THE LIFE HISTORY OF *POLISTES METRICUS* SAY: A STUDY OF BEHAVIOR AND PARASITIC NATURAL ENEMIES

by

AMANDA COLEEN HODGES

(Under the direction of KARL ESPELIE)

ABSTRACT

The ability to recognize nestmates is an integral component of eusocial insect societies. Cuticular hydrocarbon profiles are believed to be important in the recognition process and these profiles have been shown to differ by age for some *Polistes* species. The effects of age on nest and nestmate discrimination are examined for *Polistes metricus* Say (Hymenoptera: Vespidae) workers. Age affected nest discrimination. Older (10-day old) *P. metricus* workers spent significantly more time on their natal nest than than younger (3-day old) workers. However, nestmate discrimination did not occur for either younger or older *P. metricus* workers. The lack of nestmate discrimination exhibited by either age class of workers emphasizes the potential role of a social insect's environment in the recognition process. Brood stealing, parasitism pressures, resource limitations, and other environmental factors are eliminated in a homogenous laboratory setting.

Polistes wasps are considered beneficial generalist predators, and the majority of their prey consists of lepidopteran larvae. The prevalence and occurrence of parasitic natural enemies are reported for early-season collected colonies of *P. metricus*. The strepsipteran, *Xenos peckii* Kirby, the ichneumonid *Pachysomoides fulvus* Cresson, the pyralid *Chalcoela pegasalis* Walker, and the eulophid *Elasmus polistis* Burks were present in *P. metricus* colonies. *Xenos* infestations have previously been thought to be infrequent, but *X. peckii* was the most predominant parasite or parasitoid over a four-year period.

Life history information concerning the host-parasite relationship between *X. peckii* and *P. metricus* are provided. Forty-nine female and 58 male *X. peckii* were found in 51 out of 221 dissected *P. metricus* adults, and 19.6% of these parasites were visible only after dissection. Some dissected queens contained female *X. peckii*, suggesting that infested *Polistes* females may be reproductively viable. Significantly fewer parasites per host occurred when only females were present, possibly due to the female's need for host survival as an obligate endoparasitic adult. Overall development and prey consumption of *P. metricus* colonies were not affected by parasitism. These results suggest that *X. peckii* exists at low levels of infestations within a *Polistes* colony in order to minimally impact colony survival.

INDEX WORDS: *Polistes metricus* Say; Kin Recognition; Paper Wasps; Nestmate Discrimination; Parasites; Parasitoid; Stylopization; *Xenos peckii* Kirby

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AMANDA COLEEN HODGES

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AMANDA COLEEN HODGES

Approved:

Major Professor:	Karl E. Espelie
Committee:	Robert W. Matthews Kenneth G. Ross Joseph V. McHugh Brian T. Forschler

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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CHAPTER 1: INTRODUCTION

Polistes, a cosmopolitan genus comprising approximately 200 species (Carpenter 1996), has been the focus of numerous ecological and behavioral studies. Open-combed nests are constructed on various structures, trees, and shrubs. Foundresses build their nests from macerated wood and plant fibers. Adult *Polistes* forage for nectar and occasionally honeydew (Hodges and Hodges 2001). The nests of *Polistes* are suspended by a pedicel, to which the queen applies a compound that repels several species of ants (Post and Jeanne 1981). Nests are relatively small, usually with less than 250 cells for tropical species and less than 100 cells for temperate species (Reeve 1991; Pardi 1996). Colonies are founded by either one foundress (haplometrosis) or several (pleometrosis) foundresses. Temperate species are usually haplometrotic (Reeve 1991). For colonies founded by multiple females, one female will physically dominate the other foundresses and become the dominant egg layer (Pardi 1996). The presence of the dominant foundress suppresses the ovarian development of the subordinates, but their reproductive capabilities are not completely eliminated. If the dominant egg layer dies or abandons the nest, one of the subordinates will assume the role as primary egg layer.

The colony cycle of *Polistes* is divided into four phases: the founding phase, the worker phase, the reproductive phase, and the intermediate phase (Reeve 1991; Yamane 1996). The founding phase, also sometimes referred to as the pre-emergence phase, is the time period in the colony cycle from colony founding until the first worker emerges. The worker phase of the colony cycle begins with worker emergence and continues until emergence of males and gynes, which marks the beginning of the reproductive phase.

The reproductive phase ends with the dispersal of males and gynes. The intermediate phase begins with the dispersal of males and gynes and ends with the next cycle of nest founding. Temperate gynes overwinter until suitable temperatures allow for nest founding in the following spring. The majority of *Polistes* overwinter as mated gynes, and mating for most *Polistes* species studied has been shown to occur in aggregations of males at landmark-like display areas, termed leks (Beani 1996).

Eusocial animals, including *Polistes*, have classically been defined as having cooperative brood care, overlap of generations, and a reproductive division of labor (Michener 1969; Wilson 1971; Gadagkar 1996). Even though numerous debates have focused on the definition of eusociality, these criteria remain the most widely accepted (Gadagkar 1996). Highly eusocial animals have distinct morphological and physiological separations between reproductive and non-reproductive castes. The evolution of highly eusocial animals results in a completely altruistic class within a social group. In contrast, non-reproductives of *Polistes* are not obligately sterile. Due to the plasticity of the reproductive capabilities of the worker caste, *Polistes* are model organisms for the study of the evolution of eusociality.

Even though *Polistes* workers choose altruistic instead of selfish behaviors, they may have the chance to be reproductive in the absence of the dominant female. Studies of *Polistes* and other eusocial animals without sterile castes may further our understanding of the evolution of eusociality and obligate altruism. Hamilton’s inclusive fitness theory, with the ability to recognize kin as a prerequisite, has been a model for understanding the evolution of altruism in insects (Hamilton 1964a, b; Queller 1996). Under the assumptions of Hamilton’s theory, an individual will choose to help raise a relative's offspring if the fitness benefits outweigh the costs. The altruistic individual can indirectly transfer genes to the next generation through its relative's progeny. Kin recognition is an essential component of social insect societies (Michener and Smith 1987). The ability to recognize kin and the underlying mechanisms of kin recognition, and more specifically nestmate recognition, have been extensively studied in several *Polistes* species (Gamboa 1996).

Nestmate recognition cues may be used to protect a *Polistes* colony from social parasitism. Birds and several other animals are attracted to the larvae of *Polistes* as a food source. Predation is a common reason for nest failure (Yamane 1996). If a *Polistes* queen has lost her nest due to predation or some other event, she may attempt to usurp, or to claim, the nest of a conspecific. Usurping the nest of a conspecific is a type of facultative temporary social parasitism (Cervo and Dani 1996). Nest usurpation in *Polistes* is most common during the late founding phase of the colony cycle when nest predation is highest (Dani and Cervo 1992; Lorenzi and Cervo 1995). The resident queen and any brood destined to be reproductives are usually killed (Klahn and Gamboa 1983; Klahn 1988). Kin recognition cues may be used by *Polistes fuscatus* L. in order to avoid usurping close relatives (Klahn and Gamboa 1983; Klahn 1988; Gamboa et al. 1992). Interspecific parasitism also occurs within *Polistes* (Cervo and Dani 1996). A few cases of species capable of either founding their own nest, or usurping the nest of another species exist. Three species of *Polistes* are known to be obligately parasitic on other species (Cervo and Dani 1996). *Polistes* species dependent on interspecific parasitism no longer have a worker caste. Social parasitism could be an additional factor in the enhancement of kin and nestmate recognition cues.

Even though kin recognition does not always result in kin discrimination, these terms have been used interchangeably throughout *Polistes* literature (Reeve 1989; Gamboa 1996). Production, perception, and action are the three components typically involved in kin discrimination (Holmes and Sherman 1983; Gamboa et al. 1986a; Reeve 1989; Sherman et al. 1997; Starks et al. 1998). The genetic relatedness in conjunction with the origin and acquisition of recognition cues are the production components. The development and utilization of a kin template to compare to another individual's template are the perception components. The action component is the behavioral response elicited by an individual based on the perception component of discrimination.

The optimal acceptance threshold model proposed by Reeve (1989) relates to the action component of kin discrimination. Reeve's (1989) acceptance threshold predicts the level of dissimilarity that results in rejection of a conspecific and is correlated with several possible fitness-related factors. These factors include the frequency of contact with kin (or nestmates) versus non-kin (or non-nestmates) and the fitness costs of accepting or rejecting non-kin (or non-nestmates). If the acceptance threshold is increased, for example, due to social parasitism, the probability of rejecting nestmates in addition to non-nestmates also increases. Universal tolerance is predicted if fitness costs for accepting non-nestmates are minimal or do not exist.

Starks et al. (1998) tested Reeve's (1989) optimal acceptance threshold model with *Polistes dominulus* (Christ). Nestmate discrimination for *P. dominulus* only occurred in the presence of either a nestmate or a nest fragment containing brood. Also, in contrast to the results of Pfennig (1990), Starks et al. (1998) found more highly intolerant behaviors directed at neighboring versus non-neighboring colonies. Since

Polistes foundresses generally return to the approximate location of their former nest for nest founding, neighboring colonies could be related, non-nestmate kin (Pardi 1996). Fitness-maximizing factors, such as brood stealing, may have resulted in more aggressive behavior toward either related or non-related neighboring colonies for *P. dominulus* in Starks et al.'s (1998) study (Kasuya et al. 1980; Gamboa et al. 1992).

A majority of *Polistes* research has concentrated on the production and perception components of nestmate discrimination (Gamboa 1996). Most *Polistes* studies have shown nestmate discrimination for foundresses, gynes, and workers. Males have been the least studied caste of *Polistes*, but male *P. fuscatus* have been shown to be capable of recognizing their male nestmates (Shellman-Reeve and Gamboa 1985; Ryan and Gamboa 1986).

Initial studies of nestmate discrimination in *Polistes* found that sister foundresses were more tolerant of each other than non-sister foundresses for *P. fuscatus* (Noonan 1981) and *Polistes metricus* Say (Ross and Gamboa 1981). Contrary to expected results, Queller et al. (1990) found *Polistes annularis* L., a pleometrotic species, does not prefer to associate with former nestmates in nest founding. These findings suggest that *P. annularis* either lacks the ability to discriminate in favor of former nestmates or chooses to associate with less related individuals for colony founding. Pratte (1982) found that *P. dominulus* (as *P. gallicus*) foundresses did not prefer to associate with former nestmates in pleometrotic nest founding, but Pratte considered co-habitating individuals to be nestmates. Post and Jeanne (1982) found *P. fuscatus* foundresses associated with nestmates, but not with non-nestmate co-habitators in colony founding.

Nestmate recognition cues are believed to be both endogenous and exogenous for *Polistes*, but learning of nestmate recognition cues is restricted to the adult stage (Gamboa 1996). Gamboa et al. (1986b) determined that nestmate discrimination did not occur for female *P. fuscatus* reared under homogenous laboratory conditions unless the females were removed from their natal nest and nestmates for several days. Since nestmate discrimination occurred after isolation of the female *P. fuscatus*, Gamboa et al. (1986b) concluded that separation of the females from the colony allowed for degradation of the environmental masking of heritable odors. In addition to genetic and environmental components, *Polistes* acquire recognition cues from the nest comb and/or brood. *Polistes* isolated from their nest immediately upon emergence are subsequently not able to discriminate nestmate from non-nestmate. Nestmate discrimination can occur for *P. fuscatus* and *Polistes carolina* (L.) gynes if isolates are exposed to a portion of the natal nest with brood (Shellman and Gamboa 1982; Pfennig et al. 1983).

Social insects must have colony or kin specific cues in order for nestmate or kin discrimination to occur (Breed 1998). Insects should be able to determine the cues of another individual by physical contact, possibly by antennation. Cues utilized by social insects for nestmate and/or kin discrimination are usually chemical (Wilson 1971) and have been termed discriminators, recognition pheromones (Hölldobler and Michener 1980), or discrimination pheromones (Henderson et al. 1990; Downing 1991). Cuticular lipids, mainly serving as a hydrophobic barrier against dessication, have been hypothesized to be an important component of the social insect recognition process

(Blomquist and Dillwith 1985). Cuticular lipids of social insects consist mainly of hydrocarbons ranging in chain length from 20 to 40 carbon atoms and including *n*-alkanes, alkenes, and methyl-branched alkanes (Lockey 1988).

Evidence for the potential importance of cuticular hydrocarbons in communication can be illustrated by comparing the cuticular hydrocarbon profiles of parasites, social parasites, and inquilines of social insects (Blomquist et al. 1998). Some parasites of social insects apparently passively acquire the chemical signature of the host by direct contact with the host or possibly the host's nest (Vander Meer and Wojcik 1982; Franks et al. 1990; Topoff and Zimmerli 1993). Biosynthesis of the host's cuticular hydrocarbons by the parasite has also been shown to occur (Howard et al. 1982, 1990).

Species-specific and colony-specific cuticular hydrocarbon profiles in social ants, bees, and wasps provide further support for the utilization of cuticular hydrocarbon profiles by social insects (Breed 1998). Cuticular hydrocarbon profiles for all *Polistes* examined appear to be species-specific. Colony-specific hydrocarbon profiles have been shown for *P. metricus* (Layton et al. 1994), *Polistes exclamans* Viereck (Singer et al. 1992), *Polistes biglumis* (Geoffroy) (Lorenzi 1992), and *P. fuscatus* (Espelie et al. 1994). Cuticular hydrocarbon profiles for *P. dominulus* have also been analyzed, but the results did not support colony-specificity (Bonavita-Cougourdan et al. 1991). Even though Bonavita-Cougourdon et al. (1991) were not able to find significant differences in cuticular hydrocarbon compositions among colonies, they did find differences among dominant females and workers. Layton et al. (1994) also found significant differences among castes of *P. metricus*.

Espelie and Hermann (1990) hypothesized that nest surface hydrocarbons may play a role in nestmate recognition due to similarities in hydrocarbon composition between the surface of the nest and adult *P. annularis*. Subsequently, Espelie et al. (1990) showed that *P. metricus* workers were only able to discriminate between their natal nest and a foreign nest when surface hydrocarbons were present.

