HOST LOCATION BY *MELITTOBIA DIGITATA* DAHMS (HYMENOPTERA: EULOPHIDAE), A LARVAL PARASITOID OF MUD DAUBERS, *TRYPOXYLON POLITUM* SAY (HYMENOPTERA: SPHECIDAE) 

by

CHRISTIAN SHERLEY ARAÚJO DA SILVA TORRES 

(Under the Direction of Robert W. Matthews)

ABSTRACT

Signals helping parasitoids to find hosts often originate from the host and/or its habitats, providing cues for locating hosts that are often cryptic or highly dispersed. *Melittobia* are gregarious ectoparasitoids, which primarily attack *Trypoxylon politum* prepupae. How *Melittobia* locates its host is poorly known, but may involve host-related chemicals. This study investigated the roles of chemical cues and natal rearing effect in host location and recognition by *M. digitata*. In a small arena, which contained *T. politum, Megachile rotundata, Neobelleiria bullata*, empty cocoons, or nest mud, all isolated from the parasitoid, *M. digitata* spent significantly more time on host than on control patches. In olfactometer trials, *M. digitata* spent significantly more time in fields that contained hosts than on blank controls. Host cocoons elicited a positive response, but cues from nest mud and natal host fidelity were not supported. Results suggest that host-related chemicals act as arrestments for *M. digitata* females.

INDEX WORDS: parasitism, chemoreception, host location, host recognition, olfaction, olfactometer, leafcutter bee, blow fly, mud dauber.
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CHRISTIAN SHERLEY ARAÚJO DA SILVA TORRES

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by

CHRISTIAN SHERLEY ARAÚJO DA SILVA TORRES

Major Professor: Robert W. Matthews
Committee: John R. Ruberson
Karl E. Espelie

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2004
DEDICATION

I dedicate this Master Thesis to three very special people. First, to my mother, Lenilda Araújo, who gave me the support I needed in my entire life. Second, to my husband, Jorge Torres, who is unbelievably good to me. Finally, to my grandmother, Guiomar da Silva, the love of my life.
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CHAPTER 1
INTRODUCTION

A key determinant of the success of any population lies in the ability of its members to locate food sources for themselves and their offspring. The individual’s survival and reproductive success depend, in turn, on its behavior, and natural selection should favor animals with efficient foraging strategies (van Alphen and Vet, 1986; Potting, 1996). Therefore, insect parasitoids would be expected to have developed their ability to find hosts, to recognize them, and perhaps to discern those potential hosts that could best support the development of their progeny. But, how does a given parasitoid wasp actually locate a potential host? Having found one, how does the wasp recognize it? Because the answers to such questions have both theoretical and practical importance, this thesis investigated selected aspects of them, using small, cosmopolitan, gregarious external parasitoid wasps of the genus Melittobia Westwood (Hymenoptera: Eulophidae). In their natural host range Melittobia primarily parasitize solitary wasps such as the mud dauber wasps, bees, and their inquilines (Freeman and Ittyeipe, 1982; Matthews et al., 1985).

This thesis presents results of an investigation on how M. digitata females locate and recognize their hosts. I focused my research on the following questions:

a) Is the initial location of hosts by M. digitata random or cue-driven?

b) Are M. digitata females able to discern and/or recognize the presence of a potential host through chemical cues/olfactory stimuli from its nest, cocoon, or body?
c) Does a female’s natal host species influence her later attraction to a potential host upon which to rear her offspring?

The chapter two is a literature review concerning how insect parasitoids perceive their world, and the sequence of behaviors likely involved in enhancing their chances of locating a suitable host. In the succeeding chapters each of the above questions is addressed experimentally.

Finally, the overall results are summarized and conclusions are made about how *M. digitata* females locate and recognize their hosts.

**REFERENCES**


Parasitoid and host interactions are behaviorally and ecologically complex. Signals emitting from the host itself and/or host habitats such as shapes, colors, host food plants, host nest volatiles, and host feces usually help parasitoids to locate hosts in the field (Weseloh and Bartlett, 1971; Wilson et al., 1974; van Alphen and Vet, 1986). In this context, chemoreception is considered the dominant perceptive modality utilized by parasitoids, even though temperature, humidity, visual, auditory and tactile cues are also a very important part of the total sensory input to insects’ central nervous systems (Wäckers, 1994; Potting, 1996).

From the viewpoint of a parasitoid’s benefits, these chemical cues are considered kairomones, chemical substances produced or acquired by an organism that, when contacted by an individual of another species, evoke a reaction that is adaptively favorable to the receiver (Brown et al., 1970). Actually, the use of kairomones is a common phenomenon (Weseloh, 1981). For example, parasitoids of the genus *Trichogramma* are able to respond to chemical extracts of host moth scales and some braconid species are able to respond to extracts of host larval frass (Jones et al., 1973; Beevers et al., 1981; Noldus and van Lenteren, 1983, 1985).

Laing (1937), Flanders (1937) and Vinson (1975) have proposed the steps followed by a parasitoid in its host attack activity as follows: (1) attraction to the host habitat; (2) attraction to host individuals in the habitat; (3) host acceptance; (4) host suitability, and (5) host regulation. Supposedly, these steps are sequentially followed by the parasitoid to successfully attack its host.
Picard and Rabaud (1914) observed that many parasitic Hymenoptera attack species in several insect orders once the hosts feed on one species of food plant. Thus, natural enemies are often attracted to a habitat instead of directly to a host in the habitat. Besides, food of a host may positively or negatively affect its parasitoids (Lawson, 1959). Vet and Schoonman (1988) found that *Leptopilina heterotoma* (Thomson), a parasitoid of *Drosophila* spp. larvae, finds its host through volatiles from the host substrates (e.g. vegetable matter or flows of tree sap). After the parasitoid arrives at appropriate places, it initiates searching and, if successful in locating the host, it “learns” that the particular substrate in which it has been foraging yields hosts. Then, when it leaves that particular area, the “memory” of the volatile from the patch is retained and later it will prefer to search habitats with the same odor. After the host habitat is located, hosts are located by random and direct search (Ullyett, 1943; Doutt, 1959).

After the parasitoid has located the host, decisions must be made by the female parasitoid as to whether the host is acceptable for oviposition (Gordh et al., 1999). Numerous cues suggest whether the host may be suitable for attack, including host odor, size, location, shape and motion, and physiology (Legner and Thompson, 1977). For example, both physical (shape) and chemical stimuli associated with larvae of *Heliothis virescens* (Fabricius) influenced acceptability of the larvae to the parasitoid *Campoletis sonorensis* (Cameron) (Wilson et al., 1974). Therefore, under natural conditions, a parasitoid would attack only a few of the species on which development is possible (Gordh et al., 1999).

