THE JOURNAL OF RESEARCH ON THE LEPIDOPTERA

VOLUME 42 2003 (2010)

ISSN 0022 4324

THE LEPIDOPTERA RESEARCH FOUNDATION

THE JOURNAL OF RESEARCH ON THE LEPIDOPTERA

ISSN 0022 4324

PUBLISHED BY: The Lepidoptera Research Foundation, Inc.
9620 Heather Road
Beverly Hills, California 90210-1757
TEL (310) 274 1052

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FOUNDER: William Hovanitz (1915-1977)

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	Subscription Rate/ Institutions	$ 35.00 year
	Life	$ 300.00

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Journal of Research on the Lepidoptera 42: 1-4, 2003 (2010)

Differences in thermal responses in a fragmented landscape: temperature affects the sampling of diurnal, but not nocturnal fruit-feeding Lepidoptera

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Abstract. Weather is the primary determinant in butterfly activity, with temperature one of the key factors affecting the biology and behavior of most Lepidoptera. Despite evidence about the influence of temperature in Lepidoptera capture patterns, few studies have analyzed microclimatic characteristics. Most studies focused on broad geographic scales and historical climatic patterns. The present study contrasts the effect of local temperature on the capture rates of two groups of fruit-feeding Lepidoptera, butterflies (diurnal) and noctuid moths (nocturnal), in a fragmented landscape. Ten fragments with five traps each were sampled in southeast Brazilian Atlantic Forest during eight days in July-2005. We found a positive relation between mean temperature and both richness and abundance of captured butterflies, but not the noctuid moths. These differences are probably a result of the miothermic nature of moths, making the moth assemblage less dependent of solar radiation than butterflies. The differences between moths and butterflies could affect the distribution of these insects across fragmented landscapes and suggest that adult moths are probably less sensitive to changes in the amount of solar radiation than adult butterflies.

Key words: fruit-feeding butterflies, Nymphalidae, Noctuidae, temperature.

INTRODUCTION

Ehrlich (1984) stated that weather is the primary determinant in butterfly activity. Temperature is a key factor affecting most Lepidoptera because it has direct effects on larval behavior and performance, flight activity and associated behaviors such as foraging and territoriality (Broersma *et al.,* 1976; Scriber & Slansky, 1981; Hrdy *et al.,* 1996; Shirai *et al.,* 1998; Kührt *et al.,* 2005; Nabeta *et al.,* 2005; Merckx *et al.,* 2006). Many butterfly species are able to maintain their body temperature above ambient through basking and/or shivering behaviors (Kemp, 2002), such that individuals with higher body temperatures can be active for longer periods than their cooler bodied counterparts (Dudley, 1991). On sunny days some butterflies have been reported to fly for longer periods and maintain higher body temperatures than on cloudy days (Shelly & Ludwig, 1985). Temperature is also known to affect the seasonal distribution of some species (Turner *et al.,* 1987), probability of capture in temperate regions (Yela & Holyoak, 1997; Holyoak *et al.,* 1997), and the abundance and diversity patterns of both butterflies (Turner *et al.,* 1987; White & Kerr, 2007) and moths (Brehm *et al.,* 2007; Choi, 2008).

Despite the evidence that ambient temperature influences activity patterns in Lepidoptera, few studies have analyzed the effect on a microclimatic scale (e.g. Yela & Holyoak, 1997). Most have focused on broad geographic scales and historic climatic patterns (e.g. Brown & Freitas, 2000; Menéndez *et al.,* 2007). However, anthropogenic activity is known to produce major changes in microclimatic conditions (Saunders, 1991; Vitousek *et al.,* 1997) which likely in turn could affect the insects that occupy a given habitat.

Accordingly the present study attempts to test the effect of local temperature in the capture rates of fruit-feeding butterflies and moths in a fragmented landscape. Here we ask whether there are differences in the responses of diurnal versus nocturnal Lepidoptera to average temperature, linking the possible differences with the effects of forest fragmentation among these two sets of insects.

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Received: 5 March 2009
Accepted: 6 March 2009

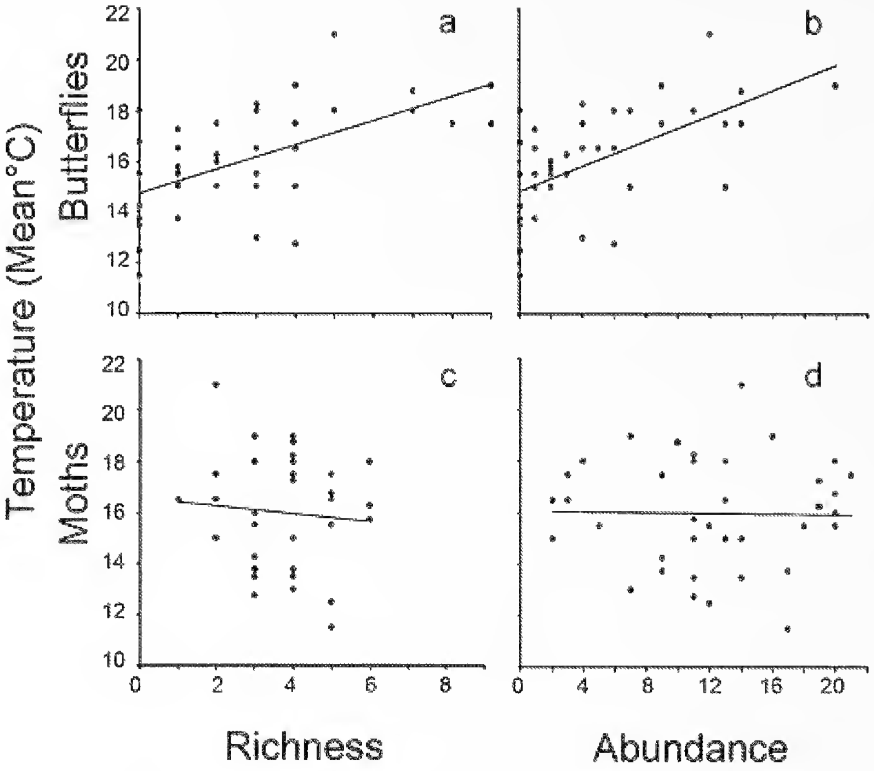

Figure 1. Linear regressions between temperature and species richness and between temperature and abundance for both butterflies and moths sampled with baited traps in a fragmented landscape in Brazilian Atlantic Forest. **a)** Linear regression between mean temperature and richness of fruit-feeding butterflies; **b)** linear regression between mean temperature and abundance of fruit-feeding butterflies; **c)** linear regression between mean temperature and richness of bait attracted moths; **d)** linear regression between mean temperature and abundance of bait attracted moths.

Materials and methods

Field work was carried out in the São Luiz do Paraitinga municipality (Fig. 1), São Paulo State, south-eastern Brazil (centered in 23°20' S, 45°20' W). The original vegetation of the area was mainly a dense humid forest (MME 1983); however, the process of forest fragmentation drastically changed the plant community cover across the region (Schmidt, 1949; Petrone, 1959; Dean, 1997). Today, large parts of the native vegetation has been removed with the resulting landscape now being composed of small patches of disturbed forest scattered in a matrix of farm fields and abandoned pastures and including some patches of *Eucalyptus* plantations.

Ten forest fragments were chosen at random for sampling. In each fragment, five traps were arranged along a linear transect for a total of 50 traps. Detailed information of sampling methods and the study site are given in Ribeiro *et al.* (2008).

Butterflies (diurnal) and noctuid moths (nocturnal) were sampled during June 2005. The traps remained in the field for eight days and were visited at 48-hour intervals. During each visit bait was replaced and each captured individual was identified, marked and released. Species not determinable in the field were taken for later identification (following Ribeiro *et al.*, 2008). All butterflies were identified to species level and the moths (all Noctuidae) were discriminated as morphospecies.

Ambient temperatures were measured with a max-min thermometer at each visit. The arithmetic mean of the maximal and minimal temperature measured during the period between visits (48 hours) was used as mean temperature.

We use simple linear regression to test if there was a relationship between abundance and richness of butterflies and moths with mean temperature.

Results

A total of 233 individuals comprising 27 species of fruit-feeding Nymphalidae and 475 individuals of nine morphospecies of noctuid moths were captured.

The mean temperatures recorded during the study period ranged between 11.5 and 21.0°C. We found a significant relation between mean temperature and species richness ($p < 0.0001$; $R^2 = 0.362$; $N = 40$; $y = 0.2481x + 14.84$) (Fig. 1a) and individual abundance ($p < 0.0001$; $R^2 = 0.362$; $N = 40$; $y = 0{,}2481x + 14.84$) (Fig. 1b) in our butterfly samples. In contrast, we did not find a significant relation between mean temperature in either richness ($p = 0.5811$; $R^2 = 0.0084$; $N = 40$; $y = -0.1608x + 16.599$) (Fig. 1c) or abundance ($p = 0.8936$; $R^2 = 0.0004$; $N = 40$; $y = -0.0079x + 16.094$) (Fig. 1d) of the moth samples.

Discussion

We found a strong relation between microhabitat temperature and richness and abundance in samples of diurnal, but not nocturnal noctuid Lepidoptera. The relation between butterfly capture and temperature is likely the result of differences in daily activity of the diurnal set of species compared with the nocturnal noctuids. Despite the great variety of behaviors related to thermoregulation in butterflies (Clench, 1966), air temperature remains important in determining butterfly activity (Douwes, 1976). Thus we would expect greater butterfly activity and capture rate on hot rather than in cold days. Although weather, especially temperature, is usually considered the most important factor determining butterfly and moth diversity (Holyoak *et al.*, 1997; Yela & Holyoak, 1997; Hawkins & Porter, 2003; Brehm *et al.*, 2007; Menéndez *et al.*, 2007; Choi, 2008), few studies demonstrate significant relations between microhabitat conditions with species richness and abundance of Lepidoptera (e.g. Yela & Holyoak, 1997; Dolia *et al.*, 2008).

The differences found in diurnal versus nocturnal fruit-feeding Lepidoptera are likely the result of the latter being miothermic and therefore independent of solar radiation to enable their activities (Daily & Ehrlich, 1996). If the conclusion is correct, we would expect that butterflies in the subfamily Brassolinae should respond similarly to nocturnal moths, since brassolines are not as dependent upon solar radiation as other butterflies are (Srygley, 1994).

The degree of fragmentation has significant influence on microclimatic conditions, as temperature, humidity and amount of solar radiation (Saunders, 1991), with many studies of diurnal fruit-feeding Lepidoptera reporting changes in the community correlated with forest fragmentation (Krauss *et al.*, 2003; Shahabuddin & Ponte, 2005; Uehara-Prado *et al.*, 2007). The change in microclimatic conditions also affects the distribution of butterflies in forest fragments, probably because fragmentation impacts the activity of these insects by being beneficial to heliophylous species that are more likely to displace shade-loving species. Another important alteration caused by microclimatic changes in temperature is the effects upon life-history traits of butterflies by changing daily fecundity and lifetime number of eggs of females (Karlsson & Van Dyck, 2005). However, the same effect may be less important in moths because they are not directly influenced by solar radiation (Daily & Ehrlich, 1996) and suffer only the indirect effects of the changes (e.g. alteration in leaf quality, resource offer and so on).

Both sample richness and abundance are positively correlated with mean temperature in the present study. Since trap capture is considered as a reliable measure of activity and density in fruit-feeding Lepidoptera, we can conclude that temperature had an undeniable effect in butterfly richness and abundance. However, in view of the great importance of temperature in determining the behavior and distribution of diurnal Lepidoptera, other studies are necessary to verify if this relation could be found in other habitats, with different climates and land covers.

Acknowledgements

We especially thank Carla Penz and Niklas Wahlberg for critically reading and commenting upon our manuscript. We also thank the several land owners who permitted field work on their properties. DBR thanks FAPESP for a fellowship (grants #03/11697-0, 07/50896-0). AVLF acknowledges the FAPESP (grants #00/01484-1 and #04/05269-9), the Brazilian CNPq (fellowship #300315/2005-8), and the National Science Foundation (DEB grant #0527441). This project is part of BIOTA-FAPESP program (grants #98/05101-8 and #02/08558-6).

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Use of sound and aerial chases in sexual recognition in Neotropical *Hamadryas* butterflies (Nymphalidae)

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Abstract. Neotropical *Hamadryas* butterflies are notorious for the clicking noise they produce in flight. Tests of the sound production capacity of nine species of *Hamadryas* and observations on aerial interactions of six species showed that, among the three species groups, only males of the *feronia* group produced sounds, whereas females of all species and males of the *februa* and *laodamia* groups never did so. Most of the aerial interactions occurred during mid-day, generally initiated by males in exploratory flights. Chasers were always males. Males engaging in chases with other males continually produced clicking sounds during the interactions whereas males chasing females usually restricted their auditory displays to the initial phase of the pursuit. In a cage containing 24 individuals of both sexes of six species of *Hamadryas*, males were involved in 100% of the aerial interactions observed, with one single male participating in 57% of them. The most chased individuals were females and males of *H. feronia*. There was no relation between the number of times an individual successfully defended a feeding perch and the number of aerial chases initiated. Sound production is probably involved in finding mates, while aerial chases may be used in the establishment of a dominance hierarchy among male *Hamadryas*.

Key words: agonistic interactions, behavior, butterflies, communication, *Hamadryas*, acoustic communication, sexual recognition, sound.

INTRODUCTION

Butterflies communicate by means of a variety of stereotyped acoustic, visual, chemical, and tactile signals (Swihart, 1967; Wickman & Wiklund, 1983; Boppré, 1984; Silberglied, 1984; Bernard & Remington, 1991; Lees, 1992). Among these, acoustic signaling is relatively infrequently cited in the literature (Swihart, 1967). The Neotropical *Hamadryas* (Hübner, 1806) butterflies, a tight-knit group containing 20 species (Jenkins, 1983), are famous for their production of loud clicking sounds during aerial chases (Bates, 1865; Darwin, 1871; Swihart, 1967; Otero, 1990). Adult butterflies feed on tree sap and rotting fruits, and individuals of both sexes may display and fight to defend feeding sites from congeners (Marini-Filho, 1996). Ross (1963) classified *Hamadryas* as 'pugnacious,' although he did not observe site fidelity or other evidence for territorial behavior. *Hamadryas* butterflies are capable of hearing sounds produced by other conspecific butterflies and consider that once these sounds are produced during social interactions, these are probably involved in conspecific communication (Yack *et al.*, 2000). Yack *et al.* 2000 also discuss that the possible origin of sound production in the Papilionoidea butterflies could be derived from bat-detection in the basal clade of Hedyloidea moths, thus being a degeneration of these former structures.

Received: 27 April 2009
Accepted: 1 May 2009

Although Seitz (1913) reported that almost all *Hamadryas* [members of the genus *Ageronia* and *Peridromia* (genus *Hamadryas* sensu Jenkins, 1983)] make sound on flight, species typical of dense tropical forests (e.g. *H. chloe*, *H. alicia*, *H. rosandra* and *H. velutina*; Jenkins, 1983) apparently do not make sounds. Otero (1986), based on the observation that males of *H. feronia* intensely produce sound when pursuing other *Hamadryas* in flight, whereas *H. februa* were 'mute' and performed a spiral flight, argues that the behaviors represent alternative means of sexual recognition in the genus. *Hamadryas* were placed by Jenkins (1983) in three species groups (subgenera) based mainly in wing venation differences (note that species names preceded by * are possible exceptions): (1) *feronia* species group (subgenus *Hamadryas*), including *H. feronia*, *H. guatemalena*, *H. iphthime*, *H. epinome*, *H. fornax*, **H. alicia*, **H. rosandra*, *H. amphinome*, *H. belladonna*, *H. arinome*; (2) *februa* species group (subgenus *Ageronia*), including *H. februa*, *H. amphichloe*, *H. glauconome*, *H. honorina*, *H. atlantis*, *H. chloe*, *H. albicornis*; and (3) *laodamia* species group (subgenus *Peridromia*), including *H. laodamia*, *H. arete*, *H. velutina*.

The mechanism of sound production by *Hamadryas* is still subject of controversy. At least seven different places on the thorax, abdomen and fore wings have been proposed as sound-producing organs (Jenkins, 1983; DeVries, 1987; Monge-Nájera & Hernández, 1991; Swihart, 1967). Otero (1990) presents good evidence that *Hamadryas feronia* produce percussive sound through the striking of the wings. He points out that the structures responsible for the loud snapping sound are the swollen veins at the distal end of the fore wing discal cell. These modifications also occur in the Australian *Hecatesia* moths and were called castanets by Bailey (1978). Some Satyrinae species in the genus *Pharneuptychia* and *Euptychoides* also seem to have identical structures associated with sound production (Kane, 1982; Murillo-Hiller, 2006). According to Otero (1990), castanets were only observed in the males of the sound-producing *feronia* species, while males and females of *H. februa* in Venezuela do not show these structures. The swellings of the sub-costal venation of the forewings have also been supposed to be associated with sound production by Monge-Nájera and Hernández (1991), however, these authors pointed that swollen sub-costal veins occur in all *Hamadryas* species they dissected (*amphinome*, *feronia*, *guatemalena*, *glauconome*, and *februa*) independent of sex. Further studies made by Yack *et al.* (2000) contradicts the percussion mechanism arguing that sound can be produced by a single forewing, thus proposing a wing deformation mechanism for the production of sound. This 'flip-flop' changes the side of the wing concavity as shown in the photographs presented by Monge-Nájera *et al.* 1998.

In the present study we evaluate the sound production capacity of nine species of the genus *Hamadryas* and analyze the hypotheses related to the possible functions of sound production and aerial chases in sexual recognition. After determining which species and sexes are capable of producing sounds, we use behavioral and morphological data to conjecture if (1) aerial interactions with sound production are used for sexual recognition between *Hamadryas*, and (2) aerial interactions are related to the establishment of a dominance hierarchy between the males present in the feeding arena.

STUDY SITES AND METHODS

Butterflies were captured using standard Van Someren-Rydon butterfly traps baited with a mixture of fermented sugarcane juice and banana (DeVries, 1987). Traps were set several times from 1992 to 2000 along the edges and interiors of forests and woodlands at eight different localities: Linhares Forest Reserve (tropical semi-deciduous forest), Linhares, ES, Brazil (19°04' S; 40°08' W); Santa Genebra Forest Reserve (subtropical semi-deciduous forest), Campinas, SP, Brazil (47°04' S, 22°50' W); Brasília Botanical Garden and Água Limpa Reserve (tropical cerrado savanna and gallery forest), Brasília, DF, Brazil (15°57' S; 47°56' W); Pipeline Road (tropical evergreen rain forest), Gamboa, Panama (09°10' N, 79°51' W); the restinga dry-forest of Praia das Neves (semi-deciduous tropical dune forest) ES, Brazil (21°18' S, 41°02' W); Ecological Station of the University of Minas Gerais (upland tropical semi-deciduous forest), Belo Horizonte, MG, Brazil (19°43' S, 43°57' W); Serra da Canastra National Park (cerrado savanna and gallery forest), MG, Brazil (20°14' S, 46°33' W); and the cerrado woodlands of Fazenda Jatobá, Correntina, BA, Brazil.

We tested freshly captured butterflies for sound production capacity by holding the hind wings closed over their backs with forceps and passing the fore wings below the hind wings, so that the fore wings were free to beat and produce their typical clicking sound (Otero, 1990).

Data on flight interactions were obtained from butterflies kept in an outdoor cage (4 x 6 m at the base and 4 m in height containing the trunks of two trees) set in a plantation of native trees in the Linhares Forest Reserve. This area was regularly used by at least five *Hamadryas* species. Caged butterflies permitted observations on butterfly social behavior during a period of *Hamadryas* scarcity between June 1993 and March 1994. Observations of flight interactions were all from October 1993 on 24 caged butterflies, comprising 13 males (m) and 5 females (f) *H. feronia*, 2 m and 1 f *H. amphinome*, 1 m *H. iphthime*, 1 f *H. arinome*, and 1 m *H. laodamia* (Table 1).

Butterflies were fed with fermented banana and sugarcane juice every morning. Each butterfly was individually numbered on the hind wing underside with India ink or marked with highly visible colored spots on the upper side of each fore wing to indicate its sex and species. For each interaction, the species and sex of both individuals and which of them was the chaser and the chased were recorded. Sounds produced and, when possible, the individual responsible for it were also recorded. Nomenclature is based on Jenkins (1983).

The non-parametric statistic Chi squared was used to test the heterogeneity of the interactions among the sexes while the binomial statistic (Z) was used to compare the distributions of probabilities of interactions among individuals of the same sex or species and those of different sex or species.

Table 1. Observed and expected [ ] number of aerial interactions from 24 caged *Hamadryas* butterflies kept in an outdoor cage (N = 129 interactions). Expected values of the main diagonal were calculated as $[n(n-1)/N] \times 129$, while the other elements were calculated as $(n^2/N) \times 129$. FR = *H. feronia*, AM = *H. amphinome*, ARI = *H. arinome*, IP = *H. iphthime*, LA = *H. laodamia*.

	FR *m* (*n*=13)	**FR *f* (*n*=5)**	**AM *m* (*n*=2)**	**AM *f* (*n*=1)**	**ARI *f* (*n*=1)**	**IP *m* (*n*=1)**	**LA *m* (*n*=1)**
FR *m*	91 [*55*]***	28 [*23*]	1 [*9*]**	0 [*5*]*	2 [*5*]	6 [*5*]	0 [*5*]*
FR *f*		0 [*7*]***	0 [*4*]*	0 [*2*]	0 [*2*]	1 [*2*]	0 [*2*]
AM *m*			0 [*1*]	0 [*1*]	0 [*1*]	0 [*1*]	0 [*1*]
AM *f*					0 [*0*]	0 [*0*]	0 [*0*]
ARI *f*						0 [*0*]	0 [*0*]
IP *m*							0 [*0*]

Hypothesis tests for sample proportion vs. hypothesized value: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Spearman rank correlation was used to assess the relationship between two discrete variables describing the number of events observed.

RESULTS

Sound production. The sound production tests carried out with recently captured butterflies showed that only males of the five species of the *feronia* group (sub-genus *Hamadryas*: *H. feronia*, *H. iphthime*, *H. epinome*, *H. amphinome*, *H. arinome*) produced sounds under the test conditions (Table 2). These were also the only species in which one can perceive swollen veins in the middle of the proximal border of the males' forewings. The *feronia* group females and individuals of both sexes of the *laodamia* (*H. laodamia* and *H. arete*) and *februa* (*H. februa* and *H. chloe*) groups never produced sounds in the hand tests (Table 2) nor in the flight cage. Males of *H. feronia*, *H. epinome* and *H. iphthime* (*feronia*-group species) produced most sounds, whether in flight interactions, when perched over food, or while walking on tree trunks. Data assembled from the literature (Table 3) suggest that there may be variation in sound production capacity of some species; however, since many *Hamadryas* species have very similar color patterns, these results need confirmation.

Flying chases. Of the 104 observed flight interactions in which the departing points of the participating individuals were observed, 74 occurred between two *H. feronia* males and 23 between a male and a female *H. feronia*. The other 7 interactions involved a male *H. feronia* and another species. The most frequent interactions between two males were initiated by a flying chaser (64%), while the most frequent interactions between a male and a female were between perched individuals (39%) (χ^2 = 58.7, d. *f.* = 3, $P < 0.0001$). Aerial chases occurred almost exclusively during periods of intense sunshine, mainly from 1130 h to 1330 h on hot windless days. *Hamadryas* males commonly made exploratory flights ca. 1.5 to 2.5 m high, surrounding the two trees inside the cage. Such behavior normally stimulated other individuals in the cage to fly.

When encountering a perched individual, flying *Hamadryas* of the *feronia*-group frequently made a pendular flight display. This display comprised a semicircular flight with constant sound production for 5 to 10 s, 10 cm below the perched individual, which could be of either sex. Some perched individuals did not respond to the display, but others took flight after or ahead of the displaying individual. When this happened with two males, a chase was normally initiated with one or both butterflies vigorously producing sounds during the first 10-20 s of the interaction. During a chase both individuals flew rapidly and performed complex aerobatic maneuvers which included downward spiral flights, fast dives-and-rises, zigzags, and sudden, momentary (<1 sec) perches.

Twenty-five percent of the aerial chases (n = 27) began when an individual walked on the trunk and found and touched another individual. Apparently individuals walking on trunks after feeding were deliberately looking for other *Hamadryas* with which to interact (and not for food). Another 33% of the aerial chases (n = 35) began with a perched butterfly darting after a hovering individual, and 18% of the chases (n = 19) began with a flying butterfly provoking a perched individual to fly using the pendular display. The other 24% of the aerial interactions (n = 26) were initiated when two individuals met in flight; these occurred mainly during the hottest hours when many butterflies were flying. Interactions between more than two butterflies were not considered.

Table 2. Number of individuals producing sound in the hand tests carried out with males and females of nine *Hamadryas* species in Brazil and Panama.

Species group (Subgenus)	Species	Sex	Number producing sound	Number soundless	Percent individuals producing sound
februa (*Ageronia*)	*februa*[3, 6, 7, 8, 9]	*m*	0	21	0
		f	0	18	0
	chloe[8]	*m*	0	3	0
		f	0	2	0
laodamia (*Peridromia*)	*laodamia*[2]	*m*	0	8	0
		f	0	4	0
	arete[2]	*m*	0	1	0
		f	0	1	0
feronia (*Hamadryas*)	*feronia*[2, 4, 5, 6, 7, 8]	*m*	35	7	83
		f	0	26	0
	iphthime[2, 4]	*m*	39	2	95
		f	0	20	0
	epinome[2, 3, 8]	*m*	17	1	94
		f	0	15	0
	amphinome[2, 7, 8]	*m*	18	3	86
		f	0	19	0
	arinome[2]	*m*	5	0	100
		f	0	5	0

1) *Sensu* Jenkins (1983). Butterflies from: (2) Linhares, ES, Brazil; (3) Campinas, SP, Brazil; (4) Balboa, Panama; (5) Brasília, DF, Brazil; (6) Praia das Neves, ES, Brazil; (7) Belo Horizonte, MG, Brazil; (8) Serra da Canastra National Park, MG, Brazil; (9) Faz, Jatobá, Correntina, Bahía, Brazil.

Table 3. Summary of published reports of sound production in *Hamadryas* butterflies based on field observations (FO) and manual tests using Otero's (1990) hand test (OHT). References: (1) Jenkins (1983); (2) Ross (1963); (3) Monge-Nájera and Hernández (1991); (4) Otero (1990).

Group/Species	Sex	Locality	Method (FO/OHT*)	Sound production	Ref.
februa species Group					
H. amphichloe	?	Dominican Republic	FO	Yes	1
H. chloe	?	? (dense rainforest)	FO	No	1
H. februa	?	Mexico	FO	Yes	2
H. februa	???	???	FO	Yes	3
H. februa	*m* and *f*	Venezuela	FO/OHT	No	4
H. guatemalena	?	Mexico	FO	Yes	2
feronia species Group					
H. feronia	*m*	Venezuela	FO/OHT	Yes	4
H. feronia	*f*	Venezuela	FO/OHT	No	4

Of the 129 aerial chases with interacting individuals of known sex, among 17 m and 7 f, 76% (n = 98) were between two males, which was more than expected (Z = 3.097, P < 0.001), 24% (n = 31) with a male chasing a female (Z = -0.600, P > 0.05), and none with a female as the chaser, which was less than expected (Z = -3.802, P < 0.001) (Table 1). *Hamadryas feronia* participated in all interactions, generally chasing a conspecific (90.1%, n = 120). Only 8 aerial interactions (6.2%) involved other species, and 7 of these were between *H. feronia* and a male *H. iphthime* (Table 1).

Chases between two male *H. feronia* occurred in a higher frequency than was expected (Z = 6.409, P < 0.001), and those involving a male and a female *H. feronia* occurred in a smaller frequency than was expected (Z = -2.721, P < 0.005) (Table 1). Chases involved fast flights and much sound production. These interactions usually ended with one of the individuals perching while the other continued flying. Interactions between a male and a female usually began with some sound production by the chasing male in a short period of fast flight, after which the male followed the female in a slow flight with little or no sound production until she perched. Frequently, the male would alight behind and court the female (described below). Individuals that were chased a lot seemed to avoid interactions and apparently flew less during the periods of greater exploratory flight activity.

The majority of the chases were carried out by only a few individuals (Fig. 1a), with one *H. feronia* (no. 89) performing no less than 57% of all chases (33 of the 58 chases by identified individuals). The next most active males initiated only four chases each. Three of the ten most chased individuals were females, but the frequency distribution of chased butterflies was much more uniform than the frequency distribution of the chasers (Fig. 1b).

Sometimes more than two *Hamadryas* engaged in aerial interactions. These interactions tended to be intense, long lasting, and difficult to keep track of the individuals. Most involved three or four individuals, but a few had up to seven. These happened when individuals met during exploratory flights in the hottest hours of the day and perched individuals joined the flying party. Some of these interactions in which all individuals could be identified consisted of two or more males chasing a female.

All *Hamadryas* species of both sexes may defend feeding sites through displays and physical interactions. However, there was no relation between the success of an individual in defending a feeding site and the number of times it chased after other individuals. Only one individual seemed to be efficient in both tasks (*H. feronia* no. 89). Of the species engaging in aerial chases, *H. amphinome* did much better at defending feeding sites than at chasing other *Hamadryas* (Table 1; Fig. 2, Spearman correlation, N = 14, $R_S = 0.25$, $t_{(n-2)} = 0.90$, $P = 0.39$). Individuals of other species participated in less chases than was expected (Table 1).

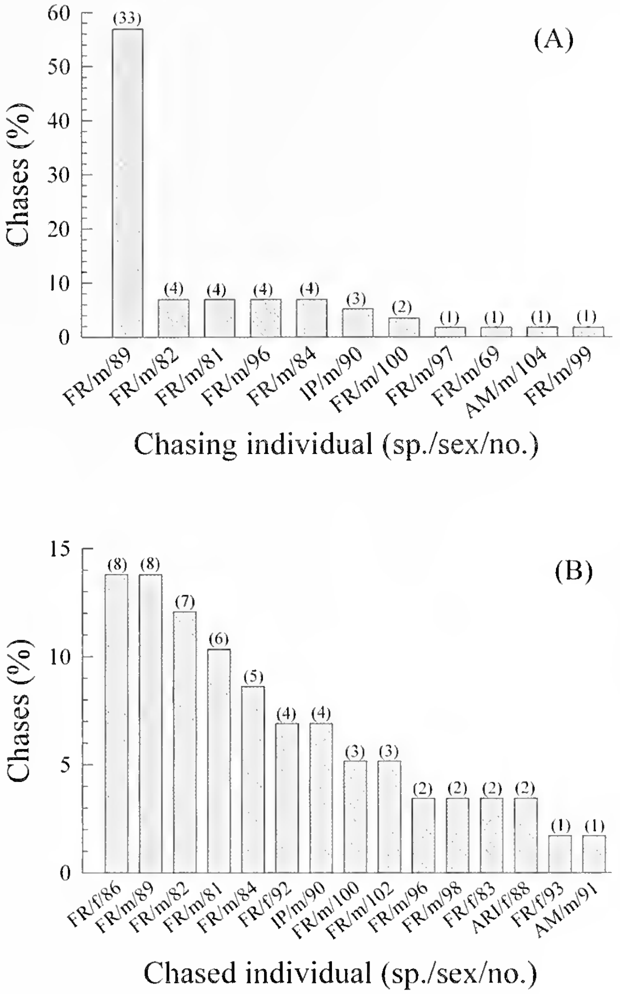

Figure 1. Frequencies of aerial chases of identified *Hamadryas* individuals in a flight cage (n = 58). **A)** Chases effected by a chasing individual. **B)** Chases suffered by a chased individual. Individuals are represented by a species/sex/number code: AM = *Hamadryas amphinome*, ARI = *H. arinome*, FR = *H. feronia*, IP = *H. iphthime*, m = males, f = females, and the capture number of the individual.

Courtship. In the cage, six observations were made on male *H. feronia* courting females perched on a tree trunk. All of them around noon. Usually a male perched 5 to 10 cm behind a female and facing her and remained 1 to 5 min slowly opening and closing

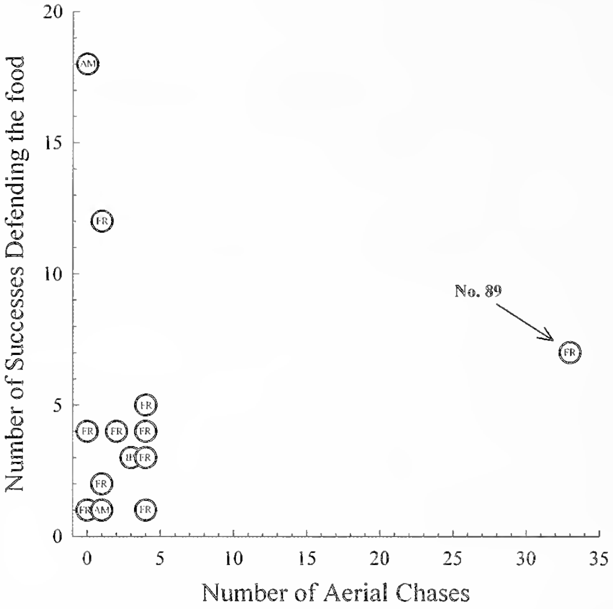

Figure 2. Relation between the number of times individuals successfully defended food and the number of aerial chases initiated by the same individuals. AM = *amphinome*, FR = *feronia*, IP = *iphthime*.

his wings while slowly approaching the female by taking a few steps at a time. The female responded to this approach with similar wing movement. When the male got close (< 1 cm), she usually walked 15 to 100 cm down the trunk. If the male touched her with his wings or legs, she usually flew to another tree trunk or to the cage wall. The male either followed her to the new perch or quit after some unsuccessful attempts. After this he would fly around the trees apparently looking for another individual with which to interact.

The first author once observed a much longer courtship in *H. epinome* in a reforested area containing *Joannesia princeps* (Euphorbiaceae) and *Senna multijuga* (Leguminosae). The butterflies behaved essentially like the *H. feronia* described above, differing principally in that when they changed perches they usually flew over a much larger area (about 20 x 20 m) from 1 to 7 m high (canopy height), and usually perched higher up (4-6 m) than did the caged *H. feronia.*

DISCUSSION

Our results, added to the observations of Jenkins (1983), Otero (1988) and Yack *et al.* (2000), indicate that sound production capacity in *Hamadryas* is restricted to the males of the *feronia* species group, with the possible exception of *H. amphichloe*, which belongs to the *februa* species group. Monge-Nájera and Hernández (1991) allude to one population of sound producing *H. februa* which disagrees with our data and those of other workers. Species of the *feronia* group are unique in their possession of swollen wing veins associated with sound production (Otero, 1988 and our observations), and the rare reports of sound production in the *februa* group (Monge-Nájera & Hernández, 1991; Ross, 1963) may result from identification errors. The five populations of *H. februa* tested in the present study did not produce sound, as is the case of the Venezuelan population studied by Otero (1988, 1990), which contrasts with the results of Monge-Nájera and Hernández (1991) and Ross (1963).

Hamadryas feronia is monomorphic, suggesting that sound production or other non-visual cues may be related to the discovery of females for mating. The absence of sound production and the presence of sexual color dimorphism in the *laodamia* species group suggest that sexual recognition between individuals of this group may have a visual component, since butterflies that possess sexual color dimorphism are supposed to recognize each other visually (Silberglied, 1984), although there is little evidence for this. The absence of both sound production and color dimorphism in the species of the *februa* species group suggests that chemical signals may be used for determination of gender.

All the species mentioned by Jenkins (1983) and Otero (1988) in which sound production has not been noted belong either to the *laodamia* or *februa* species groups. Species of the *februa* group have been reported as capable (*H. amphichloe*, and *H. februa*) or incapable of producing sounds [*H. chloe* (Jenkins, 1983) and *H. februa* (Otero, 1988)]. This suggests that there may exist different mechanisms of sexual recognition between species of the same group or that the dogma that has developed in relation to the sound production capacity of the whole group *Hamadryas* has been due to misidentifications of flying individuals because of their great similarity. In the past, this genus had a very confusing taxonomy, comprising about 100 species. Jenkins (1983) synonymized more than two thirds of these, leaving only 20 species and 21 subspecies in the genus *Hamadryas.* Probably many reports prior to the revision of Jenkins (1983) contain identification errors, which may have added to the confusion about the sound production capacity of each species.

Jenkins (1983) recorded two copulations for *Hamadryas*, one at 1130 h (*H. amphinome*) and the other at 1400 h (*H. velutina*). Most of the aerial interactions and courtship behavior in the present study occurred around noon, simultaneously with the majority of the flight interactions. The sounds were produced at will during flight interactions by male butterflies. *Hamadryas feronia* has been proven to hear the sounds in the same acoustic range they produce (Yack *et al.*, 2000). Therefore, these situations may be used to promote sexual recognition which apparently takes place quickly at the very beginning of chases carried out by males after females. Thus, males initiating chases shift to courtship behavior almost immediately upon approaching a female or proceed in aerial pursuit when finding a male. Some moths (*Hecatesia thyridion*) and Satyrinae butterflies are among the few Lepidoptera known to produce high frequency sounds, similar to those produced by *Hamadryas* males, which are voluntarily used in intraspecific communication while in courtship flight (Bailey, 1978; Kane, 1982; Murillo-Hiller, 2006). Thus, it seems difficult to ascertain what role the clicking sounds play in *Hamadryas*' behavioral repertoire. We conjecture that the clicks are used as an early recognition of sexual partners and that sound intensity may also be used by females as a means to assess the male's fitness, as bigger healthier males may produce louder clicks. Behavioral experiments are needed to provide evidence for either hypotheses.

