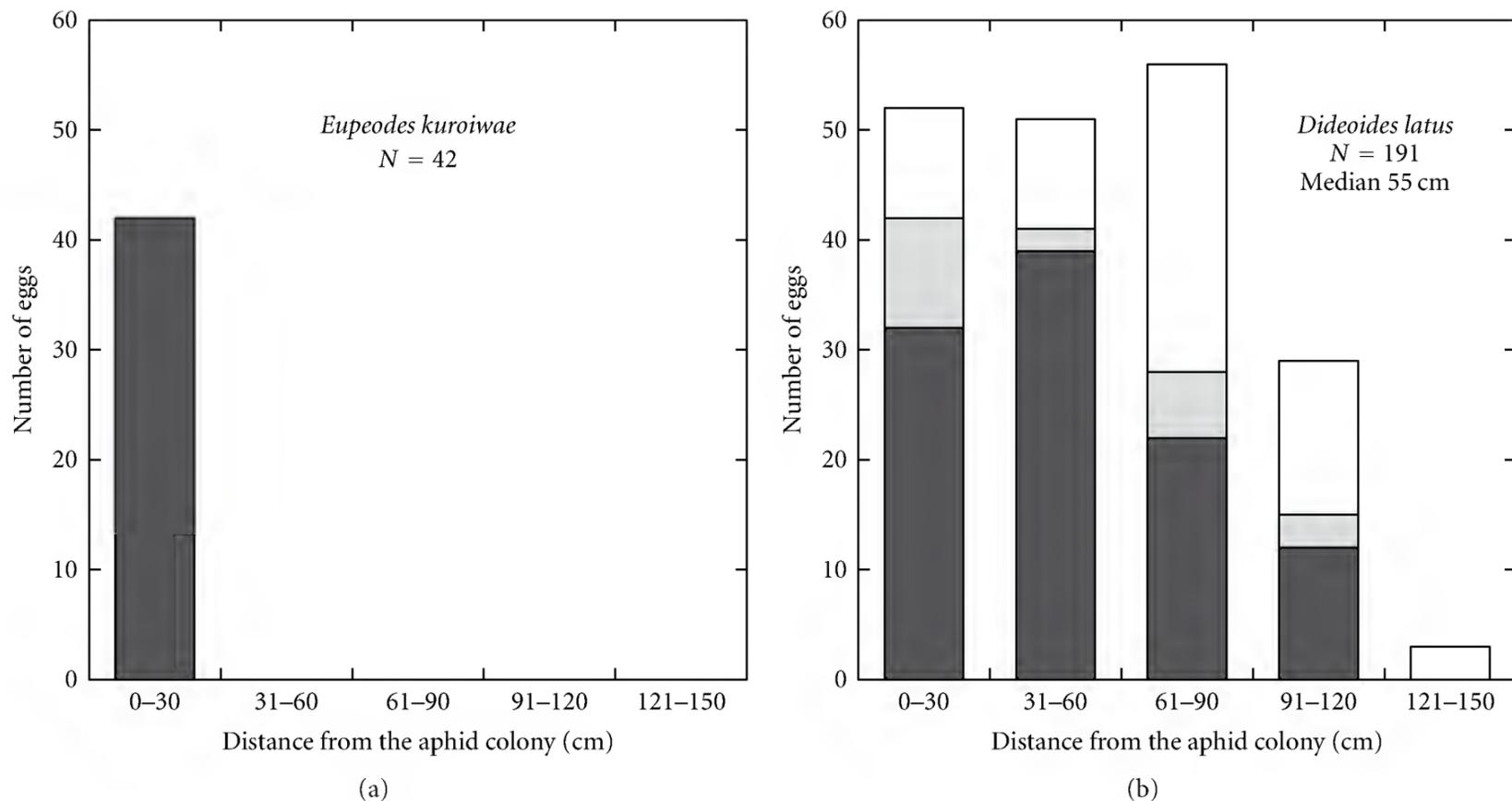


FIGURE 14: Distribution of eggs laid by two syrphid species in and around a colony of the aphid *Ceratovacuna lanigera* formed on the grass *Miscanthus sinensis*. Eggs that were and were not attacked by aphid nymphs within first three days are indicated by dark grey and white areas, respectively, and those that disappeared within first three days by light grey areas. (a) *Eupeodes kuroiwaie*. Although all laid eggs were attacked, the hatchability of attacked eggs in *E. kuroiwaie* (71.9%) was much higher than that in *Dideoides latus* (29.8%) (2×2 Fisher's exact test; $P \ll .0001$). (b) *Dideoides latus*. Eggs of *D. latus* that were laid more than 55 cm (the median) away from the colony were less likely to be attacked by aphid nymphs than those that were less than 55 cm away (2×2 Fisher's exact test; $P = .0001$). Also, the hatchability of attacked eggs (29.8%) was lower than that of unattacked eggs (70.1%) (2×2 Fisher's exact test; $P \ll .0001$) (redrawn and recalculated from data in [218]).