Oviposition by a parasitoid is not necessarily an index of host suitability. The attractiveness of the host to the adult parasitoid is often independent of its suitability for larval parasitoid development (Gordh et al., 1999). Properties of the host determine whether the parasitoid will be able to develop fully in or on the host, for example: host age and life stage, its food, its status
(health or previously parasitized), and immune response. Some host-plant species may provide a superior nutritional source for insect hosts that result in higher survivorship by parasitoids; for *Microplitis croceipes* (Cresson) larvae, the diet of their host is an important factor in their suitability (Mueller, 1983). In his study, Mueller also found that *Heliothis* spp. larvae that were reared on cotton yielded a higher survivorship of adult parasitoid wasps than hosts reared on tomato or bean.

According to Vinson and Iwantsch (1980), “parasitoidism” is a unique form of symbiosis and that is distinguished from common parasitism by the evolution of host regulation as a major feature of this relationship. The evolution of the parasitoid-host relationship in “parasitoidism” is towards control of the host for the benefit of the parasitoid’s progeny. “Parasitoidism” not only induces physiological and physical changes in the host, but the “parasitoided” host is ecologically distinct from an “unparasitoided” host (Führer, 1968). The effects of the parasitoid on the development, behavior, physiology and morphology of the host have been attributed to the feeding larva within the host, although the role of the female parasitoid in initiating some of the changes is becoming increasingly clear (Jones and Lewis, 1971; Vinson, 1972; Guillot and Vinson, 1972; Vinson and Iwantsch, 1980). These changes in the host, whether caused by chemicals injected by the ovipositing female or her progeny are considered as host regulation (Vinson and Iwantsch, 1980). As an example of developmental effect, *Trialeurodes vaporariorum* (Westwood) parasitized by *Encarsia formosa* Gahan never developed to the adult stage (Thompson and Adams, 1976).

The ability of parasitoids to locate and attack hosts is a key determinant of how well a given parasitoid population performs. The individual’s survival and reproductive success depend on its behavior; natural selection will favor animals with efficient foraging strategies (van Alphen and
Vet, 1986; Potting, 1996). Therefore, insect parasitoids are expected to forage for hosts that can directly support the development of their progeny (Potting, 1996).

Members of the genus *Melittobia* (*Mel.*) Westwood (Hymenoptera: Eulophidae) are small, cosmopolitan, gregarious ectoparasitoids that primarily attack solitary wasps, bees, and their inquilines (Freeman and Ittyeipe, 1982; Matthews et al., 1985). Most include mud dauber wasps (Hymenoptera: Sphecidae) in their natural host range.

In all stages of their development, species of *Melittobia* exhibit remarkable plasticity of behavior and adaptability to prevailing conditions. Theoretically, even unmated females can survive and eventually produce progeny of both sexes even in the absence of preferred hosts (Dahms, 1984). In the laboratory *Melittobia* accept a variety of hosts, including species of Diptera and Coleoptera (Thompson and Parker, 1927). In nature, this variety of host range probably serves *Melittobia* well, because it could reproduce both on its natural host and upon the many other predators and parasites that usually infest a mud dauber’s nest (Matthews et al., 1996).

All species of *Melittobia* are characterized by sexual dimorphism. The males are blind, have short non-functional wings (brachypterous), curiously shaped antennae, a lightly pigmented bodies, and mandibles with large teeth. Females, which are darker than males, have fully developed eyes, and are divided in two types: macropterous females, which have long wings representing the dispersing group of the population; and the brachypterous females, which have short wings and low ability to disperse but almost instantaneous progeny production (Freeman and Ittyeipe, 1976; Sudheendrakumar and Narendran, 1984; González, 1985).

Experience and age may influence host finding and oviposition behavior by parasitic wasps. Compared to a female without prior experience, a female that has already oviposited may spend
less time finding, handling, and parasitizing hosts (Ikawa and Suzuki, 1982; Kerguelen and Cardé, 1996). In contrast, in *Melittobia*, a mated female, having found and entered the mud nest of a developing host, will lay many eggs on the host surface and stay with this one host for the rest of her life. If there is a limited food supply, she may seek another host to attain her full egg laying potential, moving to a neighboring host cell by chewing through the cell wall (Dahms, 1984). However, how these species locate, assess, and recognize their hosts is unknown and little studied (Trexler, 1985; Ranger, 1996). Additionally, many factors could be involved, such as host shape, size, color, age, texture, movement, sound, electromagnetic radiation, and chemicals (Vinson, 1976, 1985; Cooperband and Vinson, 2000).

Due to its bizarre behavioral traits the genus *Melittobia* recently has become a model organism for life science classroom studies (Matthews et al., 1996), and even though several papers have been written on the methods of host finding in parasitoids, for *Mel. digitata* Dahms we still need many answers.

*Purpose of the Study*. In order to investigate the mechanism by which *Mel. digitata* females locate hosts, this study focused on various questions such as: is the initial location of hosts by *Mel. digitata* random or cue-driven?; are *Mel. digitata* females able to discern and/or recognize the presence of a potential host through chemical cues/olfactory stimuli from its nest, cocoon, or body?; and, does a female’s natal host species influence her later attraction to a potential host upon which to rear her offspring?

The literature on female dispersal in *Melittobia* is equivocal. Some authors have found that female *Melittobia* have limited dispersal capability (Balfour Browne, 1922; Buckell, 1928; Whiting, 1947; Malyshev, 1968). Others have demonstrated that female *Melittobia* are highly able to disperse (Freeman and Parnell, 1973; Taffe and Ittyeipe, 1976; González, 1994).
According to González and Terán (2001), the dispersal of macropterous females of *Mel. acasta* (Walker) is absolutely random. It may be the same for *Mel. digitata*, but females also could be attracted to host habitats by volatiles emanating from their hosts or hosts’ nest.

Because *Mel. digitata* accepts a variety of hosts in the laboratory, this study investigated whether female parasitoids used olfactory cues to initially locate and possibly prefer its natural host, mud dauber, *Trypoxylon politum* Say (Hymenoptera: Sphecidae) prepupa, compared to two suitable alternative hosts: blow fly/flesh fly, *Neobellieria (=Sarcophaga) bullata* (Parker) (Diptera: Sarcophagidae) puparium, and the alfalfa leafcutter bee, *Megachile (Meg.) rotundata* (Fabricius) (Hymenoptera: Megachilidae) prepupa. More specifically, this study investigated the roles of host chemical cues and natal rearing effect in habitat recognition by *Mel. digitata*.

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CHAPTER 3

ROLE OF Olfactory CUES IN HOST FINDING BY MELITTOBIA DIGITATA DAHMS (HYMENOPTERA: EULOPHIDAE), A PARASITOID OF SOLITARY WASPS AND BEES

Abstract - Chemical signals used by parasitoids to find hosts often originate from the host and/or its habitat, providing critical cues for locating hosts that are often cryptic or highly dispersed. *Melittobia* are gregarious ectoparasitoids, which primarily attack mud dauber (*Trypoxylon politum*) prepupae. How *Melittobia* locates its host is unknown, but may involve host-related chemical signals. Therefore, this study focused on whether host location by *Mel. digitata* is mediated by olfactory stimuli. In a small arena, which contained a choice of potential hosts (*T. politum* prepupa, *Megachile rotundata* prepupa, or *Neobelleiria bullata* puparium), empty host pupal cases, or nest mud - all of which were visually and physically isolated from the parasitoid -, *Mel. digitata* successfully located host patches, and spent significantly more time on those than on control patches. Results provide evidence for arrestment of *Mel. digitata* in response to host-related chemicals.