Although *Hamadryas* butterflies of both sexes defend feeding sites, the aerial chases observed here do not seem to be associated with resource defense or to territoriality (Ross, 1963). Food was provided *ad libitum* in two localized spots in the flight cage and the majority of the individuals had ceased feeding when aerial chases reached a climax (Marini-Filho, 1996). With the exception of one very successful male, males that had the greatest success in defending feeding sites were different from those that initiated aerial chases. While *H. feronia* was the species that performed the majority of the aerial chases, *H. amphinome* was the one that defended the feeding resource more fiercely. This is consistent with their size difference, as body size determines to a great extent the winner of those interactions (Marini-Filho, 1996).

Aerial chases apparently play two intimately related functions: the discovery of receptive females and the establishment of dominance hierarchies for mating priority, although we did not consider this during the experiment. Dominance hierarchies may be the result of natural selection over intraspecific differences in flight capacity or other fitness-related character, promoting the individual spacing in natural populations and enhancing the mating chances of hierarchically superior individuals (see Rutowski *et al.*, 1989), the priority order being generally established through previous agonistic encounters (Archer, 1988: ,114). The establishment of dominance hierarchies may come about by the recognition of the individual aggressiveness by the butterflies present in the same feeding area (usually one or a few tree trunks oozing fermenting sap). It is unlikely that monomorphic butterflies as other monomorphic insects are able to visually recognize others of the same or similar species (Ewing, 1984). Males and females of most species of the groups *feronia* and *februa* have a cryptic marbled color pattern, making them difficult to be recognized in flight. It is more likely that after a series of aerial chases the individuals present in the area can recognize behaviorally that there are other individuals more aggressive assuming then a submissive attitude, either evading chases or refusing to fly during the period of most aerial interactions. Possibly the most chased *Hamadryas* females were receptive, while non-receptive females would not stay close to the food source during the period of aerial chases, and would otherwise be looking for suitable host-plants for their larvae if they could have left the cage.

These results may also help the definition of the *Hamadryas* phylogeny once it seems that there is a high agreement between the ability to produce sounds observed on the males of the *feronia* species group and the inability to produce sound in the other two species groups (*laodamia* and *februa*). The ability of *H. amphichloe* to produce sound must be checked by hand test and further considered with the morphologic factors to find if it is an exception from this pattern or in fact more associated to the feronia species group than to the februa species group.

ACKNOWLEDGEMENTS

We thank the direction and staff of the Linhares Forest Preserve for essential logistic support during this study. We are grateful to André V. L. Freitas, Carla M. Penz, Rogério P. Martins, Douglas Yanega, Robert Srygley and Stanley Rand for discussions and/or critical comments on earlier drafts of the manuscript. Study grants to OJM-F were provided by Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Smithsonian Tropical Research Institute. Financial support for this study was provided by FAPESP to WWB.

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Ecobiology of the common castor butterfly *Ariadne merione merione* (Cramer) (Lepidoptera: Rhopalocera: Nymphalidae)

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Abstract. We describe the life history of the common castor butterfly, *Ariadne merione merione*, monthly occurrence and seasonality of early stages and larval performance in terms of food consumption and utilization, and the length of life cycle. Our study was conducted during 2002 in the Andhra University campus at Visakhapatnam (17°42' N, 82°18' E), South India. Field study indicated that *A. merione merione* was in continuous flight and reproduction, with highest densities of early and adult stages occurring during June – September, the time of the entire South-West monsoon. Occurrence of the early stages was positively, but non-significantly correlated with rainfall, relative humidity, temperature and day-length. Multiple regression analysis showed that the effect of any combination of weather parameters on the reproductive activity was less than 40%. The South-West monsoon period probably influenced the reproductive activity by promoting fresh growth of the larval host plant, *Ricinus communis*, which in turn supported development of early stages. *Ariadne merione merione* was exemplified by a life cycle of 27.4 ± 3.57 days (eggs 3-4, larvae 13-18, and pupa 6-9 days) permitting a maximum of 8-9 overlapping generations per year. The values of the nutritional indices across the instars were A.D. 87.02-95.50%; E.C.I. 3.80-20.90%; E.C.D. 4.00-24.08%, measured at 28°C in the laboratory. These relatively high values, at least partially explain the ecological success of *A. merione merione* in the urban environment.

Key words: castor butterfly, *Ariadne merione*, life history, population index, nutritional indices.

Introduction

Of the estimated 20,000 – 30,000 species of butterflies occurring globally, at least 1,500 species occur in India. Several field guides for the identification of the Indian butterflies are available (Wynter – Blyth, 1957; Haribal, 1992; Gay *et al.*, 1992; Gunathilagaraj *et al.*, 1998; Kunte, 2000 and the references therein). A list of the works giving the descriptions of the life histories was given by Pant and Chatterjee (1950), of which those of Bell (1909 – 1927) are important. However, review of these early works indicated that for many species data, particularly on the duration and phenology of early lifestages, are either absent or incomplete. Haribal (1992) noted that the life histories of nearly 70% of the Indian species require description. We began studies to address the situation. Here we describe the life history of *Ariadne merione merione* (Cramer), the common castor butterfly, of the Oriental region. It is a specific pest of the castor seed plant *Ricinus communis* (Nayar *et al.*, 1976) and the larvae also feed on the stinging nettles *Tragia involucrata* and *T. plukenetti* (Euphorbiaceae) (Kunte, 2000). Because reproductive efficiency depends on life style and feeding pattern (Boggs, 1981; Slansky & Scriber, 1985; Muthukrishnan & Pandian, 1987), we also studied larval performance with respect of food utilization by feeding them on a daily supply of pieces of fresh leaf of the castor plant.

Materials and methods

The study was conducted during the year 2002 in the Andhra University campus (168 ha) at Visakhapatnam (17°42' N, 82°18' E) situated in the east coast of India. The natural plant community of the campus was searched for the distribution and reproductive activity of the common castor butterfly *Ariadne merione merione* (Cramer). Adult butterflies were seen mostly near the larval host plant *Ricinus communis* Linnaeus. Once located detailed observations were made at 10 sites in order to observe the flight activity and abundance of adults, the period of copulation and oviposition, following which we collected fresh eggs to study the life history and the duration of early stages. After oviposition, the leaf

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Received: 24 July 2008
Accepted: 20 August 2008

with egg(s) was collected in Petri dishes (15 cm x 2.5 cm depth) and brought to the laboratory. The piece of the leaf with the egg was then placed in a smaller Petri dish (10 cm x 1.5 cm depth) the inside of which lined with moistened blotter to prevent the leaf from drying. Five such samples were placed in a cage covered with wire mesh. The laboratory temperature was 28 ± 2° C and relative humidity 80 ± 10% with normal indirect sunlight conditions that varied in duration between 12h during November/January and 14h during June/July. The eggs were then examined at 6h intervals daily for recording time to eclosion. The larvae were subsequently reared on a weighed quantity of fresh leaves supplied daily. The time of each moult was noted. The morphological characters, body measurements, body weight of each instar and the faeces egested were taken daily. The prepupal behavior of the final instar, pupal particulars and the time of adult eclosion were also recorded. Larval performance in terms of food utilization indices were calculated as described by Waldbauer (1968) as:

$$\text{Food consumption index (C.I.)} = \frac{\text{Wt. of food consumed}}{\text{Wt. of instar x No. of feeding days}}$$

$$\text{Relative growth rate (G. R.)} = \frac{\text{Wt. gained by the instar}}{\text{Mean wt. of instar x No. of feeding days}}$$

$$\text{Approximate digestibility (A. D.)} = \frac{\text{Wt. of food ingested - Wt. of faeces}}{\text{Wt. of food ingested}} \text{ x } 100$$

$$\text{Efficiency of conversion of digested food (E. C. D.)} = \frac{\text{Wt. gained by the instar}}{\text{Wt. of food consumed - Wt. of faeces}} \text{ x } 100$$

$$\text{Efficiency of conversion of ingested food (E. C. I.)} = \frac{\text{Wt. gained by the instar}}{\text{Wt. of food ingested}} \text{ x } 100$$

To determine the developmental success of each of the early stages, a number of eggs were placed in Petri dishes in each month and the number of larvae hatched, pupae formed and the adults eclosed were recorded. To record the different early stages on the natural host plant, one plant at each of the 10 study sites was thoroughly searched at 10 day intervals each month and the early stages found were enumerated and pooled for each month. During the same visits, the flight frequency of adults was also noted using the arbitrary scale of rare, less common, and common. The relation between the monthly distribution of early stages and prevailing rainfall, relative humidity, temperature, and day-length was assessed by statistical correlation and multiple regression analysis using Minitab Statistical Software 14, 2003.

RESULTS

Adult stage (Fig. 1a, b)

Both male and female adults were nearly identical, characterized by their reddish brown colored wings bearing black colored wavy lines. Copulations occurred during mid-day, mostly between 1100 – 1500 h and lasting for more than one hour. Adults were found feeding on spoiled flowers of *Lantana camara*, overripe, fallen and damaged fruits of *Annona squamosa*, *Syzygium cumini* and *Artocarpus heterophyllus*, and the sap oozing from wounds in the tree trunks of *Citrus aurantifolia*.

Egg stage (Fig. 1c)

Gravid females lay eggs singly on the under surface of the leaves of the castor plant mostly before mid-day, between 0900 –1200h. Females spread their wings during egg laying, depositing 1 to clutches of 15. There was no bias for the age of the leaf. During one survey old leaves had 1 – 7 and young tender leaves 1 – 3 eggs. The eggs were round, 0.80 – 0.90 (0.83 ± 0.04) mm in diameter. At oviposition they were white, the color changing to light brown before hatching. When first laid eggs appeared soft in texture, but within 6 – 10 seconds they became hairy. They hatched in 3 – 4 days. Soon after hatching, larvae ate their egg-shells. Each larva passed through five distinct instars over a period of 13 – 18 days.

Larval stage (Fig. 1d-h)

Instar I lasted for 2 - 3 days. Larvae were 1.8 - 2.0 (1.9 ± 0.08) mm on D1, growing to a length of 2.50 - 3.00 (2.80 ± 0.21) mm and width of 0.30 - 0.50 (0.43 ± 0.09) mm before moult. Body was somewhat rectangular in shape, but slightly narrowing posteriorly. Its color was pale brown immediately after hatching, later turning brownish green with three brown colored horizontal bands on dorsal side. Head was very minute, and brown. Instar II also lasted for 2 - 3 days and attained a length of 3.30 – 4.00 (3.73 ± 0.30) mm and width of 0.60 - 0.90 (0.73 ± 0.12) mm. Whitish green spines with branched ends appeared over the entire body. Head was brown with a pair of brown horns. There were no changes in other characters seen in instar I. Instar III lasted for 3 – 4 days. Developing to a length of 6.00 – 8.00 (7.00 ± 0.81) mm and width of 1.10 – 1.50 (1.36 ± 0.18) mm. Dorsally they had a yellowish green broad stripe with brown edge longitudinal to the body. The body spines present on the three brown horizontal bands were also brown. Head was 1 mm in size, blackish brown in color with white markings. The head horns were 0.80 – 1.00 (0.90 ± 0.08) mm long and branched. Legs were clearly visible. The larva did not move much, but moved its head continuously when disturbed. There were no changes in other characters from previous instar. Instar IV also lasted for 3 - 4 days, growing to a length of 10.00 - 15.00 (12.00 ± 0.21) mm and a width of 1.50 – 2.00 (1.73 ± 0.20) mm. Body became green in color. The dorsal stripe turned brown with yellowish cream edges. The three black horizontal bands began to disappear. Head was blackish brown in color, square shaped and measured 1.00 – 2.00 (1.53 ± 0.41) mm in diameter. There were three triangular white markings on the head. The head horns were reddish brown in color and measured 2 mm in length. Segmentation was clear. Body spines were green in color, arranged in four lines on each side of the body on all the segments. The legs were green. Instar V also lasted for 3 - 4 days. When full grown the larva was 23.0 – 30.0 (25.6 ± 0.32) mm long and 2.20 – 3.00 (2.73 ± 0.37) mm wide. Body was dark green. The dorsal stripe changed to orange with black edges showing numerous small white to cream colored spots. The dorsal three horizontal bands disappeared completely. Head was 2.00 - 3.00 (2.56 ± 0.41) mm in diameter. It had prominent white triangular markings with black border two present above and one below. The horns became orange in color, with black tips, and measured 3.00 – 4.00 (3.60 ± 0.43) mm in length. Light and dark green crossed lines developed on both lateral sides of the body. The color of spines changed to brown with black tips and with yellow to orange colored spots at their base.

Pupal stage (Fig.1i)

During the prepupal period of 1 – 2 days the full-grown larva stopped feeding, turned brown and its lateral crossed lines changed to brown and white. The body contracted and the larva attached itself to the substratum with its posterior end hanging downwards. It measured 20.00 – 25.00 (22.60 ± 0.20) mm in length and 3 mm in width. The pupal stage lasted for 5 – 7 days. The brown color changed to black with pupal maturation until adult eclosion. It measured 15.00 – 17.00 (16.00 ± 0.08) mm in length and 6.00 – 7.00 (6.46 ± 0.41) mm in width at the broadest end. The anterior end was narrow. At the broadest point both lateral sides were curved inwards, between which two pointed projections appeared on dorsal side. Average pupal weight was 202.3 mg.

Development success and population index

Hatching success varied between 40 and 100%, being highest during June to September. Both larval and pupal development success varied between 50 and 100%, (Table 1). The numerical frequency of eggs, larvae, pupae recorded on the host plants and adult abundance, along with the prevailing weather data are given in Table 2. The three early stages and adults could be found under natural conditions throughout the year. However, the period of June and September provided the highest frequency of all stages, with peak numbers in July. Correlation between the counts of early stages and monthly average temperature, average relative humidity, total rainfall, and average day-length was positive, but non-significant, the coefficient values being 0.566, 0.333, 0.468, and 0.521 respectively. The four weather variables jointly influenced the distribution of early stages to the extent of about 40%, as indicated by multiple regression coefficients, R^2 0.216 – 0.396 (Table 3). Other combinations including temperature/rainfall-/day-length, temperature/

Table 1. Hatching, larval and pupal development success of *Ariadne merione merione* in the laboratory.

Life cycle stage	Calendar months											
	J	F	M	A	M	J	J	A	S	O	N	D
# eggs incubated	4	4	5	4	5	10	17	10	6	7	5	6
# larvae hatched	2	3	2	3	3	10	17	8	5	5	5	4
# pupae formed	1	2	1	2	3	9	12	8	5	4	4	3
# adults emerged	1	1	1	2	2	9	11	8	4	3	3	3

Table 2. Distribution of early stages of *Ariadne merione merione* on *Ricinus communis* and the associated weather conditions.

Life cycle stage, weather	Calendar months											
	J	F	M	A	M	J	J	A	S	O	N	D
Early stages	7	7	9	8	24	42	117	61	33	21	14	16
Adults	*	*	*	**	**	***	***	***	***	**	**	*
Temperature(°C)	24.15	25.45	27.85	29.15	30.7	29.4	30.75	28.2	29.25	28.3	26.2	24.55
Relative humidity (%)	74	68	74	74.5	71.25	77	73	80.5	76.5	74	62.25	69
Rainfall (mm)	014.1	000.0	000.0	085.2	015.1	143.2	075.4	143.5	023.5	118.4	007.8	000.0
Daylength (h)	1112	1148	1215	1312	1337	1316	1304	1322	1232	1224	1132	1105

* Rare, ** Common, *** Very common.

Table 3. Multiple regression of the counts of the early stages in relation to the prevailing weather parameters.

Constant (A)	X_1	X_2	X_3	X_4	R^2
-229.6	7.342	0.758			0.331
-153.7	6.336		0.139		0.369
-195.5	7.707			0.009	0.321
-7.1		0.338	0.239		0.216
-208.4		0.188		0.183	0.272
-146.2			0.118	0.138	0.297
-137	6.419	-0.270	0.152		0.370
-229.6	9.48	1.094		-0.068	0.335
-87.7	11.88		0.220	-0.183	0.395
-121.1		-0.497	0.137	0.146	0.300
-101.3	12.21	0.301	0.211	-0.196	0.396

X_1 - Monthly average temperature; X_2 - Monthly average relative humidity; X_3 - Monthly total rainfall; X_4 - Monthly average daylength.

Table 4. Food consumption, growth and food utilization efficiencies of *Ariadne merione merione* larva fed with *Ricinus communis* leaves.

Instar number	Wt. of food ingested (mg)	Wt. of faeces (mg)	Wt. gained by larva (mg)	GR (mg/day/mg)	CI (mg/day/mg)	AD (%)	ECD (%)	ECI (%)
I	–	–	–	–	–	–	–	–
II	45.0 ± 10.03	2.0 ± 0.35	1.72 ± 0.16	0.42	11.02	95.50	04.00	03.8
III	150.0 ± 16.39	13.0 ± 2.16	10.85 ± 0.59	0.34	04.80	91.30	07.90	07.2
IV	250.0 ± 05.65	25.0 ± 5.09	31.00 ± 1.65	0.36	02.90	90.00	13.70	12.4
V	925 ± 22.22	120.0 ± 5.88	193.87 ± 2.61	0.45	02.16	87.02	24.08	20.9

– Indicates no data due to very small size of first instar.

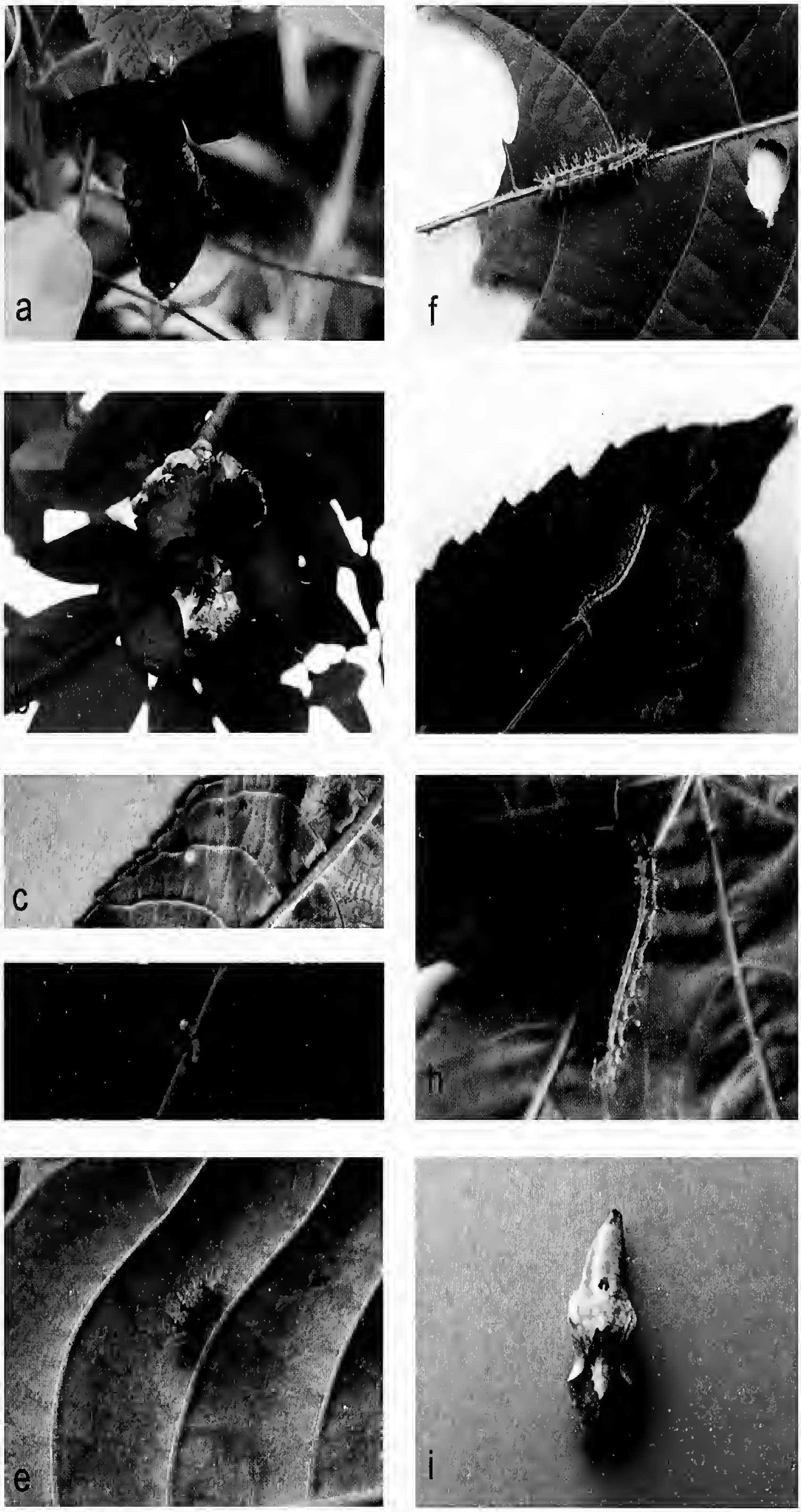

Figure 1. Photographs of the sequential stages in the life history of *Ariadne merione merione*. **a)** Adult pairing. **b)** Adults feeding on the damaged fruits of *Annona squamosa*. **c)** Egg. **d)** Instar I. **e)** Instar II. **f)** Instar III. **g)** Instar IV. **h)** Instar V. **i)** Pupa.

relative humidity/rainfall, and temperature-rainfall also had similar to lower values.

Food consumption, growth and utilization:

The data for the weight of food consumed and weight gained by the larvae are given in Table 4. The same data could not be collected for instar I due to its small size with consequent danger in handling. The amount of food consumed increased from instar to instar, the proportion of total food consumed in instars from II to V being 3.28, 10.94, 18.24, and 67.51%. Thus, there was greatest consumption in instar V. The weight gain corresponded to the food consumption trend of the respective instars. The weight gain in instar V was 81.65% of total larval weight. The weight of successive instars plotted against the food consumed indicated a clear relationship between these two parameters ($y = 0.227x$ and 18.383; $r = 0.9963$). The values of growth rate (G. R.) decreased from instar II to III and then increased to instar V, the values varying between 0.34 and 0.45 mg/day/mg. Consumption index (C. I.) progressively decreased from instar to instar, the values ranging between 2.16 and 11.02 mg/day/mg. Table 4 also includes the indices of food utilization efficiencies A. D., E. C. I., and E. C. D. The range of A. D. values was 87.02 to 95.5%, that of E. C. I. 3.8 to 20.9% and E. C. D. 4.0 to 24.08%. While E. C. I. and E. C. D. decreased, A. D. increased as the larvae progressed.

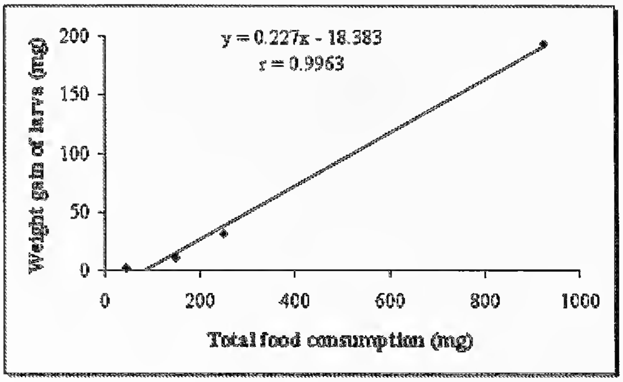

Figure 2. Relationship between food consumption and growth in *Ariadne merione merione* on *Ricinus communis.*

Discussion

The year round occurrence of early stages on the host plant *Ricinus communis* showed that *Ariadne merione merione* breeds continuously, corresponding with the usual pattern noted for most tropical butterflies (Owen, 1971; Owen *et al.*, 1972). The period of highest frequency from June to September correlates with the South - West monsoon. Thus rainfall appears to be the most important factor promoting higher reproduction rates in *A. merione merione* as is the case for both *Catopsilia crocale* (Chirstopher & Mathavan, 1986) and *Catopsilia pyranthe* (Atluri *et al.*, 2004a). However, statistical correlation of the distribution and abundance of early stages with the rainfall, though positive, was non-significant. Precipitation during the South - West monsoon likely had its influence on reproduction via the host plant. During this season, the host plant had its greatest fresh growth, a resource needed by the larvae for better performance due to the likely higher levels of nitrogen and water content (Slansky & Feeny, 1977; Scriber, 1977; Mattson, 1980). Although the host plant was available throughout the year, leaf quality in terms of nitrogen and water content might have varied through the year, hence the observed trend in the pattern of reproduction of *A. merione merione.* The work of Pullin (1987) on the growth of larvae of *Aglais urticae* fed with foliage with different water contents also suggested the likely variations in the breeding success as being due to variations in rainfall. Pollard *et al.* (1997) also examined a similar relationship. The low incidence of early stages during periods other than the South – West monsoon could have been due to a decrease in mature egg number as reported by Braby (1995) in the Satyrine butterflies, which also breed continuously.

Few other species noted at the study biotope also reproduced all year, but at a higher rate during different periods: *Pachliopta arisolochiae* April to May, and October to November (Atluri *et al.*, 2004b), *Papilio polytes* August to February (Atluri *et al.*, 2002), *Graphium agamemnon* August to December (Venkataramana *et al.*, 2003a), *Eurema hecabe* September to November (Venkataramana *et al.*, 2003b), *Euploea core* November to January (Venkataramana *et al.*, 2001). For most of India, Wynter - Blyth (1957) rated spring as the most favorable period, followed by post monsoon and South – West monsoon. In the northern western Ghats, Kunte (1997) observed highest flight activity during late monsoon (August to September) and early winter (October to November). These differences in the phenology of butterflies suggest that different species respond differently to the prevailing environmental seasonality and exhibit different life history patterns. Even different species of a genus may behave differently as observed by Jones and Rienks (1987) in the three species of the tropical *Eurema* they studied.

The overall effect of weather on population trends is complex and difficult to predict, as also expressed by Pollard (1988).

The characters of full grown larva observed in this study substantiate those given in Bell (1910) and Sevastopulo (1939) as well as pupal duration. The total development time from egg laying to adult eclosion was determined as 27.4 ± 3.57 days at about 28°C, thus permitting a maximum of 8 to 9 overlapping broods per year. This behavior is in line with the expectation of tropical butterflies to have a short life cycle, and multiple broods over the year (Owen, 1971). Since temperature influences instar duration and the overall development time (Mathavan & Pandian, 1975; Palanichamy *et al.*, 1982; Pathak & Pizvi, 2003; Braby, 2003), the brood number in other parts of *A. merione merione* distribution may vary from our records depending on the prevailing temperatures. As no temperature extremes occur at Visakhapatnam, especially at the Andhra University site, the duration of life cycle did not vary much over the overlapping seasons.

Adult feeding on the damaged and ripened fruit helps them obtain proteins and carbon sources (Levey & del Rio, 2001), with such nutrient uptake improving egg productivity (Fischer *et al.*, 2004). The larval food also appears to be highly nutritional as indicated by the observed values of assimilation efficiency (A. D.), the efficiency of conversion of ingested food (E. C. I.), and the efficiency of conversion of digested food (E. C. D.) into the body substance. The chemistry of the leaf, particularly its nitrogen and water content, influences the assimilation efficiency (Pandian & Marian, 1986). The castor leaves contain 2.54% nitrogen and 75.20% water (Senthamizhselvan & Murugan, 1988). Hence the observed high A. D. value, mean 90.97%. Such high values are characteristic of the foliage feeders (Slansky & Scriber, 1985) and indicative of their high growth efficiency (Singhal, 1980). The values of E. C. D. and E. C. I., particularly those of the last two instars, are also relatively high (12.4%, 20.9%; 13.7%, 24.1%), thus respectively indicating tissue growth efficiency and ecological growth efficiency, which enabled *A. merione merione* to thrive successfully in the urban environment.

ACKNOWLEDGEMENTS

The authors thank Professor Frances S. Chew of the Department of Biology of Tufts University, Medford, USA for appraising our work. We also thank Mr. S. Naresh of the Andhra University Statistics Department for help in the statistical analysis of the data, and the Librarian, BNHS, Mumbai, for the supply of literature. Two anonymous reviewers improved our original manuscript for which we thank them.

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Journal of Research on the Lepidoptera 42: 21-33, 2003 (2010)

Larval feeding behaviour and myrmecophily of the Brenton Blue, *Orachrysops niobe* (Trimen) (Lepidoptera: Lycaenidae)

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Abstract. The larval feeding behaviour and myrmecophily of the Brenton Blue *Orachrysops niobe*, an endangered polyommatine butterfly from Knysna in South Africa, were investigated by field observations and captive larval rearing. The aerial and subterranean parts of the *Indigofera erecta* legume host plants were searched for *O. niobe* eggs, larvae and potential host ants. Third and fourth instar larvae and pupae were found in association with *Camponotus baynei* ants on the host plant rootstock. Ant colonies in viewable artificial *C. baynei* nests were sited near host plants bearing multiple *O. niobe* eggs, but no larvae were taken into the nests. Cannibalism was observed between larvae raised in captivity on cut host plant. A third instar captive larva was enclosed with a potted host plant connected to a similar artificial ant nest. The larva disappeared and was later found feeding on the depleted plant rootstock, tended by the ants, and this behaviour was confirmed by field observations. *O. niobe*'s ant association is inferred to be obligate. Leguminous *Indigofera* host plants have amino acid enriched rootstocks, which may have pre-adapted the larval digestive system to a cannibalistic or carnivorous lifestyle. Larval growth characteristics are used to compare African polyommatine genera and *Orachrysops* is intermediate between the facultative myrmecophilous genera and the predaceous/parasitic *Lepidochrysops* species. A cladistic analysis based on host plants, ant associations and feeding behaviour leads to a hypothetical phylogeny of the African myrmecophilous polyommatines.

Key words: myrmecophily, *Orachrysops niobe*, polyommatine, phytophagy, rootstock feeding.

Introduction

South Africa has a wealth of myrmecophilous lycaenids (Terblanche & van Hamburg, 2003), many of which exhibit restricted ranges (endemism) and are Red Listed species (Henning & Henning, 1989; Henning *et al.*, 2009). The phenomena of endemism and rarity are believed to result from the narrow environmental niches available to species that require the overlapping presence of host plants and tending ants (Pierce *et al.*, 2002). Nearly all of the obligately myrmecophilous South African lycaenid butterflies are in the tribes Aphnaeini and Polyommatini (*sensu* Pringle *et al.*, 1994).

Orachrysops is a recently erected polyommatine genus (Vári & Kroon, 1986), for which the life history and myrmecophily of its 11 species and one subspecies are little known. Clark and Dickson (1971) were only able to rear larvae of *Orachrysops lacrimosa* (Bethune-Baker, 1923) to the end of the second instar, after which the larvae died. Recent work on the two most endangered species in the genus has extended this knowledge to all stages of their life history. Edge and Pringle (1996) reported that the larvae of *Orachrysops niobe* (Trimen, 1862) were phytophagous in all instars during captive rearing to the adult stage, and whilst a dorsal nectary organ (DNO) was present no ant association appeared to be necessary. Lu and Samways (2001; 2002a; 2002b) made field observations of all larval stages and pupae for *Orachrysops ariadne* (Butler, 1898) and detected an apparently obligate ant association with *Camponotus natalensis* (F. Smith).

Polyommatine larvae display a range of ant associations, including predacious parasitism, facultative mutualisms and myrmecoxeny (no ant association). Larval diets vary from phytophagy to entomophagy, or combinations thereof (Cottrell, 1984; Fiedler, 1991b; Fiedler, 1998; Pierce *et al.*, 2002). Variation is evident within genera (e.g. *Maculinea*) as well as between genera, with significant implications for the ecology and population dynamics of each species (Thomas *et al.*, 1998). Consequently extrapolations between even closely related species can be misleading, and detailed field observations as well as laboratory experiments are the only way to establish with any certainty the larval diet and the exact nature of the myrmecophily for each species (Thomas *et al.*, 1989; Elmes & Thomas, 1992).

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Received: 27 October 2008
Accepted: 5 November 2008

MATERIALS AND METHODS

Study site

The study site was the Brenton Blue Butterfly Reserve (BBBR) at Brenton-on-Sea near Knysna in the Western Cape Province of South Africa. It has a total area of 14.670 m², is centred at co-ordinates 34°04'20" S, 23°02'00" E, and lies at 90-115 metres above mean sea level on a well-drained south-facing slope with an average inclination of 1 in 3 (18°), varying between 10° and 26°. The climatic, topographical and geological features of the site and its vegetation communities have been fully described elsewhere (Edge, 2005; Edge *et al.*, 2008a).

Field observations

All *O. niobe* host plants (*Indigofera erecta* Thunberg, Fabaceae) were systematically searched for eggs between November 2001 and April 2003 and all plants with >5 eggs were searched repeatedly to detect the presence of any larvae, pupae or ants, at various times of day including the evening. The size, stage and behaviour of any larvae discovered were recorded, and samples were taken of ants for identification. Sizes were measured with a vernier scale using a hand lens. From April 2002 not only were the leaves and stems of the plants down to ground level searched, but also some of the rootstocks were carefully excavated to a depth of 2–4 cm.

Captive rearing on cut host plant

Host plant sprigs bearing eggs were cut off and placed in clear air-tight plastic containers 25mm diameter x 55 mm high, with a drop of water maintained in the bottom of the container to prevent desiccation of the plant. The oviposition date (if known), hatching date and all subsequent dates and measurements were written on labels attached to the container lids. If there were two or more ova on a sprig, the larvae were separated into individual containers on emergence. Every few days the contents were carefully removed, the larvae examined and the overall length (from the tip of the mandibles to the end of the final segment for the first instar larvae, and of the dorsal carapace for the second, third and fourth instars) measured to an accuracy of 0.5 mm with a vernier calliper. The containers were cleaned out with water and fresh cut sprigs of host plant were inserted after carefully transferring the larvae to the new leaves. From the third instar onwards, the larvae were transferred to larger flatter plastic containers 90 mm diameter x 50 mm high that would accommodate larger pieces of host plant. Upon pupation, the pupae were removed and placed on cotton wool under a netting eclosion cage. Any adults emerging were preserved as voucher specimens.

Artificial ant nests

Artificial ant nests similar to those used by Britton (1997) 300 mm x150 mm x 20 mm deep, with labyrinthine passages, were made from wood with sealed transparent tops, and covered by a detachable hardboard lid to exclude light. Three queen right colonies of the host ant *Camponotus baynei* Arnold were collected on 5 October 2002, at a location away from the BBBR, by breaking open decayed logs lying on the ground under dense bushes. Each ant colony was kept in a large plastic box 320 x 220 x 60 mm deep with fluon (active ingredient: polytetrafluoroethylene) coated walls to prevent escape, and the ants were offered access into one of the artificial nests through a translucent plastic tube. The ants quickly took up residence in the artificial nests and feeding stations were set up in the large plastic box where a 50% v/v solution of sugar, plain water and chopped up dead insects was provided. On several occasions a third instar larva of *O. niobe* on its sprig of host plant was placed in the plastic box to observe any ant interactions.

Two of these ant nests were slightly buried (covered with 10–20 mm soil) on 16 October 2002, close to host plants on which a large number of *O. niobe* eggs had been laid, to see whether butterfly larvae would be taken into the artificial ant nest. Translucent plastic tubing provided access from the ant nest to the base of the host plant. The nests were inspected every week until 27 January 2003, when one of the nests was removed to the laboratory to prepare for a captive rearing experiment (see below). The other nest remained in the field until January 2004, when it too was removed to the laboratory for another captive rearing experiment.

Captive rearing with live host plant and ants

Two *I. erecta* plants were transplanted from the field with their surrounding soil into pots 175mm diameter x 100 mm deep in December 2002, and watered regularly. An experiment was set up in February 2003 with the two potted and caged *I. erecta* plants, an artificial ant nest containing an ant colony with brood of all stages, and one of the large plastic boxes with ant feeding stations, all connected by clear 6 mm diameter plastic tubes (Fig. 1). A 3rd instar larva

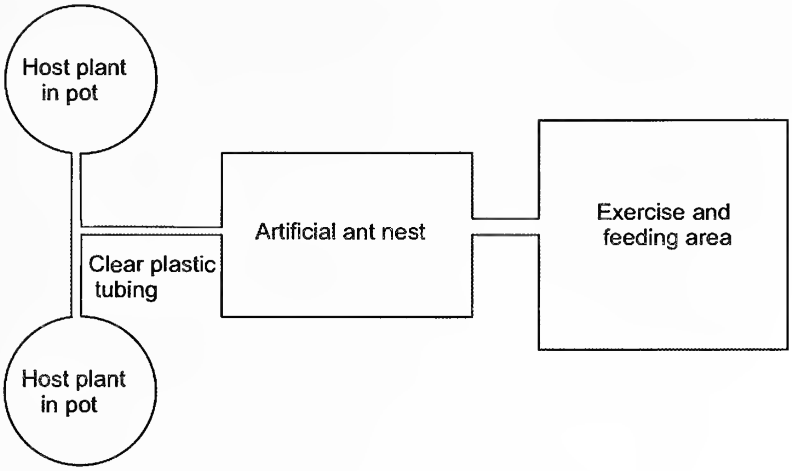


Figure 1. Experimental set up for captive rearing with live host plant and ants

(7 mm long) was placed on each of the *I. erecta* plants on 8 March 2003. The plants and the ant nest were examined regularly to detect any larval activity and any ant-larva interactions.

Morphology of the immature stages of *O. niobe*

Larvae were examined with a Wild M5 stereomicroscope at magnifications of up to 50x. The various stages were photographed under magnification with a Nikon Coolpix E4600 digital camera. Particular attention was given to the dorsal nectary organs (DNOs), perforated cupola organs (PCOs), tentacular organs (TOs) and the mandibles of the 4th instar larva.

Growth characteristics of *O. niobe* larvae and comparison with other polyommatines

Data were obtained from Clark and Dickson (1971) and Elmes *et al.* (2001) to enable a comparison to be made between the growth patterns observed in the early stages of *O. niobe* and other polyommatine species.

Host plants recorded for other *Orachrysops* species

Data were obtained from various published sources and from fellow lepidopterists of the host plants recorded for the genus *Orachrysops*. Localities for other *Orachrysops* species were visited, the females were observed ovipositing, and specimens were taken of the host plants and sent to an expert for identification. Ova of the *Orachrysops* species were collected and it was confirmed that the larvae survived and fed on the host plant on which they were laid. High magnification photographs were taken of the eggs and the larvae that hatched from them.

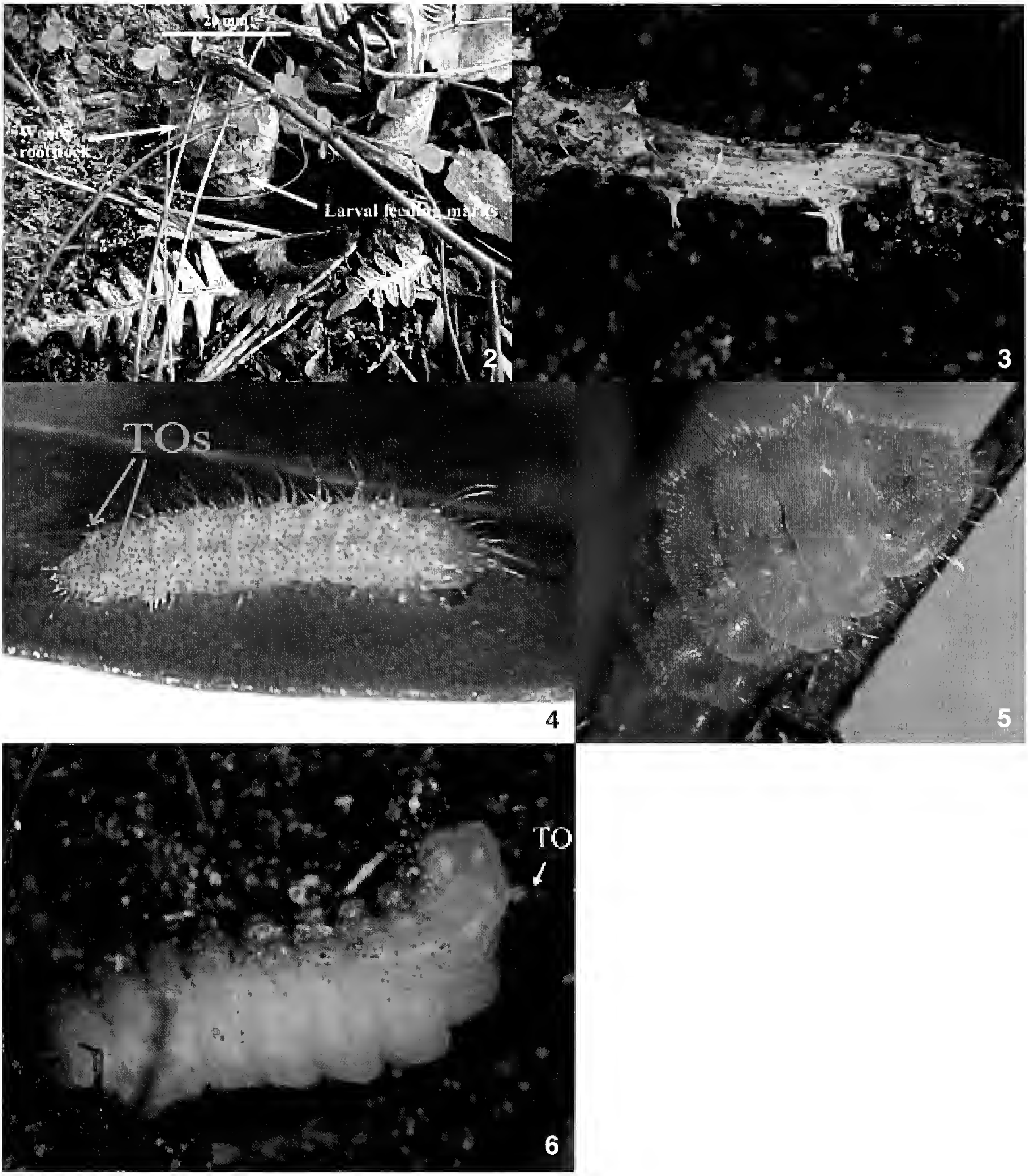
RESULTS

Field observations – larvae and pupae

The 1st and 2nd instar larvae of *O. niobe* make 0.5 mm – 1.0 mm grooves in the epidermis and palisade parenchyma of the glabrous uppersides of the leaflets of *I. erecta*. When not feeding, the larvae descend to the lowest part of the plant and rest on the stem in a head-down position, making them very difficult to find in the field.

The first 4th instar larva was discovered on 27 March 2002 at 15.50 pm on a cool, cloudy day. A vertical hole about 8 mm diameter was alongside the rootstock of this plant, from which several ants emerged. Down the hole about 20 mm deep was a 4th instar larva, which was carefully removed for measurement and found to be 18 mm long x 4 mm wide, and then replaced in the hole. A sample was taken of the ants and H. G. Robertson of the South African Iziko Museum identified them as *Camponotus baynei* Arnold. The next day the larva had pupated (dimensions 12 mm x 4 mm). A few days later the pupa could not be found, so possibly the attendant ants must have taken it deeper underground.

Most of the subsequent observations were also made later in the day and early evening, when the *C.*



Figures 2 - 6. **2.** *O. niobe* larval feeding marks can be seen on the 18mm diameter rootstock of *I. erecta* host plant, where 3 larvae were found at different times. **3.** Rootstock of *I. erecta* showing feeding damage inflicted by larva of *O. niobe* (original diameter of 6mm reduced to 2mm) (x20). **4.** 2nd instar *O. niobe* larva (2.5 mm long). **5.** 3rd instar *O. niobe* larva (7mm long) showing the head shield (Photos by D. A. Edge). **6.** 4th instar *O. niobe* larva (18mm long) showing an everted tentacular organ (TO) on abdominal segment A8 (top right) (Photo by L. du Preez).

baynei attendant ants appeared to be more active. On two occasions two fully-grown 4th instar larvae were found on the same *I. erecta* rootstock. In one instance the *C. baynei* originally in attendance were supplanted by a *Pheidole* species (in large numbers), and the two larvae could no longer be found. The ant attendants were *C. baynei* in thirteen out of fifteen observations made (seven 4th instar larvae; five pupae and three pupa cases), with *Camponotus berichti* in attendance in the other two cases.

Mature rootstocks of *I. erecta* are up to 18 mm diameter (Fig. 2). The holes alongside the rootstocks of *I. erecta* appear to be excavated by the *C. baynei* ants, but these holes do not lead to ant nests. *C. baynei* was only found to be nesting above ground in decayed dead wood with holes bored out by a beetle larva, and this was usually some distance away from the *I. erecta* plants.

Captive rearing with cut host plant

The size and duration of the early stages of *O. niobe* during captive rearing on cut host plant are summarised in Table 1. The few adults that were reared were dwarfs, notwithstanding their rarity in nature (Edge, 2008).

During the 2004 and 2005 captive rearing experiments a number of new observations were made. It was confirmed that the first and second instars (and presumably the third) normally consume their shed cuticle, including the head capsule, after ecdysis.

In April 2005 experiments were conducted whereby pairs of well-fed fourth instar larvae were placed in the same container with fresh host plant. Within 24 hours in each case one of the larvae disappeared and the survivor grew in size. In one instance the act of cannibalism was observed. Whilst the prey larva was feeding on a host plant leaf, the predator larva attacked it from behind and below, through the soft ventral parts of abdominal segments A7 and A8. After penetrating the integument with its jaws, the predator larva sucked out the prey's body contents, eventually reducing it to just a skin and head shield, which was also subsequently consumed by the predator larva.

Table 1. Summary of the size and duration of the early stages of *O. niobe*, reared on cut host plant.

Stage	Size	Duration
Ovum	0.6 dia x 0.3mm high	6 -7 days
1st instar	0.8 - 1.5mm*	5 - 6 days
2nd instar	1.5 - 3.0mm*	8 - 12 days
3rd instar	3.0 - 7.5mm*	35 - 57 days
4th instar	7.5 - 12.0mm*	26 - 61 days
Pupa	7.5 - 8.0mm	13 - 23 days
Adult	10 - 13mm	Up to 15 days

* For the larval instars the sizes are at the start and finish of the instar.

The prey larva thrashed about during the attack trying to free itself, with the tentacular organs (TOs) being very active. The predator larva increased in size from 9 mm to 11 mm before and after this attack. The attacking behaviour appears to be calculated and instinctive.

Artificial ant nests

Regular inspections of the artificial *C. baynei* ant nests showed that the ant colonies remained healthy, with stable abundances of adult ants and brood. During the 103 days that the first nest was in place, no larvae of *O. niobe* were observed inside the nest, nor were any *O. niobe* larvae seen at all. The other nest was in the field for 15 months and although the nest remained active, no larvae were observed to come into the nest. Both host plants had large numbers of *O. niobe* eggs laid on them, and there is a high probability that *O. niobe* larvae were in the immediate vicinity of the nests.

Captive rearing with live host plant and ants

The two third instar larvae that were placed on the two host plants could not be found after the first 24 hours. When the ants were given access to the plant, they were observed crawling on the plant and on the soil under the plant in the evenings, but no larvae were detected. The larvae were also not seen in the ant nest. Three months later, the plants were isolated from the rest of the experiment and the soil around the rootstock of the plants was carefully excavated. Nothing was found around the rootstock of the more healthy plant. The stems of the other plant were badly withered and many were dead. A large hole (±20 mm) was discovered alongside the rootstock. The plant became loose and it was lifted out of the hole. A final instar *O. niobe* larva (15 mm long x 4 mm wide) was clinging to the rootstock in a hunched position. The rootstock was badly damaged (reduced to 2 mm diameter from 6 mm).

This larva was observed for the next few months with ants remaining in attendance at all times. Since both the larva and the ants were photophobic, the larva was relocated to a vertical wooden box with a red perspex translucent viewing window. The box was filled with soil and a rootstock from a live plant above was placed in a groove visible through the window. Ants from the artificial nest were given access, and were observed tending the larva and imbibing secretions from the dorsal nectary organ (DNO). The larva mostly stayed on the rootstock until it became clear that the larva was eating the rootstock, cutting out deep grooves (Fig. 3). The length of the larva was now about 18 mm. The larva eventually lost interest in the rootstock, and accompanied by ants made its way to the bottom of the box where the ants had excavated a hole. The *C. baynei* ants continued to imbibe secretions from the DNO, cover the larva with soil when exposed to light, and occasionally bodily carried the larva into deeper holes that they had excavated. At no stage was there an attempt to carry the larva into the ant nest, nor were the ants observed to feed the larva by trophallaxis or any other method. When the soil was excavated again on 5 August the larva had pupated (pupa 15 mm long), still tended by the ants. The pupa was removed in mid-October and placed under a hatching cage. A full size male butterfly (forewing length 17 mm) eclosed on 3 November 2003.

Morphology of the immature stages

The following features of the morphology of the immature stages of *O. niobe* were not reported by Edge and Pringle (1996). The 2nd instar has much shorter dorsal and ventral setae than the 1st instar, and already has active TOs (Fig. 4). The head shield of the 3rd instar larva completely covers the head (Fig. 5). The 4th instar larva frequently everts its TOs (Fig. 6).

Comparative growth characteristics of polyommatine larvae

The ratios between the lengths at the finish to the lengths at the start of each larval instar are depicted in Fig. 7. Note particularly the contrast between the normally phytophagous taxa, *Lampides boeticus* (Linnaeus, 1767) and *Euchrysops barkeri* (Trimen, 1893), and the myrmecophagous species *Lepidochrysops patricia* (Trimen & Bowker, 1887), *Lepidochrysops variabilis* (Cottrell, 1965) and *Maculinea arion* (Linnaeus, 1767). The difference between *O. niobe* reared purely on leaves cut from the host plant and the same species reared on live host plant, including rootstock and with ants in attendance, is also notable, particularly in the 4th instar.

Host plants and ant associates recorded for other *Orachrysops* species

The data currently available for the host plants and known ant attendants for *Orachrysops* species are summarised in Table 2. All host plants recorded so far are in the genus *Indigofera* or the closely affiliated genus *Indigastrum* and all ant associates yet known are in the genus *Camponotus.*

DISCUSSION

Phytophagy

The life histories of several *Lepidochrysops* species have already been described:

L. patricia - by Clark and Dickson (1957).

L. variabilis - by Cottrell (1965).

L. methymna (Trimen, 1862) - by Clark and Dickson (1971).

L. trimeni (Bethune-Baker, 1923) - by Claassens (1972; 1974 and 1976).

L. ignota (Trimen & Bowker, 1887) - by Henning (1983b).

L. plebeia (Butler, 1898) - by Williams (1990).

It was generally accepted from these observations that the larvae of *Lepidochrysops* are mostly myrmecophagous.

Clark and Dickson (1971) reared *Orachrysops lacrimosa* (Bethune-Baker, 1923) as far as the start of the 3rd instar, when the larvae died. When Vári (1986) separated the genus *Orachrysops* from genus *Lepidochrysops* Hedicke on adult morphological

Figure 7. Ratios between lengths at the finish and lengths at start of larval instars for six polyommatine butterflies: *O. niobe* 1 = reared on cut host plant; *O. niobe* 2 = reared on live host plant with ants; *E. barkeri* and *L. boeticus* = Clark & Dickson 1971; *L. patricia* = Clark & Dickson 1957; *L. variabilis* = Cottrell 1965; *M. arion*= Elmes *et al.* 2001.

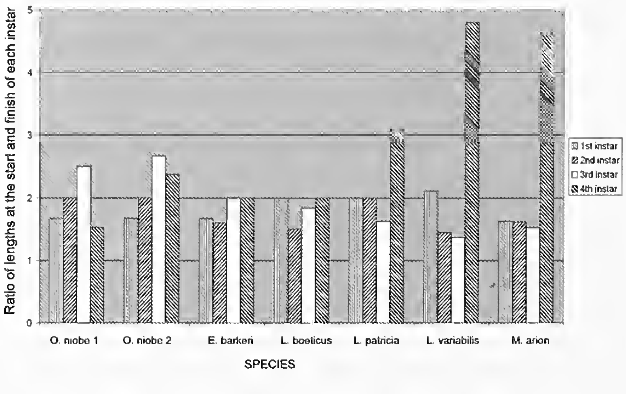


Table 2. Host plants and ant associates recorded for *Orachrysops* species.

***Orachrysops* species**	***Indigofera* host plant**	**Locality region**	***Camponotus* ant associate**	**Sources**
O. niobe (Trimen)	*I. erecta* Thumb.	Brenton S. Cape	*C. baynei* Arnold	Williams, 1996; Lubke *et al.*, 1997
O. ariadne (Butler)	*I. woodi* var. *laxa* H. Bolus	Karloof KZN	*C. natalensis* (F. Smith)	Lu & Samways, 2001
O. lacrimosa (Bethune-Baker)	*I. obscura* N. E. Br.	Greylingstad Gauteng	Not known	Edge personal observations 2004
O. lacrimosa (Bethune-Baker)	*Indigastrum fastigium* (E. Mey.)	Verloren Valei Mpumalanga	Not known	Edge personal observations 2004
O. brinkmani Heath	*I. declinata* E. Mey.	Kammanassie S. Cape	Not known	Heath 1997
O. subravus G. A. & S. F. Henning	*I. woodi* var. *woodii* H. Bolus	Wahroonga KZN	*Camponotus* sp.	Samways & Lu, 2007
	I. tristis E. Mey.		Not known	Lu, 2003
O. nasutus nasutus G. A. & S. F. Henning	*I. elandsbergensis* P. B. Phillipson	Hogsback E. Cape	Not known	Edge personal observations 2004
O. mijburghi G. A. & S. F. Henning	*I. evansiana* Burtt Davy	Heilbron OFS	Not known	Pringe *et al.*, 1994
O. near *myburghi*	*I. dimidiata* Vogel ex Walp. *sensu stricto*	Suikerbosrand Gauteng	Not known	Terblanche & Edge, 2007
O. regalis G. A. & S. F. Henning	*I. accepta* N. E. Br.	Wolkberg Limpopo	Not known	Edge personal observations 2004
O. warreni G. A. & S. F. Henning	*I. dimidiata* Vogel ex Walp. sensu stricto	Verloren Valei Mpumalanga	Not known	Edge personal observations 2004
O. montanus G. A. & S. F. Henning	*I. dimidiata* Vogel ex Walp. sensu lato	Golden Gate OFS	Not known	Edge personal observations 2004

N. B. All *Indigofera* and *Indigastrum* plant names determined by Schrire (2005a)

grounds, the larvae of both genera were still assumed to be myrmecophagous. Edge and Pringle (1996) reared *O. niobe* larvae in captivity on host plant cuttings without ants, and this discovery added a clear biological justification for the separation of *Orachrysops* from *Lepidochrysops.*

The *O. niobe* larvae reared on host plant cuttings resulted in dwarf adults. In field observations 1st and 2nd instar larvae were nearly always found feeding on the leaves of the host plant (with a single observation of a 1st instar larva feeding on the rootstock). 3rd and 4th instar larvae were always found underground feeding on the rootstock attended by ants, and have never been found feeding on the leaves. Rootstock feeding in the Lycaenidae has only been recorded once before. Jackson (1937) observed that the larvae of *Euchrysops crawshayi crawshayi* (Butler) fed "on the fleshy outer cortex of *Cynoglossum coeruleum* Hochst. *et* D.C., Boraginaceae, always below the ground; and they are attended by many species of ants." Rootstock feeding in *O. niobe* appears to be essential to produce full size adults, since there is no evidence that the diet of *Orachrysops* larvae includes any ant provided food. Rootstock feeding has also subsequently been observed in another *Orachrysops* species (Terblanche & Edge, 2007).

Rootstocks as a dietary source

Pierce (1985) noted that many myrmecophilous lycaenid larvae feed on nitrogen rich plants and nitrogen rich parts thereof (such as flowers and legume pods). More recent work has questioned this association (Fiedler, 1995; 1996), but the rootstock feeding behaviour of the 3rd and 4th instar larvae of *O. niobe* accords with Pierce (1985), if the rootstock is indeed protein rich. The rootstock of the legume *I. erecta* is a storage organ from which the plant can resprout, storing carbohydrates and bearing nitrogen fixing root nodules probably rich in amino acids and protein. Analysis of *I. erecta* leaves and rootstocks showed that although their overall amino acid content is quite similar (11600 nmol per g dry weight), the rootstocks had more than double the content of essential amino acids, particularly threonine, histidene and allo-isoleucine. These amino acids could play an important role in the development of 4th instar larvae and pupae (e.g. threonine is important for the synthesis of collagen, a basic constituent of the more complex connective tissues generated in the lepidopteran pupal stage).

Cannibalism

Cannibalism by *O. niobe* larvae is another potent source of protein. Frequently multiple eggs are laid on a single host plant (up to 31 eggs were recorded), so encounters between 1st, 2nd and 3rd instar larvae on the host plant (where attacks are most likely to succeed) must be quite frequent. A larva grows rapidly after it has successfully attacked and consumed one of its siblings, and its survival chances are considerably enhanced. The habit that the larvae have of resting on the lower thicker part of the stem no doubt protects their vulnerable ventral parts from such attacks. Larvae that survive long enough to reach and start feeding on the rootstock are much better protected from attack, since the thick dorsal integument (carapace) extends down to the rootstock on both sides.

Morphological and behavioural adaptations of the larvae of *O. niobe*

Cottrell (1984) described a number of adaptations of lycaenid larvae that enable them to pursue a myrmecophilous life style. These adaptations have great value in not only creating "enemy-free space" (Atsatt, 1981), but also by providing access to a more reliable nutritious diet resulting in more rapid growth and larger adults (Pierce *et al.*, 2002). A number of these adaptations have been observed in the larvae of *O. niobe*. They have a thick, tough integument, which defends the larvae from attack by the ants or from conspecific larvae. They have an onisciform body shape with an expansion of the dorsal and dorsolateral areas, which slope down to well-developed seta-fringed marginal ridges that can be brought in close contact with the substrate, and seal off the ventral areas including the retracted head and legs. The ants (and the larva's siblings) therefore cannot gain access to the softer ventral parts and vital organs when the larva crawls or rests on a hard substrate.

O. niobe larvae have a dorsal nectary organ (DNO) on the seventh abdominal segment in the 2nd, 3rd and 4th instars, producing a secretion that aids myrmecophily (Pierce, 1989; Fiedler & Maschwitz, 1989). They have tentacular organs (TOs) on the eighth abdominal segment that appear to excite the ants in attendance and draw them to the DNO (Claassens & Dickson, 1977; Fiedler & Maschwitz, 1987). The larvae of *O. niobe* also have a number of other adaptations not yet observed in other lycaenid larvae, including powerful mandibles, which adapt them for rootstock feeding and cannibalism, and an ability to burrow in the soil – although the attendant ants may assist this burrowing.

Larval shelter and overwintering

The subterranean, myrmecophilous lifestyle of the larvae of *O. niobe* insulates them from extremes of temperature and humidity changes; provides protection from fires; and gives them access to a food source (the rootstock) and a safe place to shelter during the winter when the growth of the host plants pauses.

Larval growth patterns

Dyar (1890) postulated that insect dimensions increased by the same factor at each moult. Phytocarniverous lycaenid larvae such as *Maculinea* that parasitise ant colonies are exceptions to this rule (Elmes *et al.*, 2001). *Maculinea* larvae show a normal growth pattern during the first three instars, but after adoption by their *Myrmica* ant hosts, in the final instar they increase in length by a factor of five, and by >50 times in mass (Elmes *et al.*, 1991; Thomas & Wardlaw, 1992). Elmes *et al.* (2001) hypothesised that these growth patterns may have evolved to make the newly moulted 3rd instar butterfly larvae approximately the same size as the ant larvae at adoption and better able to mimic the larvae of their ant hosts. Once in the ant nest and accepted by the ants they could grow to a larger size than other lycaenids because of the readily available, high quality food source.

In Fig. 6 it is clear that the phytocarnivorous larvae of *Maculinea* and *Lepidochrysops* show similar growth patterns. Whilst *O. niobe* larvae raised under artificial (1) or natural conditions (2) have a higher rate of growth in the third instar than any of the other examples, in the final instar the growth rate of *O. niobe* under natural conditions (2) is intermediate between the phytophagous larvae (*E. barkeri* and *L. boeticus*) and the phytocarnivorous larvae. Rootstock feeding may be the key to the higher growth achieved in the final instar relative to the normally phytophagous taxa.

Specialisation of *Orachrysops* associations

All the known host plants of the *Orachrysops* genus are in the genus *Indigofera* or the very closely affiliated genus *Indigastrum* (Table 2). Monophagy is common in the localised species and allows such species to

Table 3. Ant associations of southern African polyommatine butterflies.
Key to third column [coding adopted from Fiedler (1991a)]
0 = No ant relationship (myrmecoxenous)
1 = Very few ant associations reported (weakly myrmecophilous)
2 = A varying proportion of larvae attended by ants (moderately myrmecophilous)
3 = Most if not all mature larvae ant-associated (steadily myrmecophilous)
4 = Larvae dependent on ants as commensals or parasites (obligately myrmecophilous)
** = DNO + TOs
* = DNO only
= PCOs only
( ) = hypothetical
? = likely but not confirmed

Genus	Known ant associates	Degree of myermecophily	Sources
Uranothauma Butler		?	a
Pseudonacaduba Stempffer		(0/1)	c, g
Cacyreus Butler		(0/1)*	a, c
Harpendyreus Heron		(0/1)*	c
Brephidium Scudder		(1)**	c
Oraidium Bethune-Baker		(1)?	g
Tuxentius Larsen		(1)**	c
Zintha Eliot		(1)**	g
Zizula Chapman		(1)**	c
Actizera Chapman		1**	c
Leptotes Scudder	Indeterminate	1**	f
Zizina Chapman		(2)**	c
Cupidopsis Karsch		2**	a, c
Eicochrysops Bethune-Baker		2**	c
Lampides Hübner	*Camponotus* *Plagiolepis*	2**	c, f
Zizeeria Chapman	*Tapinoma*	2/3**	c, f
Azanus Moore	*Pheidole*	3**	a, c, f
Chilades Moore	*Pheidole*	3**	c, f
Tarucus Moore	*Plagiolepis* *Monomorium*	3**	c, f
Euchrysops Butler	*Monomorium*	3**	a, c
Orachrysops Vári	*Camponotus*	4**	i
Lepidochrysops Hedicke	*Camponotus*	4**	b, c, d, e

Sources: a = Jackson, 1937; b = Cottrell, 1965; c = Clark & Dickson, 1971; d = Claasens, 1974 & 1976; e = Henning, 1983a; f = Fiedler, 1991a; g = Pringle *et al.*, 1994; h = Williams, 1999; i = Lu & Samways, 2001.

avoid direct competition and co-exist at a locality (e.g. *O. lacrimosa* and *O. warreni* at Verloren Valei and *O. subravus* and *O. ariadne* at Wahroonga - Samways & Lu, 2007).

Although only two ant associations are known as yet for *Orachrysops* species, the ants concerned, *C. baynei* and *C. natalensis* have some ecological similarities (both are primarily nocturnal ants). *C. baynei* is only found in fynbos or thicket and nests in dead wood above ground level (Edge *et al.*, 2008b), whereas *C. natalensis* can be found in fynbos as well as grassland and nests in the ground (Lu & Samways, 2002a).

Ant associates of South African polyommatines

The records of known or hypothesised ant associations within the South African polyommatines [members of the tribe Polyommatini as defined by

Eliot (1973)] are listed in Table 3. (Sources: Claassens, 1974, 1976; Clark & Dickson, 1971; Cottrell, 1965; Fiedler, 1991a; Henning, 1983a; Jackson, 1937; Lu & Samways, 2001; Pringle *et al.*, 1994; Williams, 1999). The coding system used in the final column has been adopted from Fiedler (1991a).

The close affinities between *Orachrysops* and *Lepidochrysops* would have always made a species of *Camponotus* the most likely host ant for *Orachrysops*, and this has now been confirmed for two of its species. However, with only 11 out of 126 *Lepidochrysops* species ant associates known (Pierce *et al.*, 2002) and 2 out of 11 *Orachrysops* ant associates known, there is insufficient evidence to conclude that *Camponotus* species are the only ant associates for both genera.

Nature of the ant association of *O. niobe*

3rd and 4th instar *O. niobe* larvae were almost always tended by the same ant, *C. baynei*. *O. niobe*'s congener *O. ariadne* has only been found in association with one ant (*C. natalensis*) (Lu & Samways, 2001). Cottrell (1984) considered that relationships specific to one ant species tended to be obligate. Even when more than one ant species interacts with the larvae, in obligate relationships one ant species is the most successful host (Thomas *et al.*, 1989). Facultative relationships by contrast tend to be formed with several species of ant, even from different genera (Fiedler, 2001).

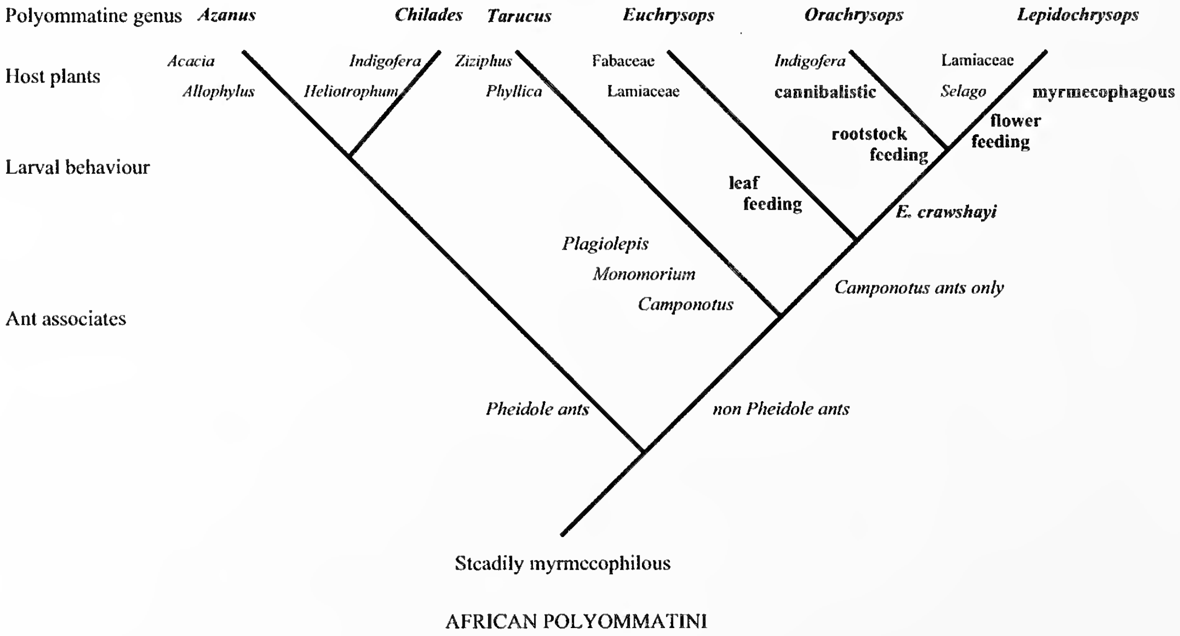
The larvae of *O. niobe* did not enter into ant nests either in the field (artificial nests) or in the laboratory. The nests of the attendant ants found to date are relatively remote from the plants on which *O. niobe* feeds, but the plants need to be within the foraging range of worker attendant ants so they can find the larvae.

No trophallaxis or other feeding of the larvae by ants has been observed. The larvae are rootstock feeders in the later instars, and appear to need the assistance of the ants to access the rootstock. After pupation, clear access to the surface through a hole or tunnel is necessary for the eclosed adult to escape and expand its wings. Ants have been observed both in captivity and in the field repositioning pupae and their assistance may be essential to place the pupa in a safe position to eclose. The balance of evidence is therefore that *O. niobe* is obligately dependent on an ant association with *C. baynei*.

Phylogenetic origins of *Orachrysops* and *Lepidochrysops*

Various authors have proposed phylogenetic hypotheses regarding the origins and evolution of lycaenid ant associations (Hinton, 1951; Eliot, 1973; Fiedler, 1991b; Pierce *et al.*, 2002). Hinton (1951) believed that the possession of a DNO was a primitive feature in the Lycaenidae, and that its absence was a

Figure 8. Hypothetical cladogram of the myrmecophilous polyommatine genera of South Africa based on genus of ant associates, larval feeding behaviour and host plant families and genera.



derived condition. Fiedler (1991b) pointed out that the lycaenid subfamilies that were apparently more primitive on other morphological considerations (Poritiinae, Miletinae and Curetinae) were not generally ant associated; whereas the more advanced subfamily Lycaeninae [sensu Eliot (1973) = Theclini + Aphnaeini + Lycaenini + Polyommatini] contained most of the myrmecophilous lineages. Within Lycaeninae *sensu* Eliot Pierce *et al.* (2002) predicted that the Aphnaeini and certain subtribes of Theclini would be shown to be basal and that Lycaenini and Polyommatini were derived groups.

In Table 3, southern African polyommatine genera are listed in ascending order of their degree of myrmecophily. A tentative cladogram of the steadily myrmecophilous polyommatine genera (denoted as 3 or 4 in Table 3), based on genus of ant associates, larval feeding behaviour and host plants is presented in Fig. 8. *Azanus* and *Chilades* are associated with *Pheidole* ants, which are the dominant ants in some habitats. *Tarucus* is associated with a few ant genera, including *Camponotus*. The genera *Orachrysops*, *Euchrysops* and *Lepidochrysops* are predominantly *Camponotus* associated. *Orachrysops* has evolved rootstock feeding, cannibalism, an obligate ant relationship and specialisation on *Indigofera*. *Euchrysops* has a looser ant association, and normal phytophagy, with the exception of *E. crawshayi*. This interesting taxon has genitalia similar to *Euchrysops* (Stempffer, 1967), facies similar to *Harpendyreus*, and larval behaviour with features found in *Orachrysops*. It is of note that this species has been placed in different genera by various authors (by Butler in *Scolitantides*; by Aurivillius in *Harpendyreus* and later *Cupido*; by Jackson in *Cyclirius*; and finally by Stempffer in *Euchrysops*).

The larvae of *Lepidochrysops* are phytophagous in the first two instars, and myrmecophagous in the last two instars (they feed on the ant brood). This is considered to be the closest ant relationship (Fiedler, 1998), with the larvae being treated as if they were ant brood within the ants' nests. It is here hypothesised that the larvae of the common ancestor of the three genera *Euchrysops*, *Orachrysops* and *Lepidochrysops* could have had rootstock feeding habits, which physiologically adapted them to a higher protein diet. Furthermore, the cannibalistic behaviour of *O. niobe* larvae may have evolved in the common ancestors. This would have enabled them to develop a taste and a need for insect protein, a trait that could have evolved in a sister lineage into the myrmecophagous behaviour of *Lepidochrysops* larvae.

A higher protein diet may have made closer ant associations possible because of the enhanced ability to produce nutritious (high protein) secretions from the DNO (Pierce, 1985). This would have enabled the larvae to attract more ant attendants and given them the opportunity to evolve more advanced chemical camouflage and signaling skills (Fiedler, 1991b; 1998). Such adaptations would have generated further selective advantages in these butterfly lineages, resulting in more complex ant associations and greater interdependence of the butterflies and their ant associates.

CONCLUSIONS

O. niobe is a specialised organism, being monophagous and having an obligate relationship with a single ant species. Its habit of rootstock feeding, which may be shared with other *Orachrysops* species, distinguishes it from nearly all other afrotropical polyommatines. A close phylogenetic relationship between the genera *Orachrysops*, *Euchrysops* and *Lepidochrysops* is inferred from a cladistic analysis.

ACKNOWLEDGEMENTS

CapeNature is thanked for allowing access to the BBBR to conduct research. The Green Trust's financial support is gratefully acknowledged, and North West University kindly provided a stereomicroscope on loan. Dr. Brian Schrire of the Royal Botanic Gardens, Kew identified the *Indigofera* and *Indigastrum* specimens.

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Journal of Research on the Lepidoptera 42: 34-49, 2003 (2010)

Life history of the Imperial Moth *Eacles imperialis* (Drury) (Saturniidae: Ceratocampinae) in New England, U.S.A.: distribution, decline, and nutritional ecology of a relictual islandic population

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Abstract. The decline and current status of *Eacles imperialis* (Drury) (Saturniidae: Ceratocampinae) in New England are reviewed, and primary data surrounding the life history and nutritional ecology presented. Though common throughout much of its historical North American range, this species declined precipitously in New England during the 20th century. Suggested explanations for this region-wide decline include the deployment of pesticides and metal halide street lamps and the introduction of parasitoid flies. The existence of a remnant population of *E. imperialis*, thought to have been extirpated from New England as early as the 1950s, is reported from Martha's Vineyard Island, Dukes County, Massachusetts, U.S.A., representing the last relict of a phenotypically, phenologically, and possibly ecologically infrasubspecific entity. Based on comparisons with museum specimens, adults from this population appear to be indistinguishable from the few historical specimens known from nearby mainland Massachusetts, smaller than those from now extirpated populations in Connecticut, New York, and New Jersey, and slightly larger than members of, the northernmost *E. imperialis* populations (*E. i. pini* Michener) found in the Great Lakes region. The Martha's Vineyard population is univoltine, peaking in late July and exhibiting a more contracted flight season than other extant North American populations. Both parentage and food plant significantly affect larval growth and development, and although larvae on Martha's Vineyard feed extensively if not exclusively on pitch pine (*Pinus rigida*) in the wild, they grow significantly faster, attain greater pupal weights, and more efficiently convert ingested and digested tissues of post oak (*Quercus stellata*) to biomass in the laboratory. Performance as measured by relative growth rate and the efficiency of conversion of ingested and digested food to biomass (ECI and ECD) are correlated with foliar nitrogen and water content: post oak foliage during the larval growth season contains more nitrogen and water than corresponding amounts of pitch pine foliage growing in the same soils. It is suggested that there exists a non-nutritional explanation for the association of *E. imperialis* with pitch pine and for its pattern of decline and persistence. The taxonomic and possible biogeographic affinities of this population are discussed from within the context of a growing understanding of New England's diverse yet threatened lepidopteran fauna, and the potential for reintroducing *E. imperialis* to mainland New England is discussed.

Key words: *Eacles imperialis*, invertebrate conservation, Martha's Vineyard, pitch pine.

INTRODUCTION

The imperial moth *Eacles imperialis* (Drury) (Saturniidae: Ceratocampinae), is one of North America's largest and familiar saturniids. Throughout its extensive range, this species exhibits considerable regional variation in coloration and size—on the basis of which three North American subspecific epithets are currently attributed—as well as variation in phenology and host plant utilization (Ferguson, 1971; Covell, 1984; Tuskes *et al.*, 1996). Considered a common moth in the southern United States, *E. imperialis*, like many large bombycoid moths, underwent a precipitous decline in much of northeastern North America during the mid-20th century (Hessel, 1976; Ferguson, 1971; Schweitzer, 1988). Ferguson (1971: 25) writes "[the imperial moth]...like some of the other large saturniids, is said to have largely disappeared from heavily populated areas such as those in Connecticut and the vicinity of New York City, where it was formerly common." Various hypotheses for such declines, ranging from the expanded use of metal halide bulbs in street lamps (Hessel, 1976) to the widespread deployment of pesticides aimed at gypsy moths (Goldstein, 1991) and to the introduction of parasitoids, specifically the tachinid fly *Compsilura concinnata*, for the same purpose (Boettner *et al.*, 2000) have been invoked to explain these declines. Believed extirpated from New England, a relict population of *E. imperialis* was verified by the author in 1982 on Martha's Vineyard Island (Dukes Co.), off Cape Cod, Massachusetts, where it quickly became of interest to conservationists. In this paper, following a

Received: 24 July 2006
Accepted: 24 August 2006

review of the taxonomy and regional variation in this species complex, I present life history observations and larval growth and performance data on this population between 1986 and 1989, discuss this species' decline by summarizing historical records and information on pesticide deployment and parasitoid impacts, and discuss the nutritional and life history requirements of this species in light of putative reestablishment on mainland New England.

Distribution and variation in the *Eacles imperialis* complex in North America

Extending from Canada to Argentina, the imperial moth *E. imperialis* is both the widest-ranging and the northernmost occurring species in its genus and one of the most widely distributed saturniids that occurs in North America (Lemaire, 1988), representing a complex of ecologically and possibly phylogenetically distinct intra-nominal entities. Authors have differed in their treatment of subspecific epithets, variously recognizing the western *E. oslari* as a full species versus a subspecies of *imperialis* and the southern *E.i. nobilis* Neumoegen as a subspecies versus a synonym of nominate *imperialis* (reviewed in Tuskes *et al.*, 1996). Not including these, two recognized subspecies of *E. imperialis* occur in North America (Lemaire, 1988; Tuskes *et al.*, 1996): the nominate subspecies and *E. i. pini*, which is distributed in the Great Lakes region and the Adirondacks.

According to Ferguson (1971: 24), *E. imperialis* "occupies nearly all of the United States east of the Great Plains, with the exception of northern New England and northern portions of Michigan and Wisconsin." Ferguson (1971: 25) further reports records "from southern New Hampshire...through New York State, the Niagara Peninsula of Ontario, and southern Michigan, westward to the eastern edge of the Great Plains, and southward to the Gulf Coast and southern Florida (but not the Florida Keys). It has not been taken in Maine." The New England states are represented by specimens in collections at the American Museum of Natural History (AMNH), the Museum of Comparative Zoology, (MCZ), the Peabody Museum of Natural History (PMNH), which houses the bulk of the F. M. Jones collection from Martha's Vineyard, and the collection of the now defunct Boston Society of Natural History (BSNH), which currently resides at Boston University (Fig. 1). A single 1900 specimen from Kittery Point (southern Maine's York County) is housed at the MCZ, and Patch (1908; cited in Tuskes *et al.* [1996: 65]) reported it from Cumberland County, ME. Beyond these records, Farquhar's (1934) thesis enumerated various other New England records, also included in Fig. 1.

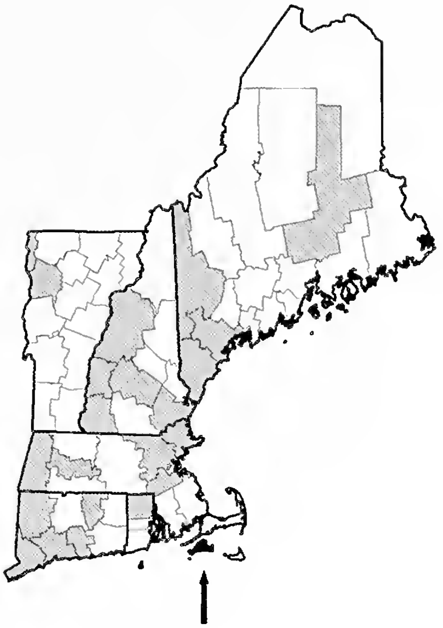

Figure 1. Historical distribution of *E. imperialis* in New England by county. Arrow indicates presence of lone extant population on Martha's Vineyard Island, Dukes Co., MA.

The species' current occurrence in the northeastern portion of its range south of New England, including Long Island, N. Y. and southern New Jersey, is associated at least in part with habitats characterized by sandy soils such as pitch pine-scrub oak barrens, where its larval host plant, pitch pine (*Pinus rigida*), abounds. Such habitats have been heavily impacted, in large part due to the ease with which sandy soils are manipulated for building and construction purposes. Sandy, well-drained soils may be a requirement for *Eacles imperialis* in that, like all ceratocampine saturniids, *Eacles* larvae do not construct cocoons but burrow into and pupate within the soil, from which the pupae themselves emerge so that the adults may eclose above-ground. The conspicuous dearth of historical records from Cape Cod, where sandy soils predominate, may be attributable to a combination of small private collections' having been orphaned.

Eacles imperialis exhibits apparently clinal variation in wing maculation and shading as well as in size and phenology, with southern populations (formerly attributed to *E. i. nobilis*) often exhibiting more intense brown postmedial shading than northern populations. It has also been observed that adult individuals of northern populations attributed to *E. i. pini* in northern Michigan , Ontario, Quebec, and New York, are markedly smaller than their southern and eastern counterparts (Ferguson, 1971; Tuskes *et al.*, 1996), and bear more intensive peppering with blackish spots; the larvae exhibit pronounced dorsolateral scoli relative to nominate *imperialis.* The maculation on specimens taken from Martha's Vineyard is consistent both with museum specimens from the island and with those taken from mainland New England prior to the species' decline there. Based on an examination of these and other specimens from the northeast, the mean forewing length for male *E. imperialis* from Martha's Vineyard was 49.64 mm (N = 60, se = .401), significantly smaller than that of 78 specimens examined from New York, Connecticut, and New Jersey (one-way ANOVA, p < .0001, DF = 1, F-ratio = 88.241; Fig. 2), and at the lower end of the range of 47.59 mm reported by Tuskes *et al.* (1996). This number is higher than the average male wing length of 47 mm reported for *E. i. pini* by Michener (1950) in the original description and out of the 42 mm - 48 mm range reported for male *pini* by Tuskes *et al.* (1996). Michener (1950) also reported an average wing length of 54 mm for male nominate *imperialis* from the vicinity of New York City; specimens I measured from this area averaged 54.92 mm (N = 36, se = .92), by way of comparison. Although female wing lengths for mainland New England specimens were not analyzed due to their scarcity in collections, Martha's Vineyard female specimens averaged 57 mm in wingspan, just below the range of 58-68 mm reported by Tuskes *et al.*, greater than the 51 mm average *pini* female wingspan reported by Michener and out of the 47 mm - 54 mm range reported for *pini* by Tuskes *et al.*

The familiar green/brown larval polymorphism of *E. imperialis* is apparent both among lab-reared caterpillars and those observed in the wild on Martha's Vineyard. Both maternity and food plant affect the expression of this polymorphism: among the larvae reared for this study and in situ (P. Goldstein, unpubl.), the lime green color form was less prevalent than the dark brown, with the majority of reared green larvae developing on pine versus oak. Ferguson (1971: 26) writes that "[a] brood from Massachusetts, described by Eliot and Soulé [1902], contained only one green larva." Harris (1890: 404), on the other hand, writes "[the caterpillars are] for the most part, of a green color, slightly tinged with red on the back; but many of them become more or less tanned or swarthy, and are sometimes found entirely brown." During the course of my rearing, I observed seven cases in which larvae switched from brown to green at one molt only to shift back at a subsequent molt; a common color morph of oak-fed larvae. The most common color morph of oak-fed larvae is orange to reddish brown, often with the spiracles and dorsolateral scoli surrounded by paler patches.

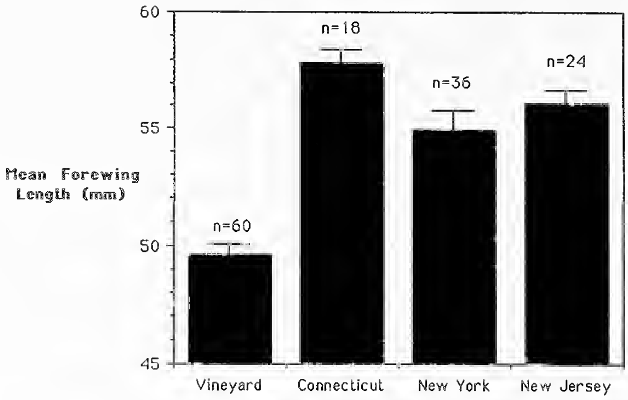

Figure 2. Mean forewing length (base to apex, in mm) of 138 male *Eacles imperialis* from Martha's Vineyard, Connecticut, New York, and New Jersey. The mean forewing length for male *E. imperialis* from Martha's Vineyard was 49.64mm (N=60, se=.401), significantly smaller than that of specimens examined from New York, Connecticut, and New Jersey (one-way ANOVA, p<.0001, DF=1, F-ratio=88.241). See text.

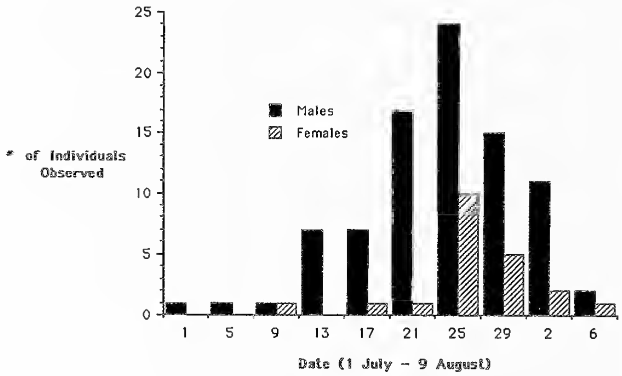

Figure 3. Flight season of *E. imperialis* on Martha's Vineyard, 1984-1989 inclusive, based on observations of 107 individuals, 86 male and 21 female (see text).

With respect to life history timing, Harris (1890: 404) writes: "The moth appears here [Massachusetts] from the 12th of June to the beginning of July, and then lays its eggs on the buttonwood [sycamore, *Liquidambar styracifolia*] tree. The caterpillars may be found upon this tree, grown to their full size, between the 20th of August and the end of September, during which time they descend from the trees to go into the ground." Phenologically, based on a sample of 107 individuals (86 male, 21 female) collected or observed on Martha's Vineyard between 1982 and 1989, the population is protandrous, and both males and females peak at the end of July: 65% (56) of the males were observed between 21 July and 1 August, and 71% (15) of the females were observed between 25 July and 1 August (Fig. 3). This flight season is consistent with the collection dates of museum specimens from the nearest known mainland historical records (e.g. Cohasset, MA; Bristol, RI), has remained predictable in the years since this study was conducted, and may represent a more contracted flight season than that reported in Tuskes *et al.* (1996) for northern *E. i. imperialis.* Most individuals appeared at lights after 2200h, and individuals were observed coming to light until 0400h.

Taken collectively, these observations hint at the possibility that northern *E. imperialis* represents at least one and possibly two biological entities distinct from southern *imperialis.* It is noteworthy that Lemaire (1988: 31) portrays the range of *E. i. pini* as crossing the Appalachian divide to northern New England. Although there exists a phenetic similarity of adult New England specimens to individuals typical of *E. i. pini* with respect to size and maculation, the diagnostic characters of adult and larval *pini* (Tuskes *et al.*, 1996: 67, 68; Pls. 1, 7, & 8) do not appear in specimens from New England. Historically, the superficially clinal nature of variation in this complex has presented obstacles to a clear understanding of what may be taxonomically and biologically distinct natural entities. Notwithstanding the perhaps controversial criteria on which the retention of saturniid subspecific epithets rely (Goldstein, 1997; but see Tuskes *et al.*, 1996 for an alternative viewpoint), the similarities between nominate *E. imperialis* and *E. i. pini* in wing maculation, size, phenology, and especially host plant use are relevant to the holobiology of this complex. It is conceivable that the small size of *E. i. pini* and the New England *E. i. imperialis* are related to the combination of a short growing season and an apparently exclusive association with pines at northern latitudes. I would recognize *E. pini* as a full species and anticipate that the northeastern, pine-feeding populations of *E. imperialis* and those comprising *pini* will ultimately be recognized as separate sister species. Since the type locality of *Eacles imperialis* is in New York, I would further anticipate that *nobilis* be resurrected.

Eacles imperialis on Martha's Vineyard: past and present

Jones and Kimball (1943), in their extensive treatment of the Lepidoptera of Martha's Vineyard and Nantucket Islands, described *E. imperialis* as occurring regularly on Martha's Vineyard, where it persists and is widely distributed on both moraine and outwash plain soils (Fig. 4). It is not known from Nantucket, and virtually all mainland New England specimens deposited in museum collections I examined were taken during the first half of the 20th century, with only a few taken as late as the early 1950s. By all accounts, this species had essentially declined dramatically in New England more than a decade before Ferguson's (1971) publication, and indeed was considered extirpated from Massachusetts during the early drafting stages of the Massachusetts Endangered Species Act (MESA; M.G.L. c. 131A and regulations 321 CMR 10.00). It is currently listed as "threatened" in Massachusetts.

Figure 4. Current distribution of *E. imperialis* on Martha's Vineyard as of 28 August, 2009. Courtesy Massachusetts Natural Heritage & Endangered Species Program. Westboro, MA.

The decline of *E. imperialis* in northeastern North America is not unique, but rather consistent with a well-known pattern of decline among saturniids (Hessel, 1976; Ferguson, 1971; Frank, 1988; Schweitzer, 1988). In fact, the three most dramatic declines of saturniids in New England have been ceratocampines: *E. imperialis*, the royal walnut moth *Citheronia regalis* (Fabricius), another of North America's most massive saturniids, and the pine devil moth *C. sepulcralis* Grote & Robinson, another barrens species that feeds on pine and the type locality of which is Andover, MA.

Martha's Vineyard appears to have served as a regional refugium for several species (e.g. *Actias luna*) that remained extremely abundant on the island while undergoing anecdotal declines—even temporary ones—on the mainland. It is not at present possible to single out any one of the various possible causes for these declines; none are mutually exclusive. Moreover the weak coincidence of the deployment of metal halide street lights and pesticides several decades following the introduction of the tachinid fly *Compsilura concinnata* (Diptera: Tachinidae) in 1906 to combat gypsy moths and other pests (Howard & Fiske, 1911) makes parsing the relative importance of these factors difficult. In hindsight, it is not surprising that the sole New England population of *E. imperialis* to persist did so on an island that was not as heavily subjected to as heavy aerial deployment of pesticides or high-wattage metal halide streetlights, or to the intentional release of parasitoids, as the mainland.

Available data on the use of DDT and other pesticides, recorded on a per-county basis between 1948 and 1965, indicate that Dukes Co. (including Martha's Vineyard) was one of four counties sprayed only once during this period, and was subjected to less intensive treatment (as measured by total spray-acres) than any other county in the Commonwealth excepting Middlesex (Bewick, 1979, reproduced in Table 1). Martha's Vineyard is approximately 100 square miles, or 64,000 acres; its single documented pesticide treatment of 17,000 acres in 1956 is less intensive, for example, than the 31,071 acres sprayed on Nantucket, whose land mass is roughly half that of the Vineyard; and far less than the hundreds of thousands of acres of maritime barrens habitats in Plymouth and Barnstable counties routinely subjected to spraying before the use of DDT was discontinued.

Prior to the widespread deployment of DDT, the parasitoid fly *C. concinnata* had become well-established in New England, shortly after its 1906 introduction (Culver, 1919). Introduced to combat the gypsy moth *Lymantria dispar* (L.), the browntail moth *Euproctis chrysorrhoea* (L.) and other pests, it quickly became apparent that this animal attacks a large and diverse assemblage of macrolepidopteran larvae (Webber & Schaffner, 1926; Arnauld, 1978). Recent observations by Boettner *et al.* (2000 and unpubl.) confirm the extraordinary impact of *C. concinnata* on several native saturniids on mainland New England and that the flies will attack *E. imperialis* caterpillars when presented with the opportunity. *Compsilura concinnata* is considered a stong candidate for playing a role in the demise of *E. imperialis* and other large moths on mainland New England (Boettner *et al.*, 2000); *Compsilura concinnata*'s occurrence on Martha's Vineyard has not been verified.

Table 1. Massachusetts DDT spray history 1948-1965 (Reproduced from Bewick, 1979).

County	Total spray-acres	# Years treated	Avg. acres sprayed per treatment year
Plymouth	525,962	5	105,193
Hampshire, Hamden, Franklin	505,952	7	72,279
Barnstable	490,130	10	49,013
Worcester, Norfolk	374,905	2	187,453
Berkshire	324,765	7	46,395
Nantucket	31,071	1	NA
Essex	29,685	2	14,823
Bristol	18,496	1	NA
Dukes	17,000	1	NA
Middlesex	16,934	1	NA

Regional variation and host plant use

Eacles imperialis feeds (and can certainly be reared) on a wide range of tree hosts (Ferguson, 1971; Stone, 1991; Tuskes *et al.*, 1996), to which it may be locally specialized, and the moth's range exceeds that of any recorded host plant species. Ferguson (1971) lists an impressive array of recorded hosts: "oak, hickory, walnut, sycamore, basswood, maple, honey locust, chokecherry, sumac, sweet gum, sassafras, elm, beech, hornbeam, birch, alder, pine, spruce, hemlock, cedar, cypress, and juniper." However, the degree to which a given population of *E. imperialis* is ever genuinely polyphagous in nature is not well studied. On Martha's Vineyard, *E. imperialis* appears to feed exclusively on pitch pine (*Pinus rigida*), a common tree associated with sandy, well-drained soils and barrens habitats. Pine-feeding is not unusual for *Eacles imperialis* elsewhere, nor is it restricted to the northernmost tier of the moth's range: Abbot and Smith (1797) observed pine-feeding in Georgia over two centuries ago, and pitch pine is a common host in the New Jersey pine barrens (D. F. Schweitzer, pers. comm.). All the recorded hosts of *E. i. pini* are conifers (Tuskes *et al.*, 1996), including jack pine (*Pinus banksiana*), which replaces *P. rigida* to the north and west of southern New England. From the perspective of consumption and digestion, conifer foliage represents a diet high in fiber, relatively low in water and nitrogen content, and possessed of allelochemical defenses such as monoterpenes stored in resin ducts (Raffa, 1991). The observation of localized conifer specialization on the part of *E. imperialis* is of interest both from the perspective of pine-herbivore biology and from that of the species' northeastern decline.

Host plant specificity among herbivorous insects in general and Lepidoptera in particular has been of broad interest to entomologists at least since the works of Brues' (1920, 1924), which were followed by seminal ecological studies and reviews highlighting general patterns in the ecology and evolution of host use breadth (e.g. Ehrlich & Raven, 1964; Futuyma, 1976; Gilbert, 1979; Cates, 1980; Jermy, 1984). Adult oviposition cues play a critical role in host plant specialization (Wiklund, 1975), and variables such as host plant chemistry and architecture (Cates & Rhoades, 1977; Scriber & Feeny, 1979; Berenbaum, 1981; Bowers, 1983, 1984; Bernays, 1989), foliar water, nitrogen and fiber content (Scriber, 1977, 1979b; Mattson, 1980; Mattson & Scriber, 1987), and maternity (e.g. Mousseau & Dingle, 1991) effect lepidopteran digestive efficiency. Population genetic and phylogenetic data have been brought to bear on the evolution of host specialization and the role of host shifts in speciation (Berlocher, 1998), and the co-cladogenesis of insects and plants (Farrell & Mitter, 1990, 1998; Futuyma & McCafferty, 1990; Funk *et al.*, 1995; Becerra, 1997; Farrell, 1998).

Studies of the ecophysiological roles of host plant growth form and seasonality (e.g. Tilton, 1977; James & Smith, 1978; Scriber, 1978) in mediating insect-plant associations are directly relevant to the evolution of pine feeding. From a nutritional perspective, folivores of trees and other woody plants represent a guild that must often contend with low nitrogen and water contents relative to graminivores or forb feeders, for example. Nitrogen is an important growth-limiting factor for many herbivores which may covary with water content (Mattson, 1980); low foliar water content can impede the growth and development of herbivores (Scriber, 1977) as well as a given herbivore's ability to utilize available nitrogen (Scriber, 1979a, b). These effects can be particularly severe with regard to the performance of tree feeders (Scriber, 1979b); since low nitrogen and water contents are typically associated with woody plants as opposed to forbs and grasses, folivores of trees often display low growth and utilization efficiencies (Scriber & Slansky, 1981).

I pursued a line of investigation towards better understanding host plant use in the relictual population of this moth, specifically the question of whether the apparent confinement to pines is nutritionally imposed or the result of some unknown non-nutrionally based or abiotic factor. This endeavor comprised laboratory-based experiments focusing on the role of food plant in influencing larval growth and development. Given the considerable range-wide variation in host use among geographically delimited populations of *E. imperialis*, the notion that such local specialization may have been accompanied by physiological adaptation is of interest from the perspective of understanding host shifts, regardless of whether they accompany speciation *per se.*

MATERIALS AND METHODS

Five treatments from four species of host plants were chosen on the basis of recorded use by *E. imperialis*, availability and, in the case of pitch pine, comparability between material from wild populations known to be used by *E. imperialis* and arboretum-raised material. Pitch pine is the only known host of *E. imperialis* on Martha's Vineyard (personal observations of both larvae and wild oviposition behavior of adult females), and was reported as the favored larval host of other Massachusetts *E. imperialis* (Eliot & Soulé, 1902). The jack pine *P. banksiana* was chosen because it is associated with *E. imperialis pini* (Michener, 1950; M. C. Nielsen, pers. comm.; B. Scholtens, pers. comm.), comprising the northernmost named subspecies of *E. imperialis* and the only other regional populations associated exclusively with conifers. Jack pine may be considered an ecological analog of pitch pine, in that it replaces the latter in habitats to the north and west characterized by granitic soils that, like maritime pitch pine-scrub oak barrens, are sandy, well-drained, and acidic soils (Little, 1979; Schweitzer & Rawinsky, 1988). M. C. Nielsen (pers. comm.) reports that *E. i. pini* appears most commonly in association with jack pine on Grayling soils. With the exception of tiny remnant stands such as that at West Rock, New Haven, CT, the post oak *Q. stellata* is represented in New England primarily as a disjunct population on Martha's Vineyard. The nearest large stands of *Q. stellata* occur on Long Island, N. Y. and in the New Jersey pine barrens, which also hosts the largest northeastern population of *E. imperialis.* The black walnut *J. nigra* was chosen because it is a recorded host of *E. imperialis*, yet does not occur regularly on Martha's Vineyard. Food plant material was harvested every five days from the Felix Neck Wildlife Sanctuary, Edgartown, MA (pitch pine and post oak) and the Arnold Arboretum, Jamaica Plain, MA (pitch pine, jack pine, and black walnut). Upon cutting, plant material was placed in water pics and maintained at 40°F until used.

Foliage fitted with water pics was placed in rearing containers, the supply of fresh food maintained, and the container cleaned of frass every five days. Since foliar water and nitrogen concentrations have been demonstrated to decrease with leaf age (Axelsson & Agren, 1979; Slansky & Scriber, 1985), it was therefore important to be as consistent as possible when selecting plant material so that foliage treatments of wildly disparate ages were not lumped under the same heading.

Gravid females from Martha's Vineyard were collected in 1988 and 1989 at light and placed in paper bags for oviposition. Ova were harvested and segregated according to parent, then maintained in 4" diameter petri dishes under constant conditions of light and temperature (per day, 14 hours light at 25°C and 10 hours darkness at 20°C). The 1988 livestock came from four females taken between 31 July and 5 August at three different sites on Martha's Vineyard: Cedar Tree Neck, West Tisbury (4 August, 110 ova); Makonikey, West Tisbury (3 and 5 August, 155 and 78 ova); and Pennywise Path, Edgartown (31 July, 107 ova).

During the first (1988) season's experiments, ova were selectively cooled at 50°F for up to four days to synchronize hatching. Larvae from each clutch were weighed upon hatching, placed on each of five of the food plant cohorts, and weighed every five days (116 hours) through day 15 in all cases and day 20 when possible. Larvae were reared individually, first in 4.0" petri dishes for their first five days, and then in plastic containers 4.5" in diameter and 3.5" in height. Humidity was maintained using 1" x 2" cut sections of paper towels secured to the container lid and moistened twice every five days. All larvae were maintained under constant conditions of temperature and lighting as described above. Since leaves that remain attached to the stem are generally less apt to lose water than if they are cut (Schroeder, 1984), food plant freshness was maintained by placing plant sprigs (or petioles, in the case of *J. nigra*) in water pics situated in larval rearing containers.

Weights of surviving larvae from different clutches and on different host plants were natural log-transformed and compared (2-way ANOVA) for each weighing interval through Day 15. The influence of food plant treatment was further analyzed (1-way ANOVA) by lumping all surviving larvae of a given food plant treatment and age regardless of parentage.

In 1989 these experiments were repeated in part and with several modifications, using ova from seven females collected between 26 and 28 July from three sites on Martha's Vineyard: Lobsterville, Aquinnah (26 July, 178 ova); Cedar Tree Neck, West Tisbury (three females, all 27 July; 55, 125, and 169 ova); and Makonikey, West Tisbury (three females, 26, 27, and 28 July; 178, 50, and 104 ova). The 1989 work focused exclusively on two food plants taken from current *E. imperialis* habitat (*P. rigida*, the native host, and *Q. stellata*, with all plant material taken from Felix Neck Wildlife Sanctuary, Edgartown, MA. Eggs were not cooled to synchronize hatching, and in order to minimize handling of young larvae, all hatchling caterpillars were started not in petri dishes but in the larger plastic containers. Given observations that hatchling larvae lost weight to desiccation quickly, care was taken to ensure that every larva was weighed and placed on the appropriate food plant immediately upon hatching. To maintain humidity, I used 1" x 1" x 2" sponges, washed at each weighing instead of paper towel sections. The sponge holds more water and for a longer period of time and does not require additional moistening between weightings. All sponge blocks were washed repeatedly prior to use to clear them of chemical additives, and thoroughly cleaned of larval frass at each weighing to avoid mold. Food plants were maintained as in 1988 at 40°F, misted with distilled water and given refreshed water pics every 24 hours. Feedings were made as close to identical as possible; all the feedings for a given weighing interval and food plant species consisted of material cut from a single tree. Sample sizes were increased to fifteen larvae per female per food plant treatment initially, for 104 larvae in total on each food plant. Color morph and instar were noted at each weighing and larvae were reared to pupation, sexed and weighed again. Weights were analyzed as described through day 20 for all larvae and pupae. Since *E. imperialis* is a sexually dimorphic species, male and female pupae were compared separately (1-way ANOVA) for each food plant cohort. Weights upon hatching were compared independent of a food plant effect (one-way ANOVA) to evaluate bias in assigning larvae to food plants. There was not a large enough sample size per sex per clutch to retrieve any significant data regarding a maternal effect on pupal weight. However, all pupal weights representing each sex were lumped for each food plant in order to test for a food plant effect via one-way ANOVA.

The gravimetric assessment of digestive and growth indices involved forty offspring of a single female on four of the 1988 food plant treatments: both wild and arboretum-grown *P. rigida*, arboretum-grown *P. banksiana*, and wild *Q. stellata*. Low survivorship on *J. nigra* precluded there being enough data to analyze. These indices were: relative growth rate (RGR); relative consumption rate (RCR); approximate digestibility (AD); efficiency of conversion of ingested food to biomass (ECI); and efficiency of conversion of digested food to biomass (ECD). The experimental regime employed here involved monitoring the food uptake and utilization during the course of a single instar, from the beginning of the third to the beginning of the fourth instar. All vegetative material used in these experiments consisted of pre-weighed individual leaves or sprigs; unconsumed food and frass was dried at 95°F and re-weighed. Control samples of plant material for each feeding were also weighed, dried, and re-weighed for the purpose of calculating conversion factors. Larvae were weighed at the beginning and end of the experiment, then frozen, dried, and re-weighed to determine the dry weight gained by each larva. Each larva thus provided its own conversion factor, its final dry weight treated as a percentage of its final fresh weight and used to estimate initial dry weight. Based on these weights of plant and larval material, the five food plant utilization measures were determined following Waldbauer (1968). For each larva used in the calculation of utilization indices, the dried control food plant material was ground and analyzed for percent-nitrogen using a Kjeltec nitrogen analysis system. Mean digestive indices were compared via one-way ANOVA.

The gravimetric assessment of performance has been reviewed and critiqued numerous times since its introduction (Scriber & Slansky, 1981; Schroeder, 1984; Agren & Axelsson, 1979; Bowers *et al.*, 1991), and a substantial body of literature has been devoted to evaluating the various sources of error associated with this method. A significant methodological source of error in the calculation of such nutritional indices derives from the indirect calculation of the dry weight of plant material and of the initial dry weight of the larva (Bowers *et al.*,1991). Since the host plants used differ in megaphyll morphology, achieving similarity between treatment and controls is difficult to standardize. This necessitated frequent feeding of fresh plant material in order to minimize the differential effects of desiccation on digestibility. For each feeding of *Q. stellata*, a single leaf was bisected, half used to determine the dry weight conversion factor. Conversion factors for *P. rigida* involved the use of individual needles excised from sheaths: for each needle-bearing sheath, a single needle was removed for immediate drying while the others were used for feeding. This method has the dual advantages of providing as accurate a control as possible, since all needles within a given sheath are the same age and size, and of minimizing damage to both treatment and control, since the sheath can be removed from its enclosed needles without tearing or severing significant mesophyll tissue.

Preliminary work showed that the short needles of *P. banksiana*, which occur in pairs, were more vulnerable to desiccation than those of *P. rigida*. To offset desiccation, single needles were therefore retained intact within bisected sheaths, the dry weight of the inedible sheath later subtracted from the estimated dry weight of the initial feeding prior to calculation of fresh weight/dry weight conversion factors.

RESULTS

Food plant and maternal effects on growth

Larval growth varied considerably across the 1988 food plant treatments. The (1988) data indicated that *E. imperialis* larvae grew more slowly at first on the deciduous plants than conifers, with growth most markedly retarded among the larvae fed *J. nigra* (Table 2, Fig. 5). Based on weight, larvae responded best to the diet of arboretum-grown *P. banksiana*, followed by that of arboretum-grown *P. rigida*, wild *Q. stellata* and finally wild *P. rigida*. However, for a period between the second and third weightings (days 5 and 10), the growth of larvae fed *Q. stellata* surpassed that of the other food plant cohorts. Most larvae fed arboretum-grown jack pine and pitch pine weighed more at Day 20 than those given other food plants. ANOVA indicated significant food plant as well as maternal effects on larval growth (Table 3), the latter far less marked than the former.

Larvae in the 1989 follow-up growth experiments, conducted under modified conditions where foliar water was more rigorously ensured, consistently gained biomass and molted earlier when fed post oak than when fed pitch pine, unambiguously demonstrating enhanced performance of *E. imperialis* on wild *Q. stellata* relative to wild *P. rigida* based on larval growth and pupal weight (Fig. 6; Tables 4, 5), again with significant differences attributable both to food plant and to maternity in each of the host plant treatments (Table 6; Figs. 7, 8).

The variation in larval growth attributable to maternal effects was, as in 1988 (Table 3), small relative

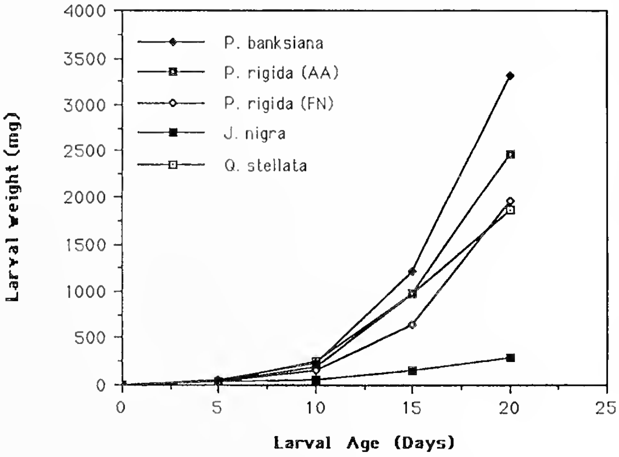

Figure 5. Fresh weight (mg) of *E. imperialis* on five food plant treatments taken at 5-day intervals, 1988. *Cf.* Table 2.

Table 2. Weights (mg) of larvae reared on five food-plant treatments, 1988. *x* = mean; se = standard error; *N* = sample size; AA = foliage used from Arnold Arboretum, Jamaica Plain, MA; FN = foliage used from Felix Neck Wildlife Sanctuary, Edgartown, MA. Cf. Fig. 5.

Age (days)	*Pinus rigida* (FN) *x*(se)	*Pinus rigida* (AA) *x*(se)	*Quercus stellata* (FN) *x*(se)	*Pinus banksiana* (AA) *x*(se)	*Juglans nigra* (AA) *x*(se)
0	9.384 (0.083) *N*=19	8.9 (0.081) *N*=19	9.47 (0.0653) *N*=23	7.632 (0.069) *N*=19	8.411 (0.083) *N*=19
5	43.947 (0.213) *N*=19	45.968 (0.21) *N*=19	40.335 (0.179) *N*=23	59.058 (0.311) *N*=19	34.211 (0.191) *N*=19
10	157.789 (.495) *N*=19	203.158 (0.45) *N*=19	246 (0.562) *N*=23	243.342 (0.556) *N*=19	68.618 (0.409) *N*=17
15	652.829 (1.279) *N*=14	988.174 (0.865) *N*=19	973.099 (1.068) *N*=19	1219.421 (1.185) *N*=19	160.769 (0.851) *N*=13
20	1964.592 (2.479) *N*=14	2471.57 (2.54) *N*=10	1867.557 (3.629) *N*=7	3320.9 (1.869) *N*=14	296.788 (1.783) *N*=8

Table 3. Analysis of variation due to food-plant and maternal effects on larval growth, 1988. NA = not applicable.

		Variation due to foodplant			Variation due to maternal effects	
Larval age (days)	DF	F-ratio	P-value	DF	F-ratio	P-value
0	4	NA	NA	3	1.08	0.362
5	4	3.988	0.005	3	6.297	0.001
10	4	22.329	<.001	3	6.259	0.001
15	4	55.356	<.001	3	4.393	0.007

Table 4. Weights (mg) of *E. imperialis* reared on *P. rigida* and *Q. stellata* at ages 0 through 20 days, 1989, followed by pupal weights for males and females. Range refers to mean values for offspring of a given female (not applicable for pupal weights). Cf. Fig. 6.

	Pinus rigida		*Quercus stellata*	
Larval age (days)	x(se)	Range	x(se)	Range
0	10.221 (0.013) N=104	8.334-11.727	10.315 (0.014) N=104	8.531-12.358
5	47.803 (0.035) N=104	41.508-58.789	59.227 (0.044) N=100	53.389-66.627
10	315.042 (0.118) N=102	229.862-468.191	555.959 (0.213) N=92	383.782-647.46
15	1516.205 (0.268) N=96	1161.357-2013.193	2195.288 (0.328) N=86	1516.931-2711.417
20	3561.943 (0.349) N=93	3007.108-4226.031	4484.786 (0.387) N=87	3517.45-5599.417
Male pupal weight (mg)	3719.304 (0.936) N=26	NA	4751.297 (0.786) N=29	NA
Female pupal weight (mg)	4473.514 (1.199) N=22	NA	5705.4 (1.277) N=22	NA

Table 5. Summary and analysis of development of *E. imperialis* in therms of instar on *P. rigida* and *Q. stellata*.

	Day 5		Day 10		Day 15		Day 20	
Instar	**1**	**2**	**2**	**3**	**3**	**4**	**4**	**5**
# Pine-fed larvae	24	80	13	89	8	88	28	65
# Oak-fed larvae	6	96	2	90	1	91	12	73
G-value	13		9		6		7	
P-value	<.001		<.005		<.025		<.025	

Table 6. Analysis of food-plant and maternal effects on larval growth, 1989. NA = not applicable; NS = insufficient data.

	Variation due to foodplant			Variation due to maternal effects		
Larval age (days)	DF	F-ratio	P-value	DF	F-ratio	P-value
0	NA	NA	NA	6	15.547	<.001
5	1	26.194	<.001	6	3.643	0.002
10	1	55.112	<.001	6	6.176	<.001
15	1	25.382	<.001	6	6.507	<.001
20	1	24.34	<.001	6	5.593	<.001
Male pupa	1	40.049	<.001	NS	NS	NS
Female pupa	1	28.115	<.001	NS	NS	NS

Table 7. Summary and analysis of nutritional indices obtained on four food-plant treatments. AA = foliage used from Arnold Arboretum, Jamaica Plain, MA; FN = foliage used from Felix Neck Wildlife Sanctuary, Edgartown, MA.

Nutritional index	*Pinus rigida* (FN)	*Pinus banksiana*	*Pinus rigida* (AA)	*Quercus stellata*	One-way ANOVA	
					F-ratio	P-value
	x(se)	*x*(se)	*x*(se)	*x*(se)		
AD	31.09 (0.6) *N*=4	31.64 (0.431) *N*=7	28.78 (0.259) *N*=9	26.5 (0.283) *N*=7	0.898	0.457
ECD	32.16 (0.714) *N*=4	39.38 (0.647) *N*=7	41.96 (0.331) *N*=9	46.22 (0.43) *N*=7	1.056	0.387
ECI	15.01 (0.304) *N*=4	16.35 (0.197) *N*=7	18.08 (0.126) *N*=9	18.48 (0.208) *N*=7	4.778	0.01
RGR	0.205 (0.04) *N*=4	0.244 (0.021) *N*=7	0.251 (0.022) *N*=9	0.264 (0.032) *N*=7	2.157	0.121
RCR	0.473 (0.072) *N*=4	0.49 (0.04) *N*=7	0.414 (0.02) *N*=9	0.425 (0.04) *N*=7	2.178	0.118
%N	5.83 (0.052) *N*=8	6.79 (0.086) *N*=8	6.84 (0.048) *N*=8	8.07 (0.091) *N*=8	48.973	<.001

to that attributable to host plant. One trend is that of the consistent relative growth of offspring of several females; mean larval weights for each of four females on post oak were more massive than on pitch pine by Day 5, remaining so at every consecutive weighting through Day 20. The offspring of each of the four females grew, relative to one another, in exactly the same order on oak and pine, with the offspring of female 5 outdistancing those of females 6, 7, and 2, respectively (Figs. 7, 8). This trend implies that there

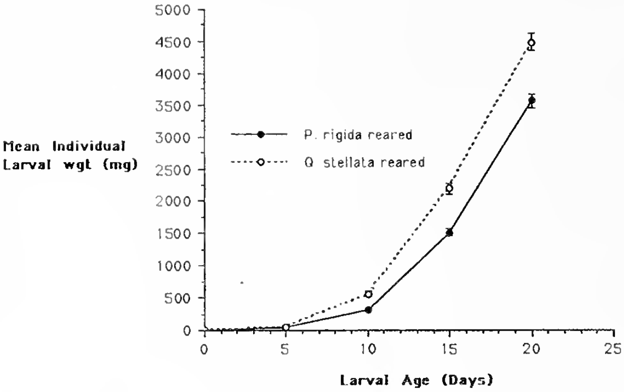

Figure 6. Fresh weight (mg) of *E. imperialis* on pitch pine *P. rigida* and post oak *Q. stellata*, 1989. Cf. Table 4.

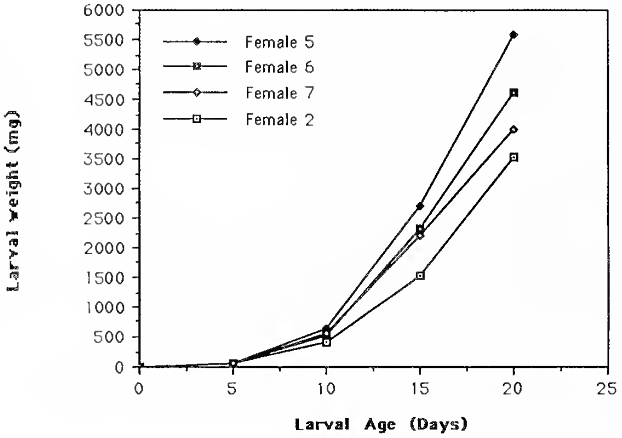

Figure 7. Fresh weight (mg) of oak-fed offspring from four female *E. imperialis*, 1989.

exists little trade-off in relative efficiency from oak to pine. That is, a larva well-equipped to utilize pine relative to another larva may be just as relatively well equipped to utilize oak. This explanation is consistent with the fact that no statistically significant interaction exists between the parental effect and the food plant effect. Significant differences appeared among mean weights of larvae from different clutches at each weighing and among mean weights of hatchling larvae, but these relative differences did not persist as such at subsequent weighings. Since there were no significant differences between mean weights of hatchling larvae given different food-plant treatments, these results can not be attributed to bias in the initial separation of larvae into treatment cohorts.

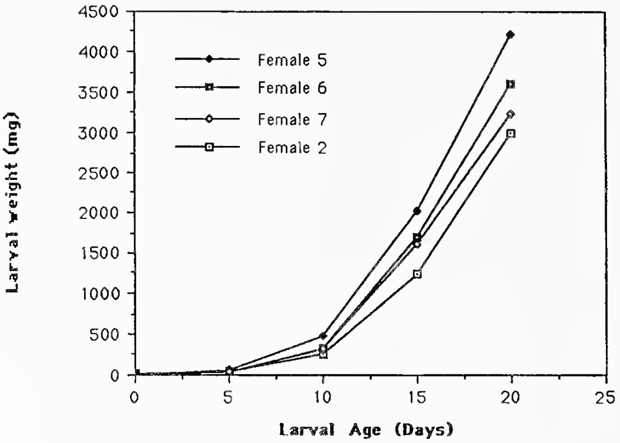

Figure 8. Fresh weight (mg) of pine-fed offspring of four females of *E. imperialis*, 1989.

A comparison of the numbers of larvae that had achieved a given instar by a given age revealed the following: significantly more of the oak-fed larvae than the pine-fed larvae molted to second instar by Day 5 (DF=1, G=12.996), third instar by Day 10 (G=8.502), fourth instar by Day 15 (G=6.162), and fifth instar by Day 20 (G=6.688). Additionally, mean male and female pupal weights were significantly higher for oak-fed larvae (4751.297 mg and 5705.4 mg, respectively) than for pine-fed larvae (3119.304 mg and 4473.514 mg, respectively).

In both years the number of larvae exhibiting the green color morph was higher for the pine-fed cohort than the oak-fed cohort. Exactly 50% (52 of 104) of all the 1989 larvae reared on pine exhibited green coloration; most of these turned green at the second molt (beginning of the third instar), and seven reverted to brown at subsequent molts, five at the third and two at the fourth. Of the 52 green larvae from both 1989 food plant treatments, only four had been reared on oak. As many as 13 of 15 and as few as 5 of 15 offspring of a given female fed pitch pine were green. Almost all larvae reared on oak exhibited a lighter brown or reddish body color.

Nutritional indices

Nutritional indices, calculated exclusively during the third instar, corroborated enhanced growth rate and efficiency on oak relative to pine, as did the higher pupal weights among oak-fed versus pine-fed larvae (Table 7). The efficiency of conversion of ingested food (ECD), the efficiency of conversion of digested food (ECI), and the relative growth rate (RGR) were positively correlated with foliar nitrogen and water content across food plants; the approximate digestibility (AD) and the relative consumption rate (RCR) were not. The only significant differences, however, were among the ECI and percent nitrogen values. The foliage of wild grown post oak and arboretum grown pitch pine contained significantly more nitrogen and water than corresponding amounts of wild pitch pine and arboretum-grown jack pine, and these numbers were paralleled by larval ECIs. (Table 7).

Discussion

The life history constraints of host specialization have been an important focus within the study of herbivore evolution. It has been hypothesized that the limits imposed by a contraction in the spectrum of potential host species are offset by an enhanced efficiency with which an herbivore utilizes that narrower host range (Brues, 1924; House, 1962; Emlen, 1973; Gilbert, 1979). The validity of this "feeding specialization hypothesis" is central to our understanding of evolved herbivory (Slansky & Scriber, 1985). Not only have consistent patterns of higher utilization efficiencies among monophagous versus polyphagous or oligophagous herbivores failed to be demonstrated, but Scriber and Feeny (1979) have contended that host plant chemistry is responsible for most of the variation seen in larval performance. That is to say the "costs" of specialization have not been well-defined or demonstrated in a broad sense because the axes along which organisms specialize may or may not intersect. It has become increasingly clear that in order to effect proper experimental and analytical procedures to test the feeding specialization hypothesis as a general paradigm, one must recognize a range of organism-specific variables, from elements in plant foliage that affect herbivore development to life history manifestations of constraints imposed by the host plant. These variables defy simple patterns, being too numerous and interdependent for their roles to be parsed except very broadly (Gaston & Reavey, 1989). Scriber (1983) suggested that one reason for our relative lack of understanding derives from the paucity of studies narrowly focused on groups of taxonomically and ecologically similar organisms. To these I would add studies of ecologically similar but phylogenetically independent and phylogenetically well understood groups.

Notwithstanding the predictable effects of nutrient rich foliage among arboretum-grown plants relative to wild foliage, the growth rates and efficiencies of consumption and digestion of oak versus the wild pine host suggest that, nutritionally, New England imperial moth caterpillars do not require pitch pine alone in order to survive. At the same time, *E. imperialis* may be adapted or pre-adapted physiologically to conifers. Conifer feeding on the part of herbivorous insects represents a nutritional dynamic different from deciduous leaf feeding. Pitch pine, in particular, is a complicated fire-adapted plant, and perhaps a more relevant comparison than that between larval performance on arboretum-grown, well-fertilized plants and performance on native hosts of disjunct moth populations might be undertaken between geographically disparate populations on the wild northern conifer hosts and among different age cohorts of pitch pine foliage growth in viable habitats of *E. imperialis*. That said, differential patterns in nutritional content between evergreen and deciduous trees have been demonstrated (Miller & Stoner, 1979), with evergreen foliage having generally lower nutrient contents. Pines and other conifers typically contain less foliar nitrogen than deciduous angiosperms under similar conditions of growth and development (Bidwell & Durzon, 1975; Slansky & Scriber, 1985), and wild pitch pine from barrens and typically nutrient-poor (Forman, 1979; Schweitzer & Rawinski, 1988). Folivores of nutrient-poor, woody plants tend to show greater breadth of dietary tolerance (i.e. be more polyphagous) than those on highly nutritious foliage (Mattson & Scriber, 1987). Mattson and Scriber (1987) cite Holloway and Hebert (1979) who found that conifer-feeding Lepidoptera "are less specific in host plant choice than species feeding on angiosperms." The data presented in this study are consistent with this claim in that the larvae of *E. imperialis* are capable of sustained development on different hosts. Both the published host records of *E. imperialis* and the results of this study support the contention that this species, including populations functionally restricted to pine, can metabolize a broad range of potential if not realized host plants.

Large body size is also considered an advantage when feeding on low-nutrient diets (Wasserman & Mitter, 1978; Peters, 1983; Mattson & Scriber, 1987), such as pines or late-season tree foliage. In fact, tree-feeding lepidopteran species active late in the growing season tend to be large (Mattson, 1980; Niemela *et al.*, 1981), as would be expected especially for those in which the adults do not feed (Slansky & Scriber, 1985) such as *E. imperialis*. There may also exist such a trend for folivores of evergreen versus deciduous plants. Opler (1978) noted that leafminers feeding on evergreen oak species tended to be larger than those on deciduous species. Although phylogenetic data were not yet available to evaluate the evolution of size in a cladistic framework, numerous authors have observed phylogenetically biased patterns in lepidopteran size associated with host plant use and life history (Mattson, 1977; Wasserman & Mitter, 1978; Niemela *et al.*, 1981; Hayes, 1983; Gaston & Reavey, 1989), and although adaptive speculation is frivolous, it is worth noting that *E. imperialis*, the most massive saturniid extant in New England, is also the latest feeding saturniid in the region, active as larvae as late as October. By this time many of the host plants utilized further south, where the flight season of *E. imperialis* is more protracted, are senescent or nearly so northward, potentially accounting in part for the more strict association with conifers northward.

In contrast to pitch pine, wild grown post oak contained significantly higher amounts of nitrogen than even arboretum grown pitch pine, even though ECI's were not significantly different for larvae fed the two food plants. This implies either more efficient nitrogen utilization of pitch pine versus post oak on the part of *Eacles* or simply that oak contains more nitrogen than *Eacles* larvae can effectively metabolize. Despite the fact that the only two plant cohorts for which nitrogen content was not significantly different were the two arboretum-grown pines, the mean ECI was significantly higher for larvae on arboretum-grown pitch pine than for those on jack pine; both were significantly higher than for wild pitch pine. The mean ECI for arboretum-grown pitch pine was comparable to (i.e. not significantly different from) that of Martha's Vineyard post oak, on which larvae had the highest ECIs, and which supported a significantly higher nitrogen content (in fact the highest of all food plants measured). Taken collectively, these results suggest a potential physiological adaptation to pine-feeding in general, and pitch feeding specifically on the part of northern *E. imperialis*.

The results presented here go to show that simply because a particular food plant species meets an herbivorous organism's nutritional requirements and the organism is physiologically capable of growth and development on that food is not an indication that it is an actual, realized host in nature. There might be any of a number of possible explanations for the fact that Massachusetts *E. imperialis* larvae appear to grow faster and more efficiently on a non-utilized host (*Q. stellata*) than on the native host (*P. rigida*). Chemical oviposition cues such as terpenes specific to conifers, selective predation of larvae on one host versus another, abiotic habitat requirements of soil pupation, and even simple availability may all play a role in the restriction of northern *E. imperialis* to conifers. For example, I observed late instars of wild-reared larvae placed on *Q. stellata* undergo heavy predation by vespid wasps (*Vespa vulgaris*; pers. obs.) relative to those reared *in situ* on *P. rigida*. The frass of oak-feeding larvae is less dry and more prone to mold than that of pine-feeding larvae, and may serve to attract predators.

The restriction of *Eacles imperialis* to the common pitch pine on Martha's Vineyard is of interest from the standpoint of conservation as well as evolutionary ecology. *Eacles imperialis* is one of 24 regionally threatened moth species occurring on Martha's Vineyard protected under the Massachusetts Endangered Species Act (MESA; M.G.L. c. 131A and regulations 321 CMR 10.00), not including at least one additional species, *Datana contracta* (Notodontidae) that appears to have been impacted severely on mainland southern New England and may be locally extirpated. As thorough an understanding as possible of why species such as these have declined—and what they require to persist—is a mission-critical prerequisite to any reintroduction and restoration effort. As conservationists consider potential sites at which to reintroduce and restore this species, we must weigh a variety of considerations, among them suitability of habitat, availability of host plant, probability of success, verifiability of historical occurrence, and legal logistics.

Biologically, the most obvious candidate sites, those showing the greatest promise for success, are barrens habitats on Cape Cod and in Plymouth County and on Nantucket Island. Ironically, historical records of *E. imperialis* from Cape Cod are lacking, and the conspicuous absence of this moth and its near relatives from Nantucket has long been noted: Jones and Kimball (1943) made the observation that although four species of ceratocampine saturniids occur on Martha's Vineyard, none were known at the time of that writing from Nantucket. Jones and Kimball speculated that such heavy bodied moths found it difficult to distribute across water barriers. As was the case during Jones and Kimball's time, four species of Ceratocampinae (*E. imperialis*, *Anisota senatoria*, *A. stigma*, and *A. virginiensis*) persist in numbers on Martha's Vineyard. *Anisota stigma*, at one point listed under the Massachusetts Endangered Species Act, occurs less ubiquitously on mainland New England than on Martha's Vineyard (Mello *et al.*, 1999). However, this species has apparently colonized Nantucket (K. Coombs-Beattie, pers. comm.; Goldstein, 1997), where it now occurs commonly, possibly obviating the argument that all ceratocampines have difficulty crossing water barriers.

Although Jones and Kimball did not discuss the historical ecology or land use history *per se* of either island, the possible role of habitat destruction and fragmentation of barrens habitats must be considered.

There can be little debate that viable habitat persists at mainland barrens sites, including the extensive maritime barrens in Plymouth County at Myles Standish State Forest (approx. 16,000 acres) and at the Massachusetts Military Reservation (roughly 15,000 acres), as well the 2,000 acre inland barrens at Montague Plain, Franklin Co., MA and on Nantucket Island. Jones and Kimball's (1943) observation that ceratocampines were absent from Nantucket during the20th century of course begs the question of whether they were ever there. The land use history of Nantucket, like that of Martha's Vineyard, involved significant alteration and conversion of forested and shrubland habitats for the purposes of agriculture (Dunwiddie, 1992). Although both Martha's Vineyard and Nantucket were part of an extensive coastal plain as recently as 10,000 years ago, Nantucket was almost completely denuded of forest during the Revolutionary War, which no doubt had an impact on the lepidopteran fauna. It may be observed that, in addition to the ceratocampines, other groups of forest tree Lepidoptera are depauperate on that island relative to Martha's Vineyard. There is a marked contrast, for example, between the islandic faunas of Limacodidae: whereas nine species of limacodids (*Euclea delphinii*, *Isa textula*, *Phobetron pitchecium*, *Prolimacodes badia*, *Apoda biguttata*, *Lithacodes fasciola*, *Packardia elegans*, *P. geminata*, *Torticidia flexuosa*) occur regularly on Martha's Vineyard, only two (*E. delphinii* and *L. fasciola*) were recorded on Nantucket by Jones and Kimball (1943: 123-125). Jones and Kimball's data also suggest a comparative dearth of leaf litter feeding deltoid noctuids on Nantucket relative to Martha's Vineyard, which would be expected following systemic deforestation.

Pitch pine, however, is now an extremely common plant on Nantucket and, ironically, it is conceivable that the introduced tachinid *C. concinnata*, to be verified from the island of Martha's Vineyard or Nantucket, may prevent the re-establishment of *E. imperialis* on mainland New England. It has yet to be determined whether or not the introduced parasitoid *C. concinnata* poses a barrier to recolonizing the mainland, whether or not the fly's absence on Martha's Vineyard is, if not an artifact of under-sampling, a reason for *Eacles*' persistence there.

ACKNOWLEDGEMENTS

Much of this work was conducted as an undergraduate thesis under the mentorship of M. Deane Bowers and James M. Carpenter. All errors, omissions and other such gaffes remain the author's sole responsibility. Many then at the Museum of Comparative Zoology and the Biological Laboratories at Harvard University are to be thanked for their help, guidance, and friendship: Eric Fajer, Ed Armstrong, Charlie Vogt, Scott Shaw, David Furth, Mark Skinner, Kathy Brown-Wing, and Peter Frumhoff. Equally important were my fellow naturalists in southeastern Massachusetts who kindly contributed field work and observations and, through the various conservation organizations for which they worked, granted permission to conduct that work on a variety of protected areas. Of particular help were Tim Simmons (Sheriff's Meadow Foundation), Gus Ben David (Massachusetts Audubon Society), and Tom Chase (The Trustees of Reservations). Mary Lesniak kindly enabled access to the collection of the Boston Society of Natural History, currently housed at Boston University. Jeff Boettner (University of Massachusetts) and Tim Simmons (Massachusetts Natural Heritage & Endangered Species Program) provided many valuable insights and clarifications. I thank Mo Nielsen (Michigan State University), Paul Opler (Colorado State University), Ray Pupedis (Peabody Museum of Natural History, Yale Univesity), and Brian Scholtens (College of Charleston) for relevant observations and collection records from their respective institutions. Rodger Gwiazdowski (University of Massachusetts, Amherst) provided real-time checking of *Eacles* data labels at UMass. I thank Sean Bober for assisting with the assemblage of figures and Mike Nelson (Massachusetts Natural Heritage & Endangered Species Program) for invaluable eleventh hour help with maps.

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Journal of Research on the Lepidoptera 42: 50-55, 2003 (2010)

Association of three species of *Strymon* Hübner (Lycaenidae: Theclinae: Eumaeini) with bromeliads in southern Brazil

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Abstract. As part of a project studying the species richness of bromeliad flower visitors and the diversity and nature of their animal-plant interactions, three species of the lycaenid butterfly genus *Strymon* were recorded as pests of bromeliad inflorescences. *Strymon ziba* fed on the fruits of *Aechmea nudicaulis*, *S. oreala* on those of *Ae. lindenii* and *Ae. caudata* and *S. serapio* on the dry capsules of *Vriesea friburgensis*. The caterpillars of *S. ziba* and *S. oreala*, pests of cultivated pineapple, were facultatively associated with ants. One *S. ziba* pupa was parasitized by a chalcidid wasp. One *S. oreala* pupa was parasitized by an ichneumonid wasp. Behaviour and life history data of the caterpillars are described and aspects of the host specificity of the lycaenids and potential pest control by parasitoid wasps are discussed.

Key words: *Aechmea*, animal-plant interactions, Atlantic rain forest, Bromeliaceae, Chalcididae, herbivory, Ichneumonidae, parasitism, restinga, *Vriesea*.

Introduction

Bromeliaceae, a neotropical plant family, can be considered keystone species by providing microcosms for the richness of tropical rain forests due to the high diversity of animal taxa, especially arthropods, associated with them (Frank & Lounibos, 2008). Among the latter, Lepidoptera are major herbivores with many caterpillars feeding upon bromeliad foliage: *Napaea eucharilla* Bates (Riodinidae) on *Werauhia sanguinolenta* (Cogniaux & Marchal) J. R. Grant (syn. *Vriesea sanguinolenta*, Schmidt & Zotz 2000), *Aechmea bracteata* Grisebach, *Ae. nudicaulis* (L.) Grisebach (Beutelspacher, 1972) and *Ananas comosus* (L.) Merrill (Schmidt & Zotz, 2000), *Caria domitianus ino* Fabricius (1793) (Riodinidae) on *Tillandsia caput-medusae* E. Morren (Beutelspacher, 1972; Frank & Lounibos, 2008), *Dynastor darius darius* Stichel and *D. macrosiris* Westwood (Nymphalidae, Urich & Emmel, 1991a, b) on *Ae. nudicaulis* and *Castnia boisduvalii* Walker (Castniidae, Biezanko, 1961; Frank & Lounibos, 2008) on *T. aeranthos* (Loiseleur) L. B. Smith. Beutelspacher (1972) also mentioned *Thecla hesperitis* Butler & Druce 1872 (Lycaenidae), which is a mistaken record of *Ziegleria hesperitis*, feeding on *Tillandsia caput-medusae*, but voucher specimens of "*Thecla hesperitis*" in UNAM (Universidad Nacional Autónoma de Mexico) examined by Robert K. Robbins are in fact *S. serapio* Godman & Salvin (1887) (R. K. Robbins, pers. comm.). Not only the plants' vegetative parts, but also their inflorescences contribute significantly to the local faunal biodiversity by providing resources for a great variety of flower visitors that act as pollinators or pollen and nectar robbers (Sazima & Sazima, 1999; Machado & Semir, 2006; Canela & Sazima, 2003; Schmid *et al.*, b, submitted). In addition to causing leaf damage, some herbivorous arthropods associated with bromeliad inflorescences directly interfere with plant reproduction by feeding on reproductive tissues of flowers and fruits, like beetles, butterflies and moths, grasshoppers and even crabs (Fischer *et al.*, 1997; Canela & Sazima, 2003; Frank & Lounibos, 2008). An example of inconspicuous herbivory affecting plant reproductive success was observed in the bromeliads *Vriesea friburgensis* Mez and *Werauhia gladioliflora* (H. Wendland) J. R. Grant whose buds are parasitized by *Eurytoma* wasps (Hymenoptera, Eurytomidae) so no fruits are formed (Gates & Cascante-Marín, 2004; Grohme *et al.*, 2007). Beyond that, cases of seed predation by *Cholus* and *Metamasius* weevils (Coleoptera, Curculionidae) (Frank 1999) and *Epimorius testaceellus* Ragonot 1887 (Pyralidae) larvae that develop in flower pods of *Tillandsia fasciculata* Swartz (1788) (Bugbee, 1975; Heppner, 1992) have been reported. New World hairstreaks (genus *Strymon*, Lycaenidae: Theclinae: Eumaeini) use ornamental bromeliads (genera *Aechmea*, *Tillandsia*) and the commercial pineapple (*Ananas comosus*) as host plants

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Received: 25 September 2009
Accepted: 15 October 2009

(Robbins & Nicolay, 2002). *Strymon megarus* Godart 1824 (syn. *Thecla basilides,* also misspelled as *T. basalides*) larvae feed on *Ananas* and other bromeliads like *Ae. bracteata* (Beutelspacher, 1972; Frank & Lounibos, 2008) and can be considered pest species. *Strymon ziba, S. serapio* (Robbins & Nicolay, 2002) and *S. oreala* (Zikán, 1956), the species examined in our study, were reported to eat bromeliads, *S. ziba* Hewitson 1868 and *S. oreala* Hewitson 1868 are known pests of *A. comosus* (Harris, 1927; Zikán, 1956). Since caterpillars and other immature stages of the Eumaeini are small and cryptically coloured, food plants have been recorded for only 25% of the species (Duarte *et al.*, 2005).

Studying the species richness of bromeliad flower visitors and the diversity and nature of their animal-plant interactions in the Atlantic rain forest of southern Brazil, we found lycaenid caterpillars attacking developing fruits. In order to assess the specificity of these associations we examined inflorescences of four common sympatric bromeliad species, *Aechmea nudicaulis, Ae. lindenii* (E. Morren) Baker, *Ae. caudata* Lindman 1891 (Bromelioideae) and *Vriesea friburgensis* (Tillandsioideae), for the presence of larvae and reared them for identification. We also recorded basic data on development, behaviour and natural enemies of the caterpillars.

MATERIALS AND METHODS

Bromeliads with inflorescences were searched for eggs and larvae between November 2006 and June 2008 at four study sites (frost-free subtropical habitats): Santo Antônio de Lisboa and the Environmental Conservation Unit Desterro UCAD (both secondary forest; 27°30'26" S, 48°30'28" W; 27°31'50" S, 48°30'50" W) (Zillikens *et al.*, 2001; Zillikens & Steiner, 2004) as well as Joaquina Beach and Campeche Beach (dune vegetation, Sampaio *et al.*, 2002; 27°40'38" S, 48°28'48" W; 27°37'37" S, 48°26'59" W), on Santa Catarina Island, southern Brazil. All bromeliads examined were growing terrestrially although *Aechmea nudicaulis* also occurs on trees. In total, 20 infested bromeliads of four species (*Aechmea nudicaulis,* n = 11; *Ae. lindenii,* n = 2; *Ae. caudata,* n = 5; and *Vriesea friburgensis,* n = 2), growing terrestrially on rocks, in sand or shallow soil, were taken to the laboratory. Presence, size and colour of eggs and caterpillars on the inflorescences were observed regularly every 1-2 days. Ants associated with lycaenid larvae were also collected. When larvae had finished feeding and retreated for pupation, the bromeliad plants were enclosed with fine gauze to capture the emerging adult butterflies.

Voucher specimens of the recorded butterfly, ant and parasitoid species were deposited in the entomological collection of J. Steiner at the Native Bee Laboratory (LANUFSC), BEG, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil.

RESULTS

Three lycaenid species of the genus *Strymon* were reared from the caterpillars found on four bromeliad species. All constitute new records of parasite/host association. Up to four caterpillars were found simultaneously on one inflorescence.

Strymon ziba (Hewitson 1868)

We found 24 larvae of *S. ziba* on 11 inflorescences of the bromeliad *Aechmea nudicaulis,* yielding an average of 2.2 caterpillars per inflorescence (range 1-4) from November 2006 to January 2007. Seventeen adults emerged in the laboratory, overall sex ratio was 0.7 (M/F). On all inflorescences we detected small white spherical bodies, the eggs from which the larvae had hatched (Fig. 1A, B).

The colour of the larvae was cryptic and changed during their growth from whitish-yellow to reddish-pink (Fig. 1C, D). The former matched well to the fruits whereas the latter matched well to the inflorescence stem. The larvae appeared shortly after the end of the flowering period and stayed close to the ripening fruits. Larval feeding behaviour consisted of gnawing a hole into the fruit base large enough for the smaller larval stages to enter the fruit completely and for the larger stages to insert the head and anterior part into the cavity. Through this hole they fed on the soft nutritive tissues of the ovary and ovules, leaving the rigid cortical outer wall of the developing fruit mostly intact (Fig. 1A, B). On an inflorescence of *Ae. nudicaulis* with two larvae feeding, 30 fruits were damaged, resulting in a mean of 15 fruits damaged per larva. Mean fruit loss per inflorescence was 84.5% (n = 2). Occasionally, the larvae drew back from the fruits to hide under the bracts for about half a day, probably for moulting.

Development in the egg took five days (n = 2). The larval phase lasted 13-15 days (n = 1). The fully grown larvae (≈12-15 mm length) moved into the bromeliad rosette where they pupated on the upper side of the leaves half way between tip and base; one male pupated on a bract of the inflorescence. Pupation took 8-11 days (n = 3). Imagines (Fig. 1E) emerged between mid November until end of January, synchronized to the flowering/fruiting period of *Ae. nudicaulis.*

In the laboratory, caterpillars were occasionally

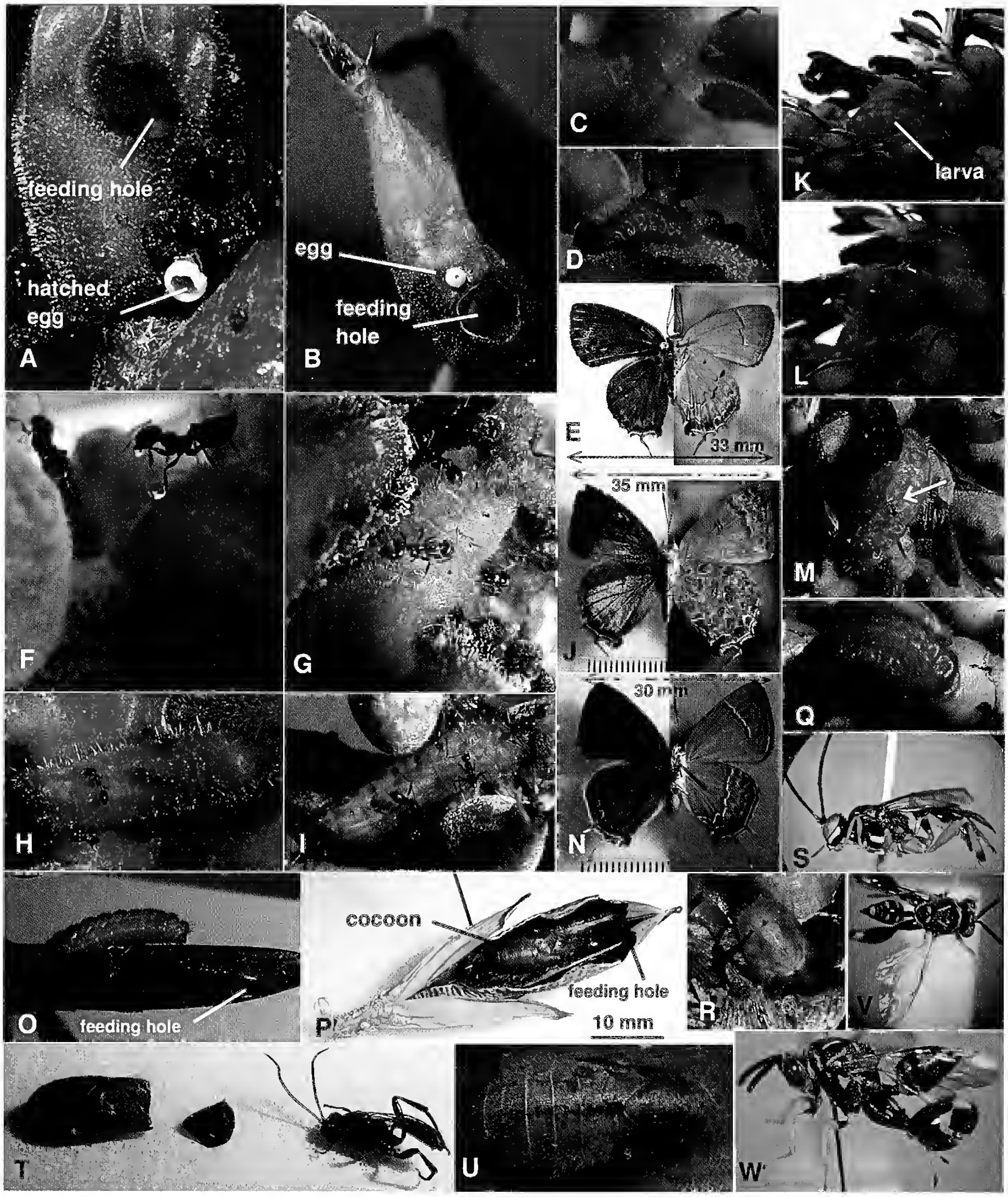

Figure 1. *Strymon* larvae on bromeliads on Santa Catarina Island, Southern Brazil.

A-P: *Strymon* larvae and imagines and associated ants

A-B: Infested fruits of *Aechmea nudicaulis*, Santa Catarina Island, Brazil. **A**: Fruit with feeding hole and larval faeces of *Strymon* caterpillar and a hatched egg of *Strymon ziba* at the base of the fruit. **B**: Fruit with feeding hole and a closed egg. **C-D**: Colour change in *Strymon ziba* larvae. **C**: Small, whitish-yellow. **D**: Larger larva after colour change to reddish-pink. **E**: *Strymon ziba* female, collected on *Aechmea nudicaulis,* Santa Catarina Island, Brazil. Right-hand side ventral view, left-hand side dorsal view. **F-I**: Ants associated with *Strymon ziba* caterpillars. **F**: *Crematogaster limata*. **G**: *Linepithema iniquum*. **H**: *Monomorium* sp. (*floricola*). **I**: *Paratrechina* sp. **J**: *Strymon oreala*, female, right-hand side ventral view, left-hand side dorsal view. **K**: *Strymon oreala* larva feeding on fruits of *Aechmea caudata*. **L**: Larva of *S. oreala* feeding on withered flower petals. **M**: *Tapinoma melanocephalum* ant on the back of a *S. oreala* larva (white arrow). **N**: *Strymon serapio*, female, right-hand side ventral view, left-hand side dorsal view. **O**: Larva of *S. serapio* on dry fruit of *Vriesea friburgensis* with feeding hole. **P**: Dry fruit of *Vriesea friburgensis* with exuvia of *S. serapio* inside. Length of exuvia: ≈ 12 mm.

Q-W: *Strymon* larvae at *A. lindenii* and parasitoids.

Q-R: *Strymon* larvae. **Q**: *Strymon* larva on *A. lindenii* feeding on fruit. **R**: *Strymon* pupa on infructescence.

S-T: *Anisobas*, a parasitoid of *Strymon* sp. **S**: Lateral view of the *Anisobas* imago that hatched from the *Strymon* exuvia. **T**: Opened *Strymon* exuvia besides hatched *Anisobas* imago. **U-W**: *Conura*, a parasitoid of *Strymon* sp. **U**: Opened pupal case of *Strymon* sp. **V**: Imago of *Conura* sp., dorsal view. **W**: Lateral view.

tended by ants of four species: *Crematogaster limata* Smith 1858, *Linepithema iniquum* Mayr 1870, *Monomorium floricola* Jerdon 1851 and *Paratrechina* sp. (Fig. 1F-I). Tending worker ants walked over the bodies of the caterpillars and took up small droplets secreted posterodorsally (Fig. 1F).

Additionally, we observed *S. ziba* imagines sucking extrafloral and floral nectar from inflorescences of *Ae. nudicaulis* and one female laying one single egg on each of two recently withered flowers, respectively. The female flew around the inflorescence and sucked nectar of several flowers before ovipositing. The initially greenish eggs turned white after a few minutes. Thereafter, the female left the inflorescence.

Strymon oreala (Hewitson 1868)

We discovered seven larvae of *S. oreala* (Fig. 1J) on five inflorescences of the bromeliad *Ae. caudata* (April 2008) and two larvae on two inflorescences of *Ae. lindenii* (August – September 2007). As described for *S. ziba* on *Ae. nudicaulis* larvae fed on the developing fruits of *Ae. lindenii* and *Ae. caudata* (Fig. 1K-M) and retreated into the rosette for pupation. Additionally, a larva was seen feeding on withered flower leaves, probably eating old reproductive structures inside the petals (Fig. 1L). On an inflorescence of *Ae. caudata* with one larva feeding, 15 fruits were damaged. The pupal stage took 15-16 days (n = 4). The larvae were reddish-pink like the inflorescence stem (Fig. 1 K-M). Occasionally, single ants of the species *Tapinoma melanocephalum* Fabricius 1793 were observed on *S. oreala* larvae (Fig. 1M).

Strymon serapio (Godman & Salvin 1887)

We detected four brownish-yellow larvae of *S. serapio* (Fig. 1N) on two inflorescences of the bromeliad *Vriesea friburgensis* in secondary forest in December 2007. The larvae chewed a hole into the hard capsule of the developing fruit (Fig. 1O) and fed on the seeds within. Pupation took place inside the empty fruit capsule (Fig. 1P), the imago emerged after 11 days (n = 1).

Parasitoids

On two occasions we found *Strymon* brood infested with a parasitoid. The first case (14 November 2005, Campeche Beach) was a larva on an inflorescence of *Ae. lindenii* (Fig. 1Q). Since the only identified *Strymon* infestation of this bromeliad was by *S. oreala* (see above) we assume that the parasitized larva belonged to the same species. The larva pupated on the infructescence on 18th November 2005 (Fig. 1R). This might, however, not be the usual location for pupating because Aurum® insect glue had been applied to the infructescence stem, thus preventing the caterpillar from moving down to the rosette. After 18 days an ichneumonid wasp of the genus *Anisobas* (subfamily Ichneumoninae) emerged from the pupa (Fig. 1S, T).

In the second case, discovered 6 January 2007 at Santo Antônio, a pupa (Fig. 1U) was located at the upper margin of a leaf of *Ae. nudicaulis* (plant with infructescence). So far, we found this bromeliad species only to be infested with *S. ziba* (see above); hence we assume that the pupa belonged to this species. On 23 January 2007, a chalcidid wasp of the genus *Conura* (subfamily Chalcidinae), "most probably of the *flava* group" (Gérard Delvare, pers. comm.), emerged from the pupa (Fig. 1V, W).

Discussion

Larval behaviour and host plants

Our findings constitute new host records for the associated *Strymon* species. The only lycaenids so far recorded on *Ae. lindenii* are larvae of an unidentified species of *Thecla* on Santa Catarina Island (Lenzi *et al.* 2006) with a similar feeding behaviour and life history data as described here for *S. oreala*. It is therefore possible that they did in fact observe larvae of a species of *Strymon*. Our observations also confirm some life history traits reported by Duarte *et al.* (2005) such as the cryptic coloration of the larvae which is well adapted to parts of the plants on which they move or feed. Besides the evident association of *Strymon* larvae with infructescences, they were even more selective in the sense that they fed only on the internal parts of the developing fruits, i.e. the ovaries, but not on leaves, sepals or other plant tissues. By hollowing out the fruits they create their own shelter for feeding or even pupal chambers (in case of *S. serapio*).

We further report here the first data on the life cycle of the three species, all of which develop within about one month and without diapause. It is therefore likely that the *Strymon* species studied by us are multivoltine having several generations per year. This is in agreement with the pattern reported for other *Strymon* species in the tropics (Opler *et al.* 2009). The choice of hosts by ovipositing females depends on seasonal availability of fruiting plants. The bromeliads studied by us all have relatively short and seasonal fruiting periods, so that only one or two generations can develop on a given plant population.

Adults emerging at the end of a flowering period have to seek for alternative hosts for egg laying. In our study, *Strymon oreala* was the only species recorded on two hosts both in the genus *Aechmea.* Interestingly, the inflorescences and flowers of *Ae. lindenii* and *Ae. caudata* are very similar in floral morphology and coloration (Kamke, pers. obs.), but *Aechmea lindenii* flowers from August to November (Dorneles *et al.*, ms) whereas *Ae. caudata* flowers from March to June without overlap, though single plants of both *Aechmea* species can be found flowering outside the main flowering period, for example *Ae. lindenii* on more open areas in restinga sites throughout the year (Lenzi *et al.* 2006) and *Ae. caudata* in secondary forest in September (Kamke, pers. obs.). Nevertheless, there is a gap of several months for which we do not yet know the host plants. We know, however, that *S. oreala* does not attack the infructescences of *Ae. nudicaulis* or *V. friburgensis*, which flower between. Therefore, a switch to another host, whether bromeliad or not, must occur in *S. oreala*, *S. serapio* and *S. ziba.*

As *S. ziba* and *S. oreala* are pests of cultivated pineapple it would be interesting to further identify alternative host plant species in order to better understand under which circumstances the larvae reach pest status and to assess their damage to the crop. In this context it is also worth emphasizing our record of a possible natural enemy of *S. ziba*, a parasitoid wasp of the family Chalcididae. Its potential as natural biological control agent should be assessed by elucidating its life history, abundance and host specificity.

Association with ants

As has been reported from other lycaenid caterpillars, the larvae of *S. ziba* possess a dorsal secretory organ, the Newcomer's gland (Malicky 1970), that might exude honey-like droplets to appease ants. Of the species recorded, *Monomorium floricola* and *Tapinoma melanocephalum* are invasive ants (Delabie *et al.* 1995; Campos-Farinha 2005) and only occurred in the laboratory. We consider the same to be true for *Paratrechina* sp. for this species was only observed in the laboratory. *Crematogaster limata* and *Linepithema iniquum*, however, were frequently found nesting in the bromeliads or visiting their inflorescences (Rosumek *et al.* 2008, Schmid *et al.* a, ms) and were thus brought to the laboratory together with the plants taken in the field. These two, at least, may be considered associated with the *Strymon* caterpillars under natural conditions, albeit only facultatively since larvae observed in the field were mostly not tended by ants.

Diversity of the *Strymon* – bromeliad association

Our finding that four species of bromeliads were parasitized in very similar ways by *Strymon* larvae is remarkable in yet another aspect. It confirms a relatively high diversity and abundance of sympatric, even syntopic, *Strymon* species in bromeliad-rich Atlantic forest and restinga habitats of southern Brazil. Thus, the fact that three co-occurring species were recorded in studies on only four bromeliad species suggests that a thorough examination of further bromeliad inflorescences might result in the finding of more *Strymon* species and underlines the importance of these plants for sustaining a high diversity of the lepidopteran fauna in the Mata Atlântica.

ACKNOWLEDGEMENTS

We thank Robert K. Robbins for lycaenid identification and valuable help with *Strymon* taxonomy and literature, as well as David Wahl and Gérard Delvare for parasitoid identification. This study is part of the Project "Internal dynamics of rain forest: specificity of animal-plant interaction" within the Brazilian-German program "Mata Atlântica", and we acknowledge the financial support by BMBF (01LB0205A1) and CNPq (590040/2006-5).

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Journal of Research on the Lepidoptera 42: 56-63, 2003 (2010)

Temporal and spatial segregation of *Battus devilliers* and *B. polydamas cubensis* (Papilionidae) in La Habana, Cuba

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Abstract. The spatial and temporal distribution of two syntopic species of the genus *Battus* (Papilionidae) that inhabit two areas in the north coast of La Habana, Cuba, is analyzed. The study was carried out from April 2006 to March 2007. Samples were taken using transects 100 m long, separated from each other by 80 m gaps. Populations of *B. polydamas cubensis* and *B. devilliers* of the evergreen forest at Boca de Canasí, the most natural habitat, had similar abundance, while in the secondary scrub at Boca de Jaruco, an extremely degraded area, *B. polydamas cubensis* was much more abundant than *B. devilliers.* Analysis of structural subniche usage by season showed significant differences in daily activity (from 9:00 am to 12:00 m and from 12:00 m to 3:00 pm) and habitat type for each species. Flight stratum had lesser influence than sunlight intensity on both butterflies. Populations of the latter seemed to be influenced by seasonality, while those of *B. polydamas cubensis* seemed more influenced by habitat.

Key words: *Battus*, Cuba, Papilionidae, segregation, syntopic species.

INTRODUCTION

Ecological roles of butterflies are important for ecosystems functioning, with their study needed to better understand their ecological interactions and functions. In Cuba, only Fontenla (1989) and Genaro *et al.* (1994) have conducted studies focusing on butterflies communities and their resource partitioning in a particular habitat. Due to their rapid reproduction and close association with specific physical factors and plant resources butterflies are highly sensitive to environmental changes, so they are good indicators of ecosystem health (Brown, 1991; Kremen, 1992; New *et al.*, 1995; New, 1997; Brown & Freitas, 2000). *Battus* presents a Neotropical distribution (Sims & Shapiro, 1983) with 12 or 14 species (Tyler *et al.*, 1994; Racheli & Pariset, 1992, respectively). The coevolutionary association of *Battus* with their host plant, *Aristolochia* spp. (Aristolochiaceae), as well as the mimetic interactions among members of this butterfly genus may be the subject of many studies on butterfly community structure (Young, 1972, 1973).

Battus devilliers (Godart, 1823) and *B. polydamas cubensis* (Dufrane, 1946) are the only members of the genus that inhabit Cuba. The former occurs in Bahamas (Knowles & Smith, 1995) and the latter was reported at Cayman Islands in 1938 (Carpenter & Lewis, 1943), but there has been no recent mention of further records for these islands (Askew, 1980, 1988; Schwartz *et al.*, 1987). On several occasions (Alayón & Solana, 1989; Racheli & Pariset, 1992; Núñez & Barro, 2003) both species were recorded in Cuba as syntopic (*sensu* Rivas, 1964). The interaction becomes more interesting considering the different habitat requirements of *B. polydamas* and *B. devilliers* mentioned by Tyler *et al.* (1994), who established that *Battus polydamas* is very common in disturbed forest, while *B. devilliers* prefer more natural seasonal forest. Since resource use for both species in Cuba is completely unknown, we undertook a study of temporal and spatial patterns of two populations on the northwestern coast of Cuba.

MATERIALS AND METHODS

Study areas

The study was conducted at two localities of the north coast of La Habana. Both areas are close to human populations and show different levels of degradation. Boca de Jaruco is 45 km east of La Habana city, at 23°11' N, 82°01' W. The site is typified by secondary vegetation with many herbs and bushes and is the most impacted area, which is why the habitat is categorized

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Received: 21 May 2009
Accepted: 30 July 2009

Figure 1. Study Sites. **A**: Secondary scrub of Boca de Jaruco. **B**: Sea grape forest of Boca de Canasí. **C**: Evergreen forest of Boca de Canasí. **D**: Summit of the hill where the evergreen forest canopy of Boca de Canasí becomes open.

as secondary scrub (Fig. 1a). The unique emergent large trees are isolated *Ficus* sp. (Moraceae). There are many invasive plants, the most abundant being *Acacia farnesiana* (L.) Willd., (Mimosaceae), *Comocladia dentata* Jacq. (Anacardiaceae) and members of the family Poaceae. Common species also includes *Lantana camara* L. (Verbenaceae) and *Viguiera dentata* (Cav.) Spreng., (Asteraceae), and vines as *Merremia dissecta* (Jacq.) Hallierf., (Convolvulaceae), *Cucumis dipsaceus* Ehrenb., (Curcubitaceae) and *Aristolochia bilabiata* L. During the dry season there are natural burns.

Boca de Canasí is 20 km east from Boca de Jaruco, at 23°09' N and 81°47' W. Two different habitat were analyzed there: a sea grape [*Coccoloba uvifera* (L.) L. (Polygonaceae)] forest (Fig. 1b), and an evergreen forest (Fig. 1c, d). The latter is crossed by several paths, but is less frequented by persons than the former. It also has higher relative humidity due to the cover of arboreal species such as *Bursera simaruba* (L.) Sarg. (Burseraceae) and *Coccoloba diversifolia* Jacq. that reduce incident sunlight. Endemic plants like *Coccotrinax borhidiana* O. Muñiz (Arecaceae), *Leptocereus wrightii* León (Cactaceae) and *Eugenia mollifolia* Urb. (Myrtaceae) are also common (Borhidi, 1996). On the summit of the hill (ca. 100 m above sea level) the evergreen forest canopy opens and bushes like *Acacia farnesiana* and *Croton lucidus* L. (Euphorbiaceae) are abundant.

Ecological counts

Boca de Jaruco was sampled from April 2006 to March 2007. At Boca de Canasí sampling started July 2006 in the evergreen forest, while in the sea grape habitat counts began on April 2006, ending on March 2007 and January 2007, respectively. The transect method was followed. Individuals were counted only when seen from the sides or the front of observer, never from behind, within a range of approximately 5 m. At least once a month, eight transects were sampled each hour, from 9:00 a.m. to 3:00 p.m., at each studied site. Transect length was 100 m, the transects separated from each other by 80 m. At the end of the study a total of 992 transects in the secondary scrub of Boca de Jaruco were covered, 352 in the sea grape and 336 in the evergreen forest.

Sunlight intensity and flight stratum were recorded for each specimen. These dimensions of structural subniche were used to analyze part of the spatial segregation. Border values of each category in a particular dimension were determined following previous Cuban ecological studies on butterflies (Fontenlak, 1989; Genaro *et al.*, 1994).

For sunlight intensity three categories were defined: sunny (when individual flies directly in sunlight, without any vegetation cover), filtered sun (when individual flies under some vegetation cover, but still in sunlight) and shading (when individual flies where vegetation cover is so dense that it is difficult for sunlight to enter). Three categories of flight stratum from ground to above 3 m were defined: first level from the ground (0 - 1.5 m), second 1.5 - 3.0 m, and third > 3.0 m. When the same individual moved from one level to another only the first stratum seen was recorded. Due to variation of climate throughout the day, the two variables were analyzed separately over a two hours range, 9:00 am -12:00 m, and 12:00 m -3:00 pm.

Relative abundance was correlated with mean precipitation of the previous month. This correlation was due to both the influence of rain on vegetation and the time it takes for plant growth (rains increase flower abundance, for example). We used a correlation between these variables based on published results showing that nectar source availability is an important condition for a well developed butterfly community (Clausen *et al.*, 2001). Values of mean precipitation per month were supplied by the Instituto Nacional de Recursos Hidráulicos.

Statistical analysis

GraphPad InStat, version 3.01 (1998) software was used for data analysis. The Kolmogorov-Smirnov test was the first step in every case to evaluate data normality ($p < 0.10$). Median and 25 and 75 percentils were calculated. A Mann-Whitney U-test was used to compare the values of the same variable due to the nonparametric nature of data. Comparison of three or more values was performed with a Kruskal-Wallis test. When the later was significant ($p < 0.05$), a Dunn's Multiple Comparisons Post-Test was applied. Correlation between two variables was analyzed with a Nonparametric Correlation (Spearman r).

RESULTS AND DISCUSSION

Spatial segregation

Habitat use. The greatest difference between populations of both species relates to their abundance in each habitat type. In the three habitats *B. polydamas cubensis* is common while *B. devilliers* is rare (Fig. 2). Human disturbance of both studied areas may be one of the factors affecting rarity, considering the habitat requirements mentioned by Tyler *et al.* (1994). Boca de Jaruco is the most disturbed site and has the highest proportional abundance of *B. polydamas cubensis* across all the sites sampled. On the other hand, the evergreen forest of Boca de Canasí is the best preserved habitat of the triad and possesses the highest proportion of *B. devilliers* individuals, even during the dry season (Fig. 3). Concerning habitat requirements, Alayón and Solana (1989) reported that both species coexist in Cuchillas del Toa, specifically in the ecotone between forest and cleared areas.

No statistical significance was found correlating precipitation mean of the previous month and species relative abundance, except in the sea grape forest (Table 1). This may be due this site being mostly a feeding area, since no host plants were found. Thus the presence of butterflies is strongly related with flower availability. Additionally, the presence, in this habitat, of non native plants probably might increase the flower availability annually (Núñez & Barro, 2003), and could be the reason why the rain acts like the primary factor influencing flowering. Although the secondary scrub presents almost the same non native plants as the sea grape forest, there is no correlation of butterfly abundance with rain. It is likely that, in this habitat, flower availability is not the primary factor

Table 1. Values of the Nonparametric Correlation (Spearman r) between relative abundance of the populations (measured as the mean of the major number of individuals in a single count) and precipitation mean of the previous month.

Species/Habitat	Secondary scrub	Evergreen forest	Sea grape forest
Battus polydamas cubensis	0.3082	0.3531	0.6786
Battus devilliers	0.3935	-0.09258	No

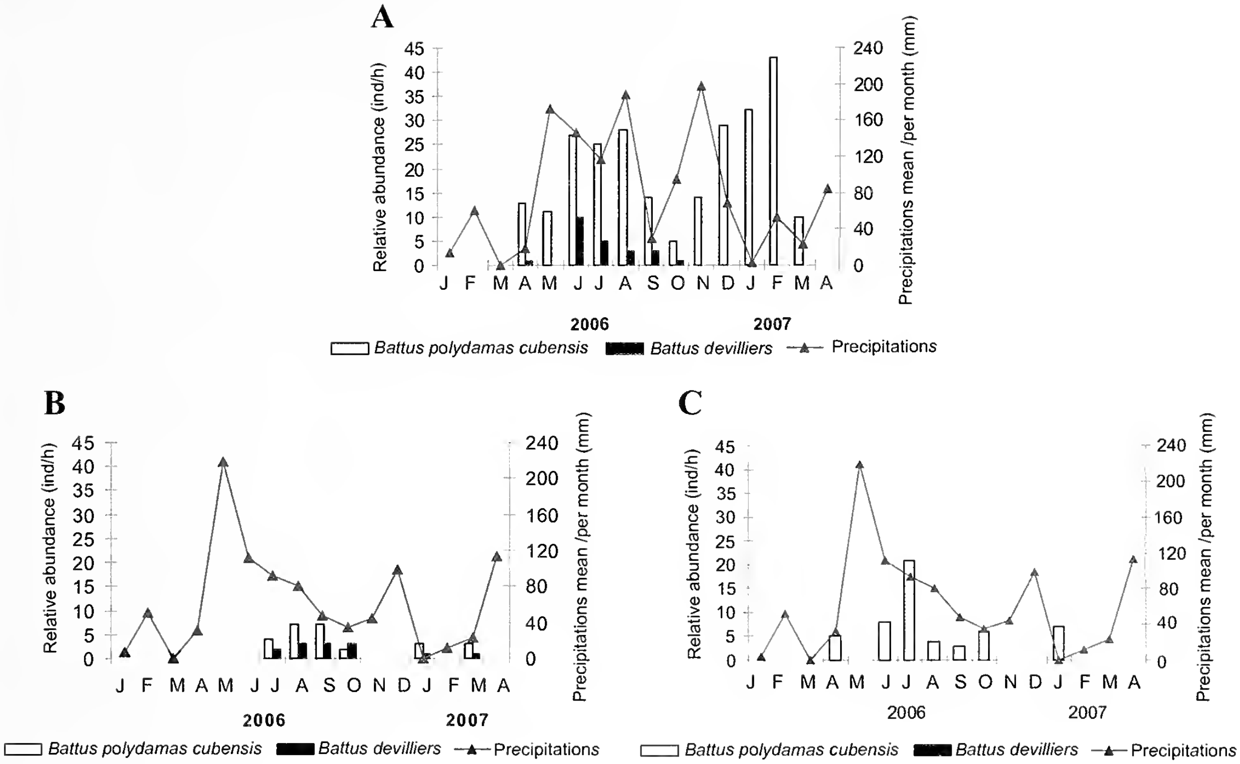

Figure 2. Population densities of *Battus devilliers* and *B. polydamas cubensis*, from April, 2006 to March, 2007. **A**: Secondary scrub of Boca de Jaruco. **B**: Evergreen forest of Boca de Canasí. **C**: Sea grape forest of Boca de Canasí. Gray line represents precipitations per month. Black squares represents relative abundance of *Battus devilliers* and the white ones those of *B. polydamas cubensis*. The blank spaces were not sampled. Relative abundance was the highest number of individuals observed in one hour.

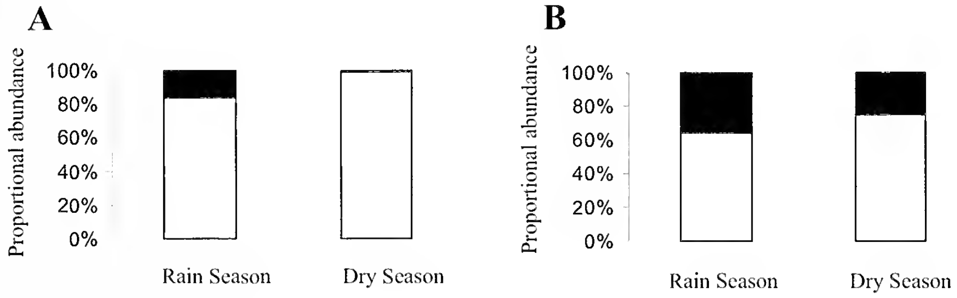

Figure 3. Proportional abundance of *Battus devilliers* and *B. polydamas cubensis* in the habitats in which they coexist. **A**: Secondary scrub of Boca de Jaruco. **B**: Evergreen forest of Boca de Canasí. The black colour represents *Battus devilliers* and white shows *B. polydamas cubensis*.

influencing the abundance of these butterflies. It may be host plant availability, for example.

At Boca de Canasí both species coexist only in the evergreen forest (Fig. 2), thus, no statistical analysis was made for the sea grape habitat. However, nearness of the two sites and the floristic diversity at the latter (Núñez & Barro, 2003) seem to support the idea of delimited feeding and breeding areas for a single population of *B. polydamas cubensis*. In this manner, the sea grape forest may be the foraging area and the evergreen forest the reproductive site (host plants observed only in the latter). This behavior was previously described for a Costa Rican population of this species by Young (1972). It may also explain the differential abundance of *B. polydamas cubensis* at each habitat, secondary scrub and evergreen forest. Another consequence of the behavior may be the similarity between relative abundance of both species in the evergreen forest during the rainy season (U = 3.5, p = 0.2469) due to an underestimation in counts of the actual number of individuals in the population of *B. polydamas cubensis* (Fig. 4). Data for the dry season were insufficient for analysis.

By contrast, populations of both species in Boca de Jaruco differed significantly (U = 1.5, p = 0.0104) during the rainy season and more so during the dry period (U = 0, p = 0.0022). *Battus devilliers* was always rare compared with *B. polydamas cubensis* (Fig. 4). However, OMR saw both species in equal proportion during the study period at another site approximately 1 km west of transects and nearest to Jaruco River, where native vegetation was best preserved.

Another reason concerning life cycle and reproductive strategy may contribute to the success of *B. polydamas cubensis.* Gregariousness of eggs and larval stages may be the most important because of the benefits described for the behavior as stated by Stamp (1980), Matsumoto (1989) and Reader and Hochuli (2003). Gregarious early stages are absent in *B. devilliers.*

Structural subniche

Sunlight intensity. Large sunny patches are common in Boca de Jaruco due to the presence of abundant herbs and bushes and only few isolated large trees. Accordingly, sunny patches were the category most used in that habitat. The Kruskall-Wallis Test shows differences among the different levels for both species. The Dunn's Multiple Comparisons post test demonstrates that in the morning those differences occur between sunny patches and the other levels ($p < 0.001$ for both species). During the afternoon differences in the use of three levels were also present. The results of post test were $p < 0.001$ comparing sunny patches with the other two, although sun filtered and shading patches usage by *B. polydamas cubensis* increases (Fig. 5a, b).

At Boca de Canasí, vegetation cover is more highly developed, so sun filtered and shading patches are more frequent than at Boca de Jaruco. Nevertheless, values of Kruskall-Wallis Test show differences among the mentioned levels. Only *B. devilliers* increased significantly the using of sun filtered sites in the same proportion as sunny patches. Statistical differences were between sunny and shading levels ($p < 0.05$) and between filtered and shading ones ($p < 0.01$) in the first hours of day, while during the afternoon the same relation occurs, but without individuals flying in shading patches (Fig. 5c, d). On the other hand, *B. polydamas cubensis* always preferred sunny patches. Differences in the morning were between first level (sunny) and the filtered and shading patches ($p < 0.001$ in both cases). During the afternoon no individuals were observed flying in shading patches (Fig. 5c, d).

Our results show that the use of this structural dimension depends not only on habitat, but on the ecological requirements of species. At Boca de Jaruco, sunny sites cover most of the study area and consequently both species made major use of them. Nevertheless, in Boca de Canasí, even when vegetation cover increased in the forest, only *B. devilliers* used filtered patches as well as sunny ones. This tendency matches with the primary habitat of each species, mentioned by Tyler *et al.* (1994), that explains why *B. polydamas* is a widespread and flexible species, whereas *B. devilliers* is sensitive and vulnerable, dependent on forested areas.

Flight stratum

In the morning, at Boca de Jaruco both species mostly fly in the two lower strata (Fig. 6a, b). *Battus devilliers* did not change this strategy during the entire all day, while *B. polydamas cubensis* started to use any stratum indiscriminately after midday. Kruskall-Wallis values were significant in the morning for both species. The post hoc test demonstrated differences between the highest stratum and the lowest one for both species ($p < 0.001$), and between the intermediate and the highest stratum with $p < 0.01$ for *B. devilliers* and $p < 0.001$ for *B. polydamas cubensis.* After midday, KW values did not show any difference among strata used by either species, although graphically we observed that *B. devilliers* keeps similar proportions to those of the morning (Fig. 6a, b).

At Boca de Canasí, there was no difference in flight stratum use during the day. *Battus devilliers* mostly flies in the lowest stratum, with patches of filtered sun. On the other hand, *B. polydamas cubensis* flies in all of the three strata. For *B. devilliers* the post test display differences between the lowest and the intermediate strata with $p < 0.01$ at both time intervals. Between the lowest and the highest strata we found the same p value for the morning, and $p < 0.001$ for the afternoon. By contrast, there was no statistical difference in the use of flight stratum by *B. polydamas cubensis* in this habitat (Fig. 6c, d).

Flight strata were more indiscriminately used by both species in the two habitats. The results demonstrate almost no selection for this structural dimension, perhaps because strata are not limiting

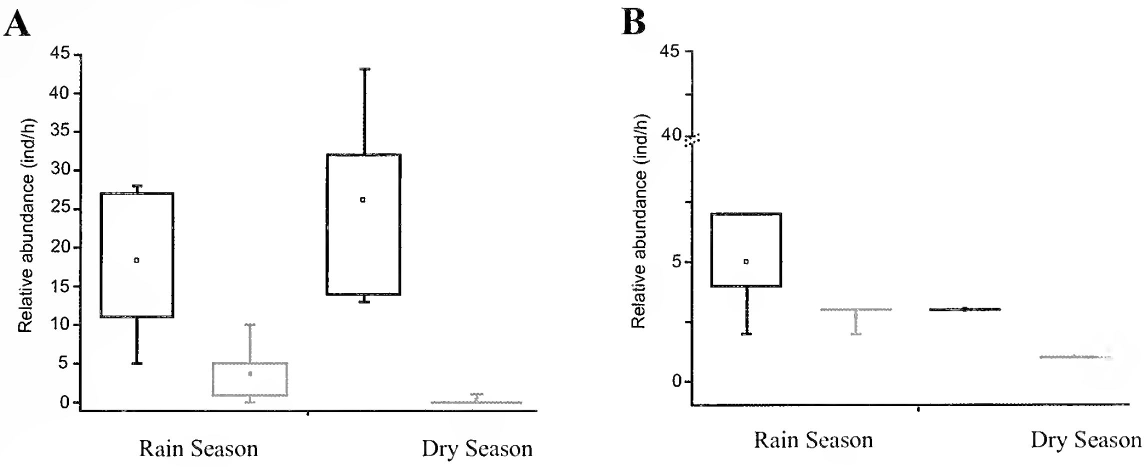

Figure 4. Relative abundance (individuals/hour) of *Battus devilliers* (gray) and *B. polydamas cubensis* (black) in the two seasons defined in Cuba as Rain (May-October) and Dry (November-April). **A**: Secondary scrub of Boca de Jaruco. **B**: Evergreen forest of Boca de Canasí. The graphic represents the median and the 25% and 75% percentils.

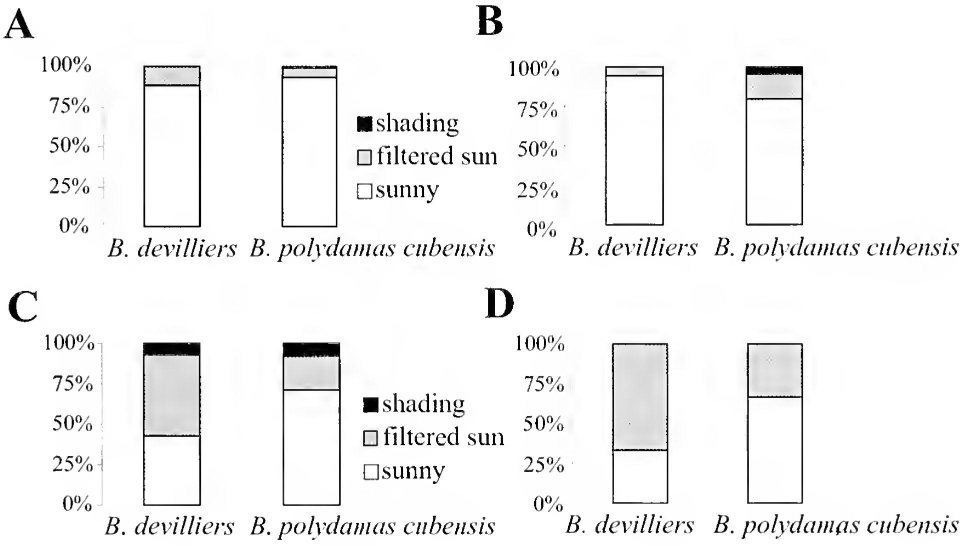

Figure 5. Spacial segregation of *Battus devilliers* and *B. polydamas cubensis* in the structural subniche, specifically in the light intensity used by each one. **A** and **B**: Secondary scrub of Boca de Jaruco (morning and afternoon, respectively). **C** and **D**: Evergreen forest of Boca de Canasí (morning and afternoon, respectively). Analysis only for the rainy season. Morning 9:00 am -11:00 am and afternoon 12:00 pm - 2:00 pm.

resources like flowers and host plant availability or because of low ecological relevance for both species. Similar results were obtained by Fontenla (1989) in a larger butterfly community.

Temporal segregation

Seasonality. Another relevant divergence in population ecology is the seasonal pattern of *B. devilliers* (U = 3.5, p = 0.0425) by contrast to the continuous dynamics of the other species (U = 10.5, p = 0.2620) (Fig. 2a, b; Fig. 4). This strategy and its presence in all sampled habitats show the greater ecological plasticity of *B. polydamas cubensis*. It is relevant that absence of *B. devilliers* in the sea grape habitat is recent, as five years ago it was observed by Núñez and Barro (2003) and again in lower frequency than *B. polydamas cubensis*. Annual fluctuations of populations, like those

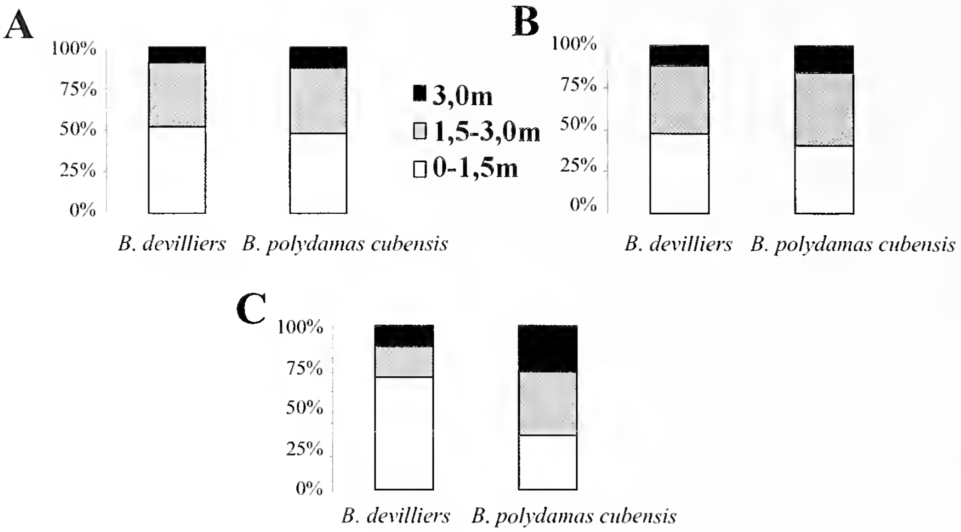

Figure 6. Spacial segregation of *Battus devilliers* and *B. polydamas cubensis* in the structural subniche, specifically in the flight height or stratum used by each. **A** and **B**: Secondary scrub of Boca de Jaruco (morning and afternoon, respectively). **C**: Evergreen forest of Boca de Canasí (there were not found significant differences in both day-hours). It was analized only rain season. Morning 9:00 am - 11:00 am and afternoon 12:00 pm -2:00 pm.

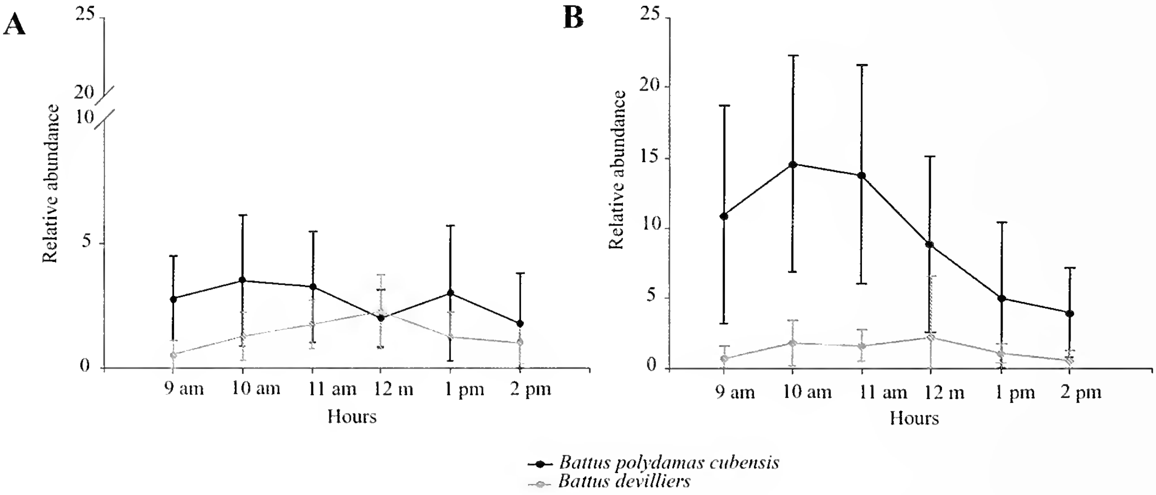

Figure 7. Daily activity of *Battus devilliers* (gray line) and *B. polydamas cubensis* (black line) for the rain season in the two studied habitats. **A**: Evergreen forest of Boca de Canasí. **B**: Secondary scrub of Boca de Jaruco.

observed in past years in Boca de Jaruco by the second author or changes in habitat structure may influence population dynamics.

Daily activity. We analyzed the daily activity for each species and habitat during the rainy season (Fig. 7). Although no statistical analyses were made with these data, two different trends were observed in each population. While the abundance of *B. polydamas cubensis* decreases during the day, with a peak of activity at 10:00 am, abundance of *B. devilliers* tends to increase until midday with the maximum number of individuals at noon. Thus at 12:00 the means of both populations were similar in the evergreen forest. At the same time, in the secondary scrub of Boca de Jaruco, although the mean values of abundance were so different, the standard deviations were too overlapped. This behavior is not surprising since *B. polydamas cubensis* flies in sunny patches more than

B. devilliers that prefers shade and is less influenced by high temperature of noon. This mechanism may affect thermoregulation permitting the species a longer diurnal flight period.

Battus devilliers and *B. polydamas cubensis* present more selectivity for the dimension sunlight intensity of the structural subniche than for the flight stratum since the late was more indiscriminate used. Temporal segregation seems to occur between these species, *B. devilliers* is influenced by seasonality while *B. polydamas cubensis* is present all the year.

ACKNOWLEDGEMENTS

We thank especially the persons who helped with the field work: Rayner Núñez, Alejandro García, Joel Lastra, Elier Fonseca and René Marzuk. We also are grateful to Ariam Jiménez who was of great help with the statistical analysis. Our gratitude to Gilberto Silva, Rayner Núñez and Oraily Madruga for their helpful comments about the manuscript and also our thanks for the botanical assistance to Iralis Ventosa and Antonio López. Three anonymous reviewers contributed to clarifying the submitted manuscript.

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A tale of two species: detritivory, parapatry, and sexual dimorphism in *Lamprospilus collucia* and *L. orcidia* (Lycaenidae: Theclinae: Eumaeini)

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Abstract. *Lamprospilus collucia* (Hewitson) and *L. orcidia* (Hewitson) are facultatively detritivorous hairstreaks. Females in nature lay eggs on dead twigs and leaves that are on or near the ground. In the lab, females oviposit readily on dead leaves. Caterpillars of both species eat dead plant material in nature and can be reared in the lab to the adult stage on artificial diet to which no plant material has been added. *Lamprospilus collucia* and *L. orcidia* have parapatric distributions; the former species is endemic to the Transandean Region and the latter to the Amazonian and Atlantic Regions. Both species have similar male behavior, which is consistent with the hypothesis that their parapatric distributions are maintained by mating interference. The sexes of *L. collucia* and *L. orcidia* have been incorrectly associated in compendia of Neotropical butterflies and are associated in this paper by geographic distribution, wing pattern similarity, and rearing data. Although *L. collucia* and *L. orcidia* have been considered to be conspecific, an analysis of geographical variation supports the hypothesis that they are distinct biological species.

Key words: Amazonian Region, biogeography, hairstreak systematics, Lecythidaceae, Transandean Region.

Introduction

Lamprospilus collucia (Hewitson) and *L. orcidia* (Hewitson) are common and widespread lowland Neotropical lycaenids (Theclinae: Eumaeini) that are biologically significant for a number of reasons. First, *L. collucia* and *L. orcidia* are ecologically unusual. Larval detritivory occurs rarely in the "Macrolepidoptera" (Powell *et al.*, 1998; Hohn & Wagner, 2002), but has been reported in *Lamprospilus* Geyer (Duarte & Robbins, in press), specifically in *L. collucia* and *L. orcidia*. Second, *L. collucia* and *L. orcidia* are biogeographically significant because they have been cited as a representative parapatric species pair with a Central/South American distribution (Robbins, 2004a). This biogeographic pattern, while well-known in forest-dwelling aposematic butterfly taxa (Brown, 1982), has not been documented previously in the Eumaeini. Third, *L. collucia* and *L. orcidia* are of taxonomic interest because they are widely misidentified in publications on Neotropical butterflies (e.g., Godman & Salvin, 1887; Weeks 1911; Draudt, 1919-1920; Kaye, 1921; Barcant, 1970; Robbins & Small, 1981; D'Abrera, 1995). Both species are sexually dimorphic. The males have similar wing patterns (Figs. 1-4, 13-16, 21-22), for which reason they have been considered to be conspecific (Godman & Salvin, 1887-1901; Kaye, 1921). Alternately, the female wing patterns (Figs. 5-12, 17-20, 23) are different from each other and from those of the males, with which they have rarely been associated (e.g., Draudt, 1919-1920).

The purpose of this paper is to address the ecology, biogeography, and taxonomy of *L. collucia* and *L. orcidia* by answering basic questions about them. Where do females oviposit? What do their caterpillars eat? When and where do males set up mating territories? What are the distributions of *L. collucia* and *L. orcidia*? In which habitats do they occur? How are *L. collucia* and *L. orcidia* distinguished? How

Received: 15 September 2009
Accepted: 12 October 2009

do they vary seasonally and geographically? On what basis are the sexes associated? What is the available evidence that they are different biological species? We add brief notes on nomenclature to confirm that we are using the correct names. The placement of *L. collucia* and *L. orcidia* in *Lamprospilus* Geyer is dealt with elsewhere (Duarte & Robbins, in press).

MATERIALS AND METHODS

Eggs were obtained in the lab following the methods detailed in Duarte *et al.* (2005). Larvae from these eggs were reared on artificial diet to which no vascular plant material was added other than wheat germ and linseed oil (Duarte *et al.*, 2005). Rearing methods for immatures collected in nature generally follow Feinstein *et al.* (2007). Depositories for vouchers are noted.

Biogeographic and taxonomic results for *L. collucia* are based on 96 males (6 genitalic dissections from Mexico, Panama, western Ecuador, and eastern Colombia) and 70 females (6 genitalic dissections from Mexico, Costa Rica, Panama, and Trinidad). Analogous results for *L. orcidia* are based on 50 males (6 genitalic dissections from Ecuador, Peru, and 3 states in Brazil) and 57 females (6 genitalic dissections from Peru and Brazil). We map the distributions of each species by sex because these distributions are evidence for associating the sexes. Although mitochondrial "barcodes" are reported for *L. collucia* and *L. orcidia* (BOLD website, http://www.barcodinglife.org/views/login.php, accessed 26 Aug 2009), the barcodes are not publically available and the "barcoded specimen" of *L. orcidia* is misidentified.

Genitalic terms follow Klots (1970), as modified for the Eumaeini (Robbins, 1991). Wing venation follows Comstock (1918), and other morphological terms follow Snodgrass (1935). Geographic distributions are mapped by gender. Months are abbreviated by their first three letters in English.

Vouchers for the distribution maps and other results are deposited in the following collections: (AA) Annette Aiello Collection, Ancon, Panamá; (BMNH) Natural History Museum, London, UK; (DZUP) Universidade Federal do Paraná, Curitiba, Paraná, Brazil; (MCZ) Museum of Comparative Zoology, Harvard University, Cambridge MA, USA; (MECN) Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador; (MUSM) Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Perú; (MZUSP) Museu de Zoologia, Universidade de São Paulo, Brazil; (RCB) Robert C. Busby Collection, Andover, MA, USA; (USNM) National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

RESULTS

Ecology and biogeography

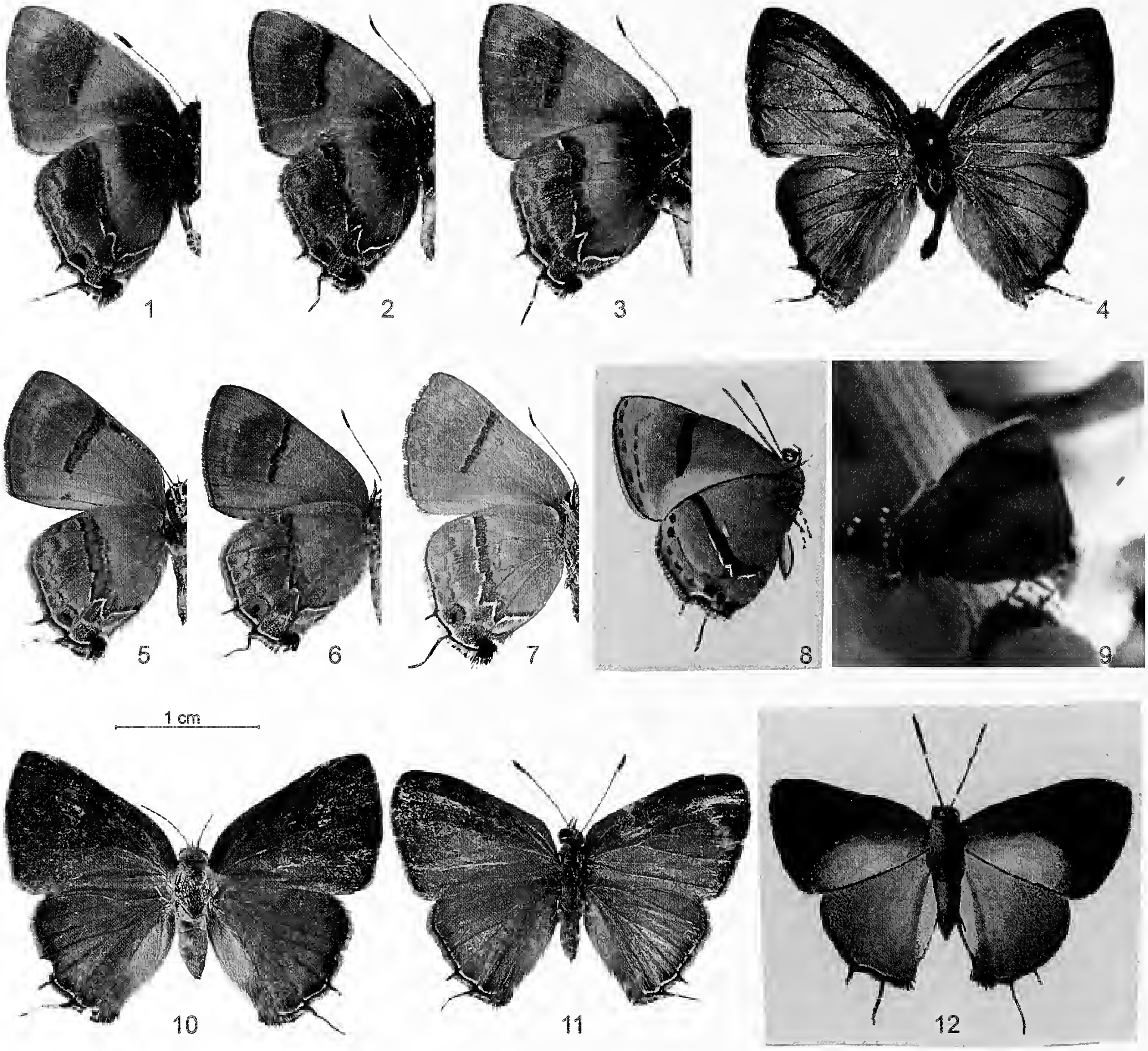
Oviposition and food "plants." As part of a study of plant fungal diseases (Davidson *et al.*, 2000), a "mostly dead" seedling of *Anacardium excelsum* (wild cashew, Anacardiaceae) was collected by Davidson about 9 Jun 1996 near the Rio Frijoles, Pipeline Road, Canal Area, Panama (see Ridgely, 1976 for information on the Pipeline Road locality). A dark reddish brown larva of *L. collucia* was found three days later eating the cotyledon of the dead seedling. Aiello fed the caterpillar the peduncle of *Anacardium occidentale* to complete its development. On 18 Jun 1996, a dark brown pupa with erect setae on the sides of the abdomen was formed. The pupa turned black on 1 Jul 1996, and a male of *L. collucia* emerged later that day. The reared adult male is deposited in AA (Aiello lot: 1996-10).

A female of *L. collucia* was collected by Robbins and Caldas on 30 Mar 2000 in Ancon, Canal Area, Panama. She laid 22 eggs over 6 days in the lab on dead leaves and on the side of a vial. Aiello reared the hatched larvae on artificial diet without any added plant material. A female emerged on 13 May 2000. The reared female and her mother are deposited in USNM.

Robbins and Caldas observed a female of *L. collucia* ovipositing on a twig on the ground in Ancon on 31 Mar 2000. After capture, the female butterfly laid another 44 eggs over the next 5 days on dead leaves in the lab. Aiello reared the resulting caterpillars on artificial diet without any added plant material, and three males and one female emerged 14-16 May 2000 (Figs. 1, 5). The mother and her reared offspring are deposited in USNM.

Robbins and Caldas observed a female of *L. collucia* ovipositing on a green leaf about10 cm from the ground on 2 Apr 2000 (Fig. 9). She was not captured and the egg was not collected.

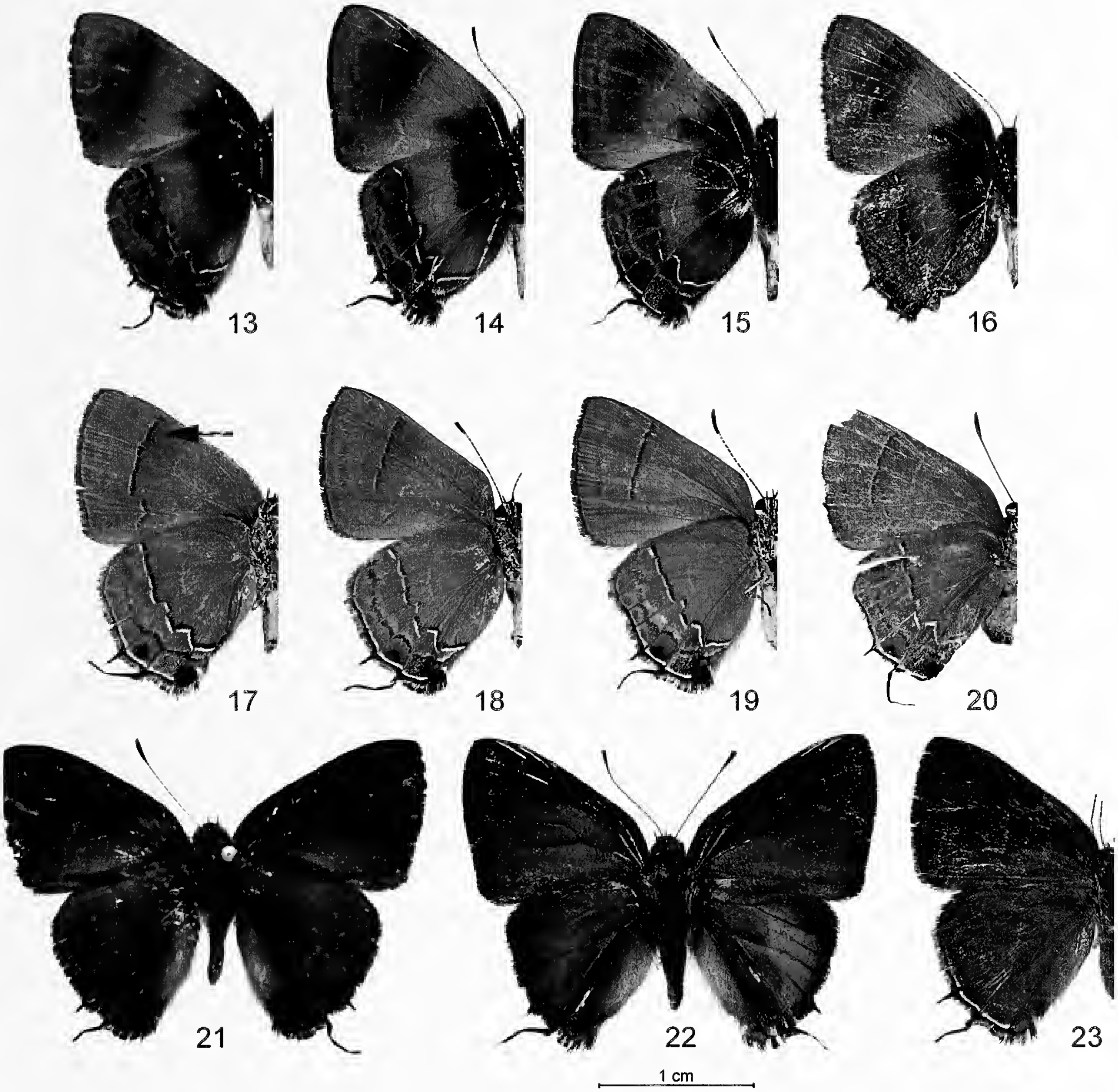
A male of *L. orcidia* was reared from the fallen androecia of *Eschweilera coriacea* (Lecythidaceae, plant vouchers deposited in New York Botanical Garden) from lowland moist forest 7 km north of Saül, French Guiana (3°37' N, 53°12' W). The androecia were collected by Berkov 21 Oct 1995 in the dry season, and the adult male of *L. orcidia* emerged 8 Nov 1995 (voucher deposited in USNM, Fig. 16). Another eight Lycaenidae that belong to another genus were also reared from these androecia (Feinstein, Robbins, & Berkov, in prep.).



Figures 1-12. *Lamprospilus collucia* adults. **1.** ♂ ventral, Panama, reared, male sibling of 5. **2.** ♂ ventral, Panama, form typically seen in the wet season. **3.** ♂ ventral, Panama, form typically seen in the dry season. **4.** ♂ dorsal of 1. **5.** ♀ ventral, Panama, reared, female sibling of 1. **6.** ♀ ventral, Panama, form typically seen in the wet season. **7.** ♀ ventral, Nicaragua, form typically seen in the dry season. **8.** ♀ ventral, no locality, reproduction of figure from the original description. **9.** ♀ ventral, Panama, female walking on a twig near the ground before laying an egg. **10.** ♀ dorsal, Venezuela. **11.** ♀ dorsal, Venezuela. **12.** ♀ dorsal, no locality, reproduction of figure from the original description.

A male and two females (Fig. 20) of *L. orcidia* (identified as Lycaenidae #2 in Feinstein *et al.* 2007) were reared from larvae found in fallen androecia of *Lecythis corrugata* (Lecythidaceae) during the first three months of 2003 in the wet season at Les Nouragues Research Station in French Guiana (4°05' N, 52°41'W; 110 km south of Cayenne). Although 17 other Lycaenidae were reared, none belong to *Lamprospilus*. Reared adult vouchers are deposited in USNM.

Male behavior. *Lamprospilus* males display "territorial" behavior that is similar to that reported in other eumaeines (e.g., Alcock & O'Neill, 1986; 1987); males wait for receptive females to fly through the territory and "defend" these areas by flying at other males that enter the territory. Males of *L. collucia* and *L. orcidia* set up mating territories in the morning on hilltops (vouchers below are deposited in USNM, observations are by Robbins; times are standard time



Figures 13-23. *Lamprospilus orcidia* adults. **13.** ♂ ventral, Brazil (Pará), presumed holotype. **14.** ♂ ventral, Peru. **15.** ♂ ventral, Peru. **16.** ♂ ventral, French Guiana, reared from fallen androecia of Lecythidaceae. **17.** ♀ ventral, Peru, arrow points to brown scales basal of the postmedian line. **18.** ♀ ventral, Peru. **19.** ♀ ventral, Peru. **20.** ♀ ventral, French Guiana, reared from fallen androecia of Lecythidaceae. **21.** ♂ dorsal, Brazil (Pará), presumed holotype. **22.** ♂ dorsal, Peru. **23.** ♀ dorsal, Peru.

at that locality).

Lamprospilus collucia in Panama, 0730-1045 hours

4 ♂ observed (2 vouchers), 5 Oct 1978, 0730-0745 hours, Canal Area, Paraiso, Cerro Paraíso.

5 ♂ (5 vouchers), 1 Jan 1979, 1000-1030 hours, Canal Area, Paraíso, Cerro Paraíso.

1♂ (1 voucher), 5 Mar 1979, 1045 hours, Canal Area, Paraíso, Cerro Paraíso.

>25♂ observed (2 vouchers), 17 May 1979, 0830-1030 hours, Canal Area, Cerro Galera.

1♂ (1 voucher), 28 Jul 1979, 1000 hours, Canal Area, Paraíso, Cerro Paraíso.

Lamprospilus orcidia in Brazil, 0904-0920 hours

1♂ (1 voucher), 18 Mar 1991, 0904 hours, São Paulo, 17 km west of Teodoro Sampaio.
1♂ (1 voucher), 24 May 1998, 0920 hours, Rio de Janeiro, Iguaba Grande.

Habitat. *Lamprospilus collucia* and *L. orcidia* occur in wet and dry lowland forest, ranging from "relatively virgin" forest (e.g., Parque Manu, Peru) to mature secondary forest (e.g., Gamboa, Canal Area, Panama) to patchy disturbed forest in urban areas (e.g., Ancon, Canal Area, Panama). We have seen no specimens of *L. collucia* collected above 1,000 m elevation in Central America, but in western Ecuador they have been found in wet forest at 1,500 m and on a ridge with dry forest at 2,100 m where there is often a strong westerly wind. Most individuals of *L. orcidia* are recorded from lowland forest, but some have been recorded from 1,000 m elevation in southern Brazil. Adults of *L. collucia* and *L. orcidia* are most abundant at the end of the dry season and beginning of the wet season in Panama and southeastern Peru, a pattern typical of the *Lamprospilus* Section (Duarte & Robbins, in press).

Distribution. Males of *L. collucia* are recorded from northeastern Mexico to Ecuador west of the Andes and to Trinidad, northern Venezuela, and central Colombia east of the Andes (circles in Fig. 24) while males of *L. orcidia* are known east of the Andes from central Venezuela to southern Brazil and Bolivia (squares in Fig. 24). Males of *L. collucia* and *L. orcidia* are not sympatric.

Females of *L. collucia* are recorded from northeastern Mexico to the northwestern tip of Peru west of the Andes and to Trinidad, and central Venezuela east of the Andes (circles in Fig. 25) while females of *L. orcidia* are known from east of the Andes from the Guianas and southern Venezuela and southern Colombia to southern Brazil (squares in Fig. 25). Females of *L. collucia* and *L. orcidia* are not sympatric.

There is one male of *L. orcidia* and one female of *L. collucia* from the Rio Suapure, Venezuela (MCZ), a tributary of the Rio Orinoco in central Venezuela (Bolivar state) that flows through llanos (savannah) and Amazonian forest habitats (arrows in Figs. 24-25). These specimens lack collection date or more specific locality data. Weeks (1911) noted only that they were collected in "the neighborhood of the Suapure River in Venezuela." It is unknown if both were collected at the same locality along the Rio Suapure, but if so, it is the only locality where both species have been found. The female from Rio Suapure was listed and illustrated as *Thecla madie* Weeks, but the male was apparently misidentified as *Thecla xenata* (a misspelling of *Thecla xeneta* Hewitson, see taxonomy section below) (Weeks, 1911). There are no males of *Calycopis xeneta* from the Rio Suapure in the Weeks Collection (MCZ).

Taxonomy

Distinguishing male characters. Location of the charcoal-black patch on the ventral forewing is the most consistent and easy way to distinguish males of *L. collucia* and *L. orcidia* (Figs. 1-3, 13-16). In *L. collucia*, this patch is distal of the postmedian line whereas in *L. orcidia*, it is distal and basal with the basal part darker in some individuals. We have not seen a male with an intermediate wing pattern. Godman and Salvin (1887-1901) and Kaye (1921) apparently considered this difference to be intraspecific variation, but Comstock and Huntington (1962) noted that the two wing patterns were distinct.

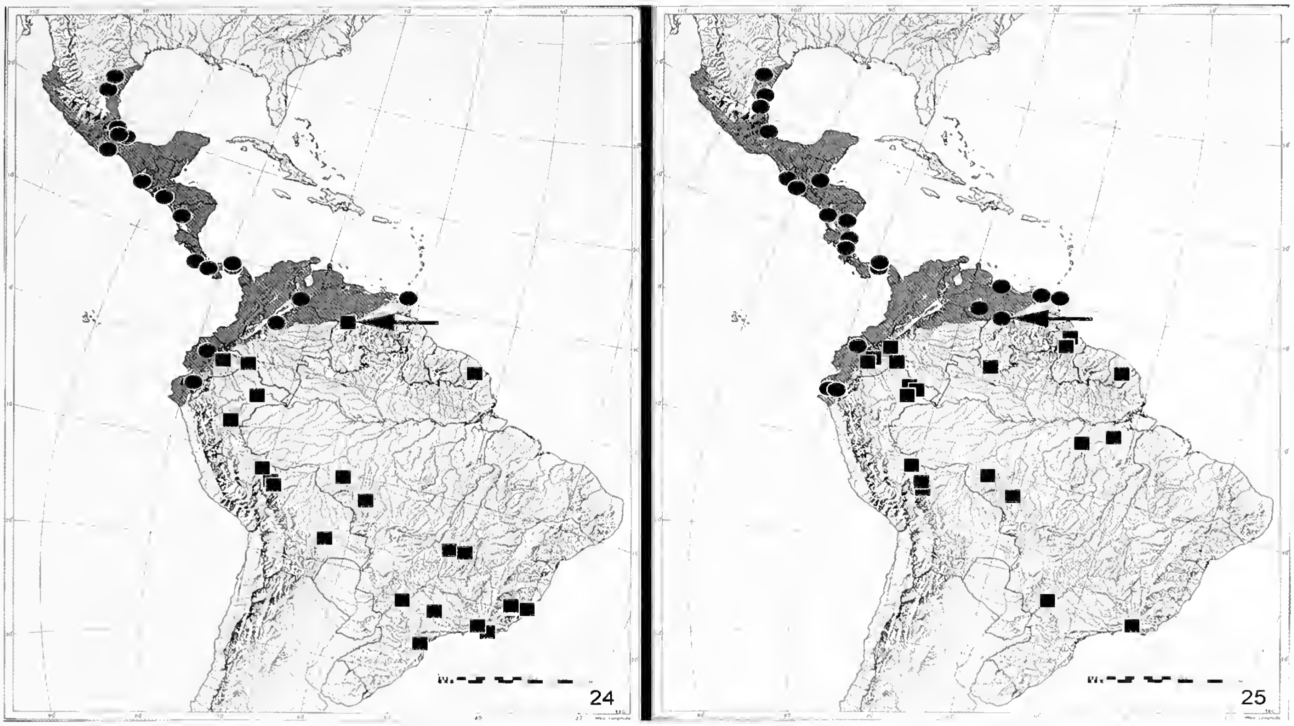
The ventral wing patterns of these males are similar to those of other species with charcoal-black patches. Males of some other *Lamprospilus* species, such as *L. coelicolor* (Butler & Druce) and *L. aunus* (Cramer), are easily distinguished by the better defined and more triangular shape of the dark brown patch on the ventral forewing (Fig. 42 in Duarte & Robbins, in press). Males of *Calycopis xeneta* (Hewitson) have a brown spot in ventral hindwing cell Cu_2-2A just distal of the postmedian line (Fig. 58 in Duarte & Robbins, in press) that is lacking in *Lamprospilus*.

Variation of male wing pattern. Wing pattern variation in male *L. collucia* is most evident on the ventral wings (Figs. 1-3). The width and exact shape of the postmedian line on both wings is perhaps the most variable element. The darkness and extent of the charcoal-black patches is also variable. Those individuals with a ventral wing pattern which is a bit lighter than average (Fig. 3) are more prevalent in the dry season, but we find no evidence for geographical variation.

Wing pattern variation in male *L. orcidia* is also most evident on the ventral wings (Figs. 13-16). Again, the shape of the postmedian line and the extent and darkness of the charcoal-black patches on both wings are the most variable elements. We do not have sufficient data to assess seasonal wing pattern variation, but find no evidence of geographical variation.

Distinguishing female characters. Shape and color of the ventral forewing postmedian line is the most consistent way to distinguish females of *L. collucia* and *L. orcidia*. This line is relatively thick and reddish to dark maroon in *L. collucia* (Figs. 5-9) and is a relatively thin black and white line with diffuse light brown scaling basally in *L. orcidia* (Figs. 17-20, arrow points to brown scaling).

The ventral wing pattern of female *L. collucia* could be confused with that of female *L. lanckena* (Schaus),



Figures 24-25. Distribution of *L. collucia* (circles) and *L. orcidia* (squares). Arrows point to possible sympatry on the Rio Suapure (Venezuela). The shaded area is an extremely close approximation to the Transandean Region of Brown (1982: 456); this area of endemism was proposed without exact borders. **24.** Males. **25.** Females.

but the later has the ventral forewing postmedian line of *L. collucia* in cell Cu$_2$-2A and more rounded hindwings. The black and white forewing postmedian line with brown basal scaling is the best way to distinguish *L. orcidia* from other hairstreak species, but this character is sometimes inconspicuous (Fig. 19). Even with genitalic dissection, some females of *L. orcidia* may be difficult to identify definitively.

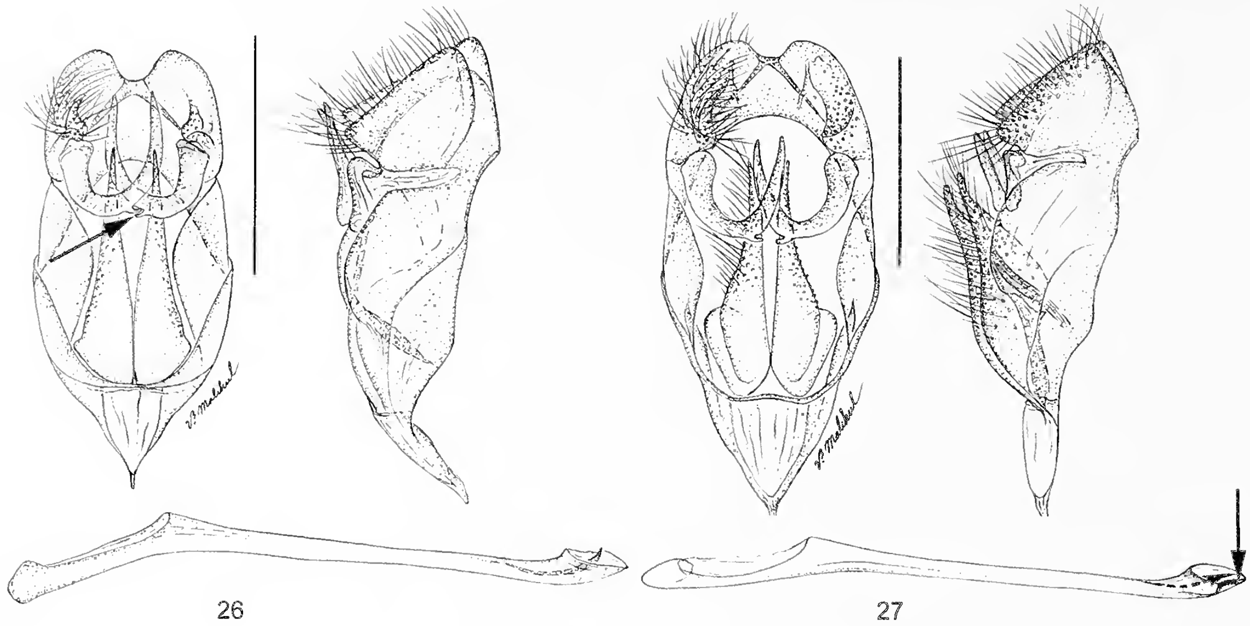
Variation of female wing pattern. Wing pattern in female *L. collucia* is quite variable. Dorsal ground color has variable amounts of blue scaling (Figs. 10-11), which varies in hue from shining blue to chalky gray. The ventral brownish-black patch of scales distal of the postmedian line varies from absent (Fig. 7) to conspicuous (Figs. 5-6). The color of the ventral postmedian line varies from reddish to dark maroon, but the thick forewing line from the costa to vein Cu$_2$ is a constant feature. As in the male, individuals with a lighter ventral wing pattern (Fig. 7) tend to be most frequent in the dry season.

Wing pattern variation in female *L. orcidia* is similarly variable. Dorsal ground color varies from blue to chalky gray. The ventral wing pattern is rather "non-descript", but the black and white postmedian line with basal brownish scaling appears to be constant, even if its expression is variable (Figs. 17-20).

Male genitalia and their variation. The male genitalia of *L. collucia* and *L. orcidia* are typical of *Lamprospilus* (Duarte & Robbins, in press) with a single medium sized tooth on each gnathos arm (arrow in Fig. 26). The only evident genitalic difference between the two species is that the penis of *L. orcidia* consistently has a small second cornutus (arrow in Fig. 27) while that of *L. collucia* may or may not (Fig. 26) have the second cornutus. Otherwise, the illustrated differences in the saccus, penis, and valvae (Figs. 26-27) fall within the range of intraspecific variation.

Female genitalia and their variation. The female genitalia of *L. collucia* are typical of *Lamprospilus* with "fan-shaped" signa (Figs. 28-29) and an inwardly curved sclerotized ridge on the distal end of the 8th abdominal tergum (illustrated in Duarte & Robbins, in press). The shape of the ductus bursae, especially the posterior end, varies intraspecifically, but does not distinguish the species. The signa of the two species differ in the sample (Figs. 28-29), but are structurally quite variable, for which reason we suspect that this difference might not be confirmed by a larger sample size.

Nomenclature. *Thecla collucia* Hewitson was



Figures 26-27. Male genitalia, ventral aspect (left), lateral aspect (right), penis in lateral aspect (bottom). **26.** *L. collucia*, Panama (Canal Area), arrow points to single "tooth" on the gnathos. **27.** *L. orcidia*, Brazil (Minas Gerais), arrow points to small second terminal cornutus. Scale 1 mm.

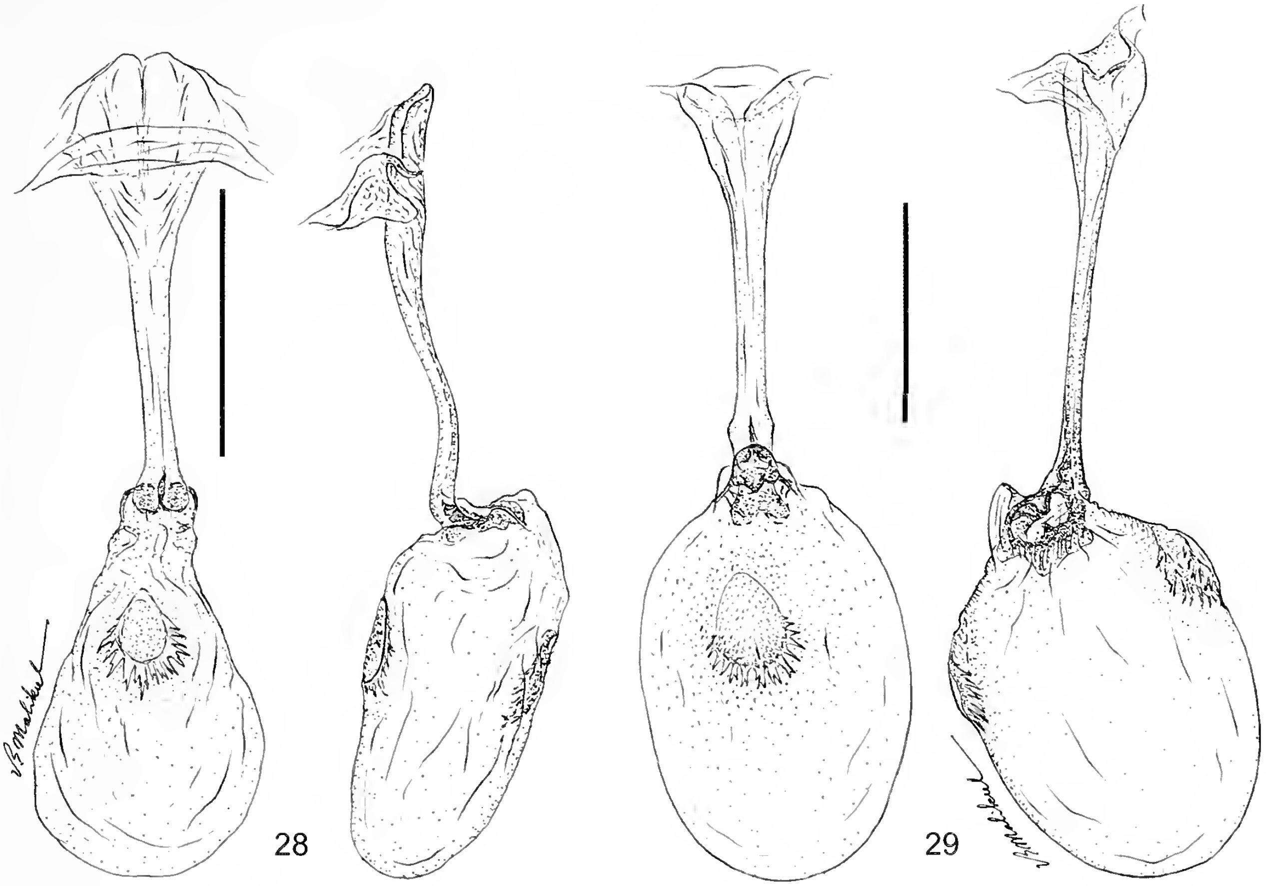
described from at least one pair in the Hewitson Collection (now in BMNH), but only the female was illustrated (Figs. 8, 12). No type locality was given. Johnson (1993: 22) designated a female lectotype (B.M. Type Rh 1010) from Esp. Santo (presumably Espírito Santo, Brazil) that fits the original illustration very well even though it is missing most of its forewings. However, there was no type locality in the original description, and a photograph of the lectotype and its labels from the 1970s shows that this specimen lacked a locality label at that time. Johnson (1993: 22) did not list Brazil as part of the South American distribution of *collucia*, so his "Esp. Santo" citation is difficult to interpret. Primary types of *Thecla collucia*'s junior synonyms have been examined: *madie* Weeks (♀, MCZ), *amphrade* Schaus (♀, USNM, the original description erroneously listed the BMNH), *iodinus* Kaye (♂, BMNH), *posetta* Dyar (♀, USNM), and *shueyi* Johnson (♂, AMNH).

Thecla orcidia Hewitson was described from at least one male in the H. W. Bates collection (now in BMNH) from the Amazon. There is one male in the BMNH that fits this description (Figs. 13, 21, B.M. Type Rh 872) and is presumed to be a holotype. Illustrations of the holotypes (by original designation) of junior synonyms *tafiensis* Johnson (AMNH), *mossi* Johnson & Kroenlein (BMNH), *silva* Austin & K. Johnson (DZUP), *rondonia* Austin & K. Johnson (DZUP), *obscura* Austin & K. Johnson (DZUP), *perplexa* Austin & K. Johnson (DZUP), and *purpura* Austin & K. Johnson (DZUP) can be found in the original descriptions (Johnson, 1993; Johnson & Kroenlein, 1993; Austin & Johnson, 1997).

The wing pattern of female *L. orcidia* is non-descript, as noted. Perhaps for that reason, a female of *L. orcidia* was included in the type series of the unrelated *Thecla ceromia* Hewitson. However, Johnson and Kroenlein (1993: 4) designated another specimen as the lectotype, which is the reason that *Thecla ceromia* is now placed in *Ziegleria* (Robbins, 2004b; Duarte & Robbins, in press).

DISCUSSION

Detritivory. Females of *L. collucia* have been recorded in nature ovipositing on dead twigs on the ground and on a leaf near the ground. In the lab, females oviposit readily on dead leaves. Caterpillars of *L. collucia* and *L. orcidia* in nature have been found eating a "nearly" dead seedling and the androecia of Lecythidaceae flowers on the ground. In the lab, larvae complete development on live and dead organic matter. Although many butterflies, including Lycaenidae, can be reared on an artificial diet to which dried, ground leaves of the food plant are added (Morton, 1981; Mark, 1993; 1995), larvae of



Figures 28-29. Female genitalia, dorsal (left) and lateral aspects. **28.** *L. collucia*, Panama (Canal Area). **29.** *L. orcidia*, Peru (Madre de Dios). Scale 1 mm.

L. collucia and *L. orcidia* readily ate and completed development on an agar-based artificial diet without the addition of leaves. These results are very similar to those reported for *Calycopis* (S. Johnson, 1985; Robbins *et al.*, 1996; Duarte *et al.*, 2005), and are consistent with the hypothesis that *L. collucia* and *L. orcidia* are facultative detritivores.

Different kinds of detritus provide different kinds of nutrition for a caterpillar. A preliminary analysis of some Lecythidaceae androecia showed that they have higher sugar and phosphorus content than "leaf litter" (nitrogen levels were variable), but a lower content of other minerals and fiber (A. Whigham pers. comm.). Detritivores may also eat micro-organisms living on detritus (Findlay & Tenore, 1982; Hohn & Wagner, 2002), but to date, the nutrition that lycaenid detritivorous caterpillars derive from different food objects is an unexplored subject.

Maximal adult abundance of *L. collucia* and *L. orcidia* at the end of the dry season and beginning of the wet season suggests that larvae find more suitable food or suffer lower mortality during the dry season. Many trees are deciduous during the dry season, but whether fungi and other caterpillar pathogens and predators are less abundant at that time is an open question.

Parapatry. Brown (1982) partitioned the distribution of Neotropical forest butterflies into four slightly overlapping "fuzzy-edged" biogeographic regions of endemism, three of which (Transandean, Amazonian, and Atlantic) consist primarily of areas under 1,500 m elevation. The biogeographic distribution of *L. collucia* is a "textbook" example of Brown's Transandean Region; this species occupies virtually the entire Transandean Region (shaded part of Figs. 24-25). The distribution of *L. orcidia* is a combination of Brown's Amazonian and Atlantic Regions. So far as we are aware, this is the first time that a clear-cut Transandean/Amazonian parapatric distribution has been documented in the Eumaeini. In most other potential cases, such as *Lamasina draudti* (Lathy) and *L. ganimedes* (Cramer) (Robbins & Lamas, 2008), species are not sufficiently well-represented in museum collections to determine whether distributions are allopatric or parapatric.

The parapatric distributions of *L. collucia* and *L. orcidia* (Figs. 24-25) are unlikely to be maintained by competition for larval food; it is difficult to visualize the dead organic matter that the caterpillars eat as a limiting resource. However, males of both species set up mating territories in the morning on hilltops and occur in similar habitats. These findings suggest the testable hypothesis that mating interference is responsible for maintaining parapatry between the two species.

Associating males and females. The evidence that males and females of *L. collucia* are correctly associated is that the distribution of each sex is almost identical (Figs. 24-25), both sexes have a dark brown patch on the ventral forewing distal of the postmedian line (Figs. 1-3, 5-9), and both sexes have been reared from eggs laid by the same mother (Figs. 1, 5). The evidence that the male and female of *L. orcidia* are the same species is that the distribution of males and females is almost identical (Figs. 24-25), both sexes have darker scales (albeit, much reduced in the female) basal of the ventral forewing postmedian line (Figs. 13-20), and both have been reared from fallen flowers of Lecythidaceae (Figs. 16, 20; no other *Lamprospilus* species were reared from these flowers). Finally, no other "unassociated" *Lamprospilus* male or female has the same distribution as either species.

Biological species. With the possible exception of the old Rio Suapure specimens mentioned above from Weeks (1911), the distributions of *L. collucia* and *L. orcidia* are parapatric (Fig. 24-25). Distinguishing characters are consistent throughout the range of each species and do not vary in the areas where the distributions meet. This evidence is consistent with the hypothesis that the two taxa do not interbreed.

Lamprospilus collucia and *L. orcidia* are likely to be phylogenetic sisters. In a phylogenetic analysis intended to determine relations among the genera of the "*Lamprospilus* Section" (Duarte & Robbins, in press), the morphological character coding for *L. collucia* and *L. orcidia* was identical. However, the coding was also very similar to that for *L. coelicolor* and *L. aunus*. For this reason, an analysis of phylogenetically informative characters among *Lamprospilus* species is needed to test whether *L. collucia* and *L. orcidia* are indeed sister species.

ACKNOWLEDGEMENTS

We are grateful to Karie Darrow for preparing the plates of adults, to Vichai Malikul for preparing the genitalic illustrations, to Brian Harris for help with technical aspects of this project, to Gerardo Lamas for providing data on *L. collucia* in Peru, and to Amie Whigham for allowing us to cite her preliminary unpublished information. For supporting travel by both Duarte and Robbins and for substantial funds, we thank Fundação de Amparo à Pesquisa do Estado de São Paulo/FAPESP (as part of the project "Systematics, Bionomy, and Evolution of Neotropical Lepidoptera"; process numbers 02/13898-0 and 03/13985-3) and Pró-Reitoria de Pesquisa da Universidade de São Paulo/USP/Projeto 1. For excellent suggestions on the manuscript, we thank Paul Goldstein, André Freitas, and Rudi Mattoni.

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Aggregated oviposition in *Actinote pellenea pellenea* Hübner (Lepidoptera: Nymphalidae)

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Abstract. The oviposition pattern of *Actinote pellenea pellenea* on *Austroeupatorium inulaefolium* was investigated in two coastal sites in SE Brazil to test if there is any pattern of preference for host plant traits. At both sites, host plants were marked and measured for height, distance of the nearest plant, number of leaves with *A. p. pellenea* immatures (eggs and first instar larvae), number of groups of immatures, and total number of eggs per plant. An apparency index was calculated relating plant size to distance from its three nearest conspecific neighbours. Total leaf area, orientation and inclination were recorded for each leaf with a group of immatures. There was no significant correlation between the number of ovipositions on each plant and habitat and plant characteristics as plant height and apparency index. The number and density of eggs by oviposition was not correlated with leaf characters as area, orientation or inclination. At both sites clusters of immatures showed a grouped distribution, with some plants having more immatures than predicted by chance. In the only case of a double oviposition on the same leaf, the later cluster was significantly smaller. The results show that distribution of eggs - reflecting female choice - was not related with the above measured plant and leaf traits. However, the results do suggest that females probably choose plants where immatures are already present, resulting in the observed grouping pattern.

Key words: *Actinote*, *Austroeupatorium*, host plant selection, oviposition.

Introduction

For most holometabolous insects in general, and with herbivores in particular, adults are more mobile than immatures, with the decisions of ovipositing females often critical for the fitness of the offspring (Doak *et al.*, 2006). For most herbivorous insects, host plant acceptability and quality vary strongly among plant species, populations and individuals and even among different plant parts (Strong *et al.*, 1984; Price, 1997; Kerpel *et al.*, 2006). The ability of females to choose among different hosts or host parts has important consequences on their offspring's performance, with the females usually using plant cues to identify the most suitable food resource. Many different cues are used by females during the oviposition process. These include secondary compounds, visual signals (plant and leaf shape), presence of natural enemies or mutualists, presence of conspecific immatures, microclimate (Rausher, 1978; Williams & Gilbert, 1981; Freitas & Oliveira, 1996) and plant vigour (Price, 1991, 1997). Furthermore, ovipositing females can also respond to indirect cues such as leaf age and size, internode length (Price *et al.*, 1987; Freitas *et al.*, 1999), plant apparency, grouping and position of host plants across the landscape (Feeny, 1976; Courtney & Courtney, 1982; Mackay & Singer, 1982).

The Neotropical genus *Actinote* Hübner, [1819] (Nymphalidae: Heliconiinae: Acraeini) has 31 described species distributed through Central and South America, reaching maximum diversity in the montane regions in the Andes and Southern Brazil (Francini *et al.*, 2004; Lamas, 2004; Paluch, 2006; Paluch *et al.*, 2006; Silva-Brandão *et al.*, 2008). All known species feed on Asteraceae and are gregarious during all stages (Francini, 1989, 1992; Paluch *et al.*, 2005; Freitas *et al.*, 2009).

The widespread *Actinote pellenea* Hübner, [1821] has 17 recognized subspecies distributed across all South America from Colombia to Northern Argentina found over a wide variety of secondary and open habitats (Francini, 1989, 1992; Paluch, 2006). In coastal Southern Brazil, *A. pellenea pellenea* Hübner, [1821] is very common with four or five generations per year. Here larvae of *A. p. pellenea* feed on three species of Asteraceae: the vines *Mikania micrantha* and *Mikania cordifolia*, and the shrub *Austroeupatorium*

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Received: 22 June 2009
Accepted: 25 June 2009

inulaefolium (Francini, 1989, 1992) (Fig. 1).

The following study describes the pattern of oviposition distribution of *A. p. pellenea* on patches of *A. inulaefolium*, and discusses the factors that may explain the observed patterns.

METHODS

Study sites

The study was carried out at two coastal sites of São Paulo State, Southern Brazil: 1) Xixová-Japuí State Park (JAPUI), São Vicente, São Paulo (23°59' S, 46°23' W), in July 1st and 2nd , 1991, and 2) the valley of the Cubatão river, (VRCUB), Cubatão, São Paulo (23°53' S, 46°27' W) during November 12, 1992. Both sites are covered by lowland subtropical forest (Ururahy *et al.*, 1984). Annual rainfall reaches 2500 mm and the average annual temperature is 21°C (Setzer, 1949; Nimer, 1989). Field work was conducted along open trail edges and in early succession stages secondary vegetation.

Sampling procedures

At each site all individuals of *A. inulaefolium* present along a previously defined linear transect of 200 m were sampled, including all plants to a 5 meters distance on both sides of the transect, including all nearby plants in the area. Immatures of *A. p. pellenea* of each host plant were recorded and all ovipositions were collected for laboratory work. Each individual plant was tagged and recorded for height (five classes of 100 cm), distance of the nearest plant (in meters), orientation in relation to the nearest plant (in degrees), number of leaves with immatures, total number of immature groups and total number of immatures. For each leaf with immatures, the area, inclination (to the horizontal), orientation (in degrees to the central axis of the plant), amount of herbivory and number of immatures per group were recorded. An "apparency index" (AI, following Feeny, 1976) was calculated for each individual plant, as: $AI = [(Ho - Ha + DA) + (Ho - Hb + DB) + (Ho - Hc + DC)] / 3$; where Ho = plant height, Ha, Hb, Hc = height of the nearest three plants of the species, and DA, DB, DC = distance of the nearest three plants of the same species present along the linear transect. This index is lowest (including negative values) when the distance between a pair of plants is less than the lower plant of the pair. The index increases with increasing distance between the two plants. To determine the distribution of the plants, the study area was included in a 108 m^2 rectangle, and this was subdivided in 27 squared sectors of 2x2 m (three rows and nine columns). The distribution pattern of plants was determined using the index of dispersion (Ludwig & Reynolds, 1988) with values < 1 indicating a uniform distribution, = 1 a random distribution and > 1 a grouped distribution.

Inclination and orientation of each leaf was estimated with compass and protractor with a precision of 1°. Each oviposition was assigned to one of three developmental stages based on predominant egg colour following Francini (1989), where Y = yellow (0-1 day after oviposition), O = orange (1-3 days after oviposition), R = red (more than 3 days after oviposition), B = black (1 or two days before eclosion). First instars were also considered in the present study, since larvae usually remain, together with their empty egg shells, on the same leaf for the first days following eclosion.

All measured leaves were scored into four categories of herbivore damage: (0) 0%; (1) up to 10%; (2) 11% to 25%; (3) 26% to 50%; (4) more than 51%. Since the leaves of *A. inulaefolium* are nearly rhombus-shaped, leaf area (in cm^2) was estimated using the formula $(L*W)/2$, where L = length and W = width.

Egg density for each oviposition was estimated from the average of five independent counts of eggs over different sections of each oviposition event, giving the number of eggs per cm^2. The number of eggs in each oviposition was estimated as the product of the average density by the area of the oviposition.

RESULTS

Host plant density. At JAPUI 23 plants of *A. inulaefolium* were inspected showing a density of 0.21 plants/m^2 with a non-random distribution (Poisson test, $\chi^2 = 4.2031$, DF = 2, $p < 0.05$). The dispersion index was 1.331, indicating that the plants are grouped. At VRCUB 11 plants were inspected with a density of 0.10 plants/m^2 with a random distribution (Poisson test; $\chi^2 = 0.7365$, DF = 1, $p > 0.70$) and dispersion index of 0.8042, which indicated a homogenous distribution.

Host plant height. At JAPUI *A. inulaefolium* height ranged from 100 to 500 cm ($\bar{x} = 213.0$ cm, SD = 109.98, n = 13), significantly lower than from VRCUB, that ranged from 300 to 700 cm ($\bar{x} = 472.7$ cm, SD = 110.37, n = 11) (t test = -6.4339, DF = 32, $p < 0.001$). At JAPUI the AI (apparency index) ranged from -100 to 1760 while at VRCUB the range was greater, from 800 to 3040, indicating that plants with high apparency co-occur with plants with low apparency. There was no correlation between the AI and plant height at either site (Spearman, r = 0.0689 in JAPUI, t = 0.3163, DF = 21, $p > 0.75$, and r = 0.1908 in VRCUB, t = 0.5831, DF

= 9, P > 0.57).

Distribution of immatures. At JAPUI 43 groups of immatures (eggs or first instar larvae) were recorded on 42 leaves of all plants with the number of groups per plant ranging from 0 to 9 ($\bar{x}$ = 1.9 ovipositions/plant, SD = 2.40, n = 23) and a dispersion index of 3.078. At VRCUB 41 groups of immatures were recorded on 41 leaves of all plants, with a range from 0 and to 11 ($\bar{x}$ = 3.7 ovipositions/plant, SD = 12.82, n = 23) and a dispersion index of 3.439. The values of the dispersion indexes indicate that distribution of immatures on the plants of both sites was grouped. Ovipositions were recorded on leaves ranging from 15 to 450 cm above ground at JAPUI and from 130 to 500 cm at VRCUB. When grouped into intervals of 100 cm, the data showed a concentration of 27 ovipositions within the interval 101 - 200 cm in JAPUI, and of 16 ovipositions in the interval 201 - 300 cm in VRCUB. A double oviposition on the same leaf was observed once during the study.

Oviposited leaves. The average surface area of leaves that showed oviposition at JAPUI ($\bar{x}$ = 2553.21 mm2, SD =1929.36, n = 58) was significantly lower than at VRCUB ($\bar{x}$ = 4549.3 mm2, SD = 2653.82, n = 25) (t test = -3.8755, p < 0.0002, DF = 81). There was no preferential orientation of oviposited leaves at either site (JAPUI χ^2 = 2.00, p = 0.57, DF = 3; VRCUB χ^2 = 3.37, p = 0.34, DF = 3). With respect to leaf inclination, most oviposited leaves were between 10° and 20° from the horizon at both sites. Considering only leaves showing oviposition, most were leaves with low herbivory damage (0 to 10% herbivory) at both JAPUI (67.6%) and VRCUB (92.6%). However, because the patterns of leaf orientation and inclination, and herbivory, were not evaluated for all plants, these data are not useful in showing tendencies or preferences by ovipositing females.

Eggs x plants. At JAPUI the number of eggs per plant ranged from 0 to 3850, with a total of 20,100 eggs from 14 of 23 plants ($\bar{x}$ = 873.9 eggs/plant, SD = 1154.42) and with a dispersion index of 1525, a significant grouped pattern. No significant correlation was determined among the following parameters: number of eggs and plant height (Spearman, r = 0.1179, t = 0.5442, DF = 21, p > 0.59), number of ovipositions and plant height (Spearman, r = 0.0982, t = 0.4521, DF = 21, p > 0.66), the AI and number of eggs per plant (Spearman, r = -0.1485, t = -0.6879, DF = 21, p > 0.49), or the AI and number of ovipositions per plant (Spearman, r = -0.3571; t= -1.7521, DF = 21, p < 0.09). At VRCUB the number of eggs per plant ranged from 338 to 4236 (all plants with at least one oviposition) giving a total of 15,880 eggs on 11 plants ($\bar{x}$ = 1443.6 eggs/plant, SD = 1399.91) and with a dispersion index equal to 1357.55. These data also revealed a significant grouped pattern. Again, as at JAPUI, no significant correlation was demonstrated between the following parameters: number of eggs and plant height (Spearman, r = 0.1908, t = 0.5830, DF = 9, p > 0.57), number of ovipositions and plant height (Spearman, r = 0.2435, t = 0.5392, DF = 9, p > 0.6), the AI and number of eggs per plant (Spearman, r = 0.3184, t = 0.31, DF = 9, p > 0.76), or the AI and the number of ovipositions per plant (Spearman, r = 0.3184, t = 1.0078, DF = 9, p > 0.33).

Figure 1. A female *Actinote pellenea pellenea* ovipositing in *Austroeupatorium inulaefolium*.

Eggs x leaves. At JAPUI the average density of eggs ranged from 111 to 470 eggs/cm^2 ($\bar{x}$ = 259.5 eggs/cm^2, SD = 68.32, n = 57) with the number of eggs per oviposition ranging from 86 to 1266 ($\bar{x}$ = 479.2 eggs, SD = 228.93, n = 56). There was no significant correlation among the following parameters: number of eggs and leaf area (Spearman, r = 0.1054, t = 0.7934, DF = 56, p < 0.43), number of eggs and leaf orientation (Spearman, r = -0.0114, t = -0.0856, DF = 56, p > 0.93), or number of eggs and leaf inclination (Spearman, r = 0.0144, t = 0.1078, DF = 56, p > 0.91). At VRCUB the average density of eggs was from 95 to 374 eggs/cm^2 ($\bar{x}$ = 238.5 eggs/cm^2; SD = 66.81, n = 27) with the number of eggs per oviposition ranging from 100 to 883 ($\bar{x}$ = 422.31 eggs, SD = 199.01 eggs; n = 26). And again there was no significant correlation among the parameters: number of eggs and leaf area (Spearman, r = -0.2736, t = -1.3644, DF = 23, p > 0.18), number of eggs and leaf orientation (Spearman, r = -0.0415, t = -0.1991, DF = 23, p > 0.84), or number of eggs and leaf inclination (Spearman, r = 0.0327, t = 1.1571, DF = 23, p < 0.87). Correlations between leaf area and egg density were also not significant at either JAPUI (Spearman, r = -0.718, t = -0.339, DF = 55, p > 0.9) and in VRCUB (Spearman, r = -0.1327, t = -0.6419, DF = 23, p > 0.52).

DISCUSSION

Our study was not conclusive in revealing any consistent pattern of oviposition in *A. p. pellenea*, except for clearly indicating that ovipositions tend to be grouped. The morphological plant traits investigated by our study were apparently not used by the females when selecting oviposition sites. There are of course several additional factors that would be important in selection by oviposition sites by *A. p. pellenea* females that were not evaluated in our study, such as: 1) other plant features, such as secondary compounds, nutritional quality and/or vigor (Kerpel *et al.*, 2006), 2) presence of alternative host plants in the same area (the common scandent vines *M. micrantha* and *M. cordifolia*) that might influence the patterns we found, and 3) a strong preference for plants previously oviposited by females (Ulmer *et al.*, 2003). Despite of which factors are influencing female choice, it is worth noting that the grouped pattern of immatures was revealed at both sites. As a result, many plants were not used for oviposition females at all, by contrast to a few that received up to nine ovipositions (> 3000 eggs). The advantages of grouped eggs are well known for many species of Lepidoptera, including protection against desiccation and predation (Stamp, 1980; Clark & Faeth, 1998). Gregarious larvae from egg clusters also benefit from increased development rates and survival as well as reduced predation and parasitism (Lawrence, 1990; Clark & Faeth, 1998; Denno & Benrey, 1997). On the other hand, as pointed out above, super-oviposition results in high mortality of small larvae which will not get enough food as we frequently observed in the field. The reasons for this grouped pattern of immatures in *A. p. pellenea* require further investigation to reveal if the advantages in many larvae feeding in the same plant are higher than the risk of death by starvation. Additionally comparisons with other species of *Actinote* are also needed to reveal if the pattern of group immatures can be generalized in this genus.

ACKNOWLEDGEMENTS

We would like to thank Renato Rogner Ramos for help in field work, and Carla Penz, Daniela Rodrigues and Rudi Mattoni for valuable suggestions in the manuscript. This study was funded by FAPESP (grants 84/0432-3, 86/0618-5, 88/3069-8 and 93/0097-9 to RBF, and grants 00/01484-1 and 04/05269-9 for AVLF) and is part of the project "Lepidoptera of São Paulo State" (BIOTA-FAPESP program – grant 98/05101-8). André V. L. Freitas thanks also the Brazilian CNPq (fellowship 300282/2008-7) and the National Science Foundation (DEB-0527441).

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Journal of Research on the Lepidoptera 42: 79-83, 2003 (2010)

NOTES

A new subspecies of *Argynnis nokomis* from the Sacramento Mountains of New Mexico (Nymphalidae)

The most vexing question in New Mexico butterfly lore long was, "Did the Sacramento Mts. ever support the Nokomis Fritillary?" At Paul Grey's instigation, I first started searching for colonies in 1963. The next 44 years produced nothing. I still believed that colonies had once existed at Bent, Otero Co., and at Ft. Stanton, Lincoln Co., but I had only utter frustration to show for it. Eventually, I reasoned where any museum specimens would most likely be, and had the AMNH collection searched accordingly. *Eureka*–two male *Argynnis nokomis* from Bent, Otero Co. This success inspired John Rawlins to search the Carnegie. Result: more specimens, including two of the magnificently colored dark yellow-green females (Holland, 2008).

Within the current concept of *A. nokomis* ssp., the Sacramento Mts. population is distinctive. This case deals with a most likely extinct, high-profile organism that may come to be called by an English name in town meetings. I would prefer that name be easily translated into Latin, with the result being near its actual Latin name. "The Tularosa Fritillary" seemed reasonable in this context. My use of *Argynnis* follows the recent phylogenetic placement by Simonsen *et al.* (2006).

Argynnis nokomis tularosa R. Holland, new subspecies

Diagnosis: The general shape of all markings, black and silver resemble typical *A. n. nokomis.* Two known female specimens lack the fulvus spot on DHW costa, DHW with reduced eyespots on the PM band, silvered spots on disc reduced, DFW and DHW with reduced black scaling along the veins.

Holotype: Female, Mescalero, Tularosa River, Otero County, New Mexico, ca. 7000', Aug. 13, 1931, leg. W. Huber, CMNH collection, ex. Philadelphia Academy of Sciences Collection.

Paratypes: 2 males, Bent, Otero County, New Mexico, ca. 6000', Aug. 12, AMNH collection, ex Paul Grey coll., ex. Paul Ehrlich coll. Year is not specified, but Paul Ehrlich was born in 1932, and Paul Grey donated his collection to the AMNH in 1948. 1 female, data as per holotype.

Identification: The key below will distinguish *A. n. tularosa* from all other subspecies based on females characters and provide my diagnosis. Males are harder to separate.

Etymology: The name is feminine, as that of the nearest town and oldest European settlement in the Tularosa Valley or Tularosa Basin. Tularosa itself is not a Spanish root, but is Nahuatl (Aztec), meaning "cattail" (Julyan, 1996). One is tempted to speculate it has the same etymology as the English word "toolies."

Habitat: The past and present habitats are described in detail by Holland (2008).

Comments: The locality where the specimens are from give encouragement to the possibility *A. n. tularosa* is not extinct. All known specimens were taken on the Mescalero Apache Indian Reservation. The Mescaleros were born xenophobic and live in alpine meadows an hour outside El Paso which has done little to make them trust dem city slicker Texan strangers. The Mescaleros

Key to the subspecies of *Argynnis nokomis*.

1. Sexually dimorphic 2
1a Not so .... ***wenona*** (dos Passos & Grey)
2. Dorsally yellow green in the lighter wing portions 3
2a These areas very blue, discal cell of VFW black and silver .... ***coerulescens*** (W. Holland)
3. DHW discal cell yellowish, silver spots large, dorsally the eyespots in the PM band reduced, fulvus spot on costa of DHW absent .... ***apacheana*** (Skinner)
3a Not so 4
4. Very black wings, terminal regions of both wings, both surfaces, almost solid black. DHW wanting a fulvous spot, VHW silvering of disc reduced .... ***nitocris*** (W. H. Edwards)
4a Not so 5
5. The typical *nokomis* cluster
5a DHW with fulvus spot on costa about 80%. DHW with large eyespots in p.m. band, silvered spots in disc large, DFW and VFW with heavy black scaling along veins, occurs in few large colonies, fore wing > 41 mm, fulvous scaling not always present in DFW yellow-green areas, yellow-green does not invade DFW cell .... Sangre de Cristo typical ***nokomis***
5b DHW with fulvus spot on costa nearly always, DHW with large eyespots in the p. m. band, silvered spots in disc large, DFW and VFW with heavy black scaling along the veins, occurs in a myriad of tiny colonies, fore wing > 40 mm, noticeable fulvus scaling in DFW yellow-green areas, yellow-green may invade DFW cell Chuska Mts., Navajo Res. .... ***nigrocaerulea*** (W. & T. Cockerell)
5c Two known specimens lack fulvus spot on DHW costa, DHW with reduced eyespots on the p.m. band, silvered spots on disc reduced, DFW and DHW with reduced black scaling along the veins; not seen in 70 years and feared extinct, wingspread closer to typical *nokomis* than *nigrocoerulea*, Sacramento Mts., Mescalero Res. .... ***Speyeria nokomis tularosa***

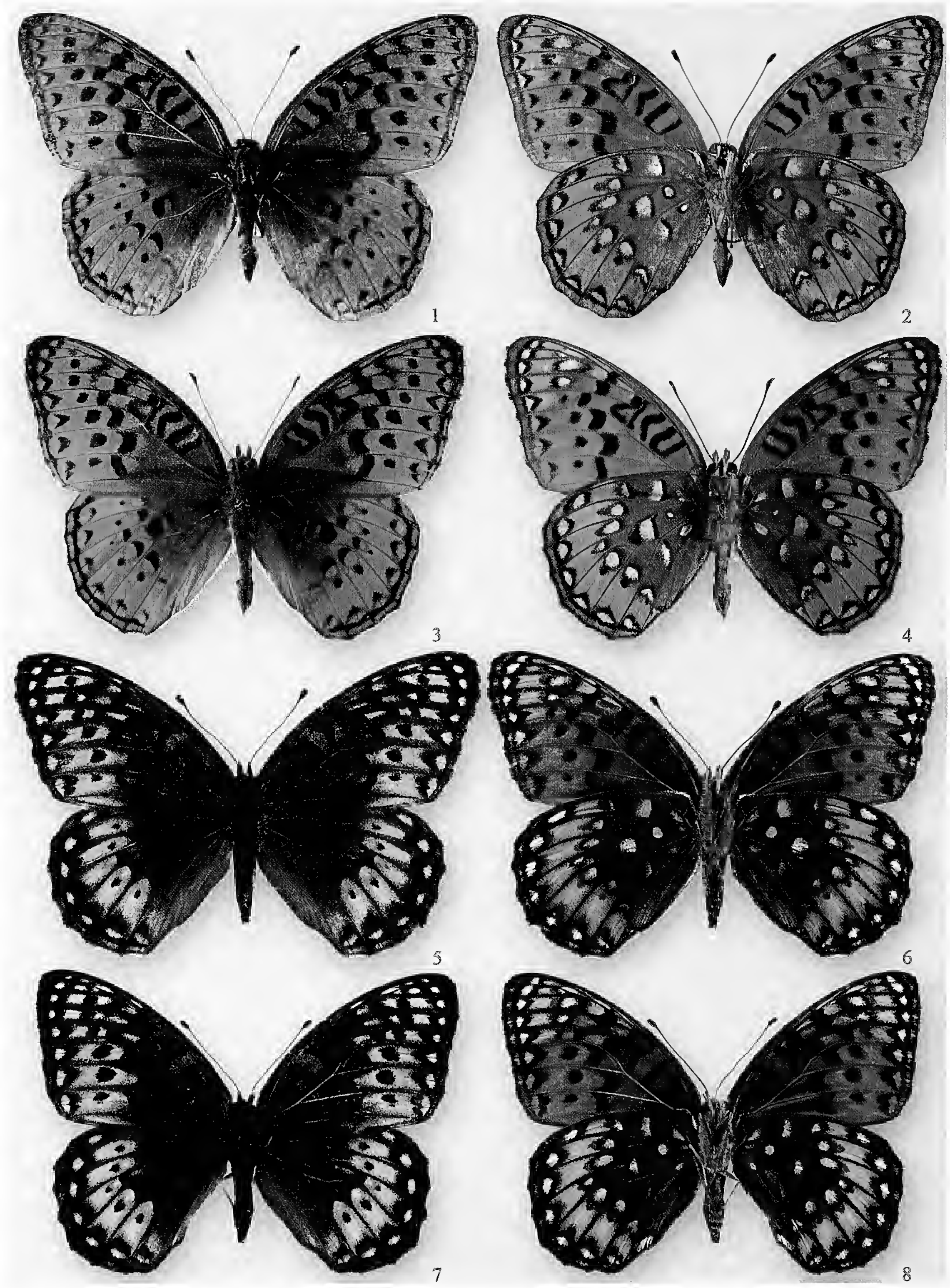

Figure 1. The Sacramento Mountains *Argynnis nokomis tularosa* population. Top two rows, males, Bent, Otero County, New Mexico, ca. 6000', Aug. 12, AMNH collection, ex Paul Grey coll., ex. Paul Ehrlich coll. Year is not specified, but Paul Ehrlich was born in 1932, and Paul Grey donated his collection to the AMNH in 1948. Bottom two rows, females, Mescalero, Tularosa River, Otero County, New Mexico, ca. 7000', Aug. 13, 1931, leg. W. Huber, CMNH collection, ex. Philadelphia Academy of Sciences Collection. The specimen in the third row is designated the female holotype. Of the two known females, it is the only one with both antennae intact.

patrol every inch of road like they were providing the Coliseum with virgins; merely stopping is forbidden. Permits to collect, survey, or just watch the wildlife are very nearly unobtainable: they don't want you spotting anything endangered, the existence of which could be cause for limiting the absolute control the tribe enjoys on its land. The endemic *Euphydryas anicia cloudcrofti* Ferris and R Holland, is known right up to the reservation line, but there is not one report from on the reservation anywhere in the public domain. The only person I ever knew to negotiate successfully to collect on the Mescalero Reservation posed *au natural* for a tribal art class in exchange.

ACKNOWLEDGEMENTS

My sincerest thanks to Jocelyn Gill of the Canadian National Museum for the magnificent photo work that can protect an irreplaceable national asset from pointless handling. All specimens of *Argynnis nokomis* from Otero Co. in either the AMNH or the Carnegie are paratypes, the holotype in the latter institution.

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Use of *Hippuris*, an emergent aquatic plant, as a larval host by the buckeye, *Junonia coenia*, in Northern California

Recent advances in DNA-sequence-based phylogeny have radically altered botanists' concepts of the relationships within the old family Scrophulariaceae and between the now-disaggregated components of that family and others previously classified in a variety of ways (Olmstead *et al.*, 2001; Kadereit *in* Kubitzki & Kadereit, 2004). In addition to DNA evidence, the distribution of characteristic secondary phytochemicals affords a partially-independent indication of plant relationships. In that vein, host-plant choices by oligophagous insects may suggest underlying chemical, and thus potentially phylogenetic, affinities among the taxa involved. The chemical basis for host selection in various Melitaeini (Nymphalidae) is the presence of the bitter compounds called iridoid glycosides (Bowers & Puttick, 1986; Gardner & Sternitz, 1988). Shapiro and Hertfelder (2009) recently reported the iridoid-selecting variable checkerspot, *Euphydryas chalcedona*, feeding spontaneously, repeatedly and successfully on the exotic garden shrub butterfly bush, *Buddleja davidii*, historically placed in the Loganiaceae or its own family Buddleiaceae but now incorporated into Scrophulariaceae.

The common buckeye, *Junonia coenia* is also a Nymphalid but not a Melitaeine, and its host range in California embraces Scrophulariaceae, the very

Figure 1. The Biggs garden pond. Emergent stand of mare's tail at right.

Figure 2. Two buckeye larvae, *Junonia coenia*, feeding on mare's tail *in situ.*

closely-related Plantaginaceae, and the genus *Phyla* (=*Lippia*) in the Verbenaceae (Shapiro & Manolis, 2007). The chemical basis for host selection in this species has been shown to involve the presence of iridoid glycosides (Bowers, 1984) although the story must be more complicated insofar as some Verbenaceous genera known to produce iridoids, such as *Lantana* (Rimpler & Sauerbier, 1986) are common in buckeye environments but never utilized.

Mare's tail, *Hippuris vulgaris*, is an emergent aquatic flowering plant with a superficial resemblance to a horsetail (Equisetaceae); it is widely distributed in the cooler parts of both North and South America but rather rare and local in California where, however, it is occasionally grown in garden ponds. It has been classified in the monotypic family Hippuridaceae, whose affinities have been obscure until recently although several authors placed it near the Scrophulariaceae. Iridoid glycosides were reported in mare's tail by the pioneering phytochemist Hegnauer in the 1970s and confirmed by Damtoft *et al.* (1994). Their importance for plant systematics was emphasized by Jensen *et al.* (1975) and El-Naggar and Beal (1980). Grayer *et al.* (1999) noted the convergence of phytochemical and molecular-phylogenetic data in the group of families around Scrophulariaceae, and subsequent authors have treated Hippuridaceae as a member of the "Scroph" clade (Kadereit, 2004).

Given these facts it was not completely surprising when one of us (KB) found common buckeye larvae feeding on mare's tail in her home pond at Sebastopol, CA (see photo)—twice in the pond's 12-year existence. We subsequently learned that Mr. Michael Koslosky found buckeye larvae on the same plant "about ten years ago while shopping at Connie's Pond Supply in Castro Valley [CA]" and reared them out successfully on it (M. Koslosky, pers. comm.). This is the only emergent aquatic plant known to be a buckeye host. It is not clear whether a larva can complete development on a single shoot or has to access an adjacent one at least once in its development; KB has seen them use downed stems as "bridges." The stems are tall enough to permit pupation and eclosion well above the water line.

The distribution of iridoid glycosides is such that many other plants not known to be buckeye hosts are potentially usable. One of us (AMS), based on the confirmed presence of iridoids in princess tree, *Paulownia* (Bignoniaceae) foliage (Lino von Poser *et al.*, 2000), has on several occasions confined buckeye females on it, obtained eggs easily, and reared the larvae through to the adult on it. The same is true on both *Catalpa speciosa* and *C. bignonioides* (currently but shakily placed in Bignoniaceae or Scrophulariaceae), which also produce iridoids (Sha'ban *et al.*, 1980; Iwaga *et al.*, 1991). All of these are trees, and there are no records of the common buckeye using any tree as a host. However, the tropical buckeye, *Junonia genoveva*, feeds on black mangrove, *Avicennia* (Avicenniaceae, sometimes put in Verbenaceae) and occasionally on *Lippia* (Scott, 1986), and these are iridoid producers. There is a suggestion that host selection by these butterflies is mediated by both apparency (growth form) and, at close range, iridoid chemistry. Clearly, we have a lot to learn.

ACKNOWLEDGEMENTS

We thank Mr. Jeffrey Caldwell for getting us in touch, and Mr. Michael Koslosky for sharing his record with us.

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EDITORIAL

Auf Wiedersehen Gutenberg

The recent trends of disappearing print media - words and images on paper - is disturbing. Many newspaper are already extinct, and excepting parts of the financial press, more are seriously endangered. Scientific print publications are also threatened by the global financial crisis as budgets everywhere are being drastically cut back. More distressing is serious economic analysis that indicates the situation is likely to become much worse, or not much better for quite some time. University and research institution libraries are shiny targets as bureaucratic managers scramble to save their jobs by demonstrating "waste." After all we can get many things on line for almost free, and if Google gets its way we might be able to abandon whole libraries with attendant savings. Who needs books? Especially as one analyst claims $130 billion a year will be saved by going wholly on line.

Elimination of transitory matter, novels, magazines and so forth will save many trees, yet all print media share one basic issue that cannot be overlooked - permanence. A good friend of mine made a documentary film a decade ago, Slow fires: on the preservation of the human record, which focused on the issue of questioning the data loss in the storage media. We all know papyrus texts survived thousands of years. There is no test of reliability of any other information storage system. As Prof. J. F. Blanchette of UCLA put it "Imagine of the only copy left of Imaging in Oncology were the Kindle version, with its garbled tables and lost color coding? Or, a more likely scenario, if several copies of the book existed in different formats, each with a different visual presentation?" Then there is long range political stability. A luddite dictatorship would have a grand following for a data farm *auto de fe*.

So science presses face an increasing problem of how to pay for printed copy when there is increasing resistance concerning cost. Our subscription base does not begin to cover costs and the base is shrinking. Institutional subscribers are also canceling. No funds. We are investigating on line publishing, whereby a small print run by a Docutech system will provide a limited press run for archival purposes, along the lines of "Zootaxa."

Lastly, and perhaps most disturbing is an observation by an academic colleague in Europe that students no longer are interested in joining scientific societies. Now if this becomes the postmodern world view, where are we headed and what is to be done? If rape is inevitable, should we relax and enjoy it?

Rudi Mattoni, Editor

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Cover: adult of *Eacles imperialis*, 7 August, 2004. © M. W. Nelson/Massachusetts Natural Heritage & Endangered Species Program.