5.4.7. Defense by Monomorphic Nymphs: Piercing with Stylets.

Tuberaphis takenouchii produces exules with no horns in its leaf roll galls formed on mistletoes of the family Loranthaceae. First-instar nymphs of the secondary-host generation have a dagger-like ultimate rostral segment and pierce insect larvae with their stylets [37].

First-instar nymphs of horned aphids may even attack predators with their stylets. *Astegopteryx bambucifoliae*, on the secondary host, produces first-instar nymphs with short horns which seem to be useless for piercing enemies. Some first-instar nymphs of this species were observed piercing insect eggs and pupae with their stylets [87]. Agarwala et al. [226] report that horned aphids of *Ceratovacuna silvestrii*, which live on bamboo in northeastern India, cause irritation to human skin. It is still unknown whether the aphids pierce human skin with their stylets.

5.4.8. Association with Ants. Cerataphidines on their secondary hosts are often associated with ants. Colonies of those species that do not produce defensive nymphs are frequently attended by ants, especially in the tropics. *Cerataphis brasiliensis* on palms [190, 227], *C. freycinetiae* on *Freycinetia* sp. [71], and *Astegopteryx* spp. on bamboos and palms [64, 70, 228] are examples. On the other hand, in those species that produce soldiers or defensive nymphs, their association with ants seems complicated. Colonies of the Siamese species

Pseudoregma baenzigeri on bamboo shoots often become huge and produce too much honeydew to be consumed by attending ants such as *Pheidologeton trechideros*, *Dolichoderus thoracicus* and *Myrmecaria brunnea*. Honeydew falling from these colonies forms a broad dark band on the forest litter [118]. This is also the case for *Pseudoregma bambucicola* on bamboo in Kagoshima, southern Japan [94], and *P. alexanderi* on *Dendrocalamus latiflorus*, in central Taiwan (our unpublished observations). Shibao et al. [99] found that many colonies of *P. bambucicola* on *Bambusa multiplex* are initially attended by the ant *Lasius japonicus* or *Crematogaster osakensis*, but most of the colonies are later abandoned by these ants. Unattended colonies, including those that were abandoned by the ants, often attain a large colony size and produce numerous soldiers. In contrast, colonies continuously attended by the ants usually remain small and have no soldiers. Shibao et al. [99] suggest that ants frequently prey upon aphids they attend, and that the aphid colony invests less in soldiers when attended by ants.

A more intimate association with ants is observed for *Pseudoregma sundanica* on ginger in the Malay Peninsula. This species is an obligate myrmecophile. Almost all the aphid colonies are tended by ants [205]. Most (ca. 80%) colonies have no soldiers, but artificial exclusion of ants results in the production of soldiers or the increase in the proportion of soldiers [205]. Schütze and Maschwitz [194]

see this ant-aphid symbiosis from a different point of view. Ants repel general predators from colonies of *P. sundanica* which they attend but do not attack lepidopteran larvae of the tribe Miletini (Lycaenidae), such as *Allotinus unicolor* or *Miletus biggsii*. These lycaenid species are specialist predators of ant-attended homopterans [229, 230] and are associated with particular ant species (e.g., *Allotinus unicolor* with *Anoplolepis gracilipes*, *Miletus biggsii* with *Dolichoderus* sp. [231]). Maschwitz et al. [229] suggest that soldiers defend their colony from miletine predators which tending ants do not attack.

5.5. Sexuparae. Sexuparae are those aphids that produce sexuals (oviparous females and males). In the subfamily Hormaphidinae, so far as is known, all sexuparae are alates and fliers. In the sister subfamily Eriosomatinae, one species (*Eriosoma rileyi*) has apterous sexuparae [232], and some (*Kaltenbachiella japonica* [233] and *Pemphigus spyrothecae* [234]) produce alate sexuparae which usually do not fly but walk to the tree trunk where their progenies mate. Such exceptions might also be found in the Hormaphidinae. Sexuparae are produced either on the secondary host (if the life cycle is host-alternating) or on the primary host (if the life cycle is non-host-alternating). They fly to leaves of the primary host, *Styrax* trees, and larviposit there. One sexupara usually (but not always) contains both male and female embryos.

5.6. Secondary Migrants. Secondary migrants are alate exules, or alates that migrate between secondary hostplants. In external morphology, they are very similar to alate sexuparae; they may have been derived from the latter. In some species such as *Ceratovacuna cerbera* [86], secondary migrants are produced only in the end of the season (in October/November) when sexuparae would be produced. In others (e.g., *Ceratovacuna lanigera* [116], *Cerataphis brasiliensis* [34]), secondary migrants are produced in various months. In species with horned exules, secondary migrants (and sexuparae) have a pair of short, often vestigial horns and can be easily distinguished from conspecific emigrants in morphology.

5.7. Sexuals. Both males and sexual (oviparous) females of the Cerataphidini are tiny and apterous. So far as is known, females each lay only one egg [17, 40, 46, 79, 80]. (Females of nipponaphidines [235] and hormaphidines [58] lay more than one egg.) Sexuals of the genera *Tuberaphis* [11, 39, 40] and *Cerataphis* [34, 46] have a rather long rostrum; it is unknown whether they need plant sap for maturation. On the other hand, sexuals of the genera *Pseudoregma* [185], *Ceratovacuna* [78, 115], and *Ceratoglyphina* [72] and females of *Astegopteryx* [16] have only a short rostrum. Males of *Astegopteryx* (at least of *A. spinocephala* and *A. bambucifoliae*) are arostrate [17] like sexuals of the Eriosomatinae [68, 236]. Females of these cerataphidine genera can mature without feeding [16, 17, 78, 79, 100]. In one species (*Ceratovacuna nekoashi*) fighting between males for a female is reported [80].

5.8. Sex Ratio. Sex ratios of the tribe Cerataphidini have not yet been fully studied. In the host-alternating species *Ceratoglyphina styracicola*, female-biased investment ratios (0.33 and 0.42) were obtained, suggesting the occurrence of local mate competition (LMC) [30]. Kurosu and Aoki [237] applied Yamaguchi's [238] ESS model, which predicts a constant number of males for each sexupara, to five cerataphidine species. Among these species, the prediction was met well in the obligately host-alternating species *Ceratovacuna nekoashi*: of 114 sexuparae dissected, most (>90%) contained eight male embryos and the others seven or nine [237]. Watase [79] obtained almost the same result with 20 sexuparae of *C. nekoashi*. However, Yamaguchi's model requires a constant number of mother sexuparae whose offspring constitutes a breeding population. Nobody has yet succeeded in explaining how this requirement can be fulfilled. In case of *C. nekoashi*, alate sexuparae larviposit on leaves of *Styrax japonicus*, and first-instar sexuals soon move to branches where mating occurs [78]. Thus, the number of mother sexuparae is likely to vary across breeding sites and across years.

In the non-host-alternating species *Astegopteryx spinocephala*, the (investment) sex ratio is almost 1 to 1 (0.464–0.519). As mentioned in Section 3.8.2, in this species, mating takes place in live subgalls that are guarded by soldiers. Most males are deposited on leaves and enter live subgalls for mating; thus some level of outbreeding is attained. On the other hand, because some (14–27%) males are deposited in the natal subgalls, LMC should bias the sex ratio toward the female. Aoki et al. [133] argue that local resource competition may counteract the effects of LMC.

5.9. Eggs. Little information is available about cerataphidine eggs. They are elongate ellipsoid in shape, pale, covered with wax secreted by the mother oviparae, and approximately 0.37 mm long and 0.16 mm wide in *Astegopteryx spinocephala* [17] and approximately 0.38 mm long and 0.22 mm wide in *Ceratovacuna nekoashi* [79]. Eggs are laid in the fissures of bark in *C. nekoashi* [78, 79], or sometimes in old dead galls in *Astegopteryx bambucifoliae* [16]. In Nipponaphidini and Hormaphidini, eggs are often laid directly onto buds of the host tree [58, 74], but in Cerataphidini no such case has yet been reported. In *A. spinocephala* (Section 3.8.2) and *Ceratoglyphina roepkei* (Section 3.8.3), eggs are laid in live subgalls defended by soldiers.

Eggs of temperate species such as *Ceratovacuna nekoashi* (Section 3.1) have to enter diapause for hibernation. Eggs of the subtropical species *Astegopteryx bambucifoliae* are thought to hatch soon after being laid without entering diapause [16]. In other subtropical or tropical species with seasonal life cycles, sexuparae are at times produced out of season (Sections 3.4.3 and 3.6). It is unknown whether eggs laid by daughters of these sexuparae hatch in appropriate months for gall formation.

6. Conclusion

In this review, we have surveyed the life cycles of cerataphidines and illustrated various patterns found in temperate,

subtropical and tropical regions. In conclusion, the life cycles of the tribe Cerataphidini are basically the same as those of the Eriosomatinae, which have been studied better [53], in the sense that they are classified into (1) obligate or facultative host alternation, (2) monoecy on the primary host, and (3) anholocycly on the secondary host. However, since many cerataphidines inhabit tropical and subtropical regions of Southeast Asia, they show some special features which have not been known in the Eriosomatinae.

Firstly, winter is mild or effectively absent in tropical and subtropical regions of Southeast Asia. The life cycles of tropical or subtropical cerataphidines are therefore not very rigidly tuned to seasonal changes in the climate if any. Migration from the primary host to the secondary host, for instance, often lasts over a few months (Section 3.4). Although this has not yet been definitely confirmed, the life cycles of some host-alternating species (including *Cerataphis brasiliensis* [34]) in tropical rainforests are alleged to be aseasonal (Section 3.7); that is, galls are formed on the primary host all the year round. The life cycles of species living in tropical monsoon and subtropical regions, on the other hand, show a clear seasonality (Sections 3.4 and 3.6). Even in tropical rainforests near the equator, the monoecious *Ceratoglyphina roepkei* has a distinct seasonal life cycle; perhaps its life cycles might not be synchronized across regions (Section 3.8.3). It may also be worth emphasizing here that all gall-forming cerataphidines hitherto known retain sex, whether they may be tropical, subtropical, or temperate species (of course, except for anholocycly on the secondary host). This fact does not contradict theories about sex [239–241], but earlier researchers (e.g., [52, 242]) tended to think that aphids in general propagate themselves exclusively by parthenogenesis in tropical and subtropical regions.

Secondly, cerataphidine galls in tropical and subtropical regions last at least several months, at times for over one year (Section 3.4). Even a temperate species induces biennial galls on a deciduous tree (Section 3.8.1). This is possible because they produce many sterile soldiers that effectively defend their galls (Section 5.3). They can produce many soldiers because their colony size becomes large [201–203], at times huge, more than 100,000 (Table 3). The colony sizes can be large because their galls become large enough to sustain many aphids. Often, cerataphidine galls are complicated in shape: twisted (Figures 6(c) and 6(d)), or coral shaped (Figures 5(a) and 12(a)), and/or with many solid projections inside (Figures 12(b)–12(e)). These features contribute to increasing the surface-to-volume ratio (cf. [243]), thus harboring more aphids per volume.

Thirdly, species of two genera of the Cerataphidini, *Pseudoregma* and *Ceratovacuna*, always and at times, respectively, produce soldiers or non-sterile nymphs that defend their colonies on the secondary host (Section 5.4.2). In addition, at least one species of *Tuberaphis* produces defensive nymphs on the secondary host (Section 5.4.7). Although soldiers are also known to occur on the secondary host in the eriosomatine genus *Colophina* [244–246], here we point out a possibility of their long-term impact upon the life cycles of Cerataphidini. The production of soldiers or defensive nymphs (as well as

association with ants) has certainly lengthened colony span on the secondary host. As a result, some clones may now persist on the secondary host over years without sex, as is suggested by the success of those species (e.g., *Pseudoregma bambucicola*, *P. panicola*, *Ceratovacuna lanigera*) that have invaded regions where their primary host is lacking. In these clones, gall-forming ability is not necessary for their short-term success, and mutations that are deleterious to the life on the primary host but neutral or beneficial to the life on the secondary host may have been accumulated. Introgression of these bad genes into the gall-forming generation through sex will decrease the rate of success in gall formation. From this “bad gene” hypothesis [237] an admittedly vague prediction is made: in tropical/subtropical regions, galls of the species that produce soldiers or defensive nymphs *on the secondary host* are in general rarer than galls of those that do not. This may be the reason why it is difficult to find galls of *Pseudoregma* in comparison with *Astegopteryx*.

Acknowledgments

This study was in part supported by a grant from Chuo University (to UK in fiscal years 2008 and 2009). Naomi Pierce and David Stern kindly permitted the authors to use the photo of Figure 12(e) and the photos of Figures 2(c) and 12(f), respectively. (All other photos were taken by the authors.)

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