Key Words - parasitism, chemoreception, host location, olfaction, flesh fly, leaf cutter bee.
INTRODUCTION

Parasitoids of the genus *Melittobia* (*Mel*) Westwood (Hymenoptera: Eulophidae) are small, cosmopolitan, gregarious external parasitoids. In their natural host range they primarily parasitize solitary wasps and bees and their inquilines (Freeman and Ittyeipe 1982, Matthews et al. 1985). *Melittobia* show remarkable plasticity of behavior, and theoretically, even unmated females can survive and eventually produce progeny of both sexes even in the absence of preferred hosts (Dahms 1984).

All species of *Melittobia* show sexual dimorphism. The males are eyeless, have short non-functional wings, curious-shaped antennae, honey/amber body, and large teeth on mandibles. Females are darker than males, have fully developed compound eyes, and exist as two forms. Macropterous females have long wings and comprise the dispersive group of the population. Brachypterous females possess short non-functional wings thereby having limited ability to disperse (Freeman and Ittyeipe 1976, Sudheendrakumar and Narendran 1984, González 1985, Cônsoli and Vinson 2002).

In the laboratory *Melittobia* accept a variety of hosts, including species of Diptera and Coleoptera (Thompson and Parker 1927). In nature, this host range probably serves *Melittobia* well, enabling it to reproduce on many of the other parasites that often infest a solitary wasp or bee nest (Matthews et al. 1996).

After finding and entering the nest of a developing host, a mated *Melittobia* female will lay hundreds of eggs on the host surface and stay with this one host all her life. In case of short food supply, she may seek another host to complete her egg oviposition process, usually moving to a neighboring host cell by chewing through the cell wall (Dahms 1984). How these species locate, recognize, and assess their host is unknown and little studied (Trexler 1985, Ranger 1996).
In laboratory colonies *Mel. digitata* accept a variety of hosts. Therefore, we investigated whether female parasitoids use host-produced olfactory cues to locate a host, and whether cues derived from its natural host, mud dauber, *Trypoxylon politum* Say (Hymenoptera: Sphecidae) prepupae, are more strongly attractive than cues from alternative hosts, puparia of the blowfly/flesh fly, *Neobellieria* (=*Sarcophaga*) *bullata* (Parker) (Diptera: Sarcophagidae) and prepupae of the alfalfa leafcutter bee, *Megachile* (*Meg.*) *rotundata* (Fabricius) (Hymenoptera: Megachilidae). Consequently, our hypotheses were if *Mel. digitata* females find their hosts by means of olfactory cues, then inside a choice arena it is more likely that they will move toward a host-odor source (nude hosts, host enclosed in cocoons, host cocoons, nest mud, and host/cocoon extracts) than to a dummy or randomly; and since mud dauber prepupae are the natural host of *Mel. digitata*, then female parasitoids will prefer their natural host compared to laboratory alternative hosts (blow fly puparia and alfalfa leafcutter bee prepupae).

**MATERIALS AND METHODS**

This study was conducted at Coastal Plain Experimental Station in Tifton, GA, Laboratory of Biological Control. Tests were carried out at normal laboratory temperature (25.2 ± 0.46°C) and humidity (41 ± 1.41%).

This study utilized *Mel. digitata* females obtained from cultures maintained in the Laboratory of Insect Behavior of the University of Georgia, Athens, GA. They were cultured on three hosts: *T. politum* prepupa, *N. bullata* puparium, and *Meg. rotundata* prepupa. *Trypoxylon* prepupae were collected from nests around Athens and Tifton, GA. Meanwhile, *N. bullata* puparia were obtained from Carolina Biological Supply Company, and *Meg. rotundata* cocoons from Pioneer Hi-Bred International, Inc., respectively.
We employed a 3-way choice arena to address our questions (Fig. 3.1). This arena apparatus consisted of three parts — a Plexiglas® base (20x20x1.2cm), a glass top (bottom of a Petri dish of 15cm diameter, Pyrex®), and a viewing mirror. A groove of the same circumference was routed into the base to receive the glass top. The Plexiglas base contained three rectangular depressions or wells (1x2x1cm), arrayed in an equilateral triangle with sides 8 cm long.

By random assignment, one well of the choice arena received one of several experimental materials — either a live host (mud dauber or leafcutter bee prepupa or fly puparium), cocoon material from one of these hosts, cocoon extract, prepupa extract, or mud fragments from a mud dauber nest. A second well was given a dummy (piece of glass rod of same approximate size as a host). The third well of the arena remained empty. The blank was used as a control for the dummy because past studies involving *Mel. digitata* host acceptance have shown that pupa-shaped glass objects were significantly more attractive to the parasitoid females than rectangular piece of glass and extract-treated glass (Cooperband and Vinson 2000). A circular filter paper 20 cm of diameter (Whatman®), placed over the arena base covered the wells to remove visual and physical cues. It was held taut by pressing it into the routed groove in the apparatus with the rim of the Petri dish. This also effectively sealed the interior of the arena preventing wasps from escaping or physically contacting a host. A single mated, inexperienced (< 5 days old) macropterous *Mel. digitata* female was first released onto the center of the filter paper with the help of a paint brush, and then the lid was pressed tightly into the base groove closing the arena. Finally, because female *Melittobia* show negative geotactic behavior (Guinan and Matthews 2000), the arena was inverted and suspended above a mirror from which each tested female parasitoid’s movements could be tracked. All trials were conducted in the dark and a flashlight
covered by red cellophane was used to observe the parasitoid and to minimize possible light influences on the parasitoid’ behavior.

For the first minute after introduction the female was left to habituate to the arena surroundings. Beginning at the second minute, the female’s first choice and the time (in seconds) spent in each treatment patch of the arena was recorded over the following 20 minutes (see Fig. 3.1). Twenty trials, each using a new female of standard age from the same source culture, were run. The positions of the host, dummy and blank were rotated after each trial, and the filter paper was changed after every third trial.

To obtain host extracts, a single host cocoon or prepupa was washed with 5 ml of hexane (Sigma-Aldrich®), for 2 minutes at room temperature. Extracts were stored in 7 ml scintillation vials (Solvent Saver®, Kimble Kontes) and put in the freezer until further experimental procedure, when 80µl of the extract was pipetted onto a 4 cm-diameter disk of filter paper (Fig. 3.1) and allowed to evaporate for 10 minutes before running a trial. A pure hexane-treated filter paper patch served as control for the solvent.

The time spent by parasitoid females around the respective treatment patches in the arena was tested for normality, using Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von, and Anderson tests (SAS Institute 2000). When parameters showed significant deviation from a normal distribution, they were transformed into square root (x + 0.5) to meet ANOVA assumptions. Then, data were analyzed using ANOVA, and Waller-Duncan’s test of mean comparisons (α = 0.05). Data for the first choice made by females was analyzed using a chi-square test (Proc FREQ of SAS).
RESULTS

Individual females of *Mel. digitata* were able to successfully locate host patches hidden under the filter paper, and spent significantly more time on these patches than on non-host, control patches in the three-way choice arena. Initially, *Melittobia* females spent considerable time walking inside the apparatus, but once they crossed the boundaries of the wells that contained the hosts, they spent significantly more time investigating such areas (Tables 3.1 and 3.2). Overall, female parasitoids spent more time in areas containing either a *Meg. rotundata* prepupa inside its cocoon or a *T. politum* cocoon than in any other treatment offered (Fig. 3.2). Empty cocoons from *T. politum* and *Meg. rotundata* were significantly more attractive to *Mel. digitata* females than the controls indicating that chemicals present in these structures may play an important role in *Mel. digitata* initial close-range attraction (Tables 3.1 and 3.2).

Females spent significantly more time on arena patches that contained *T. politum* and *Meg. rotundata* cocoon extracts, respectively, than on control patches (Tables 3.1 and 3.2); however, time spent on *T. politum* cocoon extract was significantly lower than the time spent on the real cocoon patch (Fig. 3.2).

Similarly, *Mel. digitata* spent significantly more time on patches that contained mud from the *T. politum* nest than on control patches (Table 3.1); however, when we compared the time spent by the females on nest mud patches and time they spent on patches containing either nude *T. politum* prepupa or *T. politum* prepupa extract (Table 3.1), there was no significant difference in female’s response towards nest mud (Fig. 3.2).

Results of *Mel. digitata* female first choice in the arena showed that even though they can respond to host-related chemicals, their initial distribution movement is random (Fig. 3.3). Overall, the number of times they chose a host as first choice was not significantly different from
the number of times they chose the controls, except for the *Meg. rotundata* cocoon, which was more frequently chosen at first by the females (Fig. 3.3).

**DISCUSSION**

The results provide strong support for chemical cues mediating close range host seeking behavior by *Mel. digitata* because parasitoid females successfully recognized and located host patches (*T. politum*, *Meg. rotundata*, and *N. bullata*) hidden under the filter paper, and spent significantly more time on these patches than on non-host (blank and dummy), control patches. These results and those reported by Ranger (1996) agree with the hypothesis that *Mel. digitata* females use olfactory stimuli to locate their hosts. Trexler (1985) also found evidence that females likely use some sort of odor cues to locate hosts at close range - he observed non-random selection of host-containing nests by *Melittobia* females.

Although leafcutter bees, *Meg. rotundata*, have yet to be reported as a natural host for *Mel. digitata* in the field, other species of *Melittobia* such as *Mel. australica* Girault, *Mel. acasta* (Walker) and *Mel. hawaiiensis* Perkins have been found parasitizing this bee species (Peck 1969, MacFarlane and Donovan 1989, Woodward 1994). Additionally, *Meg. rotundata* is successfully used as an alternative host for *Mel. digitata* in the laboratory (González and Matthews 2002). The fact that *Mel. digitata* spent significantly more time on *Meg. rotundata* patches than any other host or nest material offered in the arena trials (Fig. 3.2) suggests that this parasitoid could become an additional problem for alfalfa growers if it becomes a field parasitoid of *Meg. rotundata*, currently the most widely used commercially managed pollinator, after the honey bee, *Apis mellifera* L. (Kemp and Bosch 2000). Donovan et al. (1982) found that *Mel. hawaiiensis* parasitized *Meg. rotundata* prepupae in New Zealand within eight weeks of release of leafcutting bees in the field, and that the parasitism rate increased from 0.02% to 11.3% in just three years.
Similarly, Woodward (1994) reported that *Mel. australica* parasitized 19% of *Meg. rotundata* population in South Australia in 1988-1989.

Positive responses of *Mel. digitata* towards blow fly puparia in the arena could be attributed to the fact that blow flies have been extensively used in laboratory cultures of *Melittobia* as an alternative host yielding reasonable numbers of progeny per female (Matthews et al. 1996, Silva-Torres and Matthews 2003). Chemically blow fly puparia are likely to share similarities with certain dipteran inquilines, such as satellite flies and bee flies, which often occur in mud dauber nests and can also be successfully parasitized by *Melittobia* (Dahms 1984, Matthews et al. 1996).

Female *Mel. digitata* also responded positively to nest mud compared to the controls; however, this response was relatively low and similar to the response of females towards naked prepupae or prepupal extract, which were not significantly more attractive to the parasitoid females than the nest mud. Thus, these results suggest that chemicals from both mud and nude *T. politum* prepupae have a lesser role in *Mel. digitata* host finding and recognition. However, when offered as found in the natural nest, (i.e. nest mud + cocoon + prepupa), the combined chemical bouquet appears to enhance host location and recognition.

In contrast, host cocoons and their extracts were significantly more attractive to *Mel. digitata* (Tables 3.1 and 3.2) than the controls; however, response by *Mel. digitata* towards cocoon extracts was significantly lower than that elicited by the cocoon itself in case of *T. politum* (Fig. 3.2). This could have been due to the method used to obtain the extracts, if chemical contents where not completely extracted, or to store the extracts in glass vials in the freezer before use. Ranger (1996) reported similar results when experienced *Mel. digitata* females were offered *T. politum* cocoons. According to Ranger, cocoon surface hydrocarbons play a major role in arresting searching behavior in *Melittobia* females. Because viable mud dauber prepupae in their
cocoons are usually collected and stored in the refrigerator for extended periods before use, volatiles may be lost. In this study, mud dauber nests were harvested and tested or extracted during the same nesting season. Presumably any cocoon surface chemicals used in host location by *Mel. digitata* would still have been present and readily detectable. Gas chromatography mass spectrometer analysis (Ranger 1996) revealed that *T. politum* cocoon hydrocarbons are simple, common n-alkanes having 23 to 29 carbon atoms. Similar hydrocarbons can be found in cocoons of other mud daubers, e.g., *Sceliphron* spp. (Ranger 1996), which are also common hosts for *Melittobia*.

Research involving other parasitoid species has also indicated that cocoons may offer potential cues to a searching parasitoid female. For example, Weseloh (1988) reported that empty host cocoons of *Cotesia melanoscela* (Ratzeburg) were attractive to its hyperparasitoid, *Eurytoma appendigaster* (Swederus).

Interestingly, Trexler (1985) showed that the rate of *T. politum* host location by *Melittobia* in a plexiglass arena decreased with increasing host densities. One might have predicted that higher densities of hosts would release more host-related volatiles that in turn would serve to attract more parasitoids. However, Price (1975) proposed that, because parasitoid females can recognize odors of conspecifics, under restricted conditions (e.g., in a parasitized cocoon or an experimental arena), groups of female parasitoids would be more engaged in avoidance of each other and escape than in finding a host. This would result in a reduced number of parasitized hosts as parasitoid density increased.

Our results and others (Freeman and Parnell 1973, Taffe and Ittyeipe 1976, González and Terán 2001) have shown that *Melittobia* females initially disperse more or less randomly (Fig. 3.3). Perhaps, following emergence and mating, long-winged females search for suitable hosts
only in nearby areas such as neighboring cells and nests. If females perceive chemicals emanating from hosts and their cocoons, they respond to those odors and readily find the host. Failing to be arrested by host odors, females may change to a long-range dispersal behavior. Prior studies have suggested that, due to their small size, there could be passive dispersal induced by wind (Freeman and Parnell 1973, Taffe and Ittyeipe 1976, González and Terán 2001). However, further studies are needed to understand dispersal.

In summary, olfactory signals emanating from the hosts cocoons appear to be important as short-range cues used by *Mel. digitata* females to locate potential hosts. The identity and chemical characterization of these cues remain to be investigated. Because of the taxonomic diversity of their hosts it seems likely that the host recognition odors will be found to be mixtures of simple hydrocarbons commonly found in holometabolous species.

ACKNOWLEDGMENTS

I thank the Insect Behavior Laboratory discussion group (Janice Matthews, Jorge González, and Leif Deyrup) for valuable suggestions and encouragement; the summer students, Melanie McClellan and Holly Tawzer (Biological Control Laboratory, CPES, Tifton, GA) for helping with mud dauber nest hunting trips; Dr. Moukaram Tertuliano (Insect Biology and Population Management Research Laboratory, USDA, Tifton, GA) for helping with the chemical extractions.

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Table 3.1. Mean time (seconds) spent by single mated inexperienced *Melittobia digitata* females during a 20 min trial in the different arena choices containing various combinations of *Trypoxylon politum* hosts. Values followed by same letter in a column are not significantly different by Waller-Duncan's K-ratio t test at $\alpha = 0.05\%$. For all treatments followed by an asterisk $P < 0.0001$.

<table>
<thead>
<tr>
<th>Host treatment</th>
<th>Number of females responding out of 20 tested</th>
<th>Mean Time ± SE</th>
<th>Statistics ($F_{df}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mud + cocoon + prepupa</td>
<td>15</td>
<td>262.8 ± 35.99 a</td>
<td>$F_{2.44} = 33.43 \ast$</td>
</tr>
<tr>
<td>Dummy</td>
<td>17</td>
<td>52.4 ± 13.00 b</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>15</td>
<td>40.5 ± 7.24 b</td>
<td></td>
</tr>
<tr>
<td>Cocoon</td>
<td>19</td>
<td>377.6 ± 28.17 a</td>
<td>$F_{2.50} = 115.85 \ast$</td>
</tr>
<tr>
<td>Dummy</td>
<td>16</td>
<td>50.4 ± 10.69 b</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>18</td>
<td>43.5 ± 10.30 b</td>
<td></td>
</tr>
<tr>
<td>Nude prepupa</td>
<td>17</td>
<td>144.0 ± 33.33 a</td>
<td>$F_{2.44} = 2.73, P = 0.0762$</td>
</tr>
<tr>
<td>Dummy</td>
<td>17</td>
<td>53.8 ± 7.42 a</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>13</td>
<td>67.2 ± 21.36 a</td>
<td></td>
</tr>
<tr>
<td>Cocoon extract</td>
<td>18</td>
<td>138.9 ± 19.36 a</td>
<td>$F_{2.51} = 14.68 \ast$</td>
</tr>
<tr>
<td>Dummy</td>
<td>17</td>
<td>41.5 ± 4.92 b</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>18</td>
<td>56.0 ± 12.25 b</td>
<td></td>
</tr>
<tr>
<td>Nude prepupa extract</td>
<td>14</td>
<td>73.0 ± 17.80 a</td>
<td>$F_{2.42} = 1.13, P = 0.3322$</td>
</tr>
<tr>
<td>Dummy</td>
<td>14</td>
<td>76.7 ± 18.17 a</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>17</td>
<td>47.2 ± 7.61 a</td>
<td></td>
</tr>
<tr>
<td>Mud</td>
<td>18</td>
<td>145.6 ± 32.9 a</td>
<td>$F_{2.51} = 8.14, P = 0.0009$</td>
</tr>
<tr>
<td>Dummy</td>
<td>17</td>
<td>48.5 ± 6.72 b</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>17</td>
<td>36.9 ± 4.78 b</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.2. Mean time (seconds) spent by single mated inexperienced *Melittobia digitata* female during a 20 min trial in the different arena patches containing *Megachile rotundata*, *Neobellieria bullata*, or hexane extracts. Values followed by same letter in a column are not significantly different by Waller-Duncan’s K-ratio t test at $\alpha = 0.05\%$. For all treatments followed by asterisk $P < 0.0001$.

<table>
<thead>
<tr>
<th>Host treatment</th>
<th>Number of females responding out of 20 tested</th>
<th>Mean Time ± SE</th>
<th>Statistics ($F_{df}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meg. rotundata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocoon + prepupa</td>
<td>18</td>
<td>465.6 ± 70.69 a</td>
<td>$F_{2,33} = 22.72 \ast$</td>
</tr>
<tr>
<td>Dummy</td>
<td>9</td>
<td>65.2 ± 29.14 b</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>9</td>
<td>72.0 ± 15.55 b</td>
<td></td>
</tr>
<tr>
<td><strong>Meg. rotundata cocoon</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dummy</td>
<td>15</td>
<td>54.0 ± 7.40 b</td>
<td>$F_{2,47} = 26.38 \ast$</td>
</tr>
<tr>
<td>Blank</td>
<td>15</td>
<td>60.0 ± 10.34 b</td>
<td></td>
</tr>
<tr>
<td><strong>Meg. rotundata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocoon extract</td>
<td>18</td>
<td>238.9 ± 50.09 a</td>
<td>$F_{2,39} = 11.3 \ast$</td>
</tr>
<tr>
<td>Dummy</td>
<td>11</td>
<td>78.5 ± 14.14 b</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>13</td>
<td>50.9 ± 13.32 b</td>
<td></td>
</tr>
<tr>
<td><strong>N. bullata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocoon + pupa</td>
<td>15</td>
<td>234.5 ± 41.85 a</td>
<td>$F_{2,40} = 14.99 \ast$</td>
</tr>
<tr>
<td>Dummy</td>
<td>15</td>
<td>68.9 ± 22.03 b</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>13</td>
<td>47.8 ± 6.85 b</td>
<td></td>
</tr>
<tr>
<td><strong>Hexane</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dummy</td>
<td>17</td>
<td>52.2 ± 7.84 a</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>18</td>
<td>63.2 ± 10.87 a</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3.1. Choice arena used in this study. One well was filled with a host treatment; the second with a piece of glass rod (dummy), and the third was empty (the blank control). The wells and the entire arena were covered with a single piece of filter paper and one parasitoid female was introduced in the center of the arena for each trial. A female was considered to have made a choice upon entering the respective small patch (dotted line) surrounding each well. When extracts were used as odor source they were applied on the filter paper within the same patch around the well.
Fig. 3.2. Comparison among mean time spent by single mated inexperienced *Melittobia digitata* female in various areas of the arena containing a possible host source, cocoons, mud, or host/cocoon extracts. Bars followed by same letter are not significantly different by ANOVA and Waller-Duncan’s K-ratio t test. Bars followed by an asterisk differ significantly from their respective controls (dummy and blank).

Note: mud+cocoon + *Trypoxylon* prepupae (TMCP), *Trypoxylon* mud (TMUD), *Trypoxylon* empty cocoon (TC), naked *Trypoxylon* prepupa (TP), naked *Trypoxylon* prepupae extract (TNPEx), *Trypoxylon* cocoon extract (TCEx), *Megachile* prepupa+cocoon (MEG), *Megachile* empty cocoon (MEGC), *Megachile* cocoon extract (MEGCex), *Neobellieria bullata* puparia (BF).
**Fig. 3.3.** First choice made by a single mated inexperienced *Melittobia digitata* female in the arena containing a possible host and controls (average between dummy and blank) during a 20 min trial (n = 20). Bar followed by an asterisk is significantly different from the control ($\chi^2 = 0.2857, P = 0.0455$).

Note: mud+cocoon+*Trypoxylon* prepupae [Typ (M+C+P)], naked *Trypoxylon* prepupa [Typ (P)], *Trypoxylon* empty cocoon [Typ (C)], *Trypoxylon* cocoon extract [Typ (Cex)], *Trypoxylon* naked prepupae extract [Typ (Pex)], mud [Typ (M)], *Neoellieria bullata* puparia [Bf], *Megachile* cocoon extract [Meg (Cex)], *Megachile* prepupa+cocoon [Meg (P+C)], *Megachile* empty cocoon [Meg (C)].
CHAPTER 4

ROLE OF CHEMICAL CUES AND NATAL REARING EFFECT IN HOST RECOGNITION

BY THE PARASITIC WASP, MELITTOBIA DIGITATA. ¹

Abstract - Parasitoids are expected to have the ability to find, recognize, and perhaps to discern potential hosts that could best support the development of their progeny. *Melittobia* are gregarious ectoparasitoids, which primarily attack mud daubers. How *Melittobia* locates its host is poorly known, but may involve host-related chemical signals. Therefore, this study investigated the roles of host chemical cues and natal rearing effect in host recognition by *Mel. digitata*. In an olfactometer, which contained prepupae of *Trypoxylon politum*, *Megachile rotundata*, pupae of *Neobelleiria bullata*, empty or intact host cocoons, or nest mud, *Mel. digitata* spent significantly more time in arms that contained a host than in control arms. Additionally, host cocoons elicited a strong positive response; but nest mud and natal host had no attraction. Finally, *Mel. digitata* were most attracted to *Meg. rotundata* and *T. politum*.

Key Words - Olfactometer, host recognition, host location, chemoreception, olfaction.
INTRODUCTION

The success of any population lies in the ability of its members to locate food sources for themselves and their offspring. Thus, an individual’s behavior will affect its survival and reproductive success. Because natural selection favors animals with efficient foraging strategies (van Alphen and Vet 1986; Potting 1996), insect parasitoids are expected to have the ability to find, recognize, and perhaps to discern potential hosts that could best support the development of their progeny. However, how does a parasitoid wasp locate a potential host in the field? Having found one, how does the wasp recognize it? Because the answers to such questions have both theoretical and practical importance, this study investigated how parasitoid wasps of the genus Melittobia (Mel.) Westwood (Hymenoptera: Eulophidae) locate their hosts.

All 14 species of the genus Melittobia are gregarious ectoparasitoids, which primarily attack solitary wasps and bees; most North American Melittobia species include mud dauber wasps, Trypoxylon politum Say and Sceliphron caementarium Drury (Hymenoptera: Sphecidae) among their natural hosts (Freeman and Ittyeipe 1982; Matthews et al. 1985). In the laboratory, Mel. digitata Dahms will accept a variety of hosts, including species of Diptera and Coleoptera (Thompson and Parker 1927). In nature, this host breadth of tastes probably serves Melittobia well, enabling it to reproduce both on its natural host and upon parasites and inquilines that infest a mud dauber’s nest (Matthews et al. 1996).

Melittobia digitata typically have a very short (ca. 4-week) life cycle, producing hundreds of offspring that usually completely consume their host. Young mated females disperse from the host cocoon. Whether this female dispersal (and associated host-finding) is cue-driven or random is a matter of some discussion (González and Terán 2001) and no consensus has been reached regarding how Melittobia finds a new host nest.
Once *Mel. digitata* have found the host nest, decisions must be made by the female parasitoid as to whether the contained hosts are acceptable for oviposition (Gordh et al. 1999). Numerous cues are used by different parasitoids to “evaluate” whether a host is suitable for attack, including host odor, size, location, shape and motion, and physiology (Legner and Thompson 1977). For many parasitoid species, host-produced chemicals have been postulated as very important in the process of host recognition and acceptance, and in some species these odors may immediately induce probing or oviposition behavior (Corbet 1971; Vinson 1976). The ways by which *Mel. digitata* recognize and evaluate potential hosts are poorly known and little studied (Trexler 1985; Ranger 1996).

Hopkins (1917) postulated the existence of a causal link between larval feeding behavior and adult choice of oviposition sites. The main point of Hopkins’ host-selection principle is to suggest an effect on adult behavior by the chemical environment experienced as a developing larva. Neural changes in the larva that persist into adulthood are also called “memory” (Jermy et al. 1968; Jaenike 1983). Most researchers trying to identify the nature of the link have failed to distinguish among Hopkins’ “memory” principle and two other possible explanations: genotype selection, in which larva and adult behavior are both suitable for the new host; or changes in adult behavior mediated by chemical contamination carried over from the larva to the adult environment (Jaenike 1983). Similarly, Corbet (1985) proposed the chemical legacy hypothesis to account for the link between a larval chemical environment and the chemosensory responsiveness of the adult of the same individual parasitoid. Corbet’s hypothesis proposed that not only insect “memory”, but mainly a legacy of chemical cues, traces of certain host-related chemicals inside or outside the insect’ body, persisting from one stage to another, would affect the chemosensory responsiveness of a later insect stage.
In this context, this study investigated the roles of host chemical cues and natal rearing effect on host recognition by *Mel. digitata*. Two basic questions were asked:

1) If female *Mel. digitata* use chemical cues to locate a host, is attraction an additive, multi-component process?

2) Does experience during natal life affect an adult *Mel. digitata* host preference?

These questions led to the following hypotheses, respectively:

If *Mel. digitata* females are given a choice between bare hosts and hosts still inside cocoons with nest material, they will be more attracted to the cocooned hosts plus nest pieces than to the nude ones in an olfactometer assay.

If *Mel. digitata* are reared on different hosts [mud dauber, *T. politum*, blow fly/flesh fly, *Neobellieria bullata* (Parker) (Diptera: Sarcophagidae), and alfalfa leafcutter bee, *Megachile rotundata* (*Meg.*) (Fabricius) (Hymenoptera: Megachilidae)] in the laboratory, and they find their host by olfactory cues, then host-seeking adult females will be most attracted to the smell of their natal hosts in an olfactometer choice assay.

MATERIALS AND METHODS

This study was conducted at the laboratory of the United States Department of Agriculture in Tifton, GA. All experiments were conducted at ambient laboratory temperature (26 ± 1°C) and (50-70%) humidity.

Subjects

*Melittobia digitata* females were obtained from the University of Georgia Entomology Department’s Laboratory of Insect Behavior in Athens, GA, and cultured on three hosts: *T. politum* (TP), *N. bullata* (NB), and *Meg. rotundata* (MR). *Trypoxylon politum* was collected from nests around Athens and Tifton, GA. *Neobellieria bullata* was obtained from Carolina
Biological Supply Company, Burlington, NC and *Meg. rotundata* from Pioneer Hi-Bred International, Inc., respectively.

**Bioassay Setup**

To address the research questions, an olfactometer apparatus was employed. This assay system (Fig. 4.1), adapted from Vet et al. (1983), consisted of a 4-armed olfactometer with a 2-part exposure chamber (5 mm deep), which was enclosed in a cardboard box. Four odor fields were created inside the olfactometer chamber using a membrane vacuum pump/compressor, 10 l/min capacity. A reduced airflow was essential to achieve the desired speed of 7 ml/min per arm, a flow rate found to be optimal in preliminary tests because the air flew through the system and parasitoid females were able to move freely inside the chamber. Each olfactometer arm was regulated separately with its respective flow meter adjusted to equalize the airflow through the four arms. Each of the arms of the olfactometer was connected to a set of two 50 ml glass vials. The one closest to the chamber was the odor source, and the outer one contained charcoal, which filtered the incoming air. All connections were made of Teflon® tubing (Fig. 4.2).

A video camera (Panasonic®, Model No. WV-CP460) was positioned centrally above the chamber which was placed inside a cardboard box to minimize possible disturbances due to observer movements, and a monitor was used to view the behavior of the parasitoid being tested. Red cellophane was used to cover the box top to simulate the dark environment of the mud cell and avoid light interference on parasitoid choice (Fig. 4.2).

**Experimental Protocols**

In order to investigate whether female *Mel. digitata* use chemical cues to recognize a host and if attraction is an additive, multi-component process, mated inexperienced females reared upon *T. politum* that were less than five days old were utilized. *Melittobia digitata* females were
presumed to be mated because they usually engage in courtship and mating behavior with conspecific brothers soon after emergence (Matthews et al. 1996, Matthews and Matthews 2003). Parasitoid females were released individually in the center of the olfactometer and exposed to one of several host choices (6 TP prepupae inside cocoon + nest mud, 6 TP prepupae inside cocoon only, 12 NB puparia, 12 nude MR prepupae, 12 MR prepupae inside leaf-lined cocoon) versus 3 blank control choices, and observed for 10 minutes each. Preliminary tests and previous work (Chapter 3) showed that females were not responsive to nude *T. politum* prepupae and their extract. Hence, nude TP prepupae were not included in these tests.

To investigate whether natal experience affects an adult *Mel. digitata* host preference, females that had been reared from TP, NB, and MR, respectively, were utilized. Individual females were exposed in the olfactometer to a choice between 12 MR cocoons, 12 NB cocoons, 6 TP cocoons, and a blank control. For all experiments, the olfactometer was cleaned with 70% ethyl alcohol and hot water between successive trials.

Thirty trials were run for each treatment variable. The total time spent by each female in the four different odor field areas of the olfactometer (Fig. 4.1) was tallied, as well as the first and last choice made during the 10 minutes. A female was considered to have made a choice if she crossed the boundary lines of the olfactometer chamber (Fig. 4.1) to enter an odor field from a specific arm and stayed there for a minimum of at least one minute.

Statistical Analysis

Data were tested for normality, using Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von, and Anderson tests (SAS Institute 2000). When parameters showed significant deviation from a normal distribution, they were analyzed with the Wilcoxon Rank-sum statistical test. Otherwise,
parametric tests [ANOVA and Waller-Duncan’s test of mean comparisons (α = 0.05)] were used. Further details are given as footnotes to the data figures and tables.

RESULTS

When subjected to only one host at a time in the olfactometer, *Mel. digitata* females responded positively to *T. politum* prepupa + cocoon, spending significantly more time in this area of the olfactometer than in blank control areas (*Z* = 4.76, *P* < 0.001, Fig. 4.3). In contrast, parasitoid females did not show significant preference for *N. bullata* pupae or nude *Meg. rotundata* prepupae when compared to the blank control (NB: *Z* = 1.75, *P* = 0.0797; nude MR: *Z* = 1.0911, *P* = 0.2752, Fig. 4.3).

When parasitoid females were offered the hosts in tandem with components normally associated with them in nature, i.e. a combination of *T. politum* prepupa + cocoon + nest mud or a combination of *Meg. rotundata* prepupa + leafy cocoon against three blank controls, parasitoids responded significantly more to either host than to the controls (TP: *Z* = 2.92, *P* = 0.0034; MR: *Z* = 5.28, *P* < 0.001, Fig. 4.3). Although there was a significant response by females towards the combination of *T. politum* + cocoon + nest mud compared to the control, the presence of mud nest material resulted in no significant improvement in attractiveness of the host. In fact, *T. politum* + cocoon was significantly more attractive than *T. politum* + cocoon + nest mud in the olfactometer (*Z* = -2.6541, *P* = 0.0114, Table 4.1).

When *Mel. digitata* females were offered *Meg. rotundata, T. politum*, and *N. bullata* in the olfactometer assays, parasitoid females generally responded significantly more to *Meg. rotundata* and *T. politum* than to *N. bullata*, regardless of natal host (Figs. 4.4 – 4.6). Curiously, females emerged from *T. politum* responded significantly more to *Meg. rotundata* (*F* = 10.03, *P* < 0.001, Fig. 4.4) than to their natal host; but, females emerged from *Meg. rotundata* responded
significantly more to *Meg. rotundata* (*F* = 12.07, *P* < 0.001, Fig. 4.5). Finally, females emerged from *N. bullata* responded equally and significantly more to either *Meg. rotundata* and *T. politum* than to *N. bullata* (*F* = 4.51, *P* < 0.0064, Fig. 4.6).

When *Mel. digitata* females emerged from *T. politum* were offered the three hosts in the olfactometer and a blank control, only 29.63% chose their natal host as their first option (Table 4.2). Similarly, only 33.3% of *Mel. digitata* females emerged from *Meg. rotundata*, and 13.79% *Mel. digitata* females emerged from *N. bullata*, chose their natal host as first option when offered the three hosts in the olfactometer (Table 4.2). Additionally, 59.26%, 63.33%, and 51.72% *Mel. digitata* females emerged from *T. politum*, *Meg. rotundata*, and *N. bullata*, respectively, made a different last choice.

**DISCUSSION**

If female *Mel. digitata* use chemical cues to locate a host, is attraction an additive, multi-component process?

Parasitoid females responded more strongly to *T. politum* and *Meg. rotundata* than to *N. bullata* or the blank controls in the olfactometer. This response was intensified when the hosts were presented in their respective cocoons.

However the addition of mud from the host nest did not increase the female’s response. Indeed, females responded significantly more to *T. politum* prepupae + cocoon than the same complex increased by nest mud. Therefore, these and previous results (Chapter 3) suggest that *Mel. digitata* females use cues other than mud kairomones to recognize a host nest. One explanation for this result could be that not all nests have a suitable host present within. Because mud dauber nests are persistent for many years in the protected locations where nests are constructed, many nests are found abandoned or empty. Also, it could be that *T. politum* during
nest construction uses some type of glandular chemical that acts to mask the presence of a suitable host within the nest, hence reducing the chances of discovery by Melittobia. Another possibility could be that mud volatiles, if any, are only present in newly built nests, and rapidly dissipate over time (the mud used in our experiments was not from the same nest as the host, and was of unknown age).

Since attraction should be indicated by a direct movement towards the host odor source, we expected that in an olfactometer, wasps would wander about until they encountered the host’s odor plume and then they would walk towards its source. However, because a females’ first and last choice in a majority of cases were different - and earlier studies have produced similar results (Chapter 3; Ranger 1996) - arrestment rather than attraction seems to be operating here. Initial movement in the assay chamber was apparently random and undirected, but once a host odor was encountered females tended to spend more time in that region. Experiments with other parasitoid species show that host recognition and location is a very complex process. For example, Noldus (1989) found that the egg parasitoids Trichogramma spp. were significantly more attracted to their hosts’ sex pheromone than to clean air when tested in an olfactometer. However, when the parasitoids were tested in a wind tunnel, females showed up-wind anemotaxis, unaffected by host sex pheromone. In his study, Noldus concluded that Trichogramma spp. were also arrested by their host sex pheromone instead of attracted to it.

Does experience during natal life affect an adult Mel. digitata’s host preference?

Overall, natal host had no effect on the parasitoid females’ choice and appears not to play a role in adult chemosensory behavior. Generally, regardless of natal host, females preferred Meg. rotundata and T. politum over N. bullata in the olfactometer (Figs. 4.4 – 4.6). In contrast, experiments on other insects’ pre-imaginal conditioning have shown that exposing young adults
to a conditioning stimulus such as natal host-related chemicals, produced effects on adult chemosensory responsiveness (Thorpe 1938, 1939; Jaisson 1980; Jaenike 1982; Morris and Fellowes 2002). Our results could have been affected by the fact that our Mel. digitata were reared on nude prepupae, except for N. bullata. By not having to chew their way out of the host’s cocoon and nest, as would occur in nature, they may have missed experiencing one or more critical cues important for later host recognition. It may also be that the relatively broad range of solitary Hymenoptera that are acceptable to Mel. digitata as hosts has resulted in little selective pressure to retain a “memory” of the natal host. Additionally, it is possible that if parasitoid females are pre-conditioned by one or more chemicals present in the natal host, and the same chemicals are present on the other hosts in a higher concentration, then parasitoid host recognition and preference can be altered when they are offered the different hosts simultaneously.

Does a female Mel. digitata preferentially recognize a host based on its suitability for rearing her young?

Melittobia digitata females responded significantly more to T. politum and Meg. rotundata when offered in the olfactometer even though T. politum is apparently their primary natural host. Although Meg. rotundata is successfully used as alternative host for Mel. digitata in the laboratory (González and Matthews 2002), Meg. rotundata has yet to be reported as a host for Mel. digitata in the field, although other Melittobia species (Mel. australica, Mel. acasta and Mel. hawaiensis) have been found naturally parasitizing this bee species (Peck 1969; MacFarlane and Donovan 1989; Woodward 1994). In contrast, N. bullata has never been recorded as a host for Melittobia in the field, although this host has been extensively used for laboratory cultures of Melittobia as an alternative host yielding reasonable numbers of progeny.
per female (Matthews et al. 1996; Silva-Torres and Matthews 2003). In another study where female _Mel. digitata_ were placed in a small Plexiglass arena and offered a choice between the _N. bullata_ puparia, a dummy and blank controls they preferred the _N. bullata_ (Chapter 3). Successful parasitism and development in _N. bullata_, but not specific and reliable host finding, is probably attributable primarily to its similarity to certain dipteran host nest inquilines, such as satellite flies and bee flies, which often occur in mud dauber nests and can also be successfully parasitized by _Melittobia_.

Regardless of natal host, _Mel. digitata_ females’ first and last choices in over half of all trials (59.26%) were not the same when offered the three hosts and the control in the olfactometer. This finding could have several explanations. Initially, in most animals search for shelter, escape, or exploration of unfamiliar surroundings has highest priority. Later, behavioral modes switch to host searching. However, it may be that, as claimed by González and Terán (2001), _Melittobia_ females disperse randomly. Additionally, searching females under the conditions of our assay do not achieve the reinforcement normally obtained through physical contact with the host; thus they could become habituated to a particular odor, and reinitiate searching.

**Can _Mel. digitata_ disperse over long distances?**

Freeman (1977), Freeman and Parnell (1973), and González and Terán (2001) found that _Melittobia_ initial dispersal is random, and that due to their small size, they are subject to passive dispersal by wind. Furthermore, they suggested that the large numbers of progeny produced per host would increase the chances of host finding, thus host finding would be more likely an incidental by-product of high fecundity rather than an adaptation. In addition, Taffe and Ittyeipe (1976) showed that _Melittobia australica_ females search for hosts by flying to rock outcrops whereupon they would climb about until they found a host nest. A preliminary study testing _Mel.
*digitata* females in a small wind tunnel failed to elicit flight (Silva-Torres, unpublished data). The females in this study simply crawled about freely in the wind tunnel and distributed themselves apparently randomly. However, confounding factors such as light regime, female-female interference, temperature, and female confinement within the artificial environment could have affected their behavior.

Price (1975) showed that, in restricted conditions such as an experimental arena, groups of female parasitoids were more engaged in avoiding each other and escape than in host searching because parasitoid females are able to recognize the odor of conspecifics. Roland (2000) also showed that landscape composition and context, as well as the net effect of these two features combined, have a powerful impact on a parasitoid’s movement and consequently on how it finds hosts and the parasitism rate of these hosts. *Trypoxