NATURAL HISTORY OF THE MYRMECOPHILIC SPIDER, *MASONCUS POGONOPHILUS* CUSHING, AND ITS HOST ANT, *POGONOMYRMEX BADIUS* (LATREILLE)

BY

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DEDICATION

I dedicate this to my parents, Paula M. Cushing and Joseph Cushing (deceased), who encouraged my curiosity and taught me to see the world from many different angles, and to my brother, Richard J. Cushing, and my sisters, Joan D. Cushing and Patricia C. Banta, for their humor and love.

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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By

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Masoncus pogonophilus Cushing, a small (2 mm long) spider in the family Linyphiidae, spends all life stages inside the nest chambers of the Florida harvester ant, Pogonomyrmex badius (Latreille). The spiders appear to be commensals, taking advantage of the stable microclimate and abundant food available within the nests. However, the ecology of the host ant does directly affect the ecology of the spider.

The fragility of M. pogonophilus as well as an apparent female-biased sex ratio suggested that dispersal of spiders between ant nests may be uncommon. As this would affect the genetic structure of spider populations, the genetic diversity within and among three populations was measured using the PCR-based Random Amplified Polymorphic DNA technique (RAPD-PCR). Of the total genetic diversity from three populations of spiders, 77.4% was

attributable to intra-population differences, 18.2% to differences between distant populations, and only 4.4% to differences between neighboring populations of spiders. Individuals living within one ant nest are not closely related and spiders from neighboring nests are more similar genetically than spiders from distant nests. Thus, dispersal events of spiders between neighboring colonies are interpreted as occurring at a significant rate.

There is no evidence that the spiders use the trail pheromones of the ants to locate new host colonies. Although the host ants oriented significantly more to extracts of trail pheromones and to natural trails than to control trails, the spiders did not.

The success of dispersal events among the spiders is affected by dispersion of the ant colonies. Dispersion of host colonies is, in turn, affected by habitat structure, resource availability and agonistic interactions between colonies.

A detailed description is presented of the resources available to P. badius colonies in two different habitats as well as what resources (seeds) they store in their granaries. Differences in spacing patterns, densities, and aggressive interactions between P. badius nests in these two habitats are due to a complex set of factors including habitat structure and resource availability.

CHAPTER 1
INTRODUCTION

Many arthropods have evolved symbiotic relationships with ants. Some are found at the periphery of the nest, either near the entrances or on refuse piles; others are found within the chambers of the nest, either in the peripheral chambers or deeper in the nest in the brood and storage chambers (Hölldobler 1977). They range from tiny collembolans to beetles and caterpillars many times the size of their hosts (Hölldobler and Wilson 1990). The formal study of myrmecophiles began with the work of Wasmann in 1894 who developed a classification system for myrmecophiles consisting of distinct categories, each suggesting increasing specialization and integration into the host colony.

In this study, I explore different facets of the natural history and ecology of one myrmecophile and its host ant in an attempt to determine what factors are most important in the integration of this spider into the colony of its host. In general, ants live in complex societies in which only members are allowed. They communicate with their nestmates through chemical and tactile signals, and they tend to aggressively exclude intruders into their colony. The arthropod ant guests, or myrmecophiles, have evolved various adaptations enabling

them to exist in this hostile environment. Many of the myrmecophiles acquire cuticular hydrocarbons similar or identical to those of their hosts (Vander Meer and Wojcik 1982, Vander Meer et al. 1989). This allows them to become integrated with hosts that are otherwise hostile to intruders with foreign, non-colony odors. Others, such as some staphylinid beetles and lycaenid caterpillars, have evolved specialized glands that produce appeasement substances (reviewed in Hölldobler and Wilson 1990).

In many myrmecophiles, the evolution of a symbiotic association can be intimated through an examination of extant species that show varying degrees of behavioral integration (Hölldobler and Wilson 1990). For example, Akre and Rettenmeyer (1966) described species of staphylinid beetles that show varying degrees of association with army ants. Some species live only around the edges of the bivouacs or in the refuse piles but are not otherwise integrated into the colonies, others are found running along the edges and sometimes within the emigration columns of ants, and yet others are found directly in the midst of ants in the center of the emigration colonies. Some species even hitch rides on the booty or the brood carried by ants. Certain staphylinid species can only live within a narrow range of conditions found within colonies and die shortly after removal from the colonies.

If each stage in this process of gradual integration into colonies is correlated with the evolutionary history of the lineages, then the various adaptations of the myrmecophiles leading to greater integration could be viewed as characters on the evolutionary tree (Brooks and McLennan 1991). Kistner (1979) takes this idea a step further by superimposing the phylogenies of termites in the family Rhinotermitidae with their associated termitophiles in the family Staphylinidae to illustrate the evolution of host specificity. Predation pressures may have triggered greater integration into the ant and termite societies in these staphylinid species as well as in other myrmecophiles and termitophiles since association with the aggressive hosts may afford some protection to the guests. Close association with the hosts itself may have led to integration within the colonies. Stable microclimatic conditions within the ant colonies as well as an abundant food supply (either in the form of host brood or other colony guests) would select for even greater integration into the colonies.

Myrmecophilic spiders are unique because their close relatives apparently have no preadaptations to a symbiotic lifestyle. Most spiders are solitary predators and symbiosis with other arthropod groups should be rare; yet myremcophilic spiders are found in the families Agelenidae, Aphantochilidae, Clubionidae, Gnaphosidae, Linyphiidae, Oonopidae, Salticidae, Theridiidae,

Thomisidae, and Zodariidae (Donisthorpe 1927, Bristowe 1939, Noonan 1982, Porter 1985, Hölldobler and Wilson 1990, Boeve 1992). Many of these spiders are specialized ant predators, but several, such as the clubionids in the genus Phrurolithus and the Linyphiid, Masoncus pogonophilus, are found in the company of the host ants and do not feed on the ants or their brood. Such species may be occasional visitors into ant colonies, using the entrance and upper chambers as temporary refuges, or they may be commensals that have become more dependent on the conditions present within the nest and spend their entire lives within this complex ecosystem.

Within the colony chambers of the Florida harvester ant, Pogonomyrmex badius (Latreille) (Formicidae), lives a small, approximately 2 mm long, species of spider, M. pogonophilus (Linyphiidae; Erigoninae). This spider-ant association was first described by Porter (1985). All life stages of M. pogonophilus are found inside the ants' nest. They feed on collembolans (springtails) and perhaps other tiny symbiotic arthropods. All developmental stages of the spiders as well as spider eggsacs are found in all portions of the nests throughout the year (Fig. 1-1). During any given month, they are as likely to be found in shallow as in deep chambers or runways. Therefore, they are not occasional guests but true members of the nest community.

The objectives of this project were to: 1) describe this species of myrmecophile, compare it to other members of the genus, and document its life cycle and life history; 2) determine how the population structure of the host ants affects the population structure of the myrmecophilic spider; 3) determine the dispersal mechanism of the myrmecophiles; and 4) investigate the factors, such as resource availability, habitat structure, and inter-nest competition that directly affect host ant population structure and indirectly affect dispersal of myrmecophiles and integration of myrmecophiles into new host colonies.

Chapter 2 is a formal description of this previously undescribed spider. Only three other species of the genus Masoncus had been described prior to this study: M. arienus, M. conspectus, and M. dux. None of these species is known to be associated with ants. In Chapter 2 I compare M. pogonophilus with the described species and note their morphological differences. I also summarize what is known about the natural history and life cycle of M. pogonophilus based both on my own observations as well as those of Porter (1985).

Both adult and juvenile spiders can be found in P. badius nests throughout the year. The spiders are common in nests within a given area so dispersal of spiders is evidently occurring. However, due to the spiders' susceptibility to desiccation outside the nests and the

huge distances these tiny spiders would have to traverse to locate a new colony as well as to an apparent female-biased sex ratio among the spiders, I hypothesized that dispersal events are uncommon and that spider populations within ant colonies started with one or a few founding females. This type of population founding was suggested by Williams and Franks (1988) for a myrmecophilic isopod. They suggested that the myrmecophiles remained within a single colony for several generations until that colony died, at which time the myrmecophiles would disperse and a few would locate a new host nest. If this scenario were true for M. pogonophilus, then spiders within a nest should be genetically more similar to each other than to spiders from different nests. In Chapter 3, I test this hypothesis using the Random Amplified Polymorphic DNA (RAPD) fingerprinting technique.

In Chapter 4, I attempt to determine the dispersal mechanism of the spiders. Pogonomyrmex badius emigrates to new nest sites, usually less than 5 m from the old site. Such emigrations are common in this species of harvester ant (Golley and Gentry 1964, Gentry and Stiritz 1972, Gordon 1992). Between 60 and 97% of the colonies in an area migrate to a new nest site once a season, a few (under 30%) migrating two or three times (Carlson and Gentry 1973). When the ants move, so do the spiders and the symbiotic collembolans. The symbionts move in the

emigration trail on their own accord and rarely veer out of the trail. I observed several such emigrations of the myrmecophiles. These field observations suggested that the spiders might detect and follow colony odors. I hypothesized, based on these observations, that spiders were locating new colonies by following trail pheromones of the ants, perhaps by following foraging trails away from the host nests until they located the foraging trail of a neighboring colony which they then followed to the new mound. I test this hypothesis using chemical bioassays of trail pheromones as well as using bioassays with a naturally laid trail.

In Chapters 5 and 6, I shift the focus of the study toward the population structure and foraging ecology of the host ants since the population structure of the host ants indirectly affects the dispersal of the spider and integration of the myrmecophile into new nests. Closely spaced P. badius nests, for example, would be potentially easier for dispersing spiders to locate. However, if neighboring nests are aggressive towards one another and if spiders are absorbing host colony odors, then dispersing spiders arriving at a new nest may face a behavioral barrier from aggressive ants. The population structure of P. badius, in turn, is likely influenced by resource availability and habitat structure. Chapter 5 deals exclusively with resource use by the harvester ants and the effect of habitat structure on resource

availability. This information is used to explain patterns of nest dispersion and inter-nest aggression described in Chapter 6.

In Chapter 6, I explore the dispersion of P. badius colonies and the possible influence of inter-nest aggression on nest dispersion. Inter-colony competitive interactions are implicated in many studies of nest dispersion among ants (de Vita 1979, Levings and Traniello 1981, Harrison and Gentry 1981, Cushman et al. 1988). Inter-colony aggression between colonies of P. badius had not been quantitatively evaluated prior to this study. I considered this information crucial to understanding what behavioral barriers dispersing spiders might have to face from hostile ants. In Chapter 6, I also explore whether there is behavioral evidence to support the hypothesis that M. pogonophilus uses chemical mimicry to integrate itself into ant colonies.

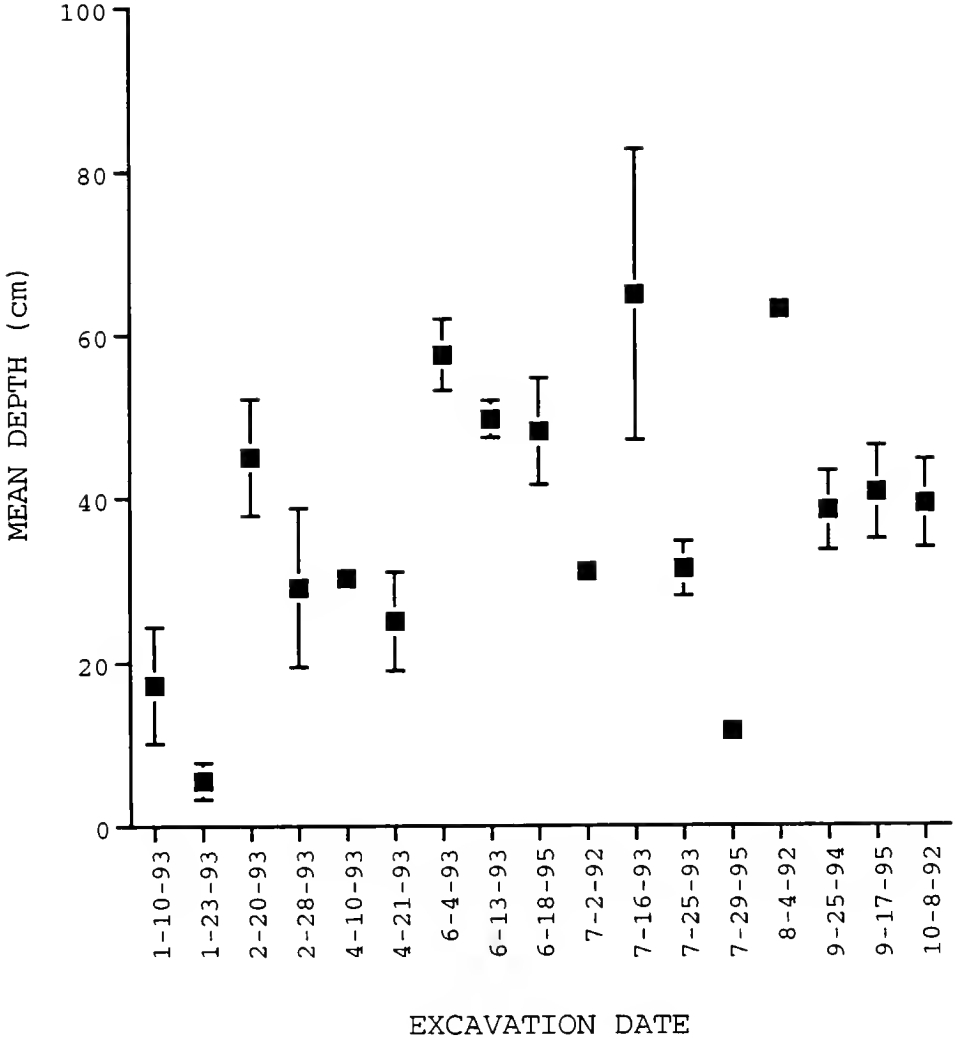


Figure 1-1. The mean depths ± standard error of samples of spiders found during 17 different excavations. The excavation dates range from January 10th to October 8th. The dates are listed in order by month rather than by actual order of excavation. The number of spiders making up each sample are shown above the error bars.

CHAPTER 2
DESCRIPTION OF THE SPIDER *MASONCUS POGONOPHILUS*, N. SP. (ARANEAE: LINYPHIIDAE) -- A HARVESTER ANT MYRMECOPHILE

Three species are included in the genus *Masoncus* Chamberlin 1948: *M. arienus* Chamberlin 1948, *M. dux* Chamberlin 1948, and *M. conspectus* (Gertsch and Davis 1936) (synonymized with *M. nogales* Chamberlin 1948 by Ivie 1967). The female holotype of *M. dux* has been lost and I was unable to locate any specimens of this species. The female holotype, male allotype and paratypes of *M. nogales* designated by Chamberlin (1948) have also been lost. However, the holotype of *Tapinocyba conspecta* is deposited at the American Museum of Natural History in New York, NY (AMNH) as are other representatives of this species. The holotype and paratypes of *M. arienus* designated by Chamberlin (1948) are also at AMNH. One male representative of *M. arienus* is deposited at the California Academy of Sciences in San Francisco, CA (CAS).

No information was recorded either in the original species descriptions or on the collecting labels of the existing specimens regarding the natural history of the described species. *Masoncus dux* was described from a single female collected in northern Manitoba, Canada. All specimens of *M. arienus* were collected in Arizona. *Masoncus conspectus* was described from the male holotype

and two male paratypes collected in Texas. Other records of this species include Arizona and Florida (the latter specimen collected by the shores of Newnan's Lake in Alachua County).

Masoncus pogonophilus n. sp. was originally collected by Sanford Porter from the nests of the Florida harvester ant, Pogonomyrmex badius (Latreille) (Hymenoptera: Formicidae) near Tallahassee, Florida in Leon County (Porter 1985). It is included in the genus Masoncus due to the presence of distinct cephalic pits and a straight, distally bifid embolic division in the males (see genus description below).

In the species description that follows, I use primarily carapace, genitalic, chaetotaxic, numeric, and palpal characters deemed most useful by Millidge (1980) for erigonine spiders. These characters include: 1) the overall conformation of the male palpal organ, 2) the shape of the embolic division, 3) the external appearance of the epigynum, 4) the number of dorsal trichobothria present on the palpal tibia of both sexes, 5) the number of dorsal tibial spines present (expressed by the formula a:b:c:d), 6) the number of dorsal metatarsal trichobothria present (expressed by the formula I:II:III:IV), 7) the relative position of the dorsal metatarsal trichobothrium on leg I (expressed by the formula TmI = distance from tibia-metatarsus joint to trichobothrium / distance from tibia-metatarsus joint to metatarsus-tarsus joint), and 8)

the relative stoutness of tibia I (expressed by the formula TibI = length of tibia / width of tibia viewed laterally). Overall body size, body color, and number of setae on the carapace are also given. Certain of these characters as well as others used in Chamberlin's (1948) descriptions or obvious on the existing specimens are of particular value in separating M. arienus, M. conspectus, and M. pogonophilus (Table 2-1). All measurements were taken directly off the specimens using an ocular micrometer in a dissecting scope. Measurements were rounded to the nearest 0.1 mm.

Masoncus Chamberlin 1948

The type species of the genus is M. arienus. The genus Masoncus is characterized by both cephalic pits in the males and a straight, distally bifid embolic division (Chamberlin 1948) (diagram of Linyphiid palpal structures in Millidge 1980).

Masoncus pogonophilus new species

(Figs. 2-1 to 2-5)

The male holotype was collected 23 cm below ground inside a nest chamber of the Florida harvester ant, P. badius in Archer Sandhills, 1.4 Km west of the Levy Co. line off of State Road 24. The female allotype was collected from the same P. badius nest. She was found in a nest chamber 46.5 cm below ground. Both were collected 25 September 1994 and both will be deposited in the Arachnological collection at CAS.

The holotype, eleven male paratypes, the allotype, and 12 female paratypes were used in this species description. The collecting information as well as the future museum destination for these paratypes are presented in Table 2-2.

Etymology. The specific epithet is derived from the generic name of the host ant.

Holotype (male). Total body length: 1.7 mm. Carapace length: 0.9 mm. Carapace width: 0.7 mm. Colors: Carapace orange; abdomen grey; legs orange; sternum orange. Number of setae along midline of carapace: three. Palp as in Fig. 2-3. Embolic division distally bifid with the proximal part of the bifurcation bent forward and extending over the most distal part (Fig. 2-4). Number of trichobothria on palpal tibia: two (Fig. 2-2). Number of dorsal tibial spines: 1:1:1:1. Number of dorsal metatarsal trichobothria: 1:1:1:0. TmI: 0.82. TibI: 7.0.

Males (general) - (n = 12). Total body length: 1.6 - 2.1 mm ($\bar{x}$ = 1.8 ± 0.14). Carapace length: 0.8 - 0.9 mm ($\bar{x}$ = 0.9 ± 0.04). Carapace width: 0.6 - 0.8 mm ($\bar{x}$ = 0.7 ± 0.05). Colors: Carapace yellow-orange to orange; abdomen grey; legs yellow-orange to orange; sternum yellow-orange to orange. The color seems to fade severely when specimens are kept in isopropanol rather than ethanol. Number of setae along midline of carapace (Fig.2-1): variable, two to four (setae easily broken in preservation). Palp as in Fig. 2-3. Embolic division as in Fig. 2-4. Number of

trichobothria on palpal tibia: generally two (Fig. 2-2), however one male had two on the left palpal tibia and three on the right and another had three on the left and two on the right. Number of dorsal tibial spines: 1:1:1:1. Number of dorsal metatarsal trichobothria: 1:1:1:0. TmI: 0.82 -0.88 ($\bar{x}$ = 0.84 ± 0.02). TibI: 6.5 - 7.7 ($\bar{x}$ = 7.0 ± 0.35).