Due to colony-specific cuticular hydrocarbon profiles in *Polistes* and the importance of nest surface hydrocarbons for nest discrimination, Singer and Espelie (1992, 1996) and Layton and Espelie (1996) focused on the importance of nest surface hydrocarbons for nest and/or nestmate discrimination in laboratory studies of *P. metricus*. These studies tested the role of nest surface hydrocarbons in the recognition process by exposing a newly emerged *P. metricus* worker isolate to her natal nest fragment, her natal nest fragment with surface hydrocarbons extracted, or her natal nest fragment with surface hydrocarbons extracted and reapplied. Singer and Espelie (1996) also included a no nest fragment treatment in their experiment. Singer and Espelie (1992, 1996) tested nestmate discrimination by using worker triplets consisting of a 4-day old test wasp, a nestmate of the test wasp, and a non-nestmate of the test wasp. Exposure of a *P. metricus* worker test wasp to a nest fragment with its surface hydrocarbons present was sufficient for nestmate discrimination (Singer and Espelie 1992, 1996). Layton and Espelie (1996) tested the role of nest surface hydrocarbons in nest and nestmate discrimination by introducing 4-day old *P. metricus* worker test wasps into their natal nest. Test wasps exposed to their nest surface hydrocarbons spent significantly more time on their natal nest. However, exposure to nest surface hydrocarbons did not affect the behaviors of the resident wasps toward the test wasp (Layton and Espelie 1996).

In order to provide further evidence for the importance of nest surface hydrocarbons in nest recognition, Singer and Espelie (1997) tested 4-day old *P. metricus* workers in a nest recognition bioassay consisting of a choice between a fragment of a natal nest and a foreign nest. Test wasps were previously exposed to either no nest fragment, their natal nest fragment, or their natal nest fragment with its surface hydrocarbons extracted. Test wasps were videotaped on two consecutive days. *Polistes metricus* exposed to their nest surface hydrocarbons spent significantly more time on their natal nest during the second day of observations. However, exposure to a nest fragment did not significantly affect time on the natal nest fragment for the first day of observation or the combined results of both days.

Although the results of Singer and Espelie (1997) and Layton and Espelie (1996) did not conclusively support nestmate and/or nest discrimination, nest surface hydrocarbons and cuticular hydrocarbon may be a component in the recognition process for *Polistes*. The age of the test wasps could have affected the results of their studies. Acceptance of foreign conspecifics upon eclosion has been shown for other social insects, including ants (Stuart 1992) and honey bees (Breed et al. 1988). For the eusocial wasp, *Ropalidia marginata* (Lep.), individuals <6 to 8 days old may be accepted into an unrelated colony (Arathi et al. 1997). Lorenzi et al. (1999) also showed that newly emerged (0-day old) *P. biglumis* are accepted on foreign conspecific colonies. *Polistes fuscatus* isolates ≤ 48 hours old are treated tolerantly by mature females, regardless of colony origin (Panek et al. 2000). Also, more colony-specific hydrocarbon profiles occurred in older (72 h) versus younger (24 h) *P. fuscatus* isolates (Panek et al. 2000). Hodges et al. (unpublished data) have shown that the cuticular hydrocarbon patterns of younger (0-5 day old) *P. exclamans* workers differ from older (10, 20, and 30 day old) workers.

Espelie et al. (1994) hypothesized that three C_{31} and C_{33} methyl-branched and dimethyl-branched hydrocarbons may serve as nestmate recognition pheromones for *P. fuscatus.* The stereochemistry of methyl-branched hydrocarbons may facilitate nestmate discrimination more than *n*-alkanes (Butts et al. 1993). Dani et al. (2001) compared the nestmate discrimination effects of adding a methyl-branched hydrocarbon, a straight chain hydrocarbon, or an alkene to a *P. dominulus* worker's cuticle. The hydrocarbons added were naturally occurring supplements (Dani et al. 2001). Increasing the amount of either a methyl-branched hydrocarbon or an alkene caused resident wasps to attack their nestmate workers. *Polistes dominulus* did not attack workers with the additional quantity of a straight chain alkane.

The combined chemical and behavioral results of Lorenzi et al. (1999), Panek et al. (2000), Dani et al. (2001), and Hodges et al. (unpublished data) suggest that *Polistes* may not develop their recognition signature until 4 or 5 days of age. Chapters 2 and 3 of this dissertation focus on comparing nestmate discrimination for younger (3-day old) and older (10-day old) *P. metricus* workers. A triplet test bioassay is utilized to test nestmate discrimination in Chapter 2. Nest and nestmate discrimination are tested in Chapter 3 by introducing a test wasp into either its natal colony or a foreign colony. If older *Polistes* contain more colony-specific hydrocarbons and if cuticular hydrocarbons as well as nest surface hydrocarbons are important for nestmate discrimination, *P. metricus* workers should recognize older nestmates more readily. More intolerant behaviors initiated by experienced wasps toward the 10-day old non-nestmates should occur. The 10-day old test wasps should spend more time on their natal nest compared to test wasps introduced into a foreign nest.

Parasitoid and parasite pressure could affect various colony behaviors, including the acceptance threshold for nestmate discrimination (Reeve 1991). Most of the reports of natural enemies of Nearctic *Polistes* emphasize the presence, occurrence, or life history of brood parasitoids (Rau 1941; Rabb 1960; Nelson 1968; Strassmann 1981). Regional and species-specific differences have been shown for these parasitoids. Members of the *Chalcoela* (Lepidoptera: Pyralidae), *Pachysomoides* (Hymenoptera: Ichneumonidae), and *Elasmus* (Hymenoptera: Eulophidae) genera are common parasitoids of North American *Polistes*.

Chalcoela pegasalis (Walker) and *Chalcoela iphitalis* Walker have similar life cycles, but *C. iphitalis* has not been shown to occur east of the Mississippi River (Rau 1941; Munroe 1972). Both *Chalcoela* parasitoids can feed directly on larvae, pre-pupae, or pupae of their *Polistes* hosts (Rau 1941; Strassmann 1981). Larval *Chalcoela* spin cocoons which result in a characteristic webbing pattern in infested *Polistes* nest cells (Rau 1941). Multiple generations are believed to occur each year, and *Chalcoela* overwinter as larvae in *Polistes* nests (Rau 1941).

Pachysomoides are parasitoids of *Polistes* larvae and pupae (Rau 1941, Strassmann 1981). *Pachysomoides stupidus* (Cresson) infests the “Canadensis group” of *Polistes*, including *P. annularis* and *P. exclamans*. *Pachysomoides fulvus* (Cresson) infests the “Fuscatus group” of *Polistes*, including *P. fuscatus*, *P. metricus*, and

P. carolina (Townes and Townes 1962; Nelson 1968). After the larvae of *Pachysomoides* pupate in their host nest cell, a distinct yellowish or orange papery material is apparent in the *Polistes* nest (Nelson 1968).

Elasmus polistis Burks was first described by Burks (1971) from the following species and locations: *P. annularis* in Georgia; *P. exclamans* in Pennsylvania; and *P. fuscatus* in Maryland. *Elasmus polistis* is a gregarious ectoparasitoid of *Polistes* pre-pupae and pupae, and in laboratory studies female *E. polistis* only laid their eggs on capped pre-pupae or non-pigmented pupae (Reed and Vinson 1979). *Elasmus polistis* overwinters as a pupa inside *Polistes* nests, and several generations occur each year. Emergence dates for *E. polistis* adults in Texas ranged from early spring (March 4) to late fall (October 2) (Reed and Vinson 1979).

Chalcoela pegasalis (as *Dicymolomia pegasalis*) has been shown to infest about 10% of *P. annularis* and *P. fuscatus* (as *P. pallipes* and *P. variatus*) colonies in Missouri, but infestations did not occur until the middle of the summer (Rau 1941). Rau (1941) also described the life cycle of *C. iphitalis* from *Polistes* nests collected in Texas and Mexico, but the percentage of infestation was not reported. *Chalcoela iphitalis* has also been reported as occurring in Kansas (Rau 1946). Records of *Pachysomoides fulvus* (as *Polistiphaga fulva*) infesting nests of *P. fuscatus* (as *P. pallipes* and *P. variatus*), *P. annularis*, and *P. carolina* (as *P. rubiginosis*) in Missouri are provided by Rau (1941).

Several species of *Polistes* in North Carolina including *P. fuscatus*, *P. exclamans*, *P. annularis*, *P. metricus*, and *P. carolina* (as *P. rubiginosus*) were studied from 1949-57 (Rabb 1960). Since biological aspects of *Polistes* were the focus of Rabb's (1960) study, the natural enemies present were recorded. The parasitoid, *P. stupidus* (as *Polistiphaga* *stupida*) was reared from *P. annularis* and *P. exclamans*. *Pachysomoides fulvus* (as *Polistiphaga fulva*) was found in 63% of 32 *P. metricus* nests as well as 59% of 73 *P. fuscatus* nests during 1956-57. Several species of *Polistes* in North Carolina contained *C. pegasalis* (as *D. pegasalis*), but infestation rates were highly variable (Rabb 1960). *Chalcoela pegasalis* was apparently a common parasitoid during 1949, but comprised less than 1% of *Polistes* nests collected during 1956-57. Some adult *Polistes* in North Carolina were apparently infested by the strepsipteran parasite *Xenos peckii* Kirby, but the percentage of individuals or colonies with infested individuals was not reported by Rabb (1960).

Nelson (1968) reared parasitoids and symbionts from field-collected nests of *P. annularis*, *P. exclamans*, and *P. metricus* in southern Illinois. *Polistes metricus* was the species with the highest percentage (70%) of nests containing either parasitoids or symbionts. *Chalcoela iphitalis* was the most common parasitoid for all species studied, and infested approximately 60% of *P. metricus* nests (Nelson 1968). Even though *P. metricus* is common in northeast Georgia, *C. iphitalis* has not been shown to occur in the Eastern United States (Munroe 1972).

After Burks' (1971) species description of *E. polistis*, records for infestation were also reported for *P. exclamans* in Texas (Gillapsy 1973), Oklahoma (Nelson 1976), and Missouri (Whiteman and Landwer 2000). The host record data for *E. polistis* have been expanded to include *Polistes dorsalis* (F.) in Florida (Macom and Landolt 1995), *Polistes major* (Beauvois) (Krombein et al. 1979), and *P. metricus* in Texas (Reed and Vinson 1979) and Missouri (Whiteman and Landwer 2000). *Chalcoela iphitalis* and *E. polistis* were found to be the most common parasitoids of *P. exclamans* in Texas (Strassmann 1981). The percentage of *P. exclamans* nests with parasitoid infestations ranged from 46% to 73% for *C. iphitalis* and from 36% to over 60% for *E. polistis* over a three year period.

Haplometrotic, founding stage *Polistes* are especially susceptible to attacks by brood parasitoids while the queen forages. According to Starr's (1976) hypothesis, parasitoid pressure may select against pleometrotic colony founding for some North American *Polistes*. However, multiple foundresses could more effectively defend the colony against brood parasitoids. A triungulin larva of the strepsipteran parasite *Xenos*, waiting at flowers for a foraging *Polistes* to carry it back to her nest, could be encountered by wasps from either haplometrotic or pleometrotic colonies (Katherithamby 1989). Pleometrotic colony members, with a larger number of individuals returning to the colony, could have a greater chance of transferring a strepsipteran larva to their colony during the founding phase of development. Triungulin larvae infest the larval and pupal stages of *Polistes* (Bohart 1941). The triungulin larva of *Xenos* is the life stage that initially attacks its *Polistes* host.

Female and male *Xenos peckii* Kirby develop inside the abdomen of their *Polistes* host. The adult female remains a larviform parasite of her *Polistes* host (Bohart 1941). The adult female and the male pupa extrude from beneath the abdominal tergites and sternites of their hosts. The *X. peckii* parasites may not be apparent upon eclosion of an adult *Polistes* host. The male puparia typically protrude from beneath the abdominal segment of the host 5 to 10 days prior to the appearance of adult females (Schraeder 1924). The male *X. peckii* emerges from his pupal stage into a free-living form. In his short adult lifespan of approximately 24 hours, the male searches for a female. In a mating process known as traumatic insemination, the male inserts his aedeagus directly into the cephalothorax of the female. Hundreds or thousands of eggs mature into triungulin larvae within the mated female *X. peckii*. The triungulin larvae are expelled directly from the female's brood passage on her cephalothorax. Even though parasitized gynes are thought to be incapable of nest founding, fertilized *Xenos* are believed to overwinter in gynes (Brues 1905; Bohart 1941; Dunkle 1979).

Chapter 4 of this dissertation focuses on reporting the occurrence and percentage of haplometrotic, early season *P. metricus* colonies with parasitoids and parasites in northeast Georgia. By rearing colonies in the laboratory, we were able to ascertain the presence of parasitoids and parasites not necessarily evident by destructive sampling. The effects of parasitism by *X. peckii* on *Polistes* colonies are unknown. The occurrence of *Polistes* colonies with parasitized individuals has previously been reported as sporadic and infrequent (Brues 1905; Schraeder 1924). Schraeder (1924) even hypothesized that a population of *Polistes* in a particular region could be driven to extinction by *Xenos*. In order to uncover more information concerning the life history of *X. peckii* in relation to its host, *P. metricus* were dissected and microscopically examined. Chapter 5 reports the sex ratio and the preferred abdominal position for *X. peckii* in *P. metricus*.

The parasite *X. peckii* was found in approximately 17% of the early season, *P. metricus* colonies collected, as reported in Chapter 4. The parasitoids *C. pegasalis*, *P. fulvus*, and *E. polistis* were also found in early season *P. metricus* colonies, but *X. peckii* was significantly more prevalent. The effects of parasitism by *Xenos* on colony development of *Polistes* are unknown. A comparison of the laboratory development of

P. metricus colonies parasitized and not parasitized by *X. peckii* is given in Chapter 6. Colony development was measured by monitoring the weekly number of eggs, larvae, and pupae present in a nest during the first six weeks of the worker phase of the colony cycle. The effect of parasitism by *X. peckii* on daily prey consumption was also measured.

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CHAPTER 2

COMPARING NESTMATE DISCRIMINATION IN THE PRESENCE OF A NESTMATE AND A NON-NESTMATE FOR YOUNGER (3-DAY OLD) AND OLDER (10-DAY OLD) *POLISTES METRICUS* SAY (HYMENOPTERA: VESPIDAE) WORKERS[1]

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Abstract

Nestmate discrimination was analyzed for 3-day old and 10-day old *Polistes metricus* Say workers in the laboratory. Bioassays consisted of worker triplets involving the test wasp, a nestmate of the test wasp, and a non-nestmate of the test wasp. Both the 3-day and 10-day old test wasps interacted with non-nestmates for the behaviors of trophallaxis and prolonged unilateral antennation significantly more than expected for two non-nestmates. The 3-day old test wasps initiated prolonged unilateral antennation significantly more with nestmates than the 10-day old test wasps. The non-nestmates initiated prolonged unilateral antennation to the test wasps significantly more than nestmates. The lack of nestmate discrimination exhibited by 3-day old and 10-day old *P. metricus* workers emphasizes the potential importance of environmental factors in the recognition systems of paper wasps.

Keywords: Paper Wasps; Kin Recognition

The first few days of adult life for social insects are believed to be important for colony members to acquire a characteristic colony odor (Arathi et al.1997). Due to the plasticity of a young adult wasp's recognition template, newly emerged adults in some species of social insects have been accepted into alien conspecific nests or allospecific nests (Carlin et al.1993, Errard 1994). For the eusocial wasp *Ropalidia marginata* (Lep.), young (<6 to 8 day-old) foreign conspecific adults are accepted into unrelated colonies, but older (>6 to 8 day-old) foreign conspecifics are not accepted (Arathi et al. 1997). Panek et al. (2000) found that mature female *Polistes fuscatus* (L.) were unable to discriminate between nestmate and non-nestmate females $\leq$ 48 hours old.

Numerous experiments utilizing wasps of the genus *Polistes* have focused on kin discrimination, and more specifically on nestmate discrimination (e.g., Ross and Gamboa 1981; Shellman and Gamboa 1982; Pfennig et al. 1983a,b; Gamboa et al. 1986a,b; Fishwild and Gamboa 1992; Singer and Espelie 1992, 1996; Layton and Espelie 1996). Reeve's (1989) optimal acceptance threshold model predicts that a social animal develops a recognition template for determining acceptance of a conspecific. The degree of dissimilarity should be less between kin than non-kin, but factors such as the amount of time an individual was previously exposed to kin are also included in the acceptance model. Starks et al. (1998) tested Reeve's (1989) model with *Polistes dominulus* (Christ) and found nestmate recognition to be context-dependent. *Polistes dominulus* exhibited nestmate recognition only when either a nestmate or nest fragment with brood was present. Starks et al. (1998) also found that *P. dominulus* behaved more aggressively towards neighboring than towards non-neighboring colonies. These results differ from the results of a previous study by Pfennig (1990). The *P. dominulus* in Starks et al.'s

(1998) study may have experienced factors that encouraged more aggression toward non-nestmate kin as opposed to alien conspecifics. For example, a colony would benefit from recognizing and defending itself against related and non-related neighbors if brood stealing occurs (Kasuya et al. 1980; Gamboa et al. 1992).

Some researchers have focused on the importance of cuticular hydrocarbons in nestmate discrimination for *Polistes* due to the occurrence of species-specific and colony-specific cuticular hydrocarbons (Singer and Espelie 1992, 1996; Singer et al. 1992; Layton et al. 1994; Layton and Espelie 1996). Singer and Espelie (1996) reported that 4-day old test wasps required previous exposure to nest surface hydrocarbons in order to discriminate between nestmates and non-nestmates. In Layton and Espelie's (1996) study, 4-day old test wasps spent more time on their natal nest if previously exposed to nest surface hydrocarbons. Hodges et al. (unpublished data) have shown that cuticular hydrocarbon profiles of younger (<5 day old) *Polistes exclamans* Viereck workers differed from those older (>5 day old) workers. Panek et al. (2000) found that more colony-specific hydrocarbons occurred in older (72 h) versus younger (24 h) isolated *P. fuscatus* females. If only older *Polistes* have cuticular hydrocarbon profiles that are colony-specific, then nestmate discrimination should be more definitive for older versus younger *Polistes* test wasps.

The objectives of this study were to examine: (1) the ability of a 3-day old and 10-day old test wasp to discriminate between a nestmate and a non-nestmate after previously only being exposed to an untreated nest fragment, (2) the ability of the nestmate of the test wasp to recognize its nestmate in the context of a non-nestmate, (3) the ability of the non-nestmate of the test wasp to recognize the test wasp as a non-nestmate in the context of a non-nestmate, and (4) the ability of the two older workers (the nestmate and the non-nestmate of the test wasp) to recognize each other as nonnestmates. Given that older wasps are believed to contain more of the colony-specific hydrocarbons, we hypothesized that the older 10-day old wasps should recognize their nestmates more readily and be more easily recognized by their nestmates.

Materials and Methods

Founding phase, haplometrotic *Polistes metricus* colonies were collected in and around Athens, Georgia, during the late spring and early summer of 1997 and 1998. Each colony was taken to the laboratory and chilled to 5-8°C in order to facilitate removal of the queen. Using a wooden craft stick and a low temperature glue gun, each nest was attached to the top of a 20 cm X 20 cm X 20 cm Plexiglas chamber as described in Singer and Espelie (1992). Each queen was marked on the thorax with a dot of yellow enamel paint and placed in the chamber with her nest. Colonies were provided with Whatman's No. 1 filter paper for nest construction. A daily supply of water, 15% honey-water, and *Spodoptera frugiperda* (Smith) larvae were provided to all colonies. Colonies found to be infested with parasites or parasitoids were excluded from this study.

A colony was chilled to 5-8°C prior to removal of the nest from the chamber once at least two workers and two pupae were present. Workers were individually marked on the thorax with enamel paint. Each pupa was excised from the nest and placed in a pharmaceutical gel capsule (Eli Lilly No. 0) as described in Layton and Espelie (1996). Each excised pupa was placed in an inverted plastic jar (9 X 6.5 cm) and provided with a piece of its nest fragment glued to the top of the inverted jar. Each nest fragment was at least the size of one nest cell in length and in diameter. None of the pupae were exposed to nest brood after excision. The remaining portion of the nest was returned to its chamber containing the queen and workers of the colony.

Each excised pupa was monitored daily for worker emergence. Following emergence, she was used as either a 3-day old or 10-day old test wasp in a nestmate recognition bioassay. The nestmate recognition bioassay was performed with worker triplets as described in Singer and Espelie (1992). Each worker triplet consisted of a 3-day old or 10-day old test wasp, a nestmate of the test wasp, and a non-nestmate of the test wasp. Both the nestmate and the non-nestmate of the test wasp were older workers, greater than 10 days old. Wasps were chilled to 5-8°C for 10-15 min, and then released into a Plexiglas chamber (15 X 15 X 15 cm) for each triplet test. Prior to the start of observations, the wasps were allowed 5 min to acclimate to the chamber. One observer performed all observations and each test lasted one hour. The identity of the nestmate and the non-nestmate of the test wasp was unknown to the observer. Thirty triplet tests from 62 colonies were performed for each age category (3-and 10-day old) of test wasps. Fifty-seven colonies were used no more than three times and 20 colonies were used only once in a bioassay. No colonies were used more than six times in a bioassay. Workers that were utilized as nestmates or non-nestmates in the triplet tests were randomly selected from colonies containing at least two workers.

The following behaviors were recorded: brief (<2-s) unilateral antennation; brief (>2-s) mutual antennation; prolonged or repeated (>2-s) unilateral antennation; prolonged or repeated (>2-s) mutual antennation; stepping or resting on another wasp; attempted trophallaxis; trophallaxis; and standing stationary (>1 min) within 2.5 cm of another wasp; chasing; fighting while falling from the top of the test chamber; grappling on the floor of the test chamber; biting; lunging; and avoiding. Each individual behavior was screened for significance across treatments by using PROC GLM (SAS Institute 1999). The treatments were as follows for both 3-day and 10-day old test wasps: test wasp to its nestmate; nestmate to the test wasp; test wasp to its non-nestmate; non-nestmate to the test wasp; nestmate to the non-nestmate of the test wasp; and non-nestmate to the nestmate of the test wasp. Behaviors which initially indicated significance at the $\alpha=0.05$ level were then analyzed with the nonparametric PROC GENMOD procedure (SAS Institute 1999). The p-values for both tests yielded similar results, but due to the nonparametric distribution of the data, the GENMOD procedure is more appropriate. Among behaviors that were found to be significant with the PROC GLM and subsequently PROC GENMOD, contrast statements were used to determine significance among treatments (SAS Institute 1999).

Results

The behaviors trophallaxis (F=4.96; df=11, 336; P<0.0001) and prolonged unilateral antennation (F=16.76; df=11, 336; P<0.0001) were significantly different across treatments by analysis of variance using PROC GLM. The behaviors trophallaxis (X^2=44.20; df=11; P<0.0001) and prolonged unilateral antennation (X^2=112.68; df=11; P<0.0001) were also found to be significant across treatments by analysis for nonparametric data using PROC GENMOD. The average number of trophallaxis and prolonged unilateral antennation initiated per treatment class are shown in Tables 2.1 and 2.2.

The treatment contrasts and the p-values obtained for trophallaxis and prolonged unilateral antennation are shown in Table 2.3. Treatment contrast A (Table 2.3) compared the behaviors involving non-nestmates and test wasps with behaviors of the older non-nestmates for both the 3-day old and 10-day old tests. Both 3-day old and 10-day old test wasps participated in trophallaxis and prolonged unilateral antennation significantly more with non-nestmates in comparison with the ‘older nonnestmates’ in the triplet tests (X^2=9.19, df =1, P=0.024; X^2=43.78, df=1, P<0.0001). (Table 2.3).

A non-significant difference occurred for (1) the 3-day old or 10-day old test wasps initiating trophallaxis to their nestmates (X^2=1.73; df=1; P=0.1879), (2) the nestmate of the 3-day old or 10-day old test wasp initiating trophallaxis (X^2=1.60; df=1; P=0.2057) or prolonged unilateral antennation (X^2=3.13; df=1; P=0.0767), (3) the 3-day old and 10-day old test wasps initiating trophallaxis (X^2=1.40; df=1; P=0.2364) or prolonged unilateral antennation (X^2=1.26; df=1; P=0.2607) with their nestmates versus their non-nestmates, and (4) the nestmates versus the non-nestmates of the test wasp initiating trophallaxis (X^2=1.69; df=1; P=0.1942) (Table 2.3). The 3-day old test wasps initiated prolonged unilateral antennation with their nestmates significantly more than the 10-day old test wasps (X^2=4.52; df=1; P=0.0334). The non-nestmates of the test wasps initiated significantly more prolonged unilateral antennation than the nestmates for both nestmate discrimination triplet tests (X^2=16.36; df=1; P<0.0001).

Discussion

Nestmate discrimination did not occur for either 3-day old or 10-day old *P. metricus* workers. Comparing the behaviors of the test wasps with their non-nestmates to the interactions of the older non-nestmates, the nestmate and non-nestmate of the test wasp, should have provided a negative control (Table 2.3, Treatment contrast A). For trophallaxis and prolonged unilateral antennation, both 3-and 10-day old test wasps interacted with non-nestmates significantly more than the two older non-nestmates did with each other. Our results should have been non-significant if these behaviors and exposure to a nest fragment alone are sufficient for nestmate discrimination. Also, the non-nestmates of the test wasps initiating significantly more prolonged unilateral antennation with the test wasps than their nestmates suggests that prolonged unilateral antennation may not be a nestmate discrimination behavior.

The uniform laboratory colony environment may have masked discrimination in our study. Gamboa et al. (1986b) found laboratory colonies to engage in less aggressive behaviors and subsequently be more tolerant of all conspecifics than field colonies of *P. fuscatus*. Even though age and nest surface hydrocarbons may be an essential component in the recognition process, our results suggest that discrimination may not occur in the absence of other recognition cues. Layton and Espelie (1996) were also unable to find differences in acceptance by *P. metricus* resident wasps of test wasps previously exposed to nest fragments with or without their nest surface hydrocarbons. Reeve's (1989) theory suggests that environment and factors including fitness consequences of accepting and the frequency of encountering kin versus non-kin should affect the threshold of acceptance. By maintaining each colony separately in the laboratory, workers no longer encountered non-nestmates during foraging activities or in defense of the colony. *Polistes* colonies reared in the laboratory may have a lower threshold for acceptance of non-nestmates than field colonies.

The presence of the nest with brood may be necessary for nestmate discrimination in laboratory-reared colonies. Further experiments could test differences between younger and older *P. metricus* workers in the context of the nest in field and laboratory settings. Laboratory nestmate discrimination bioassays may also be more effective if the amount of time a colony spends in the laboratory could be minimized. Collecting colonies during the worker phase of development and immediately excising pupae could yield more definitive results concerning nestmate discrimination and could be tested in future studies.

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Table 2.1: Mean Number of Trophallaxis in 3-day and 10-day old *P. metricus* Nestmate Discrimination Bioassays

Treatment	3-day old	10-day old
Test Wasp to Nestmate	1.47 ± 0.61	1.70 ± 0.85
Nestmate to Test Wasp	1.13 ± 0.358	1.17 ± 0.38
Test Wasp to Non-nestmate	1.80 ± 0.86	1.67 ± 0.81
Non-nestmate to Test Wasp	1.27 ± 0.44	1.13 ± 0.34
Nestmate to Non-nestmate of the Test Wasp	1.17 ± 0.38	1.17 ± 0.32
Non-nestmate to Nestmate of the Test Wasp	1.27 ± 0.44	1.13 ± 0.35

Table 2.2: Mean Number of Prolonged Unilateral Antennation in 3-day old and 10-day old *P. metricus* Nestmate Recognition Bioassays

Treatment	3-day old	10-day old
Test Wasp to Nestmate	2.10 ± 0.25	1.77 ± 0.69
Nestmate to Test Wasp	1.23 ± 0.42	1.33 ± 0.41
Test Wasp to Non-nestmate	2.10 ± 0.46	1.93 ± 0.61
Non-nestmate to Test Wasp	2.10 ± 0.34	1.33 ± 0.48
Nestmate to Non-nestmate of the Test Wasp	1.33 ± 0.48	1.27 ± 0.34
Non-nestmate to Nestmate of the Test Wasp	1.47 ± 0.51	1.37 ± 0.49

Table 2.3: Treatment Contrasts for Trophallaxis and Prolonged Unilateral Antennation

Treatment Contrast[1]	Category 1	Category 2	Trophallaxis	Prolonged Unilateral Antennation
A	Non-nestmate of the 3-day old Test wasp to the Test wasp; 3-day old Test Wasp to Non-nestmate; Non-nestmate of the 10-day old Test Wasp to the Test Wasp; 10-day old Test Wasp to Non-nestmate	Nestmate to Non-nestmate of 3-day old Test Wasp; Non-nestmate to Nestmate of 3-day old Test Wasp; Nestmate to Non-nestmate of 10-day old Test Wasp; Non-nestmate to Nestmate of 10-day old Test Wasp	P=0.024[2]	P<0.0001
B	3-day old Test Wasp to Nestmate	10-day old Test Wasp to Nestmate	P=0.1879	P=0.0334
C	Nestmate of 3-day old Test Wasp to Test Wasp	Nestmate of 10-day old Test Wasp to Test Wasp	P=0.2057	P=0.0764
D	3-day old Test Wasp to Nestmate; 10-day old Test Wasp to Nestmate	3-day old Test Wasp to Non-nestmate; 10-day old Test Wasp to Nestmate	P=0.2364	P=0.2607
E	Nestmate of 3-day old Test Wasp to Test Wasp; Nestmate of 10-day old Test Wasp to Test Wasp	Non-nestmate of the 3-day old Test wasp to the Test wasp; Non-nestmate of the 10-day old Test Wasp to the Test Wasp;	P=0.1942	P<0.0001

[1] In order to simplify references to contrasts, contrasts are assigned to treatment contrast groups.

[2] P-values indicate statistical comparisons of the behaviors in category 1 versus category 2 across rows.

CHAPTER 3

THE EFFECTS OF WORKER AGE ON NEST AND NESTMATE DISCRIMINATION FOR *POLISTES METRICUS* SAY (HYMENOPTERA: VESPIDAE)[1]

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[1] Hodges, A.C. and K.E. Espelie. 2002. To be submitted to Ethology.

Abstract

Video analysis was used to compare nest and nestmate discrimination for younger (3-day) and older (10-day) *Polistes metricus* Say workers. Prior to nestmate discrimination bioassays, test wasps were maintained in isolation and exposed only to a fragment of their natal nest. Nestmate discrimination bioassays introduced the test wasp into a colony with a queen, two workers, and a functional nest. Experimental treatments were 3-day old test wasp in natal nest, 3-day old test wasp in foreign nest, 10-day old test wasp in natal nest, and 10-day old test wasp in foreign nest. The average time the 10-day old test wasps spent on their natal nests (24.52 min) was significantly higher than time spent on the nest by the 3-day old test wasp in its natal colony (12.43 min) and the 10-day old test wasp in a foreign colony (5.62 min). Nest discrimination only occurred for older (10-day old) test wasps. The test wasp did not significantly interact with the queen across any treatments for the behaviors of mutual antennation, unilateral antennation, or trophallaxis. The 10-day old test wasps introduced into their natal colony participated in trophallaxis with workers significantly more than did test wasps in the other treatments. The 10-day old test wasps initiated unilateral antennation to workers more often in the natal versus foreign colony environment. No significant difference for unilateral antennation occurred among the treatments with 3-day old test wasps. No significant differences across treatments occurred for acceptance of the test wasp by the resident wasps. Even though age did affect nest discrimination, age did not influence overall nestmate discrimination. The lack of nestmate discrimination for both age classes of *P. metricus* workers indicates that other environmental factors may be important in the nestmate recognition process.

Keywords: Kin Recognition; Paper Wasps

The behavior of the cosmopolitan genus *Polistes* has been extensively studied in order to further understand the mechanisms and evolution of sociality. Eusocial insects, including *Polistes*, have been traditionally defined as having cooperative brood care, overlap of generations, and a reproductive division of labor (Michener 1969; Wilson 1971; Gadagkar 1996). In contrast with highly eusocial insects such as honeybees, *Polistes* lack an obligate sterile caste. Individuals belonging to the functionally sterile caste in a *Polistes* colony choose to assist a relative in raising her offspring. If a foundress in a single-foundress *Polistes* colony dies, a worker becomes the replacement queen by laying male eggs and subsequently mating with the emerging males (Yamane 1996). In addition to the plasticity of the reproductive capabilities of workers, *Polistes* are ideal for sociality studies due to their relatively small colony size, open comb, and availability (Pardi 1996).

Social insects are believed to differentiate between kin and non-kin by matching recognition cues to their own recognition templates (Holmes and Sherman 1983; Reeve 1989; Starks et al. 1998). The ability to recognize kin may lead to discrimination (Reeve 1989). Reeve's (1989) optimal acceptance threshold model predicts that acceptance of kin and rejection of nonkin is context-dependent. If the fitness costs of accepting non-kin are low, non-kin and kin may be accepted. Factors such as brood stealing and parasitoid pressure may increase fitness costs, subsequently elevating the acceptance threshold.

Extensive previous research has focused on kin and nestmate discrimination and the mechanisms underlying nestmate discrimination in *Polistes* (e.g., Ross and Gamboa 1981; Shellman and Gamboa 1982; Pfening et al. 1983a,b; Gamboa et al. 1986; Fishwild and Gamboa 1992; Singer and Espelie 1992, 1996; Layton and Espelie 1996;

Starks et al. 1998; Panek et al. 2000; Pickett et al. 2000). If exposed to a nest fragment with brood, *Polistes carolina* (L.) and *Polistes fuscatus* (L.) gynes are able to exhibit nestmate discrimination (Shellman and Gamboa 1982; Pfenning et al. 1983b). Exposing *Polistes metricus* Say workers to a fragment of their natal nest alone resulted in nest and nestmate discrimination (Espelie et al. 1990; Singer and Espelie 1996; Layton and Espelie 1996).

Identified nestmate and/or kin recognition cues utilized by social insects are usually chemical and are termed discriminators or recognition pheromones (Wilson 1971; Höllodobler and Michener 1980). Cuticular hydrocarbons have been hypothesized to be an important component of the recognition pheromone in *Polistes* due to behavioral data emphasizing nest surface hydrocarbons (Singer and Espelie 1992, 1996, 1997; Layton and Espelie 1996), and the species- and colony-specific profiles of cuticular hydrocarbons (Espelie et al. 1990, 1994; Bonavita-Cougourdan et al. 1991; Singer et al. 1992; Butts et al. 1993; Layton et al. 1994),

Triplet tests expose the test wasp to a nestmate and a non-nestmate and have been used to examine the role of nest surface hydrocarbons in nestmate discrimination for 4-day old *P. metricus* workers (Singer and Espelie 1992, 1996). Nestmate discrimination occurred for 4-day old *P. metricus* workers isolated and exposed to either the untreated nest fragment treatment or the hydrocarbons extracted and reapplied nest fragment treatment (Singer and Espelie 1996). The role of nest surface hydrocarbons in discrimination for isolated 4-day old *P. metricus* workers has also been tested in the context of their natal nest (Layton and Espelie 1996). Resident wasps did not treat test wasps exposed to various nest fragment treatments differently. However, *P. metricus* workers exposed to a nest fragment with hydrocarbons extracted spent the least time on their natal nest.

Research on the role of nest surface hydrocarbons in nestmate discrimination for *Polistes* has focused on young, 4-day old workers (Singer and Espelie 1992, 1996; Layton and Espelie 1996). In other polistine wasps, age has been shown to affect acceptance. In *Ropalidia marginata* (Lep.) individuals are not rejected from an unrelated colony before they are 6 to 8 days old (Arathi et al. 1997). Mature female *P. fuscatus* are unable to differentiate between isolated nestmates and non-nestmates ≤ 48 hours old (Panek et al. 2000). In addition to behavioral-related age changes for *P. fuscatus*, cuticular hydrocarbon profiles for *P. fuscatus* (Panek et al. 2000) and *P. exclamans* (Hodges et al. unpublished data) have been shown to change with age. Also, hydrocarbons believed to be important for colony-specificity are more prevalent in older (72 h) *P. fuscatus* (Panek et al. 2000).

The purpose of this study is to compare nest and nestmate discrimination for younger (3-day old) and older (10-day old) *P. metricus* workers. Because the nest is the center for nestmate interactions for *Polistes* (Starr 1991), nestmate discrimination bioassays were conducted in the context of a functional nest. Nestmate discrimination should be more distinct for older *P. metricus* workers. Older workers should also spend significantly more time on their natal nest than on a foreign nest.

Materials and Methods

During spring 2001, 68 founding phase pre-emergent *P. metricus* colonies were collected in Clarke, Oglethorpe, and Barrow Counties in northeast Georgia. In the laboratory, each colony was chilled at 5-8°C to facilitate removal of the queen. Chilled queens were marked on the thorax with a small dot of yellow enamel paint and returned to their respective nests. Each nest was then affixed to the top of a 20 X 20 X 20 cm Plexiglas chamber utilizing the methods of Singer and Espelie (1992). Colonies were provided with water ad libitum, 15% honey-water, and *Spodoptera frugiperda* (Smith) larvae. Whatman's No. 1 filter paper was provided for nest construction. Colonies were maintained at room temperature next to windows that provided a natural lighting cycle. Colonies were monitored twice daily for the presence of parasites, parasitoids, and worker emergence. Colonies with infestations were not utilized in the bioassay.

When at least two workers and three pupae were present in a nest, the colony was chilled at 5-8°C in order to facilitate the removal of the nest from the chamber. Each pupa was excised from the nest using dissecting scissors and placed in pharmaceutical gel capsules (Eli Lilly No. 0) (Layton and Espelie 1996). In order to facilitate worker emergence, two small holes were made near the emergence end of the gel capsule with the dissecting scissors. Each gel capsule was placed in an inverted plastic jar (9 X 6.5 cm) to provide an emergence arena. A natal nest fragment, without brood, approximately the size of one nest cell in length and diameter was attached to the top of the inverted jar. The remainder of the excised nest and adult members of the colony were returned to the original Plexiglas chamber. Prior to returning workers to their chamber, each worker was individually marked with a small dot of enamel paint on the thorax.

Excised pupae were monitored several times a day for emergence. Test wasps were provided with water ad libitum and 15% honey-water upon emergence. Test wasps were used as either 3-day old or 10-day old workers introduced into either a natal or foreign colony containing a queen and two workers (>10 days old) in a bioassay. Foreign colonies were collected from locations that were more than 9.3 km from the test wasp's colony. If more than two workers were present in a colony, these additional workers were removed prior to the nestmate discrimination bioassay. Additional workers were removed randomly from a colony using forceps after chilling the colony at 5-8°C for approximately 10-15 minutes. The queen and two workers of the colony to be tested in the nestmate discrimination bioassay were then allowed at least 30 minutes as an adjustment period following removal of additional workers.

The test wasp and the colony were chilled at 5-8°C for 15 minutes prior to the introduction of the test wasp. Wasps were allowed 5 minutes to readjust to room temperature before the start of observations. Each colony chamber was placed on a clear, clean piece of glass (48 x 34 cm) that was supported by three ring stands. The test colony was videotaped for one hour. One observer viewed all videotapes.

The total time the test wasp, queen, and each worker spent on the nest was recorded. Behavioral data focused on interactions with the test wasp while she was on the nest. Total time for the following behaviors involving the test wasp was recorded: trophallaxis (the mutual exchange of liquids), mutual antennation (two wasps reciprocally antennating each other), and unilateral antennation (one wasp antennating another). The time the test wasp spent inspecting nest cells and in trophallaxis with larvae was also recorded.

Summary statistics were prepared by using PROC MEANS (SAS Institute 1999). Due to the nonparametric distribution of the data, data were arranged by using PROC RANK and the ranks of the data were submitted to an analysis of variance using PROC

GLM (SAS Institute 1999). Mean separation by LSD was performed (SAS Institute 1999). Submitting the ranks of data values to a one-way analysis of variance is equivalent to the Kruskal-Wallis *k*-sample test (SAS Institute 1999). Resident wasps occasionally initiated the following highly intolerant behaviors towards the test wasp: chasing, biting, fighting, and lunging. A queen or worker that did not direct any of these highly intolerant behaviors at the test wasp was scored as accepting the test wasp. Chi-square analysis (SAS Institute 1999) was used to determine differences between treatments for queens or workers accepting the test wasp.

Results

The average times that test wasps, queens, and workers spent on the nest for all treatments is shown in Table 3.1. Time spent on the nest for queens (F=0.46; df=3, 69; P=0.7145) and workers (F=1.69; df=3, 142; P=0.1721) did not differ significantly between treatments. However, time spent on the nest differed significantly across all treatments for the test wasp (F=3.23; df=3, 69; P=0.0277). The 10-day old test wasps introduced into their natal nest averaged more time on nest than the other treatments, and differed significantly from the 3-day old test wasp returned to its natal colony and the 10-day old test wasp in foreign colony treatments (Table 3.1). The 3-day old test wasps in a foreign colony averaged the second highest amount of time on the nest, but did not differ significantly from any of the other treatments (Table 3.1).

The average time the test wasp spent interacting with the queen for trophallaxis (F=0.78; df=3, 69; P=0.5092), mutual antennation (F=0.97; df=3, 69; P=0.4121), and unilateral antennation (F=0.83; df=3, 69; P=0.4821) were not significant for any treatment (Table 3.2). The queen attempting unilateral antennation with the test wasp occurred too infrequently to be analyzed. Trophallaxis between test wasps and the workers was significantly different between all treatments (*F*=8.12; df=3, 142; *P*<0.0001). The 10-day old test wasp introduced into its natal colony differed from the other treatments (Table 3.3).

Test wasp antennating workers (*F*=2.99; df=3, 142; *P*=0.0331) and workers antennating test wasp (*F*=3.64; df=3, 142; *P*=0.0144) were significantly different across treatments (Table 3.3). Unilateral antennation towards workers and test wasps was initiated more by the 10-day old test wasps in the natal versus foreign colony treatment. No significant difference occurred between the 10-day old test wasp treatments in comparison with the 3-day old treatments for test wasps or workers initiating unilateral antennation (Table 3.3). Mutual antennation between the workers and the test wasp was too infrequent to be analyzed.

The average time the test wasp spent inspecting nest cells and engaged in trophallaxis with larvae is shown in Table 3.4. The amount of time test wasps inspected cells was not significantly different (*F*=2.49; df=3, 69; *P*=0.0674), but the time test wasps spent in trophallaxis with larvae was significantly different (*F*=3.46; df=3, 69; *P*=0.0210). The 10-day old test wasps in their natal nests spent significantly longer time in trophallaxis with larvae than 10-day old test wasps in a foreign nest (Table 3.4). However, neither of the 10-day old test wasp treatments differed significantly from the 3-day old test wasp treatments.

Based on the incidence of intolerant behaviors, the percentage of acceptance was high for both queen and workers for all treatments (Table 3.5). Queens (X^2=1.05; df=3; *P*=0.7890) and workers (X^2=3.1974; df=3; *P*=0.3622) did not differ significantly across treatments for acceptance of test wasps. Overall, 10-day old test wasps in a foreign colony were accepted the least (Table 3.5).

Discussion

The amount of time 10-day old test wasps spent on their natal nests in comparison with the other treatments suggests that age does affect nest discrimination for *P. metricus* workers. The 3-day old test wasps averaged more time on foreign nests (17.99 min) than natal nests (12.43 min), but the difference was not significant (Table 3.1). These results appear to contrast with Layton and Espelie's (1996) study of 4-day old *P. metricus* workers, but in their experiments all wasps were introduced into their natal nest.

Averaging less than a minute of the total observation time, antennation behaviors and trophallaxis were infrequent among the test wasps and the resident wasps (Table 3.2, Table 3.3). The total time the test wasp spent inspecting nest cells and in trophallaxis with larvae also averaged less than a minute. Acceptance of the test wasp by the resident wasps was not significantly affected by either age or colony origin of the test wasp. Although Layton and Espelie (1996) found significant differences in the amount of time a test wasp spent on its natal nest depending on exposure to nest cuticular hydrocarbons, they did not detect differences in acceptance by resident wasps of test wasps. The age of the test wasps, laboratory rearing of colonies, and utilization of only natal nests for bioassays may have affected Layton and Espelie's (1996) acceptance results.

Nest surface cuticular hydrocarbons may play an important role in nestmate discrimination, but Pickett et al. (2000) demonstrated that *Polistes dominulus* (Christ) was able to use a compound not naturally occurring in *Polistes* nests, pentacosanoic acid methyl ester, to augment nestmate discrimination. These results illustrate the potential importance of environmental components in the recognition process and suggest that nestmate discrimination in *Polistes* does not occur under the classical definitions of recognition pheromones as endogenous, learned odors (Wilson 1971; Hölldobler and Michener 1980). The laboratory maintenance of colonies in our study as well as in the study by Layton and Espelie (1996) may have affected nestmate discrimination by eliminating environmental factors such as contact with non-kin, brood stealing, predation, and parasitism. Gamboa et al. (1986) found that nestmate discrimination does not necessarily occur for *P. fuscatus* reared under homogenous laboratory conditions. All of these findings support Reeve's (1989) optimal acceptance threshold model. This model predicts that several environmental factors may influence the threshold of acceptance. Low fitness costs of accepting non-kin could result in universal acceptance. Clearly, additional work, including field observations, is needed to further understand the roles of age, cuticular hydrocarbons, and environmental factors in nest and nestmate discrimination.

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Table 3.1: Mean Time on Nest for Queens, Workers and Test Wasps

Treatment	**Queen**	**Worker**[2]	**Test Wasp**
3-day old Test Wasp, Natal Colony (n=18)	39.33 ± 23.56	26.65 ± 22.65	12.43 ± 20.49 **B**[1]
3-day old Test Wasp, Foreign Colony (n=17)	33. 64 ± 27.83	33.12 ± 20.89	17.99 ± 23.04 **AB**
10-day old Test Wasp, Natal Colony (n=20)	43.12 ± 22.12	28.69 ± 23.31	24.52 ± 24.44 **A**
10-day old Test Wasp, Foreign Colony (n=18)	35.30 ± 24.26	21.12 ± 19.87	5.62 ± 13.39 **B**

[1] Means with the same letter within a column are not significantly different at the $\alpha=0.05$ level.

[2] Time for each of the two workers per treatment was compiled separately.

Table 3.2: Mean Time the Test Wasp Interacted With the Queen in Trophallaxis, Mutual Antennation, and Unilateral Antennation

Treatment	Trophallaxis	Mutual Antennation	Test Wasp Antennating Queen
3-day old Test Wasp, Natal Colony (n=18)	0.16 ± 0.39	0.05 ± 0.13	0.05 ± 0.12
3-day old Test Wasp, Foreign Colony (n=17)	0.29 ± 0.82	0	0.17 ± 0.43
10-day old Test Wasp, Natal Colony (n=20)	0.24 ± 0.56	0.02 ± 0.05	0.15 ± 0.28
10-day old Test Wasp, Foreign Colony (n=18)	0.16 ± 0.46	0 ± 0.02	0.01 ± 0.03

Table 3.3: Mean Time the Test Wasp Interacted with the Workers in Trophallaxis and Unilateral Antennation

Treatment	Trophallaxis	Test Wasp Antennating Workers	Workers Antennating Test Wasp
3-day old Test Wasp, Natal Colony (n=18)	0.16 ± 0.55 **B**[1]	0.31 ± 1.68 **AB**	0.01 ± 0.03 **AB**
3-day old Test Wasp, Foreign Colony (n=17)	0.33 ± 0.88 **B**	0.15 ± 0.35 **AB**	0.02 ± 0.06 **AB**
10-day old Test Wasp, Natal Colony (n=20)	0.34 ± 0.61 **A**	0.24 ± 0.68 **A**	0.10 ± 0.30 **A**
10-day old Test Wasp, Foreign Colony (n=18)	0.04 ± 0.19 **B**	0.04 ± 0.11 **B**	0 ± 0.01 **B**

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[1] Means with the same letter within a column are not significantly different at the $\alpha=0.05$ level.

Table 3.4: Mean Time the Test Wasp Spent Inspecting Nest Cells and in Trophallaxis with Larvae

Treatment	Inspecting Nest Cells	Trophallaxis with Larvae
3-day old Test Wasp, Natal Colony (n=18)	0.27 ± 0.94	0.07 ± 0.21 **AB**[1]
3-day old Test Wasp, Foreign Colony (n=17)	0.63 ± 1.54	0.34 ± 1.18 **AB**
10-day old Test Wasp, Natal Colony (n=20)	0.64 ± 1.41	0.43 ± 1.45 **A**
10-day old Test Wasp, Foreign Colony (n=18)	0.09 ± 0.34	0 **B**

[1] Means with the same letter within a column are not significantly different at the α=0.05 level.

Table 3.5: Percentage of Acceptance of Test Wasp by Resident Wasps

Treatment	**Queens**	**Workers**
3-day old Test Wasp, Natal Colony (n=18)	94.4	83.3
3-day old Test Wasp, Foreign Colony (n=17)	87.5	84.4
10-day old Test Wasp, Natal Colony (n=20)	90	85
10-day old Test Wasp, Foreign Colony (n=18)	84.2	71.1

CHAPTER 4

PARASITOIDS AND PARASITES OF *POLISTES METRICUS* SAY (HYMENOPTERA: VESPIDAE) IN NORTHEAST GEORGIA[1]

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[1] Hodges, A.C., G.S. Hodges, and K.E. Espelie. Accepted by Annals of the Entomological Society of America. Reprinted here with permission of publisher.

Abstract

The strepsipteran, *Xenos peckii* Kirby, the ichneumonid *Pachysomoides fulvus* Cresson, the pyralid *Chalcoela pegasalis* Walker, and the eulophid *Elasmus polistis* Burks were recorded from 303 *Polistes metricus* Say early season colonies collected from Northeast Georgia during 1997-2000. Colonies of *P. metricus* were significantly more likely to be infested by *X. peckii* than by *P. fulvus*, *C. pegasalis*, or *E. polistis*. Both *X. peckii* and *C. pegasalis* infested colonies more frequently when either eggs, larvae and pupae or larvae and pupae were present in the nest.

Keywords: *Polistes metricus* Say; *Xenos peckii* Kirby; *Pachysomoides fulvus* Cresson; *Elasmus polistis* Burks; *Chalcoela pegasalis* Walker

Gynes of temperate *Polistes* (paper wasps) found nests in the spring. In northeast Georgia, nest founding for *Polistes metricus* Say is typically haplometrotic (single-foundress) but has been reported to be both haplometrotic and pleometrotic (multiple-foundress) in other regions (West-Eberhard 1969). The adult female *Polistes* build their nest by macerating wood or paper products with enzymes in their saliva. After forming a nest pedicel, the females begin building nest cells and laying eggs. In cases of pleometrotic founding, one of the foundresses will physically dominate the other foundresses and subsequently become the dominant egg-layer.

When only the foundress(es) are present, the colony cycle is considered to be the founding phase of the colony cycle (Reeve 1991; Yamane 1996). Once the first worker emerges, the colony is in the worker phase of the colony cycle. The beginning of the reproductive phase of the colony cycle is marked by the emergence of gynes and males. During the reproductive phase of the colony cycle, the queen's dominance over the other colony members subsides and the workers will frequently lay sterile male eggs. The intermediate phase of the colony cycle is the time period between the reproductive phase and the next founding phase. The adult female gynes and the adult males disperse to mate. *Polistes metricus* gynes overwinter as fertilized females.

Polistes wasps are commonly seen visiting garden flowers or searching for prey in urban and agricultural settings. These wasps prey upon a variety of different types of insects that they use to feed their larvae. A founding stage, haplometrotric colony is vulnerable to attack by brood parasitoids, such as *Chalcoela*, *Elasmus*, and *Pachysomoides*, while the queen forages. *Chalcoela pegasalis* Walker (Lepidoptera: Pyralidae), *Pachysomoides fulvus* Cresson (Hymenoptera: Ichneumonidae), and *Elasmus* *polistis* Burks (Hymenoptera: Eulophidae) complete their development by feeding on brood in the nest cells of their *Polistes* host. Parasitoids emerge from the nest cells as adults. *Chalcoela pegasalis* and *P. fulvus* adult females lay their eggs on larvae or pupae of *Polistes*, but *E. polistis* females only oviposit in *Polistes* pupal cells (Rau 1941; Strassmann 1981). Both haplometrotic and pleometrotic *Polistes* colonies could encounter a triungulin larva of the parasite *Xenos* during foraging. *Xenos* are believed to be capable of infesting the larvae and pupae of *Polistes* hosts by waiting at flowers for a foraging wasp to carry it back to her nest (Katherithamby 1989). Interactions with parasites and parasitoids can affect foraging efficiency and other colony behaviors of *Polistes*. Starr (1976) hypothesized that parasitoid pressure may be an important factor selecting against pleometrotic colony founding for some North American *Polistes*.

Multiple foundresses could negatively affect the colony if one of the foundresses was infested by a mated female *Xenos peckii* Kirby (Strepsiptera: Stylopidae). Both male and female *X. peckii* develop within the abdomen of *P. metricus*, but the female remains a larviform parasite within the body of her host. The adult male emerges from his pupal stage as a free-living form. Mating between the male and female *X. peckii* culminates with traumatic insemination by the male inserting his aedeagus into the cephalothorax of the female (Bohart 1941). Once the female has mated, thousands of eggs mature within her, eventually being expelled from the female's brood passage on the cephalothorax as triungulin larvae. The triungulin larvae of *Xenos* infests the larval and pupal stages of *Polistes*. The adult female and the male pupa *X. peckii* exert from between the abdominal tergites and sternites of their *Polistes* host. According to Schraeder (1924) male *X. peckii* pupae (as *Acroshismus wheeleri* Pierce) exert approximately 5 to 10 days prior to adult females. Infestation by *X. peckii* is not always apparent upon emergence of a *Polistes* adult. *Xenos peckii* are believed to overwinter as fertilized females, but infested *P. metricus* are not believed to be capable of haplometrotic colony founding.

Few comprehensive reports of natural enemies of Nearctic *Polistes* exist (Rau 1941; Rabb 1960; Nelson 1968; Strassmann 1981). These reports emphasize the presence of brood parasitoids and indicate that there are regional differences in the prevalence or occurrence of parasitoids in different *Polistes* species. For example, *C. iphitalis* was the most common parasitoid found in *Polistes metricus* Say (Hymenoptera: Vespidae) nests in Nelson's (1968) study in southern Illinois but has not been shown to occur in the eastern United States (Munroe, 1972). *Chalcoela iphitalis* and *E. polistis* were the most common parasitoids in Strassmann's (1981) study of *Polistes exclamans* Viereck (Hymenoptera: Vespidae) in Texas. *Elasmus polistis* was not even known to occur in North America until Burks (1971). Rau (1941) found 10% of 200 *Polistes annularis* (L.) and *Polistes fuscatus* (F.) (as *P. pallipes* and *P. variatus*) colonies were infested by *Chalcoela pegasalis* Walker (as *Dicymolomia pegasalis*) in Missouri and noted that no moth infestations were found during the early part of the summer. Rabb (1960) recorded the parasitoids *C. pegasalis* (as *D. pegasalis*), *Pachysomoides stupidus* Cresson (as *Polistiphaga stupida*), and *Pachysomoides fulvus* Cresson (as *Polistiphaga fulva*) from *Polistes* in North Carolina. Although Rabb noted the presence of some adult individuals infested with the strepsipteran *X. peckii*, the percentage of colonies with *X. peckii* infestations was not reported. We report the identity and prevalence of parasitoids and parasites of early season *P. metricus* colonies from northeast Georgia. In order to assess parasitism levels that were not necessarily apparent by immediate destructive sampling, colonies of *P. metricus* were maintained in the laboratory after collection in order to retrieve the subsequently emerging parasites and parasitoids.

Materials and Methods

Polistes metricus colonies were collected during 1997, 1998, and 1999 from April through June. During 2000, *P. metricus* colonies were collected only during April and May. Colonies were collected from the Georgia counties of Clarke, Barrow, and Oglethorpe. Colonies were collected from structures either early in the morning or in the evening, when *Polistes* are least active. Following collection, *P. metricus* colonies were taken to the laboratory and chilled for approximately 5 min to 5-8°C to facilitate removal of the queen (and any other adults if present). Each *P. metricus* nest was affixed to a wooden craft stick which was glued to the top of a 20 x 20 x 20-cm Plexiglas chamber using a heated low temperature glue gun (Singer and Espelie 1992, 1996). The queen (and other adults if present) was marked with a dot of enamel paint and placed in the chamber with the nest. All colonies were individually housed and each was provided daily with water, 10% honey-water, Whatman No. 1 filter paper for nest construction, and a late instar *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) caterpillar. Colonies were maintained at room temperature with a natural lighting cycle provided by windows adjacent to the colonies.

Of the 303 *P. metricus* colonies collected over all years, 285 colonies were in the founding phase of the colony cycle. Fifteen of the remaining colonies were in the worker stage of the colony cycle, and three colonies were collected with pupae and no adults.

The numbers of cells, eggs, larvae, and pupae present in the nest were recorded. Based on the presence of eggs, larvae, and/or pupae, founding phase *P. metricus* were divided into the following developmental stage groupings: eggs only (n = 60); eggs and larvae only (n = 92); eggs, larvae, and pupae (n = 82); and larvae and pupae only (n = 21). The remaining 30 founding stage *P. metricus* were collected with larvae only, eggs and pupae, or pupae only.

Infestation by parasites or parasitoids was generally not apparent upon collection. *Polistes metricus* colonies were monitored daily for adult emergence, appearance of *X. peckii* parasites between the abdominal segments of wasps, and parasitoid emergence. *Pachysomoides fulvus* identifications were determined using keys in Townes and Townes (1962). *Elasmus polistis* identifications were determined by using Burks' (1971) species description. Specialists confirmed identifications of *X. peckii* and *C. pegasalis*. Voucher specimens of all species associated with this study are deposited in the Georgia Museum of Natural History in Athens, GA.

The arcsine transformation for percentage data was applied to the percentage of *P. metricus* colonies infested with parasitoids or parasites (Zar 1984). Transformed data were analyzed using an analysis of variance test (PROC GLM) (SAS Institute 1999). Due to significant differences across percentages of colonies infested with parasites or parasitoids at the α=0.05 level, mean percentages of colonies with parasites or parasitoid were then compared using Tukey's Studentized Range Test.

Data for percentage of infested *P. metricus* colonies collected at the developmental stage groupings within the founding phase of the colony cycle were also converted to the arcsine transformation for percentage data (Zar 1984). Transformed data for the developmental stage groupings were then submitted to an analysis of variance test (PROC GLM) (SAS Institute 1999). Significant differences for parasitoids and parasites occurred across developmental stage groupings, but interaction between parasitoids and parasites with the developmental stage groupings occurred. Subsequently, each parasite and parasitoid was analyzed separately for significance across developmental stage groupings. When a parasite or parasitoid showed significance across developmental stage groupings at the $\alpha=0.05$ level, Tukey's Studentized Range Test was used to separate the mean percentage of colonies in each grouping.

Results

Only *X. peckii* and *C. pegasalis* were found during each year of the study (Table 4.1). Table 4.2 shows the mean percentages of *X. peckii*, *P. fulvus*, *C. pegasalis*, and *E. polistis* differed significantly ($F=10.08$; df=3, 12; $P=0.0013$). Comparisons among colonies showed that the mean percentage of colonies infested with the parasite *X. peckii* was significantly higher than for the parasitoids *P. fulvus*, *C. pegasalis* and *E. polistis,* but that there were no significant differences in the incidences of the three parasitoids among colonies.

Significant differences occurred for developmental stage groupings ($F=5.21$; df=3, 48; $P=0.0034$), and for the interaction between parasites and parasitoids with developmental phase groupings ($F=2.11$; df=9, 48; $P=0.0465$). Analysis of each species separately revealed non-significant differences for *P. fulvus* ($F=0.63$; df=3, 12; $P=0.6104$) and *E. polistis* ($F=1.00$; df=3, 12; $P=0.4262$), but significant differences for *X. peckii* ($F=6.04$; df=3, 12; $P=0.0095$) and *C. pegasalis* ($F=4.47$; df=3, 12; $P=0.0251$). Results from Tukey's Studentized Range Test showed that for both *X. peckii* and *C.*

pegasalis significantly fewer colonies collected with only eggs present were infested (Table 4.3). For colonies infested by *X. peckii*, eggs, larvae and pupae or larvae and pupae were more likely to be present upon collection. Colonies collected with eggs and larvae were infested by *X. peckii* more than colonies with eggs only, but less than colonies with larvae and pupae or eggs, larvae and pupae. A non-significant difference occurred for colonies infested with *C. pegasalis* between eggs and larvae and eggs, larvae, and pupae (Table 4.3). Colonies collected with larvae and pupae resulted in the highest mean percentage of colonies infested for *C. pegasalis*.

Discussion

Polistes metricus colonies utilized in this study were all haplometrotic and collected in the early stages of colony development, such that visible evidence of parasitoids or parasites was generally not apparent. Although four species of parasites and parasitoids infested nests at this early stage of development, because no pleometrotic nests were collected our results are uninformative for testing Starr's hypothesis. However, the availability of only haplometrotic *P. metricus* colonies at the collection sites in this study could have resulted from parasite and/or parasitoid pressure.

Our study indicates that *X. peckii* is more common in *P. metricus* colonies in northeast Georgia than previous reports of parasites and parasitoids of *Polistes* would suggest. We are the first to show that *X. peckii* is the most prevalent parasite or parasitoid present in a population of *Polistes* during the founding phase of the colony cycle. Also, the developmental stage of the colony within the founding phase of the colony cycle was important for *X. peckii* and *C. pegasalis*. Even though *P. metricus* colonies with *X. peckii* parasites were collected at all developmental stage groupings within the founding phase of development, infestation was more likely to occur if colonies had eggs, larvae and pupae or only larvae and pupae. Linsley and MacSwain (1957) showed that *Stylops pacifica* L. larvae penetrate the chorion of its host, *Andrena complexa* Viereck. The ability of *X. peckii* to infest *P. metricus* at the egg stage has not been shown, but our data suggest that colonies collected with only eggs may already have been infested by *X. peckii* in the field. Previously, *Xenos* has been thought to only infest the larval and pupal stages of *Polistes* (Schraeder 1924).

This is also the first report of *C. pegasalis* in Georgia (Munroe 1972). Colonies infested with *C. pegasilus* were not collected when only eggs were present in the nest, and *C. pegasalis* is not believed to be capable of infesting the egg stage of *P. metricus*. Colonies infested with *C. pegasalis* were more likely to occur when eggs, larvae and pupae or larvae and pupae were present.

Previous researchers have concluded that the occurrence of stylopized *Polistes* is infrequent and localized to a particular area (Brues 1905, Schraeder 1924). Schraeder (1924) even hypothesized that the localized nature of parasitism by *Xenos* should drive a local population of *Polistes* to extinction. However, the persistent presence of infested early season colonies each year of this study despite destructive sampling of colonies suggests that a relatively stable balance between the two species exists. The extent to which *X. peckii* also attacks other *Polistes* species in northeast Georgia remains to be investigated, but other species could harbor reservoir populations that serve as sources to reinfest *P. metricus*.

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Table 4.1: Incidence of Parasites and Parasitoids in Early Season

***P. metricus* Colonies**

Year	***X. peckii***	***P. fulvus***	***C. pegasalis***	***E. polistis***
1997 (n=99)	12	2	2	0
1998 (n=83)	7	3	1	0
1999 (n=68)	13	4	4	2
2000 (n=53)	19	0	1	0
Total (n=303)	51	9	8	2

Table 4.2: Mean Percentage of Early Season *P. metricus* Colonies Infested by Parasites or Parasitoids

Parasite/Parasitoid	**Percentage**
X. peckii	17.849 ± 2.286 (A)[1,2]
C. pegasalis	2.50 ± 0.350 (B)
P. fulvus	2.080 ± 1.10 (B)
E. polistis	0.186 ± 0.741 (B)

[1] Data values not transformed are shown. Statistical analyses were performed on transformed data values.

[2] Means with the same letter within a column are not significantly different at the $\alpha=0.05$ level using Tukey's Studentized Range Test (SAS Institute, 1999).

Table 4.3: Mean Percentage of *P. metricus* Colonies Infested by *X. peckii* and *C. pegasalis* at Different Developmental Stages

Developmental Stage	***X. peckii***	***C. pegasalis***
Eggs	1.185 ± 2.462 (B)[1]	0±0 (B)
Eggs, Larvae	13.591 ± 2.058 (AB)	2.755 ± 1.099 (AB)
Eggs, Larvae, Pupae	23.999 ± 2.353 (A)	3.842 ± 2.153 (AB)
Larvae, Pupae	35.617 ± 4.745 (A)	11.884 ± 5.577 (A)

[1] Data values not transformed are shown. Statistical analyses were performed on transformed data values.

[2] Means with the same letter within a column are not significantly different at the $\alpha=0.05$ level using Tukey's Studentized Range Test (SAS Institute, 1999).

CHAPTER 5

SEXUAL DISTRIBUTION OF *XENOS PECKII* KIRBY (STREPSIPTERA: STYLOPIDAE) ON *POLISTES METRICUS* SAY (HYMENOPTERA: VESPIDAE)[1]

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[1] Hodges, A.C., Hodges, G.S. and K.E. Espelie. 2002. To be submitted to the Journal of Entomological Science.

Abstract

Some contributions to the life history of *Xenos peckii* Kirby (Strepsiptera: Stylopidae) and its *Polistes metricus* Say (Hymenoptera: Vespdiae) host are provided. A total of 221 *P. metricus* adults were microscopically examined and dissected. Of the 107 adult *Xenos peckii* Kirby (Strepsiptera: Stylopidae) present, 45.8% were female and 54.2% were male. Both male and female *X. peckii* protruded more often from between tergites than sternites. The preferred position for extrusion was beneath gastral tergite 5 for females and gastral tergite 3 for males. Females are approximately twice as large as males, and size could influence the preferred gastral tergite for extrusion. The parasite level is significantly lower when only female *X. peckii* are present in a host, possibly due to the obligate endoparasitic female adult stage. Two dissected queens containing female *X. peckii* suggests that an infested queen may be capable of colony founding. Female *Polistes* infested with *Xenos* have previously been believed to be reproductively inviable.

Keywords: Stylopization; Parasitism; Paper Wasps

Xenos peckii Kirby is a strepsipteran parasite of numerous *Polistes* species (Schraeder 1924). The Palearctic, Oriental, Neartic, and Neotropical regions comprise the distribution of *Xenos* with *Polistes*, *Vespa*, and *Mischocyttarus* species included in its host range (Bohart 1941). Strepsiptera is one of the few insect orders with two distinct larval types in their developmental cycle, a phenomenon termed hypermetamorphosis. The active, triungulin larva of *X. peckii* infests *Polistes* larvae and pupae. After entering the body of its host, a triungulin larva transforms into a vermiform larva. Male and female *X. peckii* complete their development within the abdomen of *P. metricus*. The female protrudes from between her host's tergites or sternites as a larviform adult, but the male protrudes only as a pupa. Even though the female sometimes can be seen emerging from beneath the abdominal segments of her host, she may not always be apparent externally (Schraeder 1924). After the male metamorphoses into an adult, the pupal case is still apparent on the host. The purpose of the free-living adult male form, which only lives for a few hours, is to locate and mate with a female by traumatic insemination (Bohart 1941). Hundreds or thousands of fertilized eggs will develop into triungulin larvae within the female *X. peckii*. Triungulin larvae are expelled directly from the female's brood passage on her cephalothorax. The reproductive organs of both male and female *Polistes* are diminished by *Xenos* parasitism, but only the male remains reproductively viable (Schraeder 1924; Bohart 1941). Fertilized *Xenos* females are believed to overwinter within infertile *Polistes* gynes (Brues 1905; Bohart 1941; Dunkle 1979).

Location and season affects rates of infestations by *Xenos* in *Polistes* (Schraeder 1924). Schraeder (1924) found levels of *X. peckii* infestations ranging from 0 to 25%

depending on location. Even though the overall sex rato for *X. peckii* collected in *Polistes* in the northeastern United States was 1.03:1 for males to females, males predominated in September and only females were collected during May-June and October-November (Schraeder 1924). Brues (1905) found higher proportions of male versus female *Xenos pallidus* Brues infesting *Polistes annularis* (L.) in Texas during July (96.8%) compared to October (64.8%). For *P. annularis* males collected during October and November in Florida, the sex ratio of male to female *X. pallidus* was 1:1.83 (Dunkle 1979). Also, *X. pallidus* females preferred exerting beneath tergite 5 and males beneath tergite 4. We report the preferred abdominal position of *X. peckii* and the percentage of male and female *X. peckii* from early season collected, laboratory-reared *P. metricus* colonies.

Materials and Methods

Ninety-nine and 68 *P. metricus* colonies were collected during April through June of 1997 and 1999, respectively. Ninety-four in 1997 and 56 in 1999 of the colonies were collected from Clarke Co., Georgia. The remaining colonies were collected from the Georgia counties of Barrow, Fulton, and Cherokee during 1997 and Oglethorpe and Harris during 1999. Colonies were taken to the laboratory and chilled for approximately five min to 5-8°C in order to facilitate removal of the adult wasp(s). The nest was then glued to the top of a 20 X 20 X 20-cm Plexiglas chamber utilizing the methods of Singer and Espelie (1992). The queen was marked on the thorax with a dot of yellow enamel paint and placed in the chamber with her nest. Each colony was provided with water, 15% honey-water, and a late instar *Spodoptera frugiperda* (Smith) larva daily. Whatman No. 1 filter paper was provided for nest construction.

Polistes metricus colonies were monitored daily until August 1 for the appearance of *X. peckii*. Of the colonies collected, twelve in 1997 and thirteen in 1999 contained colony members infested with *X. peckii*. Adult *P. metricus* from infested colonies were frozen post-mortem in 1997. The 1999 colonies were frozen in late August through September during the reproductive stage of colony development. A total of 221 *P. metricus* adults, 171 workers, 25 queens, 18 gynes, and 7 males, were microscopically examined externally and internally. The position and gender of the externally visible *X. peckii* parasites were recorded.

Data were transformed by taking the square root of the data values plus 0.5 (Zar 1984). Transformed data values were subjected to an analysis of variance using PROC GLM (SAS Institute 1999) in order to determine if significant differences occurred between the average parasite load of male versus female *X. peckii* when only males or females were present on a *P. metricus* host. Transformed data values were analyzed to determine if the average parasite load of male versus female *X. peckii* differed when male and female *X. peckii* were present on a *P. metricus* host. Transformed data for the preferred abdominal position of male and female *X. peckii* within their host were also tested for significance with an analysis of variance. Mean separation by Tukey's Studentized Range Test was performed to determine if male and female *X. peckii* were more prevalent in specific abdominal segments. Orthogonal contrast statements were used to determine if significant differences occurred for male and female *X. peckii* located between tergites versus sternites.

Results

Fifty-one *P. metricus* adults contained *X. peckii* males and/or females. Forty-nine female (45.8%) and 58 male (54.2%) *X. peckii* were present. Fifteen female (3.1%) and six male (1.0%) *X. peckii* were visible only after dissection.

Two queens and a gyne did not contain externally visible adult *X. peckii*, but were found to have large numbers (hundreds) of triungulin *X. peckii* larvae inside a concealed *X. peckii* female. Also, a queen was found to have a vermiform stage larva of *X. peckii* upon dissection. Four workers without externally visible *X. peckii* did have one or a few encysted, aborted triungulins apparent upon dissection. Eleven *P. metricus* individuals that contained *X. peckii* also had triungulin larvae revealed upon dissection. A worker, without externally visible *X. peckii* parasites, was found to have two vermiform immature larvae upon dissection. A worker containing a *X. peckii* female was also found to have a vermiform *X. peckii* upon dissection.

Nineteen *P. metricus* were parasitized by only female *X. peckii* and their average parasite load was 1.10 (± 0.46). Ten *P. metricus* were parasitized by *X. peckii* males only and their average parasite load was 2.10 (± 1.20). The average parasite load for *P. metricus* parasitized by males only was significantly higher than that of *P. metricus* parasitized by females only (*F=11.31;* df=1, 27; *P=0.0023*).

Twenty-two *P. metricus* were parasitized by both male and female *X. peckii*. When both female and male *X. peckii* were present, the average number of females was 1.27 (± 0.55), and the average number of males was 1.68 (± 0.89). There was not a significant difference between the average number of male and female *X. peckii* when both sexes were present (*F=3.30;* df=1, 42; *P=0.0762*).

The distribution of *X. peckii* females and males inside their hosts is presented (Tables 5.1, 5.2) in comparison to the total *X. peckii* parasite load within their *P. metricus* hosts. Female *X. peckii* were exerted beneath gastral tergites 2, 3, 4, 5, and 6 and gastral sternites 3, 4, 5, and 6. Male *X. peckii* were exerted beneath gastral tergites 1, 2, 3, 4, and 5 and gastral sternites 2, 3, 4, and 5. A significant difference occurred between the average segment of extrusion for female ($F=5.69$; df=8, 243; $P<0.0001$) and male ($F=9.33$; df=8, 270; $P<0.0001$) *X. peckii*. A significantly greater proportion of female ($F=5.79$; df=1, 243; $P=0.0169$) and male ($F=16.33$; df=1, 270; $P<0.001$) *X. peckii* were exerted between the tergites instead of the sternites.

The average number of male and female *X. peckii* exerted beneath each abdominal tergite or sternite is shown (Table 5.3). Female *X. peckii* were exerted primarily beneath tergite 5 and secondarily beneath tergite 4. A non-significant difference for extrusion of females beneath the remaining abdominal segments occurred. *Xenos peckii* males occurred most frequently beneath tergite 3, then tergite 4. Sternites 4 and 5 and tergite 1 were used the least by male *X. peckii* for extrusion.

Discussion

Schraeder (1924) hypothesized that the sex ratio of *X. peckii* should be approximately 1:1 if seasonal variation was considered, and obtained a ratio of 50.8 % males to 49.2 % females over a three year collection period. We found a sex ratio of 1:1.18 in favor of the male. Our results are relatively close to Schraeder's 1:1 sex ratio hypothesis in comparison with seasonal records of *Xenos* infesting *Polistes* (Brues 1905; Dunkle 1979; Katherithamby 1989). In contrast to destructive sampling, rearing the *P. metricus* colonies in the laboratory allowed for the development of the *X. peckii* parasites within a colony. Subsequently, the adult *X. peckii* sex ratios present should more accurately represent the average ratios present throughout the colony cycle instead of at one point during the colony cycle.

Hubbard (1892) observed *P. annularis* nests in the field parasitized by *X. pallidus* and he claimed that *X. pallidus* extruded from the abdomen of the wasps prior to their emergence. For the *P. metricus* in our study, parasitism by *X. peckii* was not always apparent upon emergence of a worker. Occasionally, parasitism was not evident until 10-20 days after emergence. Males typically became apparent before females. These observations agree more with Schraeder's (1924) observations of *X. peckii* (as *Acroschismus wheeleri* Pierce) than with Hubbard's (1892) observations of *X. pallidus*. Schraeder (1924) also stated that the exact number of *X. peckii* in a population of *Polistes* could not be ascertained without dissection of each wasp's abdomen. Our study supports this conclusion because 19.6% of the male and female *X. peckii* were only visible after dissection.

Mated female *X. peckii* are believed to overwinter inside gynes that enter winter diapause (Brues 1905). These parasitized gynes are not believed to be capable of founding nests and laying eggs in the following spring; therefore, the exact mechanism of transfer of triungulin *X. peckii* larvae to the first *Polistes* brood remains uncertain. The dissected queens that contained a female *X. peckii* with triungulins suggests that an infested queen may be able to found a colony. However, queens were not directly observed founding the colony or laying eggs in the nest. The parasitized queens may have usurped the nest from a conspecific or may have acted as a co-foundress helper to a conspecific. The presence of the females containing triungulins in the queens and in the 1997 gyne indicates that mated *X. peckii* females overwinter inside female *P. metricus*.

A larger percentage of female *X. peckii* occurred with only females (38.8%) as compared with male *X. peckii* occurring only with males (17.2%) in *P. metricus* hosts. When only male *X. peckii* infested a *P. metricus,* the average parasite load was twice as high as compared to a female only load. However, when males and females infested individuals there was not a significant difference between the average sex-specific parasite loads. Possibly, the presence of the females prevents males from attaining high rates of infestation on a particular wasp's abdomen. Male *X. peckii* were located more anteriorly than females on the host's abdomen. Dunkle (1979) suggested that the male *X. pallidus* were located more anteriorly than the female *X. pallidus* due to the fact that the females were larger than the males. Female *X. peckii* (6.25-9mm) are also larger than males (3-4.5mm), and size could affect the location of the parasite within its host (Brues 1903).

In order to further understand the host-parasite relationship between *Xenos* and *Polistes*, future research could focus on the number of infested *Polistes* within a colony and the sex ratio of their *Xenos* parasites. Hodges et al. (2002) found *X. peckii* to be the most prevalent parasite or parasitoid in early season *P. metricus* colonies in northeast Georgia. Previous researchers have not reported the percentage of colonies in a region infested by *Xenos* or the percentage of individuals within a colony infested (e.g., Brues 1905, Schraeder 1924, Dunkle 1979, Kathirithamby 1989). Future studies could also focus on developmental and behavioral effects of parasitism by *Xenos* on colonies of *Polistes.*

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Table 5.1: Distribution of *X. peckii* Females by Location and Parasite Load in Gaster

Location	**1**[1]	**2**	**3**	**4**	**5**	**6**
Tergite 2	0	0	1	0	0	0
Tergite 3	0	1	0	0	0	0
Tergite 4	3	2	4	2	0	0
Tergite 5	5	4	1	5	0	2
Tergite 6	5	1	0	0	0	0
Sternite 3	0	0	0	1	1	0
Sternite 4	0	0	1	1	0	0
Sternite 5	1	1	1	0	0	1
Sternite 6	1	0	0	0	0	0

[1] Indicates the total number of *X. peckii* parasites present.

Table 5.2: Distribution of *X. peckii* Males by Location and Parasite Load in Gaster

Location	**1**[1]	**2**	**3**	**4**	**5**	**6**
Tergite 1	1	0	0	0	0	0
Tergite 2	0	1	3	2	0	0
Tergite 3	1	6	6	8	1	1
Tergite 4	1	6	1	3	1	0
Tergite 5	0	0	0	4	1	0
Sternite 2	0	2	0	0	0	2
Sternite 3	0	1	1	1	1	0
Sternite 4	0	0	0	2	0	0
Sternite 5	1	0	0	0	0	0

[1] Indicates the total number of *X. peckii* parasites present.

Table 5.3: Mean Number of Female and Male *X. peckii* by Location in Gaster[1]

Location	Female *X. peckii* (n=28)	Male *X. peckii* (n=31)
Tergite 1	N/A[2]	0.03 ± 0.18 C
Tergite 2	0.04 ± 0.19 B[3]	0.19 ± 0.48 BC
Tergite 3	0.04 ± 0.19 B	0.74 ± 0.73 A
Tergite 4	0.32 ± 0.55 AB	0.39 ± 0.5 AB
Tergite 5	0.5 ± 0.64 A	0.16 ± 0.45 BC
Tergite 6	0.07 ± 0.26 B	N/A
Sternite 2	N/A	0.13 ± 0.43 BC
Sternite 3	0.07 ± 0.26 B	0.13 ± 0.34 BC
Sternite 4	0.07 ± 0.26 B	0.07 ± 0.25 C
Sternite 5	0.14 ± 0.36 B	0.03 ± 0.18 C
Sternite 6	0.04 ± 0.19 B	N/A

[1] Data shown are non-transformed values. Statistical analysis was performed on transformed data values.

[2] N/A=*X. peckii* not occurring at this location.

[3] Means with the same letter within a column are not significantly different at alpha=0.05 using Tukey's Studentized Range Test.

CHAPTER 6

EFFECTS OF PARASITISM BY *XENOS PECKII* KIRBY (STREPSIPTERA: STYLOPIDAE) ON COLONY DEVELOPMENT OF *POLISTES METRICUS* SAY (HYMENOPTERA: VESPIDAE)[1]

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[1] Hodges, A.C., and K.E. Espelie 2002. To be submitted to the Journal of the Kansas Entomological Society.

Abstract

The effects of parasitism by *Xenos peckii* (Strepsiptera: Stylopidae) on colony development were determined by daily prey consumption and weekly measurements of the number of eggs, larvae, and pupae present in colonies over a six-week period. Prey consumption was not significantly affected by *X. peckii* parasitism. Unparasitized colonies (n=18) had significantly more pupae present during week 3 than parasitized colonies (n=23). Parasitism did not significantly affect the number of eggs or larvae present in colonies. Parasitism did not significantly affect overall colony development or prey consumption. *Xenos peckii* may only minimally impact *Polistes* colonies due to its need for host survival. The only free-living stage of the parasitic *X. peckii* is the adult male, with the remaining life stages requiring a host.

Keywords: Paper Wasps; Stylopization

Polistes wasps, commonly found in temperate and tropical regions, have been the focus of numerous behavioral and ecological studies (Rau 1941; Rabb and Lawson 1957; Rabb 1960; West-Eberhard 1969; Strassmann 1981; Gamboa et al. 1986; Singer and Espelie 1992; Layton and Espelie 1996; Starks et al. 1998). A majority of behavioral research on this group has focused on elucidating aspects of nestmate and/or kin discrimination (Pfennig et al. 1983a, b; Gamboa et al. 1986; Singer and Espelie 1992; Layton and Espelie 1996; Arathi et al. 1997; Starks et al. 1998). *Polistes* are also important as beneficial generalist predators in agricultural situations (Rabb and Lawson 1957, Rabb 1960). Specifically, Rabb and Lawson (1957) found that lepidopteran larvae comprised most of the prey consumed by *Polistes fuscatus* (F.) and *Polistes exclamans* Viereck. The effects of parasite and parasitoid pressure on prey consumption by *Polistes* are unknown. Except for Strassmann's (1981) ecological study of *P. exclamans*, the majority of research with parasites and parasitoids of North American *Polistes* has focused on the occurrence and/or life history of the parasites or parasitoids (e.g., Brues 1905; Schraeder 1924; Bohart 1941; Rau 1941; Rabb 1960; Nelson 1968; Burks 1971; Reed and Vinson 1979; Macom and Landolt 1995; Whiteman and Landwer 2000; Hodges et al. 2002).

Immatures of *Xenos* are obligate endoparasites of *Polistes, Vespa, and Mischocyttarus* (Bohart 1941). The adult female *Xenos* remains a larviform, obligate parasite of her host, but the adult male is non-feeding and free-living (Bohart 1941; Katherithamby 1989). Mating occurs by traumatic insemination of the male's aedeagus into the female's cephalothorax. A female *Xenos* gives birth viviparously by expelling hundreds or thousands of triungulin larvae from the brood passage located on the cephalothorax. Triungulin larvae of *Xenos peckii* Kirby search for a *Polistes* larva or pupa to infest, and complete their development inside their host (Bohart 1941; Katherithamby 1989). *Xenos peckii* protrude as adult females or male pupae from between the abdominal tergites or sternites of their *Polistes* host. Most studies of *X. peckii* have focused on collecting *Polistes* to determine individual infestation levels (e.g., Bohart 1941; Katherithamby 1989). However, Schraeder (1924) extensively studied the reproductive development of *X. peckii* (as *Acroschismus wheeleri* Pierce) by infesting and raising *Polistes* larvae in the laboratory. Schraeder (1924) hypothesized that infestation by *X. peckii* would eventually lead to extinction of a population of *Polistes*.

Hodges et al. (2002) found *X. peckii* to be the most prevalent parasite or parasitoid in early season, *Polistes metricus* Say colonies in Georgia. The impact of *Xenos* on *Polistes* colonies is unknown. The purpose of the following study was to compare the development and prey consumption in laboratory-reared colonies of *P. metricus* parasitized with colonies that are not parasitized by *X. peckii*. According to Schraeder's (1924) hypothesis, the development of *P. metricus* colonies should be negatively affected by *X. peckii* parasitism.

Materials and Methods

Polistes metricus colonies in the founding phase of the colony cycle were collected during April through June in 1999 and 2000. Fifty-six of the 68 1999 colonies were collected in Clarke Co., Georgia. The remaining 1999 colonies were collected in Oglethorpe and Harris Counties in Georgia. Twenty-eight of the 53 2000 colonies were collected in Clarke Co., Georgia. Twelve colonies were collected in Barrow Co., Georgia and 13 colonies were collected in Oglethorpe Co., Georgia during 2000. The colonies were subsequently chilled for approximately 5 min at 5-8°C in order facilitate removal of the queen from the nest. The nest was then affixed to the top of a 20 X 20 X 20-cm Plexiglas chamber using the methods of Singer and Espelie (1992). The queen was marked on the thorax with a dot of yellow enamel paint and placed in the chamber with her nest. Each colony was placed in its own 20 X 20 X 20-cm Plexiglas chamber, and provided daily with water, 15% honey-water, and a late instar *Spodoptera frugiperda* (Smith) larva. Whatman No. 1 filter paper was also provided for nest construction.

Parasitism of *P. metricus* by *X. peckii* was not apparent upon collection. *Polistes metricus* colonies were monitored daily for adult wasp emergence, appearance of *X. peckii* parasites (male or female) between the abdominal segments of wasps, and emergence of *X. peckii* males. *Xenos peckii* parasites are not necessarily evident upon emergence of *Polistes* adults; therefore, daily monitoring of *Polistes* adults for *X. peckii* parasites is required (Schraeder 1924). Colonies with evidence of any parasitoids or other parasites were not used in this study.

Weekly colony observations began within 3 days after first worker emergence and continued for a six-week period. *Polistes metricus* colonies parasitized (n=23) and unparasitized (n=18) by *X. peckii* were observed. Weekly observations were taken by chilling a colony at 5-8°C for approximately 5 minutes. Chilled adults were removed during 1999 in order to record nest observations. During 2000, chilled adults were removed for weekly observations only if the nest brood were not visible. Otherwise, a clear Plexiglas bottom (slightly over-sized compared to the 20 X 20 cm dimensions of the bottom of the cage) allowed the observer to invert the cage and view the nest without removing adult wasps. The number of eggs, larvae, and pupae present in a nest were recorded weekly. Daily observations of *S. frugiperda* eaten were also recorded.

Data for weekly number of eggs present were transformed by taking the square root of eggs plus 0.25 using SAS (SAS Institute 1999). The correlation matrix of the autoregressive I model on weekly number of pupae indicated weeks 1, 2, and 3 were independent of weeks 4, 5, and 6. Subsequently, analysis of covariance using PROC MIXED COVTEST in SAS (SAS Institute 1999) on the repeated measures autoregressive I model were performed on transformed egg data, weekly number of larvae, and each grouping of weeks for number of pupae. If the effect of parasitism*week was significant for egg, larva, or pupa data, then the differences of least squares means was used to determine significance at specific weeks. Percentages of prey eaten over the observational period were transformed by using the arcsine transformation for percentage data as described in Zar (1984). Transformed prey data were submitted to PROC GLM analysis using SAS (SAS Institute 1999)

Results

For eggs present in *P. metricus* colonies, the effects of parasitism (*F*=0.00; df=1, 1; *P*=0.9948) and parasitism*week (*F*=0.22; df=5, 227; *P*=0.9532) were non-significant. Significant differences for eggs did occur across weeks (*F*=5.35; df=5, 5; *P*=0.0447), and the average number of eggs per week for *P. metricus* colonies is shown in Table (6.1). The least number of eggs were present in colonies during weeks 1 and 2. Weeks 4 and 5 had the highest proportion of eggs.

Parasitism (*F*=0.14; df=1, 1; *P*=0.7702) and parasitism*week (*F*=0.57; df=5, 227; *P*=0.7214) had no significant effect on number of larvae in *P. metricus* colonies. Table

6.2 shows the average number of larvae in *P. metricus* colonies by week and week effect (*F*=12.03; df=5, 5; *P*=0.0081) was significant. The number of larvae in colonies increased from week 1 to 6.

The average number of pupae present in *P. metricus* colonies by week is shown (Table 6.3). The effects of parasitism (*F*=0.41; df=1, 1; *P*=0.6367) and week (*F*=6.70; df=2, 2; *P*=0.1299) were not significant, but parasitism*week (*F*=2.39; df=2, 113; *P*=0.0966) was significant at the α=0.10 level for the number of pupae present in *P. metricus* colonies during weeks 1, 2, and 3. Since parasitism*week was significant, parasitized colonies were compared with unparasitized colonies at each week. A non-significant difference occurred for week 1 (*t*=-0.36; df=113; *P*=0.7203) and week 2 (*t*=0.41; df=113; *P*=0.6838). Colonies not parasitized by *X. peckii* had significantly more pupae present in their nest during week 3 (*t*=1.66; df=113; *P*=0.0998) at the α=0.10 level. The effects of parasitism (*F*=3.24; df=1, 1; *P*=0.3227), week (*F*=0.56; df=2, 2; *P*=0.6402), and parasitism*week (*F*=1.30; df=2, 113; *P*=0.2759) were not significant for the number of pupae present in *P. metricus* colonies during weeks 4, 5, and 6.

Both parasitized (76.5 ± 24.9) and unparasitized colonies (77.1 ± 27.7) consumed a high proportion of their prey. Parasitism by *X. peckii* did not significantly affect prey consumption for *P. metricus* colonies (*F*=0.02; df=1, 37; *P*=0.3209).

Discussion

If parasitism by *Xenos* impacts *Polistes* as severely as Schraeder (1924) hypothesized then queen fecundity, development of eggs into larvae, development of larvae into pupae, and prey consumption should have been affected during this study. Contrary to Schraeder's hypothesis, overall colony development and prey consumption of

P. metricus was not affected by parasitism of *X. peckii*. *Polistes* queens and workers occasionally removed larvae from their nest, but the number of larvae present in parasitized colonies was not significantly different from unparasitized colonies. The only significant difference between non-parasitized and parasitized colonies occurred for the number of pupae at week 3. Possibly, the presence of *X. peckii* in *P. metricus* larvae resulted in slower development and fewer pupae.

Even though our study suggests that *X. peckii* has no significant effect on *P. metricus* colony development, several factors could have influenced these results, including the number of parasitized individuals within a colony. Approximately 78% of colonies parasitized had 25% or less of their colony members infested by *X. peckii*. The laboratory rearing of the colonies could have affected the results. In a more natural setting, colonies with parasitized workers may not have been able to forage as efficiently, resulting in fewer brood for the colony.

Future studies could compare colony development and prey consumption of unparasitized and parasitized colonies in the field. The effect of parasitism on the emergence of viable reproductive *Polistes* from a colony is unknown. Future studies could also explore the behavioral responses of *Polistes* to parasitized colony members. Some of the parasitized *P. metricus* colonies in the laboratory seemed more aggressive than unparasitized colonies. The response of *Polistes* to *Xenos* appears to be variable. Hubbard (1892) observed *Polistes* attacking and eating male *Xenos*, while Brues (1903) reports *Polistes* behaving passively towards male *Xenos*. Further research on the effects of *Xenos* on *Polistes* colonies is needed in order to increase our understanding of this host-parasite relationship.

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Table 6.1: Mean Number of Eggs Per Week for Unparasitized and Parasitized *P. metricus* Colonies by *X. peckii*

Week	Unparasitized (n=18)	Parasitized (n=23)
1	6.11 ± 5.71	6.09 ± 4.98
2	8.17 ± 7.69	8.09 ± 5.26
3	10.33 ± 6.60	10.43 ± 6.56
4	12.72 ± 9.59	11.00 ± 8.53
5	12.44 ± 8.51	11.17 ± 8.39
6	9.56 ± 8.26	8.91 ± 7.27

Table 6.2: Mean Number of Larvae Per Week in Unparasitized and Parasitized *P. metricus* Colonies by *X. peckii*

Week	**Unparasitized (n=18)**	**Parasitized (n=23)**
1	7.11 ± 2.87	5.83 ± 3.37
2	8.67 ± 6.41	7.04 ± 3.47
3	9.67 ± 6.10	9 ± 4.38
4	12 ± 9.24	11.70 ± 6.57
5	14.83 ± 9.81	14.30 ± 9.03
6	17.73 ± 11.42	14.91 ± 7.67

Table 6.3: Mean Number of Pupae Per Week in Unparasitized and Parasitized *P. metricus* Colonies by *X. peckii*

Week	Unparasitized (n=18)	Parasitized (n=23)
1	4.39 ± 2.57	4.65 ± 2.57
2	3.77 ± 2.16	3.48 ± 2.30
3	4 ± 2.17	2.78 ± 2.07
4	3.83 ± 2.01	2.87 ± 1.74
5	3.94 ± 2.24	3.39 ± 2.06
6	4.39 ± 2.57	3.09 ± 2.00

CHAPTER 7

SUMMARY

Due to the plasticity of a young adult's template, some newly emerged social insects have been accepted into foreign conspecific colonies (Carlin et al. 1993; Errand 1994). Specifically, age has been shown to affect nestmate discrimination for the polistine wasp *Ropalidia marginata* (Lep.) (Arathi et al. 1997) and *Polistes fuscatus* (F.) (Panek et al. 2000). The results from Chapter 3 also suggest that age may influence nest recognition for *Polistes metricus* Say. Older (10-day old) *P. metricus* workers spent more time on their natal nest than on a foreign nest. Older workers also spent more time on their natal nest than younger (3-day old) workers. Younger workers averaged more time on a foreign nest than on their natal nest.

By using the laboratory bioassays presented in Chapters 2 and 3, age did not affect nestmate discrimination for *P. metricus*. The absence of nestmate discrimination demonstrated in Chapters 2 and 3 suggests that exogenous, environmental odors may be important in the nestmate recognition process. Gamboa et al. (1986) found that a homogenous laboratory environment could mask nestmate recognition. Even though workers and queens were least accepting of foreign 10-day old test wasps, no significant differences occurred across treatments and overall acceptance of all test wasps was high (Chapter 3, Table 3.3). Reeve's (1989) optimal acceptance threshold is an additional explanation for results from Chapter 2 and 3. Reeve (1989) predicted that fitness-maximizing factors affect the acceptance of conspecifics. The laboratory rearing of the *P. metricus* colonies minimized potential threats of predators, parasitoids, usurping conspecific, or brood-stealing conspecifics. Consequently, the cost of accepting foreign conspecifics may have been limited.

The parasitoids *Chalcoela pegasalis* Walker, *Pachysomoides fulvus* (Cresson), *Elasmus polistis* Burks, and the strepsipteran parasite *Xenos peckii* Kirby were the only parasites or parasitoids found in early season *P. metricus* colonies in northeast Georgia during 1997-2000 (Chapter 4). Chapter 4 presents the first report of the percentage of *Polistes* colonies in a region infested by *Xenos*. *Polistes metricus* colonies were significantly more likely to be infested by *X. peckii* than any of the parasitoids. The collection of infested *P. metricus* colonies with eggs only suggests that triungulin *X. peckii* may also infest the egg stage of *Polistes*. *Xenos peckii* has previously only been reported to infest larval and pupal stages of *Polistes* (Schraeder 1924).

A total of 107 *X. peckii* were present in 51 out of 221 dissected *P. metricus* adults (Chapter 5). The sex ratio of female to male *X. peckii* was 1:1.18. Female and male *X. peckii* preferrentially extruded beneath tergite 5 and tergite 3, respectively. Lowest parasite levels occurred when females only were present in a host. According to Schraeder (1924) the exact number of *Xenos* parasites cannot be ascertained without dissection of a wasp's abdomen. Even after microscopic examination, 19.6% of adult male and female *X. peckii* were not visible until after dissection. Two queens and a gyne containing gravid female *X. peckii* upon dissection support hypotheses of mated females as the overwintering stage (Brues 1905). Due to the fact that all colonies collected had one foundress, the results suggest that infested *Polistes* may be capable of founding a nest.

The effects of parasitism by *Xenos* on *Polistes* colony development are unknown. Parasitized and non-parasitized *P. metricus* colonies were compared in the laboratory.

Polistes are considered generalist predators, with much of their prey consisting of lepidopteran larvae (Rabb and Lawson 1957; Rabb 1960). Parasitized *Polistes* colonies had fewer pupae present in their colonies during week 3 out of 6 weeks of observation. Parasitism possibly resulted in a slower initial development for colonies. The results of Chapter 6 suggest that parasitism does not severely affect *Polistes* development or prey consumption. These results contradict Schraeder's (1924) hypothesis that parasitism by *Xenos* could result in extinction of *Polistes* colonies in a particular region.

In order to further elucidate aspects of nestmate discrimination and parasitoid/parasite effects for *Polistes*, future research could focus on field studies. The results of Chapters 2 and 3 emphasize the potential of environmental components. Parasitoid and parasite pressure could certainly affect colony behaviors for *Polistes*, including nestmate discrimination. Additional studies on the parasitoids and parasites of *Polistes* in Georgia could focus on rearing colonies collected during the worker and reproductive phases of the colony cycle. Behavioral effects of parasitism by *Xenos* on *Polistes* could provide interesting information concerning the host-parasite relationship as well as a colony's response to parasitized individuals.

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