Females - (n = 13). Total body length: 1.5 - 1.9 mm ($\bar{x}$ = 1.8 ± 0.13). Carapace length: 0.8 - 1.2 mm ($\bar{x}$ = 0.9 ± 0.11). Carapace width: 0.6 - 0.9 mm ($\bar{x}$ = 0.7 ± 0.09). Colors: same as males. Number of setae along midline of carapace: variable, two to five; females also had smaller setae scattered on either side of midline. Epigynum as in Fig. 2-5. Number of trichobothria on palpal tibia: generally three, however one female had two on both palps, three other females had three trichobothria on the left palpal tibia and two on the right. Number of dorsal tibial spines: 1:1:1:1. Number of dorsal metatarsal trichobothria: 1:1:1:0. TmI: 0.58 - 0.87 ($\bar{x}$ = 0.81 ± 0.09). TibI: 6.5 - 7.9 ($\bar{x}$ = 7.1 ± 0.38).

Diagnosis. The carapace of male M. pogonophilus most resembles that of M. conspectus (Fig. 2-6 from Chamberlin 1948 and Fig. 2-1). In both species, the cephalic pits extend beneath the posterior median eyes (p.m.e.) whereas in M. arienus the cephalic pits open behind the p.m.e. The embolic division of male M. pogonophilus n. sp. most resembles M. conspectus (Fig. 2-11 from Chamberlin 1948 and

Fig. 2-4) in that both are distally bifid with the proximal part of the bifurcation bent forward and extending over the most distal part of the bifurcation. However, in M. pogonophilus the most distal part of the bifurcation is, itself, bifurcated, whereas in M. conspectus it is flattened (although Fig. 2-11 from Chamberlin 1948 shows it to be pointed). In M. arienus the embolic division is also bifid, but the bifurcation begins very close to the tailpiece and each segment of the bifurcation is coiled (see Fig. 2-14 from Chamberlin 1948). The male palpal tibia of the new species, as with M. conspectus and M. arienus, is fringed laterally with long setae (Fig. 2-2). Chamberlin (Fig. 2-15, 1948) does not show this fringe of setae on his drawing of M. arienus but it is evident on the preserved specimens. All three species have two black-tipped processes on the distal edge of the palpal tibia (Fig. 2-2). These processes are more widely spaced in M. arienus than in either M. conspectus or in M. pogonophilus. The black-tipped process in M. conspectus is found on a slight ridge that extends away from the surface of the tibia (Fig. 2-10 from Chamberlin 1948). Interestingly, M. conspectus is the only one of the three previously described congeners whose known distribution extends into northern Florida. The new species can be separated from the congeners based primarily upon characters described in Table 2-1 as well as upon overall size; the new species being somewhat smaller than M. dux,

M. arienus, and M. conspectus which are all between 2.10 and 2.65 mm in length according to Chamberlin (1948) and Gertsch and Davis (1936).

Natural History. Masoncus pogonophilus new species. lives within the nest chambers of the Florida harvester ant, P. badius. It is about one-quarter the size of its 7 - 9 mm long host and feeds on collembolans found throughout the 1 - 3 m deep subterranean nests (Porter 1985). The ant nest provides a stable microclimate as well as an abundant food source for the spider. The spiders have never been collected away from the ant nests and cannot survive if placed on a hot substrate (such as the sand outside the nest in the middle of the day) or if placed in a vial without a constant supply of moisture. They appear, therefore, to be obligate ant symbionts, or myrmecophiles.

Immigration to new nest sites is common in P. badius (Gentry and Stiritz 1972, Golley and Gentry 1964, Gordon 1992). While observing six such colony migrations, each occurring either just after a summer shower, in the early morning when the surface temperature was cool and the humidity high, or during an overcast day, I saw both adult and immature spiders as well as collembolans moving from the old colony site to the new amidst their host ants within the emigration trails. None of these emigrations was over 5 m. Neither the spiders nor the collembolans veered out of the emigration trails suggesting that they were either able to visually follow their hosts to the new

nest sites (unlikely for either of these myrmecophiles) or they were following a trail pheromone laid by the ants (see Chapter 4). Analysis of the genetic structure of spider populations (Chapter 3) indicated that spiders disperse between neighboring ant nests.

Both sexes of M. pogonophilus build prey capture webs in the lab and I have seen webs inside the ant nest chambers. Both males and females produce sticky silk. Therefore, males presumably retain the aggregate and flagelliform glands into adulthood; most adult male araneoid spiders lose these glands during the terminal molt and cannot subsequently produce sticky silk (Kovoor 1987). Maintaining the ability to produce sticky silk as adults may be common among male erigonine Linyphiids as I have observed such behaviors among other (unidentified) male erigonines.

Female M. pogonophilus lay one to six eggs in a disk-shaped eggsac deposited in a depression in the wall of a nest chamber ($n = 9$ eggsacs, $\bar{x} = 2.9 \pm 1.5$ eggs/eggsac). The eggsac is flush against the surface of the chamber walls. Juvenile spiders molt once inside the eggsac and pass through three additional molts before reaching maturity. Since males and females do not differ significantly in size, both sexes probably pass through an equal number of developmental stages and have similar life spans. The longest-lived adult kept in the lab was a mature male that lived for three months before escaping.

He was fed mites and collembolans placed in his web. I succeeded in raising one spider from just after hatching through maturity. It was also fed collembolans and mites placed in its web. This spider died after 2.5 months in captivity, soon after maturing. If these spiders are representative of free-living spiders and if males and females do have similar lifespans, then the minimum lifespan of individual M. pogonophilus is between five to six months.

Juveniles are present inside the ant nests during all months of the year (Porter 1985, pers. obs.). Porter reported a 4:1 female-biased sex ratio among the spiders, while I have found an even more extreme 7.5:1 female-biased ratio (n = 53). Due to the difficulty of raising spiders in the laboratory, it has not been possible to determine whether this is a primary sex-ratio bias.

Table 2-1. Morphological characters most useful in separating three of four Masoncus species. (All specimens of M. dux are lost, and the species description is based solely on the female holotype.) pme = posterior median eyes.

CHARACTERS	M. ARIENUS	M. CONSPECTUS	M. POGONOPHILUS
Location of cephalic pits in males	Pit opens back of posterior eyes; not extending under pme	Pit opens and extends beneath pme	Pit opens and extends beneath pme
Cheliceral spurs towards distal end	Present on males and females	Present on males; reduced to very small black spurs on females	No cheliceral spurs on males or females
Setigerous nodule or spur anterior to fang groove	Nodule present on males and females	Spur on males; lacking on females	No setigerous nodule or spur on males or females
Endites with small spur on ectal side of tip	Present on males and females	Present on males and females although less distinct on latter	No spurs on endites of males or females
Shape of palpal tibia	Widely spaced black tipped processes on distal edge flush w/ surface of tibia; long setal fringe on lateral edge	Closely spaced black tipped processes on distal edge extending slightly away from surface of tibia; long setal fringe on lateral edge (fig. 2-10 from Chamberlin 1948)	Moderately spaced black tipped processes on distal edge flush w/ surface of tibia; long setal fringe on lateral edge (see fig. 2-2)

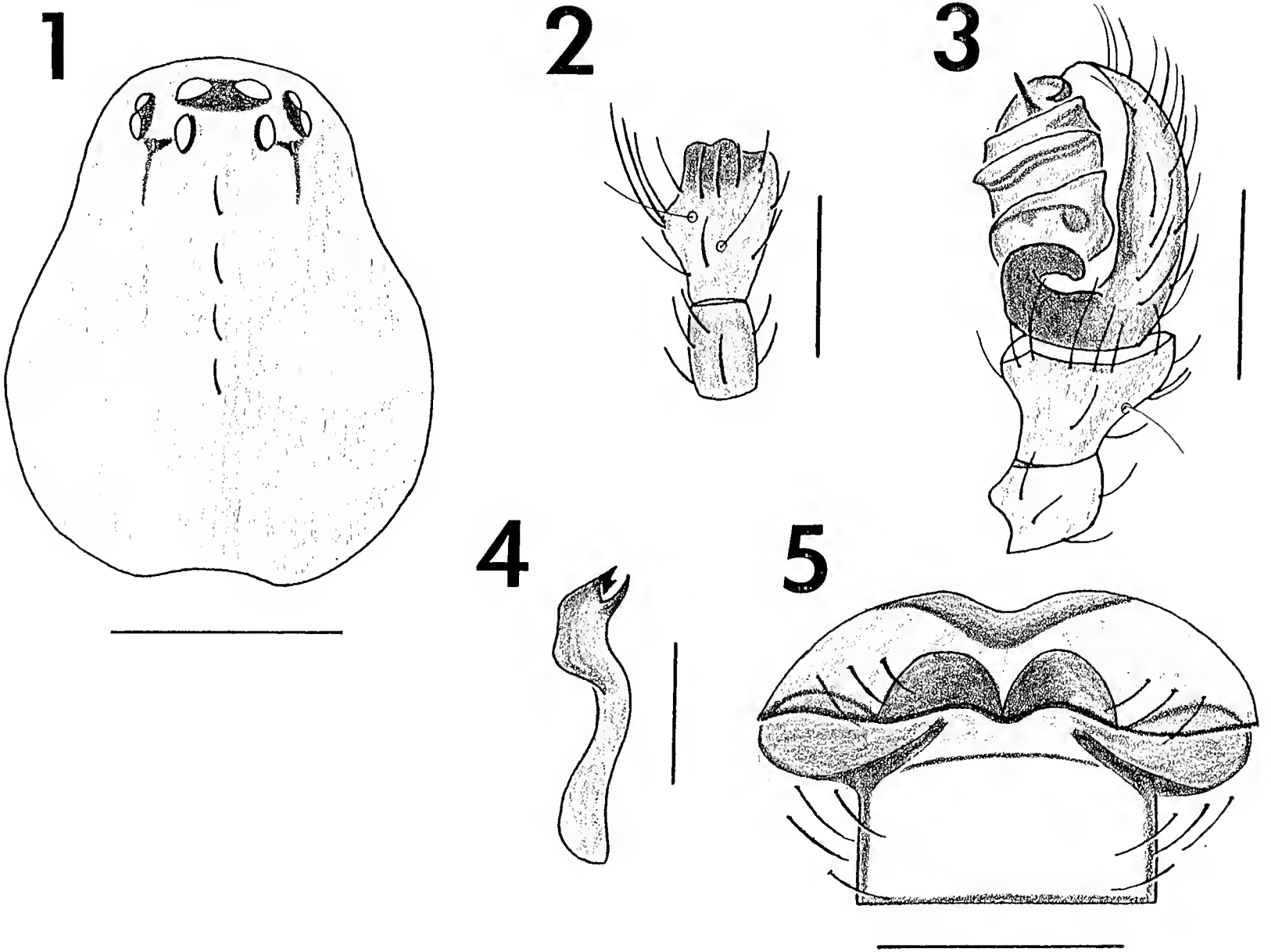
Table 2-1 -- continued.

CHARACTERS	M. ARIENUS	M. CONSPECTUS	M. POGONOPHILUS
Embolic division	Bifurcation begins close to tail-piece; each segment of bifurcation coiled (fig. 2-14 from Chamberlin 1948)	Distally bifid w/ proximal part of bifurcation bent forward and extending over most distal part which is, itself, squared off (fig. 2-11 from Chamberlin 1948 shows it pointed)	Distally bifid w/ proximal part of bifurcation bent forward and extending over most distal part which is, itself, bifurcated (see Fig. 2-4)

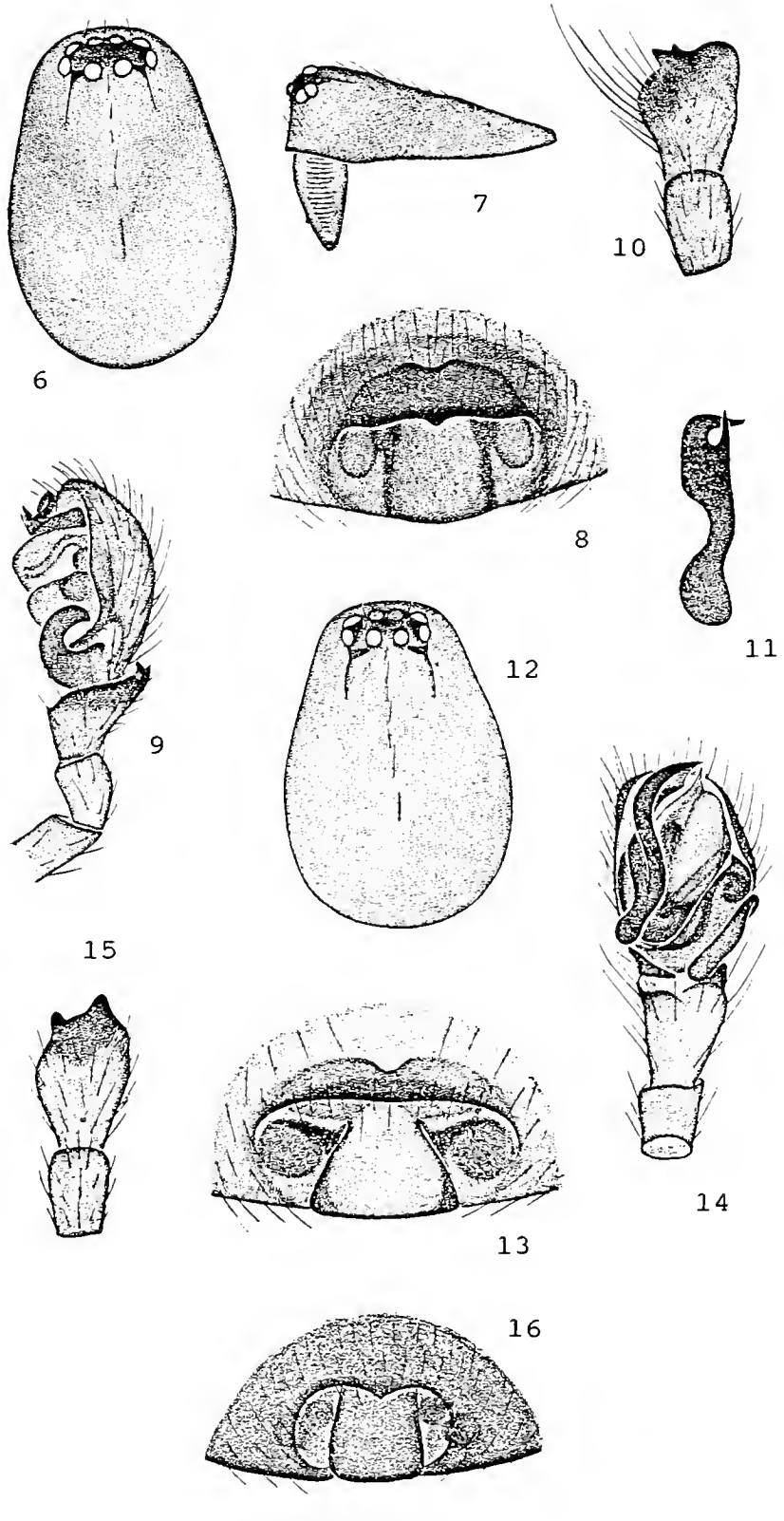
Table 2-2. Collection information and museum destination for the 23 paratypes of *M. pogonophilus*. All were collected from the nests of the Florida harvester ant, *P. badius*. MCZ = Museum of Comparative Zoology, Cambridge, Massachusetts; DPI = Division of Plant Industry, Gainesville, Florida; AMNH = American Museum of Natural History, New York, New York; CAS = California Academy of Sciences, San Francisco, California.

COLLECTION DATE	FLORIDA COUNTY	COLLECTOR	NUMBER OF SPECIMENS	MUSEUM
Males				
10- XI-1982	Leon	S.D. Porter	1	MCZ
9- XI-1984	Leon	S.D. Porter	1	MCZ
13- I-1990	Walton	Skelley, Turnbow, & Thomas	2	DPI
14- I-1990	Okaloosa	Skelley, Turnbow, & Thomas	1	DPI
9- V-1992	Leon	P.E. Cushing	1	DPI
26- V-1992	Levy	P.E. Cushing	2	AMNH
8- X-1992	Levy	P.E. Cushing	1	AMNH
20- II-1993	Levy	P.E. Cushing	1	CAS
25- IX-1994	Levy	P.E. Cushing	1	CAS
Females				
3- I-1990	Okaloosa	P. Skelley	1	DPI
13- I-1990	Walton	Skelley, Turnbow, & Thomas	1	DPI
9- V-1992	Leon	P.E. Cushing	1	DPI
28- II-1993	Putnam	P.E. Cushing	4	AMNH
25-VII-1993	Putnam	P.E. Cushing	1	MCZ
25- IX-1994	Levy	P.E. Cushing	4	CAS

Figures 2-1 to 2-5. Masoncus pogonophilus new species. 1, male carapace, dorsal view (scale = 0.4 mm); 2, tibia and patella of left male palpus, dorsal view, trichobothria in circular pits (scale = 0.2 mm); 3, male palpus, prolateral view (bifurcation of embolic division just visible distally) (scale = 0.2 mm); 4, embolic division of left male palpus, mesoventral view (scale = 0.1 mm); 5, epigynum, ventral view (scale = 0.1 mm).


1
2
3
4
5

Figures 2-6 to 2-16 (from Chamberlin 1948). 6, Masoncus nogales new species. Cephalothorax, dorsal view. 7, Masoncus nogales new species. Cephalothorax and chelicerae, lateral view. 8, Masoncus nogales new species. Epigynum. 9, Masoncus nogales new species. Male palpus, lateral view. 10, Masoncus nogales new species. Patella and tibia of male palpus. 11, Masoncus nogales new species. Mesoventral view. 12, Masoncus arienus new species. Cephalothorax, dorsal view. 13, Masoncus arienus new species. Epigynum. 14, Masoncus arienus new species. Male palpus, ventral view. 15, Masoncus arienus new species. Patella and tibia of male palpus, dorsal view. 16, Masoncus dux new species. Epigynum.



(from Chamberlin 1948)

CHAPTER 3
GENETIC DIVERSITY WITHIN AND AMONG POPULATIONS OF *MASONCUS POGONOPHILUS* USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) FINGERPRINTING

Introduction

Many arthropods have evolved close symbiotic relationships with ants. These are referred to as myrmecophiles. Some are found at the periphery of the nest, either near the entrances or on the midden (refuse pile); others are found within the chambers of the nest, either in the peripheral chambers or deeper in the nest in the brood and storage chambers (Hölldobler 1977). In general, ants live in complex closed societies in which only members are allowed. They communicate with nestmates through chemical and tactile signals, and tend to aggressively exclude intruders. Much work has been done studying the various adaptations myrmecophiles use to integrate into the hostile environment of an ant nest (reviewed in Kistner 1979 and Hölldobler and Wilson 1990). However, little has been done to investigate the influence host population structure has on that of its guests, though Kistner (1982) proposed that the spacing and abundance of host nests in an environment plays an important role in the presence, abundance and population structure of myrmecophiles.

My objective was to investigate the population structure of a myrmecophilic spider by examining genetic diversity within and among different populations and to determine the extent to which the distribution of the host ant nests influences the population structure of the myrmecophile. The spider Masoncus pogonophilus Cushing (Linyphiidae) (Chapter 2) lives within the nest chambers of the Florida Harvester ant, Pogonomyrmex badius (Latreille) (Hymenoptera: Formicidae). It is about one-quarter the size of its 7-9 mm long host and feeds on collembolans found throughout the 1-3 m deep subterranean nests (Porter 1985). The ant nest provides a stable microclimate as well as an abundant food source for the spider. The spiders have only been collected outside the ant nests when the hosts are migrating to a new nest site. The spiders are extremely susceptible to desiccation when removed from the nests. Therefore, they appear to be obligate ant symbionts.

The following features of the natural history of the myrmecophile and its host suggest that inbreeding among spiders within colonies may be high (and, thus, genetic diversity low) and that dispersal of spiders between neighboring colonies may be low (Chapter 2): 1) The spider exhibits an extreme female-biased sex ratio ranging from 4:1 (Porter 1985) to 7.5:1 (Chapter 2). Female-biased sex ratios among diploid organisms are often associated with a high level of inbreeding (Hamilton 1967, Rowell and Main 1992). A female-biased sex ratio in such systems reduces

local mate competition between closely related males. 2) The host ants aggressively defend their territory against conspecifics from other colonies (Gentry 1974, Hölldobler 1976). Pogonomyrmex badius nests tend, therefore, to be overdispersed or evenly spaced in the landscape (Harrison and Gentry 1981, Chapter 6). Colonies are usually spaced between 8-16 m from one another (Harrison and Gentry 1981, pers. obs.). The high dispersion of P. badius nests coupled with the spiders' susceptibility to desiccation outside the nest would make dispersal of spiders a high risk activity during the daytime in the xeric environments in which their host is found. 3) Dispersal to or from nests at night is prevented by the host's habit of closing the nest entrance at that time. Williams and Franks (1988) suggested that a myrmecophilic isopod (also with a female-biased sex ratio) may remain within a host nest for several generations until the nest senesces and dies, at which time the isopods pulse out into the environment and become established in new nests. Queens of P. badius can live at least 15 years and some western congeners can live up to 30 years (Gentry 1974, Porter and Jorgensen 1988). It is possible, therefore, that a population of M. pogonophilus, remains inside a single colony for several generations dispersing to a new colony only when the host nest begins to senesce.

However, M. pogonophilus are consistently found within mature P. badius nests in a given habitat so dispersal events, although perhaps infrequent, must be

occurring. Each P. badius nest is established by a single inseminated queen; the colonies do not split into new colonies (Cole 1968). Therefore, unless the spiders are phoretic, hitching rides on the bodies of their hosts (an unlikely dispersal mechanism for reasons explained in Chapter 4), they must be finding their way to new colonies by other means--perhaps by eavesdropping on the chemical signals of their host ants and following trail pheromones (see Chapter 4). Nevertheless, dispersal events among the myrmecophiles could be infrequent (i.e., occurring only when the host nest senesces) yet still maintain a relatively high occurrence of spiders among nests as spiders dispersing from the dying host nest find their way to new colonies. Given the potential lifespan of a single colony and the short generation time of spiders (Chapter 2), inbreeding among the spiders should be high.

To test the hypothesis that dispersal events of myrmecophilic spiders between neighboring nests are infrequent and that genetic diversity within nests is low (perhaps due to inbreeding), I used the PCR-based Random Amplified Polymorphic DNA, or RAPD's, technique (Williams et al. 1990, Welsh and McClelland 1990, Hadrys et al. 1992) to measure the genetic diversity among spiders within P. badius nests as well as among spiders from different nests.

If dispersal of spiders between nests is infrequent and inbreeding among spiders within each nest is high, then I would expect genetic diversity between individuals within

each nest (i.e., within each population) to be low. Furthermore, I would expect genetic diversity between spider populations from neighboring nests as well as between populations from distant nests to be approximately equal and to account for a greater percentage of total genetic diversity than within-population differences.

Materials and Methods

Population Sampling

In June 1993, three P. badius nests were excavated at two sites in north Florida. Nests 1 and 2, approximately 12 m apart, were located at Archer Sandhills (ASH1 and ASH2, respectively) 25 km west of Gainesville, FL in Levy County. Nest 3 was located 55 km east of Archer Sandhills at the Katherine Ordway Preserve/ Swisher Memorial Sanctuary (ORD3) in Putnam County FL. Approximately 1 m deep excavation pits were dug adjacent to the nest entrance. The soil was scraped away from the pit wall to expose the nest chambers and the spiders within. Fifteen adult spiders were collected from ASH1, 16 from ASH2, and 9 from ORD3.

DNA Isolation

Spiders were placed in vials and chilled at -80 ^{o}C for a few seconds to kill them. Each was washed with 20 µl of sterile STE buffer (pH 8.0) (Sambrook et al. 1989) to remove any sand grains or debris, then transferred to a new sterile microcentrifuge tube and homogenized with the rounded end of a sterilized glass pipette in 50 µl of cold

(1 - 2 ^{o}C) STE buffer. Tubes were incubated overnight (15.5 hrs.) at 55 ^{o}C with 2.5 µl 20% SDS and 2.5 µl of Proteinase K (50 µg/ml) (Sambrook et al. 1989). Samples were then extracted with phenol: chloroform (50:50) and the DNA precipitated with 95% ethanol. DNA was resuspended in 20 µl of 0.1 X TE (pH 8.0) (Sambrook et al. 1989) and concentration determined spectrophotometrically. Each sample was diluted with buffer to give a final DNA concentration of 5 ng/µl.

DNA Amplification

A Perkin Elmer Cetus DNA Thermocycler was used for DNA amplification. The cycling protocol was 1 min. at 94 ^{o}C; 1 min. at 50^{o}C; and 2 min. at 72 ^{o}C for 45 cycles. Each reaction was carried out in a total volume of 50 µl containing 0.5 X Stoffel buffer (Perkin Elmer Cetus), 100 µM of each dNTP, 1.75 mM $MgCl_2$, 0.05 µM of primer, 0.05 U/µl of ampliTaq DNA polymerase Stoffel fragment (Perkin Elmer Cetus), and 5 ng/µl of template DNA. DNA amplification bands were separated in 1.2% agarose gels in 1.0 X TBE buffer (Sambrook et al. 1989). Bands were visualized under UV light after staining with ethidium bromide at a final concentration of 0.5%.

Eight random 10-mer primers (DNA Synthesis Lab, Gainesville, FL) were screened (Table 3-1). Each amplification with one primer was replicated three times. Representative products from ASH1, ASH2, and ORD3 were run side by side. Negative controls containing all the reagents

except the template DNA for each primer were also conducted to ensure the fidelity of the results. For each spider, bands were scored as present (1) or absent (0). RAPD bands generated by the 8 primers that were consistently reproduced in at least two replicate PCR reactions were counted in the final analysis.

Statistical Analysis

The Analysis of Molecular Variance technique (AMOVA) was used to analyze genetic diversity within and among the three populations (Excoffier et al. 1992, Huff et al. 1993). This analysis was designed to handle different types of molecular data and uses no a priori assumptions regarding gene flow or population structure (Excoffier et al. 1992). Although first used to analyze mitochondrial DNA haplotype data, it has since been applied to RAPD data (Huff et al. 1993). The analysis is based upon pairwise comparisons of banding patterns between all 35 spiders. These genetic distances were expressed as Relative Band Distances = 100 * [(# different bands)/ (total # bands)] for each pair.

Results

Three of the 15 ASH1, two of the 16 ASH2, and one of the nine ORD3 spiders were males. All 15 of the ASH1 spiders, 11 of the 16 ASH2 spiders (including one male), and all nine of the ORD3 spiders were used for the final analysis. The depths of the chambers or runways from which the spiders were drawn are shown in Table 3-2.

Each spider yielded between 35 - 300 ng/µl of total DNA ($\bar{x} \pm$ s.d.: 116.3 $\pm$ 53.7). The individual primers yielded between 5 to 19 polymorphic bands (Table 3-1). Primer #3 resulted in three different sets of individual banding patterns for the three replications. Since no individual showed consistent banding patterns at least twice, I excluded these data from the analysis. Primer #5 resulted in a monomorphic pattern across all three populations. Since this provided no useful information about population subdivision (i.e., a highly conserved region of the genome was amplified), I also excluded these data from the final analysis. Six of the eight primers screened yielded a total of 67 bands. Of these, 14 were monomorphic for all 35 spiders and were excluded from the final analysis. Of the 53 polymorphic bands, 10 (19%) were unique to ORD3. Six bands (11%) were found in both ASH populations but were absent in the ORD3 population. Thus, 16 bands (30%) distinguish the populations at the ASH site from the population at the ORD site. In contrast, only two bands (4%) were unique to ASH1 and only three bands (6%) were unique to ASH2. One band was present in one individual from ASH2 and three individuals from ORD3.

The relative band differences between spiders within each population as well as between individuals from different populations are presented in Tables 3-5 to 3-10. A summary of the pairwise intra-nest and inter-nest relative band distances are presented in Table 3-3. An

unbiased estimate of the standard error of the mean, corrected for the nonindependence of pairwise comparisons, was calculated based upon the formula in Miyamoto et al. (1994) as modified from Lynch (1990):

$$SE = 100\ ((2\bar{D}[1-\bar{D}][1+\bar{D}]) / (\bar{n}[3+\bar{D}]))^{0.5}.$$

In this equation, $\bar{D}$ equals mean relative band distances for all possible pairs in the analysis and $\bar{n}$ refers to the average number of scored bands per individual.

The AMOVA results (Table 3-4) indicate significant genetic differences between the ASH and the ORD sites ($p < 0.005$) as well as between populations at the ASH site ($p < 0.04$). There is also significant genetic diversity within each of the three populations ($p < 0.005$). Of the total genetic diversity, 77.4% was due to individual differences within the three populations; 18.2% was due to differences between ASH and ORD; and 4.4% was due to differences between ASH1 and ASH2 (i.e., within their region).

To further segregate the patterns of genetic differences among the three populations, three separate pairwise comparisons of the populations were conducted (Table 3-4). For all of these comparisons, the largest component of genetic diversity is attributable to within population differences (75 -94%, $p < 0.03$). Although between 19 - 24% of the total genetic diversity is attributable to differences between distant nests, only about 6% of the total genetic diversity is attributable to differences between the neighboring nests.

Discussion

All three nests show high intra-nest genetic diversity. Only 14 of the 67 total bands (or 20.9%) were monomorphic for all three populations. The variation among the remaining 53 polymorphic bands results more from intra-nest genetic diversity rather than inter-nest diversity. However, genetic diversity between either of the ASH populations and the ORD population is higher than the genetic diversity between the ASH1 and ASH2 populations. This suggests that gene flow may be great enough to offset the diversifying effects of genetic drift between neighboring nests in contrast to the geographically separated populations (Slatkin 1994).

If the neighboring populations had been separated for many generations and if one or both resulted from a single foundress (perhaps arriving from a neighboring colony), then the foundress effect and genetic drift should have decreased intra-population genetic diversity and increased inter-population genetic differences. Instead, these data indicate that dispersal events of spiders between neighboring nests in the same habitat are occurring at a significant rate within the lifetime of an ant colony. Spiders and ants may take advantage of cool mornings in the winter or periods after summer showers to disperse. Spiders migrating with host ants to a new nest site may wander off the emigration trail and find their way to a new host nest instead. It may be that dispersing spiders

follow foraging or orientation trail pheromones to the edge of the hosts' territory and then search for the trail of a neighboring colony which they then follow until they get to the new nest. The existence of chemical trails for foraging, recruitment, and homing has been well documented in various species of Pogonomyrmex ants including P. badius (Hölldobler 1971, Hölldobler and Wilson 1970, Regnier et al. 1973). Observations of spiders emigrating with their hosts or moving from the periphery of a foreign P. badius mound directly to the mound entrance suggest that spiders may have evolved the capacity to follow chemical trails of the ants (see Chapter 4). Dispersal to new ant nests may be a mechanism for avoiding inbreeding depression. Or, it may be triggered by conditions, such as increased resource competition, within the nest. Since the ecosystem in which spiders live is destined to go extinct upon the death of the queen ant, it may be adaptive for some individuals in the population to risk the hazards associated with dispersal in order to locate potentially younger ecosystems (i.e., ant nests with a relatively longer life expectancy due to the presence of a younger queen).

If it is primarily male M. pogonophilus that disperse to other colonies, then high mortality of the dispersers when they venture into the xeric environment in search of a new colony 10-15 m away would explain the apparent female-biased sex ratio. However, a model proposed by Bulmer and Taylor (1980) suggests that the sex ratio should

be biased in favor of the dispersing sex. Because only five of the spiders used for the present study were males, I could not determine whether there were significant differences in banding patterns between the males and females within a single nest. It is common among male web-building spiders for mature males to wander about in search of females. Such wandering often leads to apparent sex ratio bias due to high mortality among the males (Vollrath and Parker 1992). However, in such species, the sexes are usually dimorphic, the male maturing at a much smaller size and after fewer molts than the female (Vollrath and Parker 1992), and the males are unable to build prey capture webs (Kovoor 1987). Neither trait holds true for M. pogonophilus males (see Chapter 2).

If, however, the female-biased ratio is real and not an artifact of differential mortality, then the traditional explanation of such ratios--high inbreeding leading to reduced local mate competition through production of fewer sons than daughters--is not supported since the RAPD's data do not indicate high levels of genetic relatedness within spider populations. Instead, this system may fit a model proposed by Colwell (1981, 1982) and Wilson and Colwell (1981) in which female-biased sex ratios are established regardless of the level of inbreeding or local mate competition. Colwell shows that in a sub-population made up both of females that skew the sex ratio of their offspring towards daughters, "Hamiltonian females", as well

as females that produce an equal number of sons and daughters, "Fisherian females", the Fisherian females will have greater fitness within a sub-population (in this case, within a single ant nest) but Hamiltonian females will have greater fitness at the larger scale of the population as a whole because they produce a greater number of dispersing daughters or foundresses. The greater the number of Hamiltonian daughters dispersing to establish new sub-populations, the greater the frequency of Hamiltonian females even within any given sub-population (within an ant nest). Therefore, the female-biased sex ratio observed among M. pogonophilus may be the result, not of inbreeding and local mate competition, but of the greater fitness of "Hamiltonian females" due to the dispersal patterns of spiders between ant nests.

Table 3-1. Eight 10-mer primers screened. Primers 1, 2, 4, 6, 7, and 8 generated the 53 polymorphic bands used in the final analysis.

PRIMER	NUCLEOTIDE SEQUENCE 5′ to 3′	NUMBER OF POLYMORPHIC BANDS
1	CTGAAGCGGA	19
2	ATCAAGCTGC	5
3	AGCTGAAGAG	*
4	GCCCTGATAT	5
5	CAGGACATCG	*
6	ACAGGGAACG	10
7	GACCCAGAAG	5
8	CGACCAGAGC	9

* The bands resulting from amplification with primers 3 and 5 were excluded from the data set (see text).

Table 3-2. Depths of the subterranean chambers or runways from which the spiders used in the analysis were drawn. Numbers in parenthesis indicate how many of the spiders collected from that location were males.

Nest	Depth of Chamber or Runway (cm)	#Spiders (#Males)
ASH1	30.5	1
ASH1	39.0	2
ASH1	46.5	3(1)
ASH1	55.0	1
ASH1	55.0	1
ASH1	70.0	3
ASH1	71.0	1
ASH1	94.0	1(1)
ASH1	bottom of exc. pit	2(1)
ASH2	23.0	1(1)
ASH2	35.0 - 41.0	8
ASH2	50.0	1
ASH2	54.0	1
ORD3	3.5	1
ORD3	13.0	1(1)
ORD3	30.5	1
ORD3	37.0	1
ORD3	37.5	3
ORD3	47.0	1
ORD3	80.0 - 84.0	1

Table 3-3. Summary of means ± standard errors and ranges for the intra- and inter-population Relative Band Distances.

POPULATION COMPARISON	#PAIRS	$\bar{X}$ ± SE	RANGE
I Within Populations			
Intra-ASH1	105	29.56±8.24	0.00-70.00
Intra-ASH2	55	35.40±9.03	5.26-78.26
Intra-ORD3	36	35.92±8.54	3.57-60.00
II Between Populations			
ASH1 vs ASH2	165	34.38±8.75	0.00-75.00
ASH1 vs ORD3	120	43.83±9.10	19.05-75.00
ASH2 vs ORD3	99	44.41±9.30	15.79-76.00

Table 3-4. Analysis of Molecular Variance (AMOVA) results for the 26 individuals from Archer Sandhills and the 9 individuals from Ordway Preserve. One AMOVA (A) compared the genetic diversity between Archer Sandhills and Ordway. The genetic diversity was partitioned into between regions, between populations within regions, and between individuals within populations. Three additional AMOVA's (B - D) were performed comparing each pair of populations, partitioning the genetic diversity into differences between populations and differences between individuals within populations. SSD = Sum of Squared Differences; MSD = Mean Squared Differences

SOURCE OF VARIATION		df	SSD	MSD	VARIANCE COMPONENT	% TOTAL*	P-VALUE**
A.	ASH vs ORD	1	77.94	77.94	3.88	18.21	<0.005
	Populations/regions	1	28.49	28.49	0.95	4.44	<0.04
	Individuals/populations	32	527.60	16.49	16.49	77.36	<0.005
B.	ASH1 vs ASH2	1	28.49	28.49	0.98	5.80	<0.03
	Individuals/populations	24	383.93	16.00	16.00	94.20	
C.	ASH1 vs ORD3	1	73.36	73.36	5.10	24.26	<0.005
	Individuals/populations	22	350.60	15.94	15.94	75.74	
D.	ASH2 vs ORD3	1	61.18	61.18	4.38	19.74	<0.005
	Individuals/populations	18	320.67	17.82	17.82	80.26	

* Percentage of total variance contributed by each component.

** Probability of obtaining a more extreme variance component by chance.

Table 3-5. Relative band distances between all pairs of M. pogonophilus individuals from ASH1. The individual identification numbers are listed along the horizontal and vertical axes.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0														
2	53	0													
3	52	19	0												
4	50	16	10	0											
5	52	19	0	10	0										
6	57	20	23	27	23	0									
7	52	15	0	5	0	23	0								
8	50	19	13	16	13	10	14	0							
9	23	42	42	33	42	45	40	37	0						
10	52	29	13	16	13	14	10	13	37	0					
11	58	44	39	41	39	33	29	33	42	39	0				
12	70	40	41	45	41	21	41	30	58	33	52	0			
13	55	26	15	12	15	10	14	9	38	9	39	29	0		
14	52	24	13	16	13	30	10	19	37	24	39	48	21	0	
15	44	36	31	28	31	29	32	26	33	26	47	40	22	31	0

Table 3-6. Relative band distances between all pairs of M. pogonophilus individuals from ASH2. The individual identification numbers are listed along the horizontal and vertical axes.

	16	17	18	19	20	21	22	23	24	25	26
16	0										
17	64	0									
18	32	56	0								
19	43	62	32	0							
20	38	60	35	30	0						
21	38	69	35	27	27	0					
22	32	47	29	15	10	10	0				
23	35	64	32	27	38	13	5	0			
24	22	70	20	37	38	24	11	24	0		
25	30	70	27	24	34	26	19	26	16	0	
26	31	78	45	28	45	41	28	45	41	42	0

Table 3-7. Relative band distances between all pairs of M. pogonophilus individuals from ORD3. The individual identification numbers are listed along the horizontal and vertical axes.

	27	28	29	30	31	32	33	34	35
27	0								
28	50	0							
29	31	57	0						
30	56	39	35	0					
31	39	58	25	42	0				
32	27	49	24	46	14	0			
33	29	50	27	47	17	4	0		
34	33	50	25	47	28	21	23	0	
35	50	60	32	44	22	33	30	29	0

Table 3-8. Relative band distances between all pairs of M. pogonophilus individuals from ASH1 and ASH2. The individual identification numbers are listed along the horizontal and vertical axes.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16	44	28	38	35	38	26	38	26	38	30	50	39	33	38	18
17	40	70	64	65	64	59	50	65	63	64	62	75	68	69	70
18	55	25	35	32	35	24	35	24	50	27	45	43	30	35	41
19	50	22	3	13	3	27	5	16	45	16	42	45	18	16	34
20	52	42	27	34	27	23	0	38	52	32	43	41	38	32	45
21	52	34	24	26	24	23	0	34	52	29	44	41	31	29	50
22	47	6	10	5	10	24	10	15	33	19	30	43	23	19	33
23	50	29	24	21	24	27	5	34	47	29	48	45	31	29	46
24	47	34	34	26	34	15	19	24	42	29	44	35	26	34	41
25	52	31	21	18	21	14	10	16	44	16	41	33	12	26	33
26	40	35	30	32	30	48	35	35	50	41	57	60	41	35	38

Table 3-9. Relative band distances between all pairs of M. pogonophilus individuals from ASH1 and ORD3. The individual identification numbers are listed along the horizontal and vertical axes.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
27	70	46	36	38	36	42	30	46	59	36	56	58	42	41	57
28	71	66	55	60	55	61	52	59	65	55	62	75	59	59	66
29	62	29	23	26	23	32	19	34	43	29	45	50	35	29	37
30	65	53	44	46	44	44	40	44	58	40	55	60	45	49	42
31	53	31	26	23	26	36	24	31	39	26	47	55	32	31	39
32	64	35	25	22	25	42	23	35	43	25	50	58	31	30	42
33	64	37	27	24	27	42	23	37	45	27	52	58	33	32	44
34	59	41	31	33	31	44	26	41	50	36	52	60	42	31	44
35	55	42	38	39	38	39	27	42	52	42	53	57	47	42	45

Table 3-10. Relative band distances between all pairs of M. pogonophilus individuals from ASH2 and ORD3. The individual identification numbers are listed along the horizontal and vertical axes.

	16	17	18	19	20	21	22	23	24	25	26
27	57	75	39	34	40	36	39	41	46	38	53
28	74	76	64	54	60	59	59	62	66	60	67
29	40	64	29	27	32	39	20	34	44	41	47
30	45	68	42	47	53	53	42	49	53	50	56
31	37	61	41	29	40	36	16	31	36	38	38
32	50	67	46	28	39	35	24	30	40	37	47
33	50	68	46	30	41	37	24	32	42	39	48
34	52	57	35	29	34	41	27	36	46	43	48
35	40	57	43	41	36	47	20	42	47	49	50

CHAPTER 4
THE ABILITY OF THE MYRMECOPHILIC SPIDER TO FOLLOW THE TRAILS OF ITS HOST ANT

Introduction

Masoncus pogonophilus Cushing (Linyphiidae; Erigoninae) is a common symbiont within the nest chambers of the Florida seed harvesting ant, Pogonomyrmex badius (Latreille) (Porter 1985). Adult spiders are 1.8 mm in length (Chapter 2), about one-third the size of the formicid host. All developmental stages of the spider, as well as the eggsacs of M. pogonophilus can be found throughout the chambers of the subterranean nest (Chapter 2).

A study by Cushing (Chapter 3) using the Random Amplified Polymorphic DNA (RAPD) fingerprinting technique indicated that spiders from neighboring P. badius colonies were genetically similar. This finding suggested that dispersal of these small spiders between nests was more frequent than expected. This study was an attempt to determine the mechanism by which spiders disperse.

Dispersal among many species in the family Linyphiidae, especially among the tiny spiders in the subfamily Erigoninae, is by ballooning. Juvenile and adult spiders climb up to a high point--a blade of grass or a fence post --and release a strand of silk (Gertsch 1979).

The silk acts as a kite, lifting the spiderling into the air. Species in the family Araneidae that disperse by ballooning normally produce large numbers of offspring--more than 1000 per female (Tolbert 1977, Reichert and Gillespie 1986). This high fecundity presumably offsets the risks of this dispersal strategy. However, M. pogonophilus produces, at most, six eggs per eggsac with a mean of 2.9 eggs (Chapter 2). Most araneoid species that produce multiple clutches, produce no more than five (Vollrath 1987). If M. pogonophilus females produce multiple clutches, their lifetime reproductive output would still only average 15 offspring. Such low fecundity is common among the tiny erigonine spiders of the family Linyphiidae (Bristowe 1958, Roberts 1995). In general, fecundity in spiders is positively correlated with the body size of the female (Peterson 1950, Kessler 1973, Wise 1975).

The risks of ballooning are high in areas where suitable habitat is patchy. In such cases, ballooning is rare and has been selected against as a viable dispersal mechanism (Janetos 1986). The ant nests where M. pogonophilus makes its home are spaced an average of 12.1 m from one another according to a study by Harrison and Gentry (1981). At my study sites, Archer Sandhill in Levy County, Florida and the Ordway-Swisher Preserve in Putnam County, Florida, the mean nearest neighbor distances were 11.45 m and 20.00 m respectively (Chapter 5). Due to the

low fecundity of M. pogonophilus, the high spacing between P. badius nests, and the susceptibility of M. pogonophilus to desiccation outside the nests, ballooning is an unlikely dispersal mechanism for the spiders.

I hypothesized that the spiders were, instead, locating new nests by using the chemical signals laid down by the host ants. Although neighboring P. badius colonies, as well as colonies of western species of Pogonomyrmex harvesters, often partition their foraging territories and locate their foraging trails in such a way as to reduce contact between foragers from different colonies (Hölldobler 1976, Harrison and Gentry 1981), foraging trails of neighboring colonies sometimes do intersect (personal observation). I hypothesized that dispersing spiders follow foraging trails away from their host nests until they located, through random searching, the foraging trail of a neighboring nest. Although P. badius does not produce the distinct trunk trails seen in western species of the genus (Hölldobler 1974, Hölldobler 1976), it does produce three or four primary trails that persist for several months (Harrison and Gentry 1981). The ability to follow host trails has been demonstrated for a variety of myrmecophilic arthropods (Moser 1964, Akre and Rettenmeyer 1968, Schroth and Maschwitz 1984).

Materials and Methods

Pheromones from the poison gland and Dufour's gland are involved with trail marking, recruitment to food

sources, and homing in P. badius (Hölldobler and Wilson 1970, Hölldobler 1971). The chemical composition of the poison gland secretion was determined by Schmidt and Blum (1978). It is an enzyme rich substance containing high concentrations of phospholipase A_2 and B, hyaluronidase, acid phosphatase, lipase, and esterases (Schmidt and Blum 1978). The composition of the Dufour's gland secretion (for other members of the genus Pogonomyrmex) was determined by Regnier et al. (1973) who found it to consist of various hydrocarbons. There is no evidence of colony specificity for either of these glandular secretions (Hölldobler and Wilson 1970, Hölldobler 1971). Therefore, no effort was made in the following experiments to control for nest identity of the ants from which glands were excised or the ants used in the trials.

Ants were collected from two field colonies and one laboratory colony for use in the following experiments. Ten spiders were collected from an excavated colony at Archer Sandhills in Levy County. These included two females, seven males, and one juvenile. (This was the only excavation in which more males than females were collected.) Eleven spiders were collected from the emigration trail of another P. badius colony at the Ordway-Swisher Preserve in Putnam County which included five females, one male and five juveniles.

To test whether M. pogonophilus could follow the trail pheromones of its host, I conducted three types of

experiments: olfactometer experiments using poison gland and Dufour's gland extracts; choice experiments using artificial trails laid with gland extracts; and choice experiments using naturally laid trails. Due to difficulties in keeping the spiders alive in the lab, only 16 were available for the artificial trail experiments and only 12 were available for the natural trail experiments. The spiders used for each experiment were quite active when removed from their vials. Any spiders that were listless or appeared to be sick or dying were not used for the experiments. Before experimenting with the spiders, I conducted bioassays with the host ants to ensure that the host ants, themselves, would respond to these chemical cues.

Olfactometer Experiments

I dissected poison glands and Dufour's glands from P. badius workers. These glands are located near the tip of the abdomen and release their contents through the sting. A drawing of these two glands is reproduced from Schmidt and Blum (1978) in Fig. 4-1. The olfactometer consisted of Y-shaped glass tubing with two enlarged bulbs on the arms of the Y (Fig. 4-2) (Vander Meer et al. 1988).

For each trial, I made one-gland equivalent and 0.1-gland equivalent solutions of the poison gland and of the Dufour's gland using hexane as a solvent. The one-gland equivalent solution consisted of one crushed gland per 10 µl of hexane; the 0.1-gland equivalent

solution consisted of one crushed gland per 100 µl of hexane. These solutions were in the same range as those used by Hölldobler and Wilson (1970) and Hölldobler (1971). For each trial, 10 µl of one solution or the other were pipetted onto a 3 X 7 mm rectangle of absorbent paper. Ten ul of hexane were used as a control. The paper saturated with the sample was placed in the enlarged bulb of one arm of the Y and the paper saturated with the control was placed in the other arm (see Fig. 4-1). An equal current of air was blown through the arms of the Y.

Sixty ants were used for each trial. Ten ants at a time were placed in a small holding vial with rubber tubing at one end which could be fitted onto the long arm of the Y. I waited a few minutes for the ants to settle down before joining the holding vial to the glass tubing of the olfactometer. The ants then left the holding vial and, when they reached the junction of the Y, they either chose one arm or the other immediately or paused and antennated in both directions before choosing a side. The arm chosen was recorded. In a few instances, one or two ants refused to leave the holding vial; data for these individuals were not included. After every group of 10 ants, the olfactometer was rinsed with acetone, fresh extracts of sample and control were pipetted onto new pieces of paper, and the side of the Y-arm in which the sample and control were placed was reversed to eliminate directional bias. The same procedure was followed for the spiders except the

spiders were introduced into the apparatus individually. After each introduction, I attempted to sweep out at least the long stem of the Y-tube with a camel-hair brush to remove any stray draglines. After 4-5 spiders, the apparatus was cleaned and the locations of the sample and control were reversed.

Artificial Trail Experiments

Spiders not only can detect airborne chemicals via tarsal organs on their legs but can also detect chemicals from the substrate via contact chemoreceptors, or taste hairs, on the distal segments of the legs and palps (Foelix 1982). I tested whether the spiders could detect trail pheromones from the substrate by laying an 11 - 12 cm trail of 0.1 gland-equivalent poison gland extract on a piece of chromatographic paper. An 11 - 12 cm hexane trail was laid at approximately 45^{o} from the poison gland trail as a control.

As with the olfactometer experiments, I first ran bioassays with P. badius to ensure that such substrate-bound trails were biologically meaningful. Four groups of 10 ants were placed in plastic vials and allowed to calm down since agitated ants tend to move randomly and do not readily orient towards trails (Hölldobler and Wilson 1970, personal observation). Each vial was then tipped on its side and opened, allowing the ants to move out onto one or the other trail.

After each group of ants, the chromatographic paper was changed, the metal tray in which the experiment was conducted was wiped with acetone, and the location of the trails was reversed. I repeated the experiment using the 16 spiders still alive (from the original 21). The same procedure as above was followed, except the spiders were allowed to choose trails individually. The paper was changed, the tray cleaned, and the trails reversed after every four spiders. For both the ants and the spiders, a choice was considered a distinct directional movement along one trail or the other.

Natural Trail Experiment

Although the poison gland has been shown to be a major source of the trail pheromones of P. badius, it is possible that the trails are actually a mixture of glandular secretions from both the poison and Dufour's glands. To test whether the spiders could follow naturally laid trails of P. badius, I collected between 90 - 100 P. badius workers from a field colony and established them in a 45 X 65 cm tray. The ants moved readily into a test tube half-filled with water, plugged with cotton and covered with red acetate. On the opposite side of the tray from the test tube, I placed two flat metal dishes, each 6.5 cm in diameter, on 1.4 cm high pedestals. On one of these elevated dishes, I placed seeds and on the other I placed a mixture of the Bhatkar ant diet (Bhatkar and Whitcomb 1970). Since P. badius are not adept at crawling up

vertical surfaces, the only way they could reach the food was to travel up a 1.5 X 9 cm strip of chromatographic paper that served as a bridge. The paper bridge was kept in place for four days, and the ants readily used it to forage. As the number of ants moving from the opposite side of the tray directly to and up the bridge increased over that time period, I assumed they had marked a trail both on the tray surface as well as on the paper bridge.

To test this, I positioned two glass tubes in a metal tray at approximately 45^{o} from one another. In one of the tubes, I placed a strip of chromatographic paper cut the same size as the strip used for the bridge. In the other, I placed the bridge. Both strips extended 1 cm beyond the end of the glass tubes and both ends were folded down so the paper was flush with the tray surface. Six groups of 10 ants were placed in vials and allowed to calm down. As with the artificial trail experiments, each vial was then tipped on its side and opened. I recorded which glass tube the ants entered. After every group of 10 ants, I cut a new strip of control paper, rinsed the glass tubes and the metal tray with acetone, and reversed the placement of the paper strips.

The experiment was repeated with the 12 remaining spiders. As before, the spiders were allowed to choose individually. To prevent the tiny spiders from evading both tubes altogether, I built a small arena (approximately 3 X 3 cm) out of pieces of stiff acetone placed in front of

and between the two tubes. The spiders were allowed to move about in the arena until they moved up one piece of paper or the other. After each spider, I brushed the arena with a camel hair paint brush to remove stray dragline silk. After six spiders, I cut a new strip of control paper, rinsed the tray and the glass tubes with acetate and reversed the order of the paper strips. For all experiments, the data were analyzed using X^2 tests.

Results

Ants were highly attracted to extracts of the poison gland (Table 4-1). These results are consistent with those of Hölldobler and Wilson (1970), Hölldobler (1971) and Regnier et al. (1973). However, my results indicate that P. badius is not attracted to extracts of the Dufour's gland (Table 4-1). These results contradict the published accounts of the above authors. However, Hölldobler (personal communication) assured me that orientation towards the poison gland extract and not towards the Dufour's gland extract is in accord with his expectations for Pogonomyrmex harvester ants. Since my olfactometer experiments indicated that the Dufour's gland extract was not a useful bioassay for trail-following, it was not used for any subsequent experiments with the ants or spiders.

Since the 1-gland equivalent poison gland extract was found to be a biologically meaningful concentration for the host ants, I used this higher concentration extract first in my olfactometer experiment with the spiders. I reasoned

that if either the 1-gland equivalent or the 0.1-gland equivalent mixtures were not biologically (physiologically) meaningful concentrations, I would have seen some indication of this in the behavior of the ants in the form of reduced orientation towards the sample (Attygalle and Morgan 1985). Since both the 1-gland equivalent and the 0.1-gland equivalent poison sac extracts elicited significant orientation responses from the ants, I considered both concentrations biologically meaningful. The spiders, unlike their hosts, did not orient more towards the poison gland extract than towards the control (Table 4-2).

Since the myrmecophilic spiders may respond more readily towards substrate-bound chemicals than towards airborne odors, I used the artificial trail experiment to determine if they might be better able to follow such a trail. I used the 0.1-poison gland equivalent solution for this experiment. Although the host ants showed a clear preference for the artificial trail over the control trail (Table 4-1), the spiders did not (Table 4-2).

Because the spiders were not attracted to the pure poison gland extracts however the extracts were presented to them, I thought they might, instead, be responding to some other chemical element of the natural trails. However, the experiment with the natural trail indicated that this was not the case (Table 4-2). Although the host

ants oriented towards the natural trail (Table 4-1), the spiders did not.

In all experiments, the numbers of males, females, and juvenile spiders orienting towards the sample versus the control was approximately equal. There was also no correlation between spiders collected from the emigration trail versus spiders collected from inside the nests and their orientation towards either the extracts or the controls.

Discussion

The results do not support the hypothesis that M. pogonophilus spiders use trail pheromones of host ants to disperse from and locate new host nests. However, the context of laboratory experiments may not be adequate for testing this hypothesis. Perhaps the spiders do not respond in captivity as they would otherwise respond in the field. Or perhaps they are only responsive to host pheromones during certain times in their life. However, if this were the case, the spiders collected from the emigration trail should have been more sensitive to either the artificial or natural trails than the spiders collected from inside the excavated nest. This was not the case. Rather than cueing in on trail pheromones, spiders may locate new colonies by sensing airborne colony odors (osmochemotaxis). Hölldobler (1969) demonstrated that a myrmecophilic staphylinid beetle, Atemeles pubicollis, was able to locate new host colonies via such airborne cues.

The spiders are able to follow the host ants from the old nest sites to the new nest sites when the hosts emigrate (Chapter 2). I believe this is the time when spiders disperse from the host colony and make their way to new colonies. The present study suggests that the spiders may not be using trail pheromones to accomplish either feat. It is highly unlikely that they are following the host ants visually since the majority of araneoid spiders have notoriously poor eyesight (Foelix 1982). If M. pogonophilus is an exception to this rule, I should have seen some evidence of visual acuity in the prey capture behavior of the spiders. However, when I feed collembolans to the spiders in the lab, they only attempt to capture the springtails that have either directly contacted the spider or wandered next to the spider (or been caught in the webs many of the spiders build in the lab). These observations suggest that the spiders respond primarily to vibratory, rather than visual stimuli.

If the spiders are not responding to trail pheromones or visual signals when locating a new nest site, then they might simply be following the vibrations or movements of the host ants themselves--literally swept along with the ants in the emigration trails or in the foraging trails. However, I have seen spiders in emigration trails moving towards the new nest sites even when no host ants are in their immediate vicinity. I have never seen spiders in foraging trails.

Another possible mechanism of dispersal is phoresy, or hitching a ride on the body of a newly inseminated queen or a forager. Phoresy has been seen in other ant symbionts such as many species of mites (Hölldobler and Wilson 1990). Some species of pseudoscorpions are phoretic on a variety of other arthropods as well as on vertebrates (Weygoldt 1969). However, it is unlikely that M. pogonophilus is using phoresy to disperse from nest to nest. Since each P. badius nest is established by a single inseminated queen, the spider would have to ride on the body of a queen as she flies away from the natal nest to find a nest site of her own. Immediately prior to leaving the natal nest, the female alate is mated by both siblings as well as males flying onto the nest from surrounding colonies (however, personal observations of mating in P. badius indicates that most matings are between siblings). Mating among male and female P. badius alates occurs on the surface of the mounds. Several males surround and clamber onto the bodies of females who often try to dislodge the males (Turner 1909, Van Pelt 1953, Harmon 1993, personal observation). In the mating activity I witnessed, I saw no symbionts --collembolans, mites, or spiders--clinging to the bodies of the females. The spiders are so delicate and so unprotected by any type of thickened cuticle that I find it unlikely that, if they were phoretic, they would survive the mating frenzy of the alates. The spiders may be phoretic on the bodies of foragers. However, after hundreds

of hours of observing ants in the field, I have never seen a single such phoretic spider.

The ants are probably not actively transporting their guests themselves. After observing many emigrations of P. badius, all the M. pogonophilus spiders I saw in the emigration trails were moving of their own accord; none were being carried by the host ants.

In sum, the mechanism by which M. pogonophilus is dispersing to new host nests remains a mystery. Although this study argues against the hypothesis that the spiders are using the chemical signals of the host ants, it may be that the laboratory bioassays, although adequate for the host ants, were not meaningful for the symbionts. For example, the texture of the substrate may be of critical importance in cuing the spider in to the presence of a foraging or emigration trail.

Table 4-1. Results of the olfactometer, artificial trail, and natural trail experiments using *P. badius* workers.

COMPOSITION OF SAMPLE	# TO SAMPLE	# TO CONTROL	P-VALUE
Poison Gland: 1-gland equivalent	49	10	<0.001
Poison Gland: 0.1-gland equivalent	45	15	0.002
Dufour's Gland: 1-gland equivalent	26	32	0.622
Dufour's Gland: 0.1-gland equivalent	29	29	0.793
Poison Gland Trail: 0.1-gland equivalent	34	6	0.002
Natural Trail	45	15	0.002

Table 4-2. Results of the olfactometer, artificial trail, and natural trail experiments using *M. pogonophilus* spiders.

COMPOSITION OF SAMPLE	# TO SAMPLE	# TO CONTROL	P-VALUE
Poison Gland: 1-gland equivalent	11	10	$0.5<p<0.9$
Poison Gland Trail: 0.1-gland equivalent	8	8	>0.90
Natural Trail	7	5	$0.5<p<0.9$

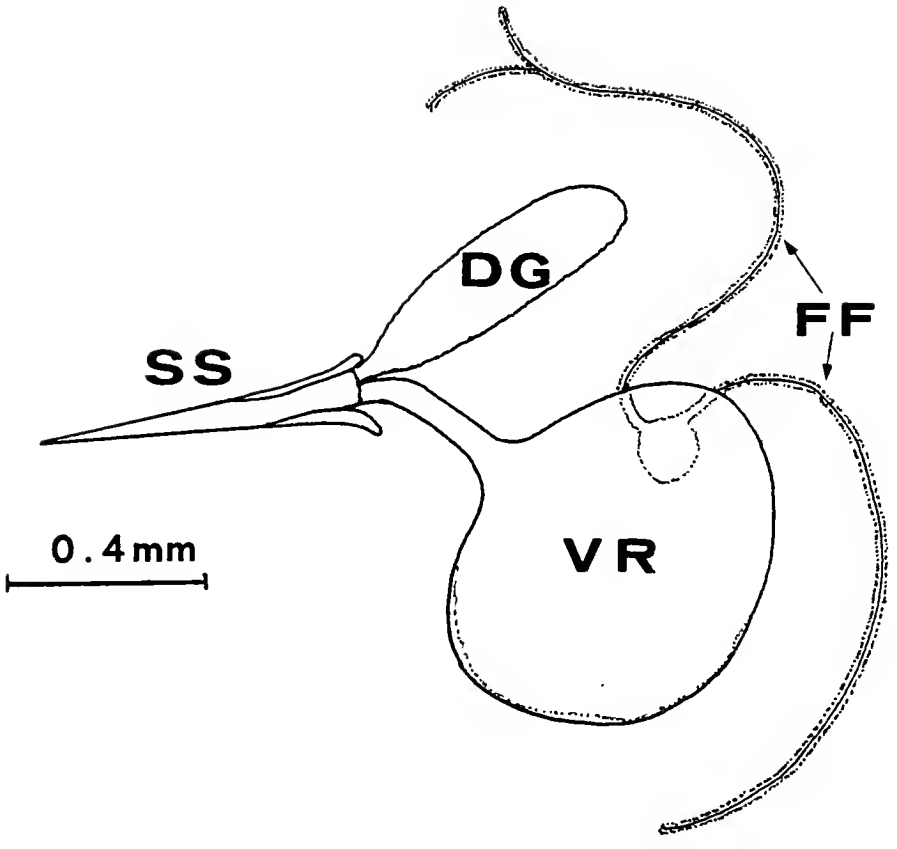


Figure 4-1 (From Schmidt and Blum 1978). Venom apparatus of the harvester ant P. badius. Abbreviations: DG, Dufour's gland; FF, free filaments; SS, sting shaft; VR, venom reservoir.

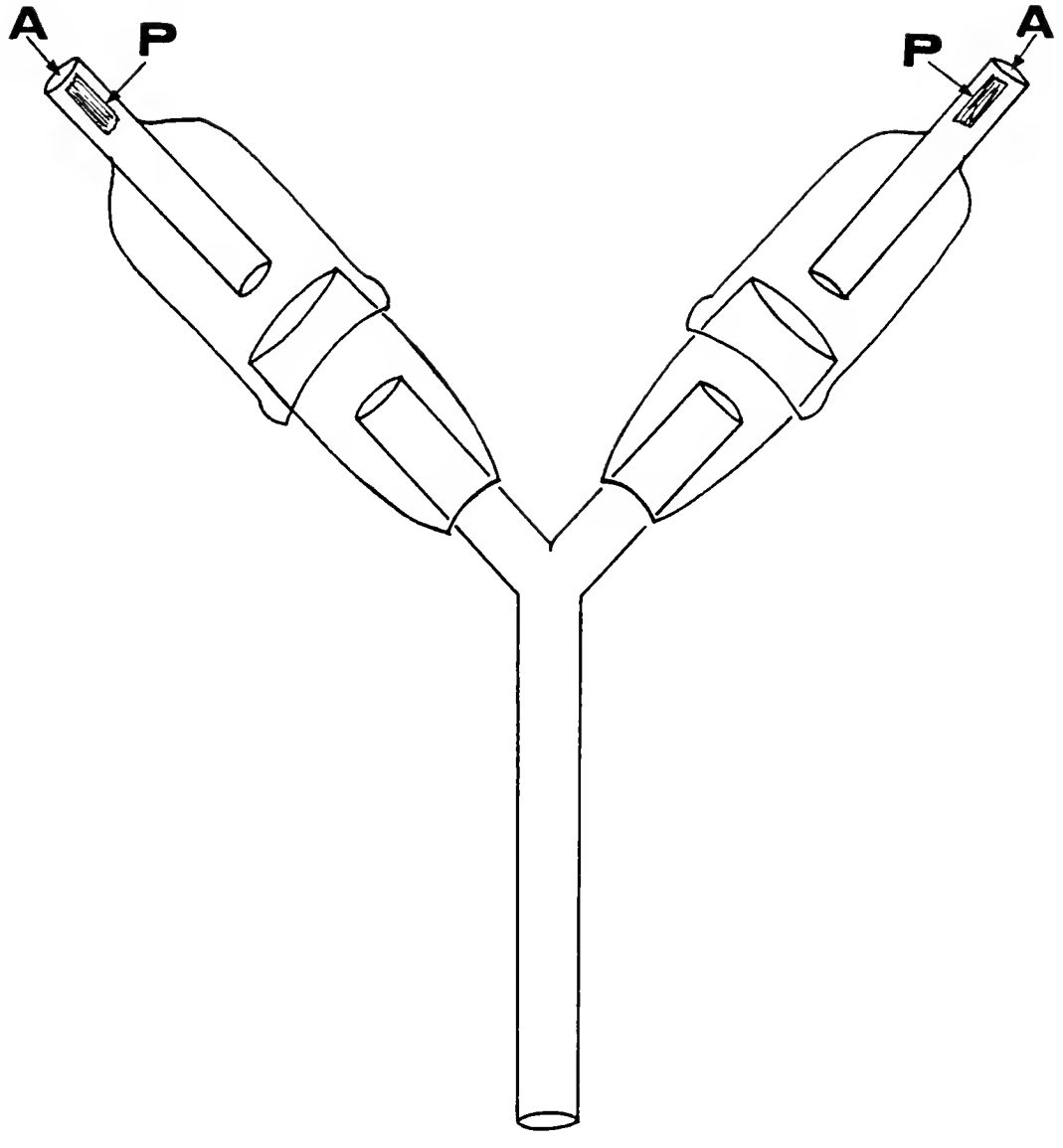


Figure 4-2. Y-shaped Olfactometer. A, air flow over the sample or over the control. P, chromatographic paper saturated with sample or with control. Ants or spiders are introduced into the long stem of the Y. Drawn approximately to scale.

CHAPTER 5
FACTORS AFFECTING SEED SELECTION BY THE FLORIDA HARVESTER ANT, POGONOMYRMEX BADIUS, AT TWO NORTH FLORIDA SITES

Introduction

Although it is widely acknowledged that granivorous ants influence habitat structure (Coffin and Lauenroth 1990, Crist and MacMahon 1992, Gentry and Stiritz 1972, Harmon and Stamp 1992, Hobbs 1985, Kelrick et al. 1986, Rissing 1981), there is little agreement as to what influence habitat structure has on resource use by granivorous ants. My objective was to investigate the influence of habitat structure on seed use by the Florida harvester ant, Pogonomyrmex badius (Latreille) (Formicidae), and to determine if seed selection is correlated with seed nutritional quality. Pogonomyrmex badius inhabits the Gulf Coastal states of the U.S. east of the Mississippi River from Louisiana to North Carolina. It is found in more xeric habitats with well-drained soils and is an important arthropod granivore in these environments (Cole 1968).

Fewell (1988) showed that when resources are clumped, P. badius moves its foraging trails to maximize resource use. Such clumping of resources may be reflected in the proportional representation of that plant species harvested by the ants (Davidson 1977, Whitford 1978).

Where seed sources are more variable or more evenly dispersed, the proportions of different seeds brought into each colony may reflect this difference in resource availability (Fewell 1988). Although some researchers have found such a correlation (de Vita 1979, Whitford 1978), others have not (Crist and MacMahon 1992, Gordon 1993, Hobbs 1985).

Seed selection can also be influenced by seed characteristics such as shape (de Vita 1979, Pulliam and Brand 1975, Whitford 1978), size (Bailey and Polis 1987, Rissing 1981), and nutritional quality (Fewell 1990, Gordon 1980, Kelrick and MacMahon 1985, Kelrick et al. 1986). However, researchers do not agree on which, if any, of these factors are most important in seed selection by ants. Some have indicated either no correlation or negative correlation between size or quality and seed selection by ants (Crist and MacMahon 1992, Pulliam and Brand 1975).

Here, I was specifically interested in addressing the following questions: 1) Are there attributes of habitat structure and seed availability in different habitats that influence seed selection by ants? 2) Are there differences in seed selection by colonies found in the same habitat that may reflect either seasonal differences in resource availability or differences in seed availability within the different foraging ranges of

the colonies? and 3) Does the nutritional quality of seeds influence seed selection by the ants?

The study was conducted at two different sites in north Florida: Archer Sandhills (ASH) in Levy County and the Ordway-Swisher Preserve (ORD) in Putnam County. The habitat at ASH is dominated by Florida rosemary, Ceratiola ericoides (Empetraceae), whereas the ORD site consists of an old field habitat with no one plant species predominating. This field was characterized by a wide variety of grasses and forbs (Franz and Hall 1991). If the apparent difference in habitat structure between these two sites is reflected in the resources available to the ants from the seed bank (i.e., dropped seeds lying in the soil), and if the ants are selecting seeds based on availability, a preponderance of rosemary seeds should be found in the seed chambers, or granaries, of the P. badius colonies at ASH and a more variable representation of seeds in the granaries of the colonies at ORD. In addition, a measurement of the proportional availability of seeds in the seed bank should correspond to the proportions of these seeds collected by the ants. (Reichman (1979) has shown that Pogonomyrmex harvester ants primarily collect seeds from the surface.)

Furthermore, if individual foragers or individual nests specialize on certain types of seeds, then differences in seed selection should be observed between colonies within a site. Such differences in seed selection

may also result from seasonal differences in seed set by plants within the each colony's foraging range. Finally, if the ants are selecting seeds, not based on availability but on quality, then correlations should exist between seeds harvested and some measure of quality (e.g., seed size, caloric, lipid, protein, or carbohydrate content).

Materials and Methods

Seed Collection and Comparison of Seeds Harvested

Seven P. badius nests were excavated at Archer Sandhills (ASH) in Levy County, Florida 26 Km SW of Gainesville on 8 Oct 1992 (ASH15), 20 Feb 1993 (ASH18), 10 Apr 1993 (ASH20), 21 Apr 1993 (ASH21), 4 June 1993 (ASH20B), 13 June 1993 (ASH22), and 25 Sept 1993 (ASH24). ASH20 and ASH20B represent two excavations of the same nest. Four P. badius nests were excavated at the Katherine Ordway Preserve-Swisher Memorial Sanctuary (ORD) in Putnam county, Florida 35 Km SE of Gainesville on 10 Jan. 1993 (ORD16), 23 Jan. 1993 (ORD17), 28 Feb. 1993 (ORD19), and 25 July 1993 (ORD23B). ORD17 and ORD23B represent two excavations of the same nest. The contents of two to five granaries were collected from each nest, dried, sorted, and counted. If several granaries were encountered during an excavation, we collected several at different depths. Florida rosemary seeds, C. ericoides, were so abundant in the ASH nests that we estimated the number present in each granary using the weight of 1000 seeds. Rosemary seeds were stored both as individual

seeds as well as fruits (each of which contains two seeds). (Harvester ants were observed to collect both individual seeds as well as fruits.) Therefore, fruits were counted as two seeds. A little over half of the 1000 seeds weighed were in the form of fruits since this is the approximate representation of fruits versus individual seeds found in the granaries. The seeds were identified, as far as possible, using the seed collection at the University of Florida Herbarium as well as floral lists of ASH and ORD (Franz and Hall 1991 and W. Judd, pers. comm.). Representatives of all seed types collected were then sent to the USDA APHIS Seed Examination Facility in Beltsville, Maryland for further identification or verification. Voucher specimens of all seed types will be kept for future reference. The average seed weight was determined for each species using at least 10 seeds (when possible). All seeds were kept under the same storage conditions throughout the study.

Chi-square analyses were used to determine if any differences existed in the proportions of different seed species harvested between the ASH nests or between the ORD nests. For each X^2 analysis, seed types that had expected numbers of five or fewer were combined. Since the nests were excavated at different times of the year, I suspected that seasonal differences in seed availability might be reflected as differences in granary contents between the nests.

Measuring Seed Availability

To determine whether seeds harvested were correlated with seed availability, six samples from the seed bank at ASH and six samples at ORD were collected in May 1995. Each sample was collected by placing a ring 17 cm in diameter onto the soil and collecting the soil within the ring down to a depth of 0.75 cm. Three samples at each site were collected 2 m from a P. badius nest while the other three samples were collected 4 m from the same three nests. Therefore, all six samples were collected well within the foraging range of the ants. The three nests chosen were greater than 30 m from one another in order to sample a broad range of resources available to the ants in that habitat. The samples were dried and sifted through successively smaller mesh sizes, the smallest being 0.50 mm. Coffin and Lauenroth (1989) found seasonal differences in the seed composition of the seed bank. To reduce the potential temporal bias inherent in collecting seed bank samples only one time of the year, seed fragments were included in the samples as long as the fragments were identifiable. However, to be conservative in the estimates of seed numbers present, seed fragments were counted as 1/2 a seed. The data from the six samples at each of the two sites were pooled and Spearman's rho was used to determine if overall proportions of different seeds from the ASH and from the ORD nests were correlated with seed availability.

Measuring Nutritional Value of Seeds

Nutritional value was determined for only the ten seed species for which sufficient material was available. Of those ten, six were among the most abundant species listed in Table 5-2. The remaining four species represented very minor portions of the contents of the ASH and ORD nests. However, these seeds were abundant in the granaries of a nest excavated at a separate site in North Florida (San Felasco Preserve) and were included in the nutritional analyses because they were part (if only a minor part) of the contents of the ASH and ORD nests. Insufficient material (< one gram) was available to run nutritional analyses for the following seeds, listed in Table 5-2 as being some of the most abundant species collected by the ASH and ORD nests: golden aster, Pityopsis (Chrysopsis) cf graminifola (Asteraceae); centipede grass, Eremochloa ophiuroides (Poaceae); an unidentified species of grass (Poaceae); and jointweed, Polygonella sp. (Polygonaceae). The nutritional quality of the euphorb Crotonopsis linearis, or rushfoil, was also measured. This species was abundant in the San Felasco nest and was used for a seed choice experiment (see below).

Caloric content was measured using a Parr Model 1261 Isoperibol Calorimeter (Paine 1971, Parr Instrument Co. 1984). Between 0.25 - 0.65 g of macerated material was used for each run. For each run, benzoic acid was used as

a standard. Two bombs were used alternately. Bomb #1 had a relative standard deviation of 0.130827. Bomb #2 had a relative standard deviation of 0.120286. I did not correct for ash content since all samples were less than one gram. Sufficient material was available to do two or three replicate runs for five of the 11 major seed types. For these replicates, the largest measurement of caloric content did not differ from the smallest measurement by more than 1.27%. Therefore, I was confident that even for the six seed types for which no replicates were made, the measure of caloric content was accurate. The caloric content per gram of material was converted to calories per seed using the seed weights. For the five seed types for which replicates were made, the mean value for all analyses was used.

An ether extraction technique was used to measure the lipid content of the 11 major seed species according to the procedure described in AOAC (1990). Lipid content was recorded as % lipid / dry weight. For this technique, sufficient material was available to run replicates of five seed species. The higher replicate for each species did not differ by more than 1.10% from the lower value. Therefore, the mean % lipid was used for all analyses.

A protein digestion technique using a Technicon Auto Analyzer was used to determine % Crude Protein in the 11 seed species following the protocol in Gallaher et al (1975) and Hambleton (1977). Approximately 0.25 g of

macerated material was used for each sample. This analysis was conducted by the Forage Evaluation Support Laboratory at the University of Florida.

Once the total caloric density of the seeds was determined, as well as the % lipid and % crude protein composition, a crude estimate of % carbohydrate content was calculated using values of the catabolic yield for seed carbohydrates, lipids and proteins: 4100 cal/g, 9300 cal/g, and 4200 cal/g respectively (Cristian & Lederle 1984, Hill 1976).

Spearman's nonparametric rho (Sokal and Rohlf 1981, Siegel 1956) was used to test for correlations between seed size (i.e., average seed weight) and caloric content, % lipid, % crude protein, and % carbohydrates. Rho was also used to determine if there were any correlations between seeds harvested (i.e., proportions of each seed species collected by the ants) and any of the following measures of seed value: size, caloric content, % lipid content, % crude protein, and % carbohydrate.

Seed Choice Experiment

Because of the preponderance of rosemary seeds, both in the granaries as well as in the seed bank samples at ASH, I decided to do a seed choice experiment to determine if individual foragers will choose seeds other than rosemary if given a choice. Thirteen foragers from eleven nests were presented with a choice of one rosemary seed, one rushfoil (C. linearis) seed and one unidentified sedge

seed (Cyperus sp.#2, Cyperaceae). The rushfoil and sedge seeds were considered novel since neither had been found in any of the excavated ASH nests. Rushfoil was a larger seed than either the sedge or rosemary seeds and had higher nutritional values than rosemary seeds for all measures of nutritional quality except estimated % carbohydrates (Table 5-4). The sedge was smaller than the rosemary seed and had lower values than rosemary caloric content, % dry matter, and % lipids. It had slightly higher % crude protein and estimated % carbohydrates (Table 5-4).

A different cluster of the three seeds were used for each trial so that no seed was used twice. The three seeds were placed atop a small amount of sand on a 2.5 cm^2 cardboard held in front of a forager with forceps. In all trials, the three seeds were tightly clustered to increase the likelihood that the foragers would contact all of them. The foragers were from 0.7 - 5.25 m from their respective nests when intercepted. The ants did not appear to be disturbed by the presence of the cardboard and readily walked onto its surface. Only instances when a forager chose a seed were recorded. Foragers often walked over the cardboard without inspecting the seeds. In addition to recording what seed was chosen by a forager, I also recorded the order of inspection (i.e., antennation).

If individual foragers prefer novel seeds when encountered as Fewell and Harrison (1991) suggest, then

the rushfoil and sedge seeds should be chosen more often than the rosemary seed. If, however, individual foragers form a search image and specialize on seeds commonly encountered (Briese and Macauley 1981, Crist and MacMahon 1991, Hobbs 1985, Whitford 1976) then the rosemary seed should be preferred over the rushfoil or sedge seeds. Finally, if individual foragers select seeds of high nutritional quality (when presented with a choice), as suggested by several authors (Cristian and Lederle 1984, Fewell 1990, Gordon 1980, Kelrick and MacMahon 1985, Kelrick et al. 1986, Whitford 1978) then the rushfoil seed should be chosen over either the sedge or the rosemary seeds since it was both of higher nutritional quality as well as a larger seed than either the rosemary or sedge seeds.

Results

Seed Collection and Comparison of Seeds Harvested

The contents of 29 granaries, ranging in depth from 24 - 94 cm, were collected from the seven ASH nests. The granaries each contained from 155 - 18000 seeds. The contents of 13 granaries, ranging in depth from 28 - 124 cm, were collected from the four ORD nests. They contained from 527 - 4767 seeds. Fifty species of seeds in at least 19 different families were identified from the granaries (Table 5-1). At ASH, Florida rosemary made up 94.2 - 99.9% of the seeds collected by the seven nests

(Table 5-2). At ORD, the colonies showed a more varied selection (Table 5-2).

Differences existed in the proportions of the various seed species collected by the seven ASH nests (X^2=11163.12, P<<0.001). Differences also existed in the proportions of the various species collected by the four ORD nests (X^2=13436.53, P<<0.001). Separate analyses were made to determine if ASH20 and ASH20B and ORD17 and ORD23B (the same nests excavated at two different times of the year--after the colonies had repaired damage from the first excavations) contained similar proportions of the various seed species. They did not (X^2=58.52 and 916.98 respectively, P<<0.001). The probability that the same granaries were collected during the second excavation of either of these pairs of nests is minimal. Two pairs of nests at ASH (ASH20 and ASH21 as well as ASH20B and ASH22) were analyzed that were excavated during the same month. Both pairs differed in the proportions of seed species collected (X^2=248.47 and 514.04 respectively, P<<0.001). ORD16 and ORD17 were also compared. These nests, too, were excavated during the same month. They also differed in their granary contents (X^2=789.48, P<<0.001).

Measuring Seed Availability

Four of the six species of seeds found in the seed bank samples at ASH were also found in the granaries (Table 5-3): Florida rosemary; twining milk-pea, Galactia volubilis (Fabaceae); threeawn grass; and arrowfeather

threeawn grass, A. purpurascens (Poaceae). These four species made up 99.8% of the seeds in the seed bank. The remaining seeds in the seed bank were two small unidentified species. Rosemary seeds accounted for 98.9% of the seeds in the seed bank samples and 98.0% of the seeds found overall in the granaries. No significant correlation existed between the proportions of the four species found in the seed bank samples and the overall proportions of these species represented in the ASH nests (R_S=0.800, P>0.05). However, this lack of correlation was due entirely to differences in the abundance of the minor seed species.

Sixty-five percent of the seeds found in the seed bank samples from ORD were also species collected by the ants (Table 5-3). The remaining 35% consisted of six unidentified species, two of which accounted for 26% of all the seeds found. These two seed species had distinctive feathery awns which de Vita (1979) suggested made seeds difficult for seed harvesting ants to transport. In fact, none of the species collected by the ASH or the ORD nests had any such awns. Fifteen species found in the granaries were also represented in the soil bank and included the following most collected species: sedge, Cyperus sp. #2; threeawn grass, Aristida sp.; centipede grass, Eremochloa ophiuroides; Pensacola bahia grass, P. notatum; jointweed, Polygonella sp.; and Poor Joe, D. teres (Table 5-2). A significant positive

correlation existed between the proportions of these 15 species found in the seed bank samples and the overall proportions of these species represented in the ORD nests (R_S=0.509, P<0.05).

Measuring Nutritional Value of Seeds

The nutritional characteristics of six abundant seed species (Table 5-2) as well as four additional seed species that make up only a small proportion of the granaries of the ants are summarized in Table 5-4. Seed size (i.e., seed weight) is positively correlated with calories / seed (R_S=0.997, P<0.01). Seed size is not correlated with any other measure of nutritional quality.

The measures of the nutritional quality of the seeds were compared with the relative proportions of those species found in each of the eleven nests. Since seed weights had been recorded for all the species found in each of the nests, these data were used to determine if any correlations existed between seeds harvested and seed size. The proportions of seeds collected by the ants were negatively correlated with seed size for ORD17 (R_S= -0.348, P<0.05); ORD23B (R_S= -0.418, P<0.05); ASH20 (R_S= -0.743, P<0.05); and ASH22 (R_S= -0.628, P<0.05). In ASH18, proportions of seeds collected were negatively correlated with % crude protein (R_S= -1.00, P<0.05) and positively correlated with estimated % Carbohydrates (R_S=1.00, P<0.05). In ASH21, proportions of seeds were also negatively correlated with % crude protein (R_S=

-1.00, $P<0.05$). No other significant correlations were found between the proportions of seed species harvested and any measure of seed quality. By chance alone, a significant difference is expected in 5% of the correlation tests. Chance would account for some but not all instances in which significant correlations were found. The various measures of nutritional quality were converted to absolute amounts (i.e., total dry matter by weight, total lipids by weight, etc.) and these values compared with the proportions of the seeds harvested by the ants. No significant correlations were found.

Seed Choice Experiment

For the seed choice experiment conducted at ASH, 12 of the 13 foragers selected the rushfoil seed and one forager chose a rosemary seed ($X^2=20.62$, $P<0.001$). Of the 12 foragers that chose the rushfoil seed, nine clearly antennated at least one other seed before making a choice. Three appeared to select the rushfoil immediately upon encountering it. The ant that chose the rosemary seed antennated only the sedge seed before making its choice.

Discussion

The two habitats explored in this study were characterized by different overall structure and floral composition. The resources available to P. badius at the Archer Sandhills site were clumped with seeds concentrated under rosemary bushes rather than scattered evenly throughout the habitat, and were homogeneous, consisting

predominantly of the seeds of Florida rosemary. In contrast, the Ordway site was much more heterogeneous with a greater variety of seed plants available to the ants.

The lack of correlation between the seed bank sample and the granary contents at ASH was due to differences in the abundance of those seed species making up less than 3% of either the seed bank samples or the granaries. No appreciable difference was found between the proportion of rosemary seeds found in the granaries and the proportion found in the seed bank. At the ORD site, a positive correlation existed between the seeds found in the seed bank samples and the seeds found in the granaries (when the data from all the nests were pooled). These data support the hypothesis that this species of harvester ant is selecting seeds based primarily upon availability.

However, 26% of the seeds found in the seed bank at ORD were never found in any of the granaries. The bulk of these seeds had structures which are apparently difficult for ants to handle (de Vita 1979). Therefore, the ants probably do reject certain seeds due to morphological characteristics.

The fact that significant differences were found among the nests at each site supports the hypothesis that the timing of seed set and seed deposition into the seed bank by different species of plants has a significant impact on resource availability (Coffin and Lauenroth 1989). This is further supported by the observation that

the nests (one at ASH and one at ORD) which had each been excavated twice differed significantly between the first and second excavations. The most likely explanation for this is a seasonal difference in resource availability as suggested by Coffin and Lauenroth (1989). These observations further support the hypothesis that ants select seeds based primarily upon availability.

Significant differences in the granary contents among nests excavated during the same month (but at different locations in the habitat) suggest a difference in seeds available to the colonies within each of their foraging ranges. In fact, when the seed bank samples collected 2 and 4 m from the same P. badius nests are pooled and the resulting three seed bank data sets (at each site) are compared, a significant difference is found in seeds available to different P. badius nests (X^2 = 1119.3, $p << 0.001$ for ORD samples; X^2 = 32.4, $p << 0.001$ for ASH samples). For both these analyses, all seed types with expected values < 5 were collapsed into one category. Therefore, plant phenology as well as the structure of the habitat (in terms of where different seed plants are growing in relation to the colonies) influences resource use and seed selection.

Finally, the colonies, in general, did not seem to be cuing in on any measure of seed quality. In fact, when significant correlations were found, these were primarily negative correlations. This contradicts the observation

by many workers that harvester ants do use measures of nutritional quality when selecting seeds (Fewell 1990, Gordon 1980, Kelrick and MacMahon 1985, Kelrick et al. 1986). However, Fewell (1990) and Kelrick et al. (1986) determined seed preference by offering ants clumps of seeds from which to choose (analagous to our seed choice experiment except we offered only three seeds to individual foragers rather than dishes of many seeds of different species to foragers as a whole). As pointed out by Crist and MacMahon (1992), individual foragers probably rarely have the opportunity to choose among a variety of seed species of varying quality. Therefore, at the level of individual foragers, it is unrealistic to present ants with a choice of seeds and assume that their choice of higher quality seeds means that, in their daily foraging bouts, they are actively using some criteria of seed quality in their selection of resources. The seed choice experiment leaves little doubt that, when presented with a choice, harvester ants are able to discriminate among seeds based on size or some measure of quality. This may be important on occasions when foragers encounter patches of seeds. However, this ability to discriminate is probably not relevant to the decisions the majority of foragers make when they encounter seeds since our data indicate they are basing choice more on availability than on quality.

Table 5-1. Identity and proportional representation of seeds collected from the granaries of the Archer Sandhills (ASH) and Ordway Preserve (ORD) P. badius nests.

FAMILY	SPECIES	COMMON NAME	SITE	%
Amarantaceaeae	Froelicha floridana	Cotton Weed	ORD	0.08
Anacardiaceae	Rhus sp. #1	Sumac	ASH	0.03
"	R. sp. #2	Sumac	ASH	0.01
"	R. sp. #3	Sumac	ASH	<0.01
Asteraceae	Pityopsis (Chrysopsis) cf. graminifola	Golden Aster	ASH	0.41
Brassicaceae	Brassica sp.	Mustard	ORD	<0.01
Cactaceae	Opuntia humifusa	Prickly-pear cactus	ORD	0.08
Convolvulaceae	unidentified		ASH	0.02
Cyperaceae	Cyperus sp. #1	Sedge	ORD	0.01
"	C. sp. #2	Sedge	ORD	15.13
Empetraceae	Ceratiola ericoides	Florida Rosemary	ASH	98.03
Euphorbiaceae	Cnidoscolus texanus	Nettle	ORD	0.04
"	Croton sp.	Doveweed	ASH/ORD	<0.01/0.10
"	unidentified		ASH	<0.01
Fabaceae	Aeschynomene sp.		ASH	<0.01

Table 5-1 -- Continued.

FAMILY	SPECIES	COMMON NAME	SITE	%
Fabaceae	A. viscidula	Jointvetch	ORD	0.95
"	Chamaecrista nictitans	Sensitive Plant	ORD	0.13
"	Crotolaria rotundifolia	Rabbit-bells	ORD	0.01
"	Desmodium tenuifolium	Tick-trefoil	ORD	0.01
"	Galactia volubilis	Twining Milk-pea	ORD	0.01
"	Lupinus sp.	Lupine	ORD	0.14
"	Shrankia (?) sp.	Brier	ASH	<0.01
"	Tephrosia crysophylla	Golden Hoary Pea	ORD	0.28
"	unidentified #1		ORD	0.01
"	unidentified #2		ORD	0.18
Phytolaccaceae	Phytolacca americana	Poke Weed	ASH/ORD	<0.01/0.32
Pinaceae	Pinus palustris	Longleaf Pine	ORD	0.02
Poaceae	Andropogon sp.	Bluestems	ASH	<0.01
"	Aristida sp.	Threeawn	ASH/ORD	0.08/15.38
"	A. purpurascens	Arrowfeather threeawn	ASH	0.90
"	Eremochloa ophiuroides	Centipede grass	ORD	1.53

Table 5-1 -- continued.

FAMILY	SPECIES	COMMON NAME	SITE	%
Poaceae	Panicum sp. #1	Panicum	ORD	0.69
"	Pan. sp. #2	Panicum	ASH/ORD	0.01/0.72
"	Paspalum notatum	Pensacola Bahia Grass	ORD	42.16
"	Pas. setaceum	Stiff Paspalum	ASH/ORD	<0.01/0.46
"	Sorghastrum secundum	Lopsided Indian Grass	ORD	0.01
"	unidentified		ASH/ORD	<0.01/2.03
Polygonaceae	Polygonella sp.	Jointweed	ORD	1.84
Rosaceae	Rubus cunefolius	Blackberry	ASH	0.49
Rubiaceae	Diodia teres	Poor Joe	ORD	17.54
Smilaceae	Smilax auriculata or S. glauca	Wild-bamboo or Wild Sarsaparilla	ASH/ORD	<0.01/<0.01
Turnaceae	Piriqueta caroliniana var. glabra	Smooth Stem Piriqueta	ORD	0.02
Vitaceae	Vitis sp.	Grape	ORD	0.01
Unidentified #1			ASH/ORD	<0.01/<0.01
Unidentified #2 - #5			ORD	0.07
Unidentified #6 - #7			ASH	<0.01

Table 5-2. Percentages of the most abundant seed species collected by ants from the seven ASH excavations and the four ORD excavations. *, species analyzed for nutritional content.

	NEST#						
SPECIES	ASH15	ASH18	ASH20	ASH21	ASH20B	ASH22	ASH24
Pityopsis graminifolas	0	0	0	0	0	0	3.2
*Ceratiola ericoides**	98.2	99.0	99.6	99.5	99.9	95.9	94.2
*Aristida purpurascens**	0	0.1	0	0.4	<0.1	3.8	1.4
	ORD16	ORD17	ORD19	ORD23B			
Cyperus sp. #2*	0	0	61.1	<0.1			
Aristida sp.*	1.8	22.5	22.1	8.1			
Eremochloa ophiuroides	1.0	3.2	<0.1	1.3			
*Paspalum notatum**	77.3	42.5	0	62.4			
Unident. Poaceae	0.1	3.3	2.3	1.3			
Polygonella sp.	0	0.3	6.9	0.1			
*Diodia teres**	17.9	22.3	5.4	21.7			

Table 5-3. Seeds collected from the seed bank samples at ASH and ORD.

SPECIES	SITE	#COLLECTED
Froelicha floridana	ORD	14
Opuntia humifusa	ORD	1
Cyperus sp.	ORD	109
Ceratiola ericoides	ASH	2165
Chamaecrista nictitans	ORD	3
Galactia volubilis	ASH	1
Lupinus sp.	ORD	1
Pinus palustris	ORD	3
Aristida sp.	ASH/ORD	11/153
Aristida purpurascens	ASH	7
Eremochloa ophiuroides	ORD	274
Panicum sp. #1 and #2	ORD	457
Paspalum notatum	ORD	32
Paspalum setaceum	ORD	12
Sorghastrum secundum	ORD	32
Polygonella sp.	ORD	23
Diodia teres	ORD	19
Unidentified	ASH	5
Unidentified	ORD	610

Table 5-4. The nutritional characteristics of six of the most abundant seed species as well as four additional species found in ASH and ORD nests. Values represent averages except where insufficient material was available for replicate measurements. C. linearis was used for the seed choice experiment (see text).

SPECIES	SEED WT (mg)	CALORIES PER SEED	%DRY MATTER	%LIPID	%CRUDE PROTEIN	ESTIMATED CARBOHYDRATES
Cyperus sp. #2	0.4	1.89	92.62	8.24	3.16	84.65
Ceratiola ericoides	0.8	4.01	93.65	14.35	1.90	82.08
Galactia volubilis	11.6	46.98	92.16	1.84	26.99	74.68
Tephrosia crysophylla	7.7	36.73	94.66	11.61	35.41	52.44
Aristida sp.	0.4	1.72	92.04	4.16	22.91	69.85
A. purpurascens	0.6	2.68	97.50	4.08	19.20	74.15
Paspalum notatum	1.4	6.18	93.10	2.94	4.45	84.17
P. setaceum	1.0	4.35	92.15	2.56	8.18	94.84
Rubus cunefolius	1.5	6.98	93.66	16.65	5.87	81.36
Diodia teres	3.2	16.41	92.89	6.28	15.80	93.09
Crotonopsis linearis	2.3	12.65	94.96	24.32	25.49	49.39

CHAPTER 6
NEST DISPERSION AND INTER-NEST AGGRESSION OF *POGONOMYRMEX BADIUS* AND THE EFFECT OF HOST POPULATION STRUCTURE ON SPIDER INTEGRATION INTO COLONIES

Introduction

The spatial arrangement of ant colonies in a habitat may provide insight into intraspecific competition for shared resources. Many workers have found that intraspecific ant colonies as well as interspecific colonies of ecologically similar species are overdispersed (de Vita 1979, Levings and Traniello 1981, Harrison and Gentry 1981, Cushman et al. 1988). This overdispersion probably reflects interference and exploitative competition (Hölldobler and Wilson 1990).

Resource requirements of intraspecific colonies will overlap to a greater extent than resource requirements of interspecific colonies found in the same habitat. The more limited the resource, the greater the advantage of niche partitioning (i.e., increasing colony spacing). Therefore, it would be adaptive for colony members to be able to differentiate between competitors (intraspecific neighbors or interspecific neighbors who share a limited resource) and non-competitors (members of more distant colonies or interspecific foragers of a non-competitor). In fact, Gordon (1989) found that western harvester ants of the species *Pogonomyrmex barbatus* can distinguish

between a neighboring colony's foragers (competitors) and a more distant colony's foragers (stragglers or lost foragers, i.e., non-competitors). When neighboring ants were encountered in the foraging trail of a nest, foraging intensity dropped to a greater extent than when ants from a more distant colony (strangers) were encountered in the foraging trail. Gordon cited foraging intensity as a measure of the colony's reaction to the presence of alien ants. Thus, she concluded that encounters with neighbors deterred foraging to a greater extent than encounters with strangers.

Many of the studies investigating ant colony spacing and its relation to competition have focused on seed harvesting ants of the genus Pogonomyrmex whose colonies are often overdispersed (de Vita 1979, Levings and Traniello 1981, Harrison and Gentry 1981). Inter-colony worker-worker aggression has been reported in harvester ants (Hölldobler 1976, de Vita 1979, Gordon 1991) as has aggression between workers and non-colony queens (Hölldobler 1976). Inter-colony worker-worker avoidance seems to be another common method of ensuring maximum colony spacing (Harrison and Gentry 1981, Gordon 1991).

The purpose of this study was to investigate the factors that affect colony spacing of the Florida harvester ant, P. badius and to explore what effect this underlying population structure has on the integration of the myrmecophilic spider, Masoncus pogonophilus, into the

ant colonies. If, as Gordon suggested (1989), harvester ants can distinguish neighbors, or potential competitors, from strangers, or non-competitors, due, perhaps, to the greater frequency of encounters with foragers from neighboring colonies, then this difference may be reflected by differences in the level of aggression shown by ants towards neighbors versus strangers introduced onto their mounds. In addition, patterns of aggression towards neighboring colonies should influence colony dispersion if dispersion is a mechanism for reducing competition between nests. If the frequency of encounters between foragers from neighboring nests is high, then aggression towards neighbors introduced onto the nest mound should also be high and the population should tend towards even dispersion as a mechanism for reducing competitive interactions. Conversely, if encounters between foragers from neighboring nests are low, perhaps due to super-abundant resources or resources that are clumped very close to each nest, then aggression towards neighbors introduced onto the mound should be low and dispersion of nests should be random.

Ants distinguish nestmates from non-nestmates through chemically-based phenotype matching (Hölldobler and Wilson 1990). These chemical recognition cues can be genetically derived, acquired from the queen, acquired from other nestmates, and/or derived from the environment (Carlin and Hölldobler 1986, Hölldobler and Wilson 1990).

Workers antennate each other and match the cuticular cues of the encountered individual with a sensory template based upon a learned set of cues likely to be possessed by nestmates (see Hölldobler and Wilson 1990 for an extensive coverage of this topic and associated references).

Many myrmecophilic arthropods take advantage of this system of nestmate recognition among their hosts by mimicking host colony odor (probably through passive absorption into the cuticle). Such chemical mimicry has been documented in a myrmecophilic beetle (Vander Meer 1982), a parasitoid wasp (Vander Meer et al. 1989), syrphid fly predator of a formicine ant (Howard et al. 1990), and another syrphid fly predator of a myrmicine ant (Howard et al. 1990). If the myrmecophilic spider, M. pogonophilus also absorbs the colony odor of its host ants, then spiders introduced onto the mounds of neighboring (non-host) nests should be recognized and treated as intruders whereas spiders re-introduced onto the mounds of their host colonies should not be treated with aggression. If true, then host population structure and aggression between neighboring colonies would prove a formidable barrier to dispersing spiders.

Materials and Methods

Site Descriptions

This study was conducted at two different sites in North Florida: Archer Sandhills in Levy County and the Ordway-Swisher Preserve in Putnam County. These sites

differed both in floral composition as well as in overall habitat structure (see Chapter 5). The Archer Sandhills site was dominated by dense stands of Florida Rosemary bushes (Ceratiola ericoides) and scattered turkey oaks (Quercus laevis). Rosemary seeds made up between 94 - 99% of the seeds found in the nest granaries (see Chapter 5). In contrast, the Ordway Preserve site was an old field habitat characterized by many species of grasses and herbs with no dominant species (Franz and Hall 1991). This more varied resource base is reflected by the seeds stored in the nest granaries (see Chapter 5).

Nest Dispersion

Nearest neighbor distances of P. badius nests were measured in a 60 X 60 m plot at Archer Sandhills and a 110 X 110 m plot at the Ordway Preserve. Bordering areas around the demarcated plot were searched for other P. badius colonies that might be nearest neighbors to those within the plots to avoid bias from edge effects (Krebs 1989). Distances were accurate to within 0.5 m. The Clark-Evans Nearest Neighbor Method was used to determine whether the dispersion pattern was random, clumped, or overdispersed (Krebs 1989).

Inter-Nest Aggression

To test the hypothesis that P. badius workers can distinguish potential competitors (near neighbors) from non-competitors (workers from more distant nests), cross-introduction experiments using four focal nests at

Archer Sandhills and four focal nests at Ordway Preserve were performed. For each of these focal nests, the behavioral responses of ants to individual P. badius workers from either the nearest neighbor nest or a distant nest placed on the mound of the focal nest were recorded. Each of the focal nest/stranger nest pairs had at least one other P. badius nest located between them. For each pair of nests, 20 total cross-introductions of individual ants were performed; i.e., 20 neighboring ants and 20 strangers were introduced onto the mound of the focal nest. The order of introduction onto the mound was: 1) neighboring ant, 2) ant from the distant mound, and 3) a control ant from the focal nest that was collected and reintroduced onto the mound. The reintroduction of a nestmate back onto the mound was used to control for the possible effects of general alarm, or aggression on the part of the focal ants in response to disturbance during the experiment. Behavioral responses were recorded as non-aggressive if the ants only antennated each other but showed no other interest or if the focal ants oriented suddenly towards the introduced ant with their mandibles agape but did not proceed to bite or attempt to remove the introduced ant from the mound. This latter behavior (sudden orientation with mandibles agape) was recorded as non-aggressive because it was often shown towards a control ant in response to the control ant's (nestmate's) agitation (and presumed release of alarm pheromone).

Behavioral responses were recorded as aggressive if, after antennation, the ants bit or grappled with one another or if the host ants picked up the introduced ant with their mandibles and proceeded to remove the foreign ant from the mound. Introduced ants were removed only after antennating at least 3 host ants (unless an earlier encounter resulted in biting and grappling). Vials used to capture and introduce ants onto mounds were wiped out with an ethanol-soaked cloth to reduce the chances of passive absorption by ants of non-colony odors. Each of the eight sets of neighbor versus stranger pairs was analyzed using a contingency table analysis (Sokal and Rohlf 1981). Two linear regression analyses were also performed to determine if aggression towards neighbors and towards strangers decreased with increased nest spacing. Distances between neighbors or between strangers were used as the independent variables with the arcsin transformed proportion of aggressive responses as the dependent variables. Transformation of the dependent variable was necessary since it was not a continuous variable (Kleinbaum and Kupper 1978, Sokal and Rohlf 1981).

Response of Ants Towards Introduced Myrmecophilic Spiders

Masoncus pogonophilus spiders were removed from their host colonies and subsequently reintroduced to those same colonies and/or to neighboring colonies known to be aggressive towards the host colony. If the spider had been separated from their host colonies for more than one

week, they were housed in a vial closed only with metal screening and cheese cloth (to allow free air--and odor--flow) and the vial placed in a closed plastic shoebox housing 60 - 70 ants from the host nest as well as material from the mound of the host nest which Gordon (1984) has shown is saturated with colony odor. The host ants and the spiders were kept together in this manner for at least two days to allow possible reabsorption of the colony odor into the spider's cuticle. After this time, the spiders were reintroduced to the mounds of the host colony and/or introduced to mounds of neighboring colonies. All spiders housed with ants were placed in fresh vials before introducing them to an ant mound to ensure that any colony odor lingering on the vial, itself, would not trigger a behavioral response from the ants.

A total of seven spiders were reintroduced to host nests and 11 spiders were introduced to a neighboring nest. Of the 11 spiders introduced onto foreign nests, three had previously been re-introduced to their host nests and subsequently recaptured. The behavior of the ants to the spider was recorded as non-aggressive if the ants antennated or contacted the spider but showed no reaction to the presence of the spider. The behavior of the ants was recorded as aggressive if the ant attacked and attempted to bite the spider with its mandibles or if it made a sudden movement toward the spider with its mandibles agape. Care was taken to ensure that the ants

showed no visible signs of agitation or alarm prior to introduction of the spiders. The latter behavior on the part of the ants (sudden orientation with mandibles agape) was regarded in this experiment as aggressive because it was a distinct change in the behavior of an ant that, prior to encountering the spider, was antennating the midden or going about some other nest maintenance task.

Results

Nest Dispersion

Eleven P. badius nests were found in the 60 X 60 m plot at Archer Sandhills resulting in a nest density of 0.003 P. badius nests/m^2. The mean nearest neighbor distance was 11.45 m ($\pm$ 2.35). The index of aggregation, R (Krebs 1989), was 1.27 which was not significantly different from 1 ($0.05 < p < 0.1$ for a one-tailed test). Therefore, the dispersion of P. badius colonies at this site was random.

Sixteen P. badius nests were found in the 110 X 110 m plot at the Ordway Preserve for a nest density of 0.001 nests/m^2. The mean nearest neighbor distance was 20.00 m ($\pm$ 9.29). The index of aggregation was 1.45 which was significantly different than 1 ($p < 0.005$ for a one-tailed test). Therefore, P. badius nests at the Ordway site are over-dispersed.

Inter-Nest Aggression

At Archer Sandhills, three of the focal nests were significantly more aggressive toward workers from

neighboring colonies than workers from distant colonies (X^2 = 4.51, 8.18, and 10.23 respectively, $p < 0.05$, Table 6-1A). However, one focal nest (#26ST in Table 6-1A) was relatively unaggressive towards workers from both the neighboring nest as well as toward workers from the more distant nest ($X^2 = 0.11$, $p > 0.5$). At Ordway Preserve, none of the focal nests showed higher aggression towards neighbors than towards strangers (all $X^2 < 2.0$, $p > 0.2$, Table 6-1B). The closer the eight focal colonies were to their nearest neighbors, the more aggressive focal ants were towards foreigners introduced onto their mounds (r^2 = .567, $t = -2.804$, d.f. = 6, $p = 0.031$, Fig. 6-1). However, distance was not a good predictor of aggression towards strangers introduced onto the mounds of the focal colonies ($r^2 = 0.175$, $t = -1.131$, d.f. = 6, $p = 0.301$, Fig. 6-2).

Response of Ants Towards Introduced Myrmecophilic Spiders

Of the seven spiders reintroduced to their host nests, two (29%) elicited an aggressive response from the hosts. Of the eleven spiders introduced to aggressive neighboring nests, seven (64%) elicited aggressive responses from the non-host ants (Table 6-2). One of the spiders was attacked and killed by a non-host worker. The difference in behavioral responses elicited by host ants versus non-host ants, although suggestive, was not significant ($X^2 = 0.935$ $p = 0.334$, Table 6-2).

Discussion

Dispersion of P. badius nests at these two sites is not a good predictor of inter-nest aggression. At Ordway, the nests are over-dispersed. If this over-dispersion was triggered by more frequent encounters between neighboring nests, i.e., higher competitive interactions between neighboring nests, as suggested by Harrison and Gentry (1981), then neighboring nests should be more aggressive towards one another than nests spaced further apart. This is not the case: the four focal nests at Ordway showed less aggression overall towards foreign ants (neighbors or strangers) introduced onto their mounds than three of the four focal nests at Archer Sandhills.

The greater aggression of focal ants at Archer Sandhills towards neighbors than towards strangers (at least for three of the four focal nests) would suggest a higher frequency of encounters of neighbors (competitors) than of strangers (non-competitors) as suggested by Gordon (1989). If these competitive interactions trigger colonies to migrate away from one another to reduce the overlap of foraging ranges (as suggested by Harrison and Gentry 1981) then nests at Archer should be overdispersed. This is not the case.

However, the linear regression analyses suggest that, at least for neighboring nests (potential competitors), distance is a good predictor of aggression (Fig. 6-1). The aggression of the four focal nests at

Ordway towards their neighbors may be low simply because these nests are spaced further than the neighboring nests at Archer. This is further supported by the fact that the focal nest at Archer spaced furthest from its nearest neighbor (#26ST, Table 6-1) is the least aggressive of any of the four focal nests towards their neighbors.

The lack of correlation between aggression of focal ants towards foreigners from distant colonies (Fig. 6-2) and the relatively low proportions of aggressive responses of focal nests towards strangers (Table 6-1) suggests that, once a colony is outside the foraging range of the focal nest, distance is no longer a good predictor of aggression. It suggests that if the foraging ranges of two nests do not overlap, it is irrelevant to what extent they do not overlap. However, it is important to note that three of the eight focal nests (#26ST, #6, and #R1, Table 6-1) reacted somewhat more aggressively towards strangers than towards neighbors. Nevertheless, of these three, two (#26ST and #R1) showed little aggression towards any ant, neighbor or stranger, placed on the mound and none of the three were significantly more aggressive towards strangers than towards neighbors.

The difference in dispersion patterns at these two sites can be explained both by the differences in nest densities as well as by differences in habitat structure and resource availability. The density of nests at Ordway (0.001 nests/m^2) is three times less than the density of

nests at Archer Sandhills. At Archer Sandhills, there is a superabundance of rosemary seeds available to the ants; 98-99% of the seeds in the seed bank are rosemary seeds (Chapter 5). The higher density of nests at Archer Sandhills may be explained by the availability of this predictable and extremely abundant resource. Rosemary seeds are also particularly high in lipid content (Chapter 5) and are, therefore, not only an abundant resource but a nutritionally high quality resource. At Ordway, there is considerably more variation in the seeds available to the harvester ants and more variance in the nutritional quality of the seeds available at any given season (Chapter 5). This seasonal variation in resources available to the ants at Ordway may increase the mortality of incipient P. badius nests, thus keeping the density of nests low (as compared to the Archer Sandhills population). At Archer Sandhills, the apparent availability of clear, open areas of sand for nest construction is less than the apparent availability of areas for nest construction at the open field habitat of Ordway due to the density of rosemary bushes at Archer and the lack of shrubs or bushes at Ordway (P. badius does not establish nests beneath trees or bushes--it requires open areas with well-drained soils). If the habitat at Archer Sandhills was more open, I contend that the nest dispersion would be more even.

The "passivity" of nests at Ordway towards ants from neighboring mounds may simply be a reflection of the low density and increased spacing of colonies in this habitat. In other words encounters, even between the closest nests, are probably infrequent.

The results of the spider introduction experiments are ambiguous. Although some non-host ants reacted quite aggressively towards the spiders (one ant even killing a spider), many others showed no reaction to the myrmecophile. And not all the host ants reacted to the spiders as if they "belonged" on the mound; a few of the hosts even reacted aggressively towards them. The data do not preclude the possibility that the spiders absorb host colony odors. However, if they do, there may be a wide variation in the extent to which the hydrocarbons are absorbed into the cuticle of different individuals. I believe the spiders become integrated into colonies, not through chemical mimicry, but by being quick and sneaky. They are adept at evading the ants, moving rapidly away when contacted by a leg or an antennae. When placed on a P. badius mound, they wander around apparently aimlessly until they reach an area where the sand slopes downward towards the colony entrance, at which point they move directly downward and enter the colony. They move into the entrance when no ants are either entering or leaving the nest and usually enter upside down, walking on the ceiling of the entrance tunnel rather than on the floor

(where they would be more likely encountered by a worker). Once inside the nests, where they are surrounded by air saturated with the colony odor, absorption of colony odor may then play a role in maintaining the integration of the spiders with the hosts. If this is the case, a dispersing spider arriving on the mound of a neighboring colony may be recognized as an intruder and attacked before it can make its way to the nest entrance and sneak inside.

Table 6-1. Distances between neighboring nests and distant nests for each focal pair. The proportion of aggressive encounters out of the 20 total encounters between neighbors or strangers is presented as are the X^2 values. A. Archer Sandhills nests. B. Ordway Preserve nests.

	COLONY PAIRS	DISTANCE (m)	PROPORTION AGGRESSIVE RESPONSES	X^2
A.	25R/23R	7.05	.90	4.51^{*}
	25R/24R	11.50	.55	
	26ST/OAK1	14.80	.30	0.11^{ns}
	26ST/13	23.00	.35	
	23/20	5.85	.70	8.18^{**}
	23/12	20.75	.20	
	12/15	11.80	.85	10.23^{**}
	12/17	34.10	.30	
B.	27/26	8.35	.55	0.00^{ns}
	27/37	22.20	.55	
	19/20	15.00	.35	0.12^{ns}
	19/10	30.90	.25	
	6/14	10.20	.45	1.64^{ns}
	6/UNK	27.45	.70	
	R1/R2	16.25	.15	0.16^{ns}
	R1/18	50.65	.25	

* significant at $p<0.05$
** significant at $p<0.005$
ns not significant

Table 6-2. Chi-Square table showing the responses of host and non-host ants towards M. pogonophilus spiders introduced onto their mounds.

	#AGGRESSIVE RESPONSES	#NON-AGGRESSIVE RESPONSES
HOST ANTS	2	5
NON-HOST ANTS	7	4

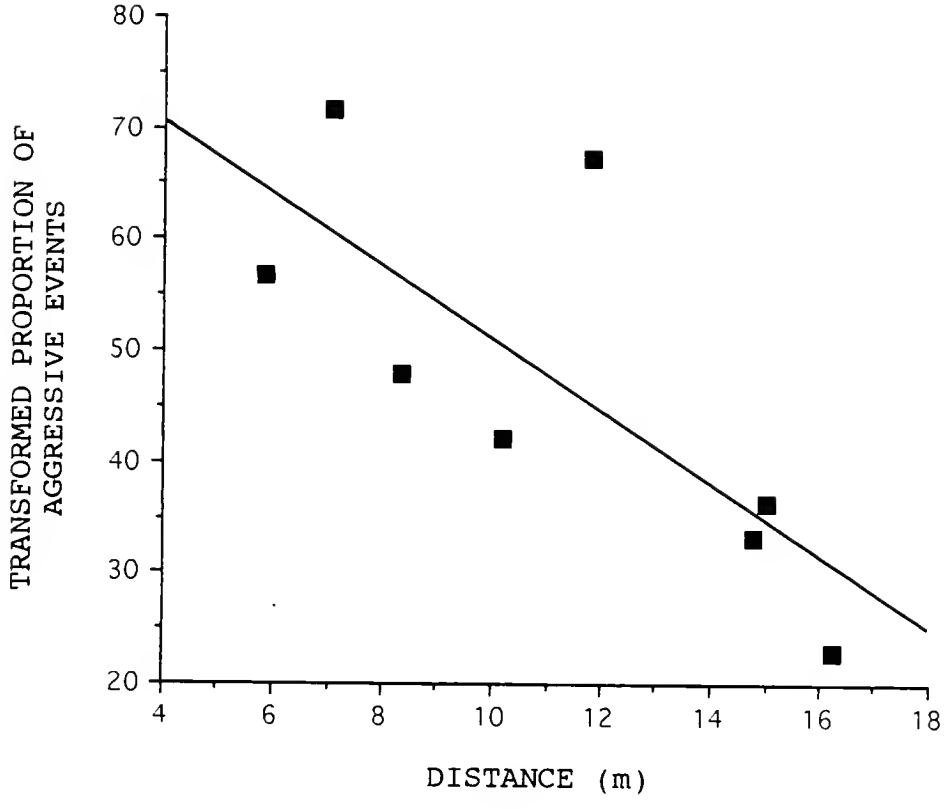


Figure 6-1. Linear regression of the distances between focal nests and their nearest neighbors and the arcsin transformed proportion of aggressive responses of focal ants towards their neighbors (r^2 = 0.567, t= -2.804, d.f. = 6, p = 0.031).

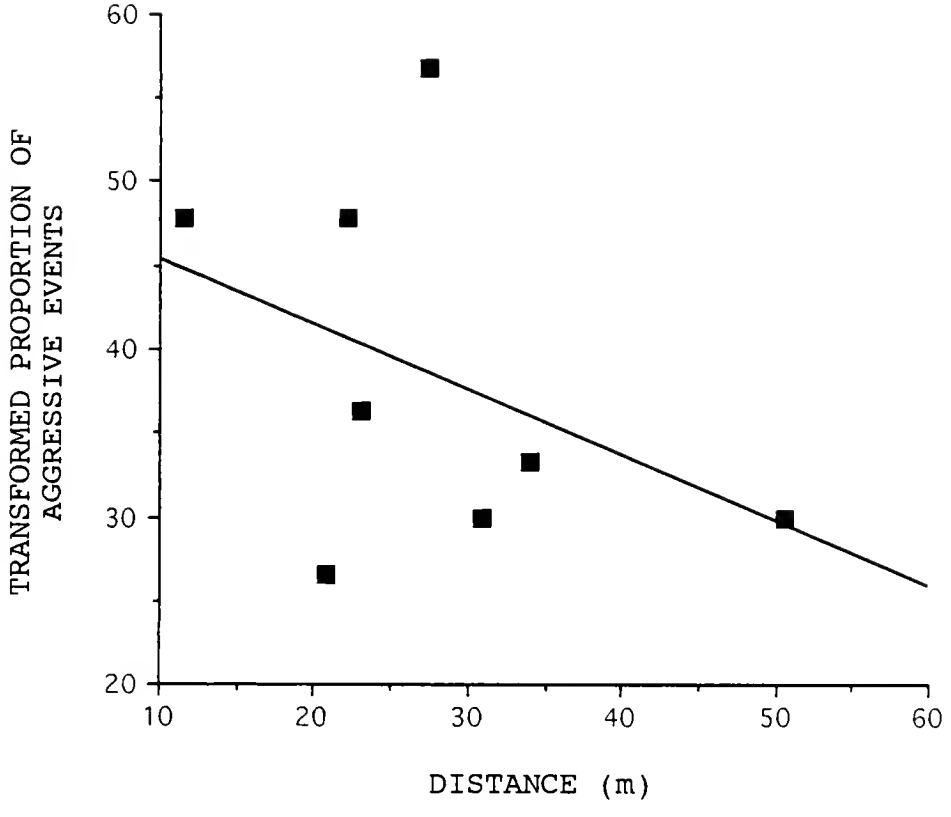


Figure 6-2. Linear regression of the distances between focal nests and strangers, or more distant nests, and the arcsin transformed proportion of aggressive responses of focal ants towards strangers (r^2 = 0.175, t = -1.131, d.f. = 6, p = .301).

CHAPTER 7
SUMMARY AND CONCLUSIONS

As Hölldobler and Wilson (1990) propose, an ant colony can be considered an isolated ecosystem. Arthropods that have evolved mechanisms for integrating themselves into this specialized community are greeted with a stable microclimate, abundant food, and protection from predators and parasites. This study has investigated two members of one such ecosystem: the myrmecophilic spider, M. pogonophilus, and its host ant, P. badius. I have determined some of the adaptations involved in this association and have raised many additional questions.

I demonstrated that M. pogonophilus is morphologically distinct from previously described congeners and is dependent upon the ant nest ecosystem (Chapter 2). These myrmecophiles deposit their eggsacs in depressions in the ceilings of the nest chambers and spend all stages of their lives inside the nests. Furthermore, when the host colony emigrates to a new nest site, the myrmecophilic spider moves with them.

I rejected the hypothesis that spider populations within ant nests represent semi-isolated demes, or metapopulations (Chapter 3). Gilpin (1991) described metapopulations as isolated local populations whose heterozygosity is low because of decreased gene flow

between demes. Masoncus pogonophilus, in contrast, shows high genetic variation within local populations (i.e., among spiders within nests) and low genetic variation between local populations indicating high rates of dispersal between neighboring ant nests within the lifetime of the host nests.

I used the Random Amplified Polymorphic DNA, or RAPD, fingerprinting technique to measure the degree to which populations of spiders were isolated. The RAPD technique proved effective in testing questions concerning the isolation of separate populations. However, it proved less useful in determining the extent of gene flow per generation, or NM (Wright 1951). Because RAPD markers are inherited as dominant alleles, it is difficult to estimate heterozygosity since banding patterns represent either homozygote dominants or heterozygotes (Welsh and McClelland 1990, Williams et al. 1990). Without an adequate measure of heterozygosity, it is difficult to estimate population subdivision, or F_{ST} (Wright 1951). Lynch and Milligan (1994) described algorithms for measuring F_{ST} with RAPD markers. However, their technique required an assumption of Hardy Weinberg equilibrium which was not a valid a priori assumption for my study. Their technique also estimated heterozygosity using an assumption that "null" alleles (or absence of bands at a locus) represent recessive alleles. However, even Milligan and Lynch (1991) admit that "null" alleles may have multiple causes such as

loss of primer sites or insertions. Despite these limitations, RAPD's is a useful molecular technique for measuring the degree to which populations are isolated.

The mechanism of gene flow or dispersal of spiders between local populations remains unknown. Laboratory experiments did not support the hypothesis that spiders were able to follow trail pheromones (Chapter 4). However, it may be that, although the host ants readily follow artificial and natural trails in the laboratory, the spiders require additional stimuli that are missing in the laboratory before they can cue in on these chemical signals. Alternatively, it may be that M. pogonophilus locates new colonies, not via trail pheromones, but by sensing airborne colony odors (osmochemotaxis). The ability to locate new host colonies via airborne cues was documented by Hölldobler (1969) for the myrmecophilic staphylinid beetle, Atemeles pubicollis.

The dispersion of host nests in an environment may affect the ability of dispersing spiders to become integrated into new host colonies in that more distant colonies may be more difficult for spiders to locate and colonies located very close to the former host colony may be more aggressive towards the spiders. Aggressiveness of P. badius colonies towards one another is correlated with proximity of and, presumably, with increased competitive interactions between neighboring colonies (Chapter 6). Aggressiveness of ants towards the spiders depends on the

ability of the ants to recognize the spiders as intruders. I presented circumstantial evidence that dispersing spiders may be recognized as intruders by ants of neighboring, non-host colonies and may, therefore, absorb colony odors into their cuticles. However, the data are too ambiguous to strongly support this hypothesis (Chapter 6).

The dispersion of P. badius nests seems to be influenced primarily by habitat structure and resource availability. However, interference competition between neighboring nests may play a secondary role in nest dispersion. Resource availability, itself, varies depending upon the floral composition of the habitat in which populations of P. badius are found. I presented detailed information about resource use by colonies of P. badius at two different sites in north Florida and showed that foragers are collecting seeds (their primary food source) based primarily upon availability rather than on some measure of seed quality (Chapter 5). These data suggest that this species of seed-harvesting ant is not following predictions of optimal foraging theory which state that organisms should choose resources based upon some assessment of the costs and benefits of collecting that resource (Krebs and Kacelnik 1991).

Coevolutionary scenarios

The host ant, P. badius is the only member of this seed-harvesting genus found east of the Mississippi (Cole 1968). During the late Pliocine and early Pleistocene,

about 10,000 years ago, a continuous band of arid habitats linked Florida with western North America (Webb 1990). During this time, present-day western relicts such as western pocket gophers, Thomomys spp., and scrub jays, Aphelocoma coerulescens coerulescens, reached Florida (Webb 1990). It was probably also during this period that the ancestor of P. badius became established in Florida. The closest relative of P. badius is P. comanche whose range is contiguous but not sympatric with that of P. badius (Taber 1990). P. comanche has been found in western Louisiana, Texas, western Kansas, western Oklahoma, and western Arkansas (Cole 1968). P. badius has been found only east of the Mississippi in Florida, Alabama, Mississippi, Louisiana, Georgia, North Carolina, and South Carolina (Fig. 7-1, Cole 1968). The development of extensive wetlands around the Mississippi basin by the mid-Pleistocene, due to the rise in sea level during interglacial periods, likely served to divide an ancestral population of Pogonomyrmex. If this is true, then allopatric speciation led to the evolution of P. comanche and P. badius. Therefore, P. badius could be considered a geologically young species.

I had hoped to be able to construct a phylogeny of the spider genus Masoncus. However, too few specimens of the previously described congeners, M. arienus, M. conspectus, and M. dux, were available to make this goal feasible. However, from the collection locales of the few

existing specimens, I can propose two intriguing scenarios. Of the three previously described congeners, I was able to compare the morphological traits of two, M. arienus and M. conspectus, with the newly described species, M. pogonophilus. Of these two, M. pogonophilus most closely resembles M. conspectus in several features: the location of the cephalic pits, the shape of the embolic division, and the shape of a black-tipped process on the distal edge of the palpal tibia (Chapter 2). If this morphological resemblance reflects evolutionary relatedness, then M. conspectus may be the sister species of M. pogonophilus. It is also the only one of the three previously described congeners whose known range overlaps that of M. pogonophilus (see Fig. 7-2). One of the two scenarios I propose here is that a sub-population of the direct ancestor of M. conspectus established a symbiotic relationship with P. badius after P. badius itself had become established as a distinct species (i.e., after the Pleistocene allopatric speciation event). In other words, the morphological characters that distinguish M. pogonophilus from M. conspectus were due to genetic drift and evolved after a sub-group of the ancestor of these two species became established as a symbiont inside the nests of P. badius.

If, however, the symbiotic association between ant and spider became established prior to the allopatric speciation event that resulted in the evolution of P.

comanche and P. badius, then excavations of the nests of extant P. comanche might very well reveal a fourth member of the spider genus Masoncus. Unfortunately, very little information is available in the literature concerning the natural history, nest structure, or symbiotic associates of P. comanche.

If the first scenario is true, that the symbiotic association between P. badius and M. pogonophilus was established after P. badius had speciated, then the lack of certain integrative mechanisms in M. pogonophilus may be related to its relatively brief (in evolutionary terms) association with P. badius. In other words, the apparent inability of spiders to follow trail pheromones and the lack of other integrative mechanisms, may be related to its brief association with the host ant.

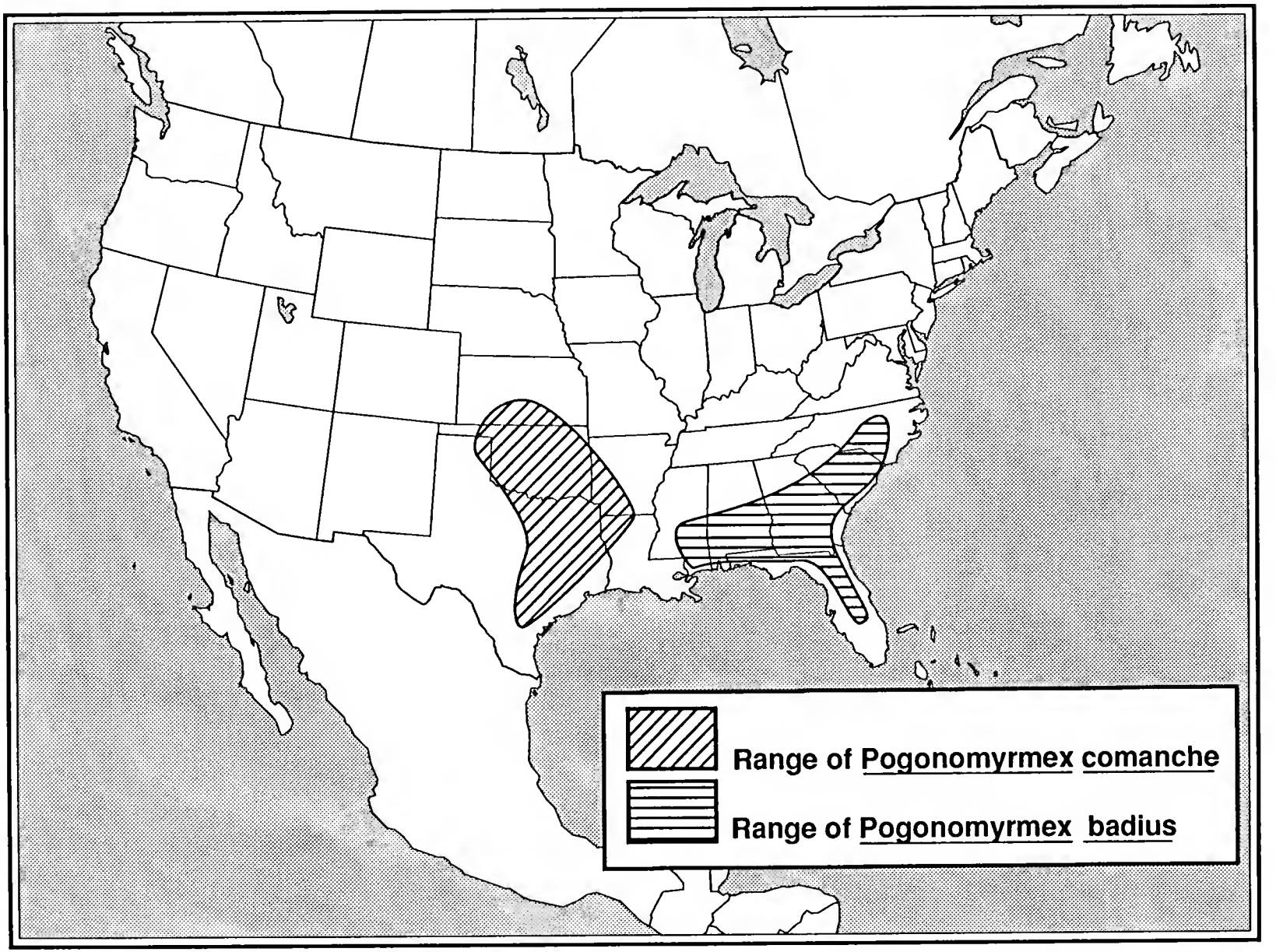


Figure 7-1 (adapted from Cole 1968). Ranges of P. comanche and P. badius.

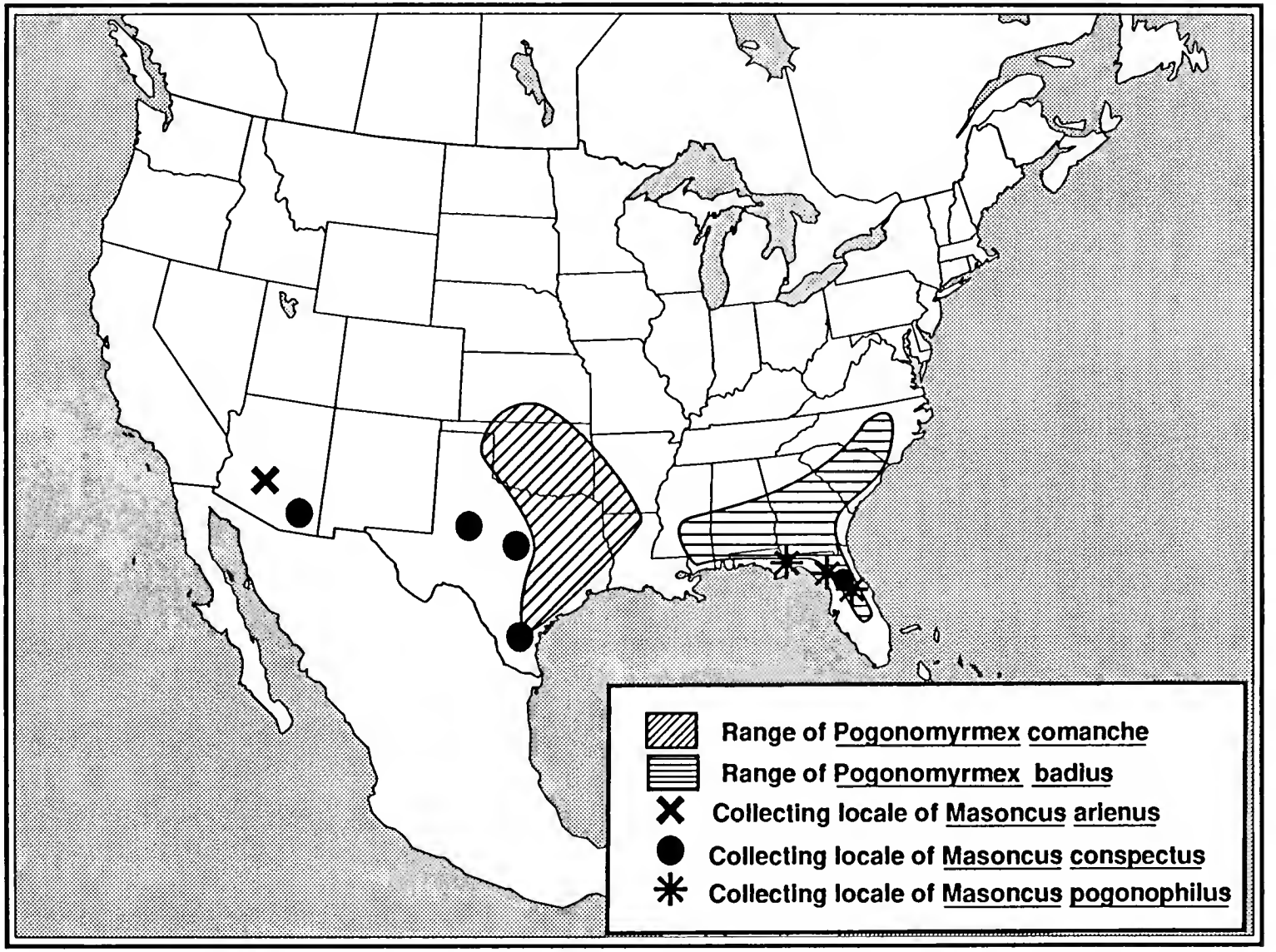


Figure 7-2 (adapted from Chamberlin 1948 and Cole 1968). Ranges of P. comanche, P. badius, M. arienus, M. conspectus, and M. pogonophilus.

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BIOGRAPHICAL SKETCH

Paula E. Cushing was born in Alexandria, Virginia, on February 17, 1964, to Paula M. and Col. Joseph Cushing. When she was in high school, she decided to become a biologist and to go as far as she could in the pursuit of her interests. At 17, she told herself that she would receive her Ph. D. by the time she was 30, and she almost made it.

She graduated from high school in 1982 and received her Bachelor of Science degree in biology from Virginia Polytechnic Institute and State University in Blacksburg, Virginia in 1985. She remained at V.P.I. and S.U. for her Master of Science degree in zoology under the tutelage of Dr. Brent D. Opell. For her master's degree, she investigated disturbance behaviors in spiders and their possible role as predator avoidance strategies. It was then that she first came to be known by family and friends as the "Spider Lady." She received her master's degree in 1988.

For two years she assuaged her wanderlust by traveling to Europe, living and working in Panama, and visiting the western United States. In between trips, she worked as a research assistant for Dr. Opell and published her master's thesis. In August 1990, Paula entered the

Doctor of Philosophy program at the University of Florida in Gainesville under the tutelage of Dr. Jonathan Reiskind. She looks forward with enthusiasm to her career as a professional biologist. She intends to go even further in pursuit of her interests.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jonathan Reiskind, Chairman
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John Anderson
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Assistant Professor of
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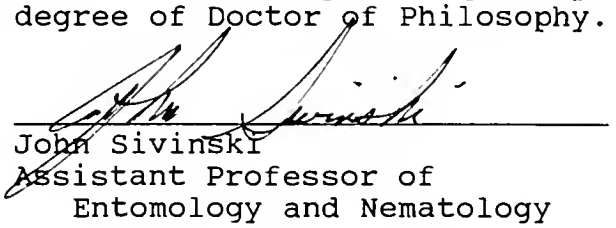

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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1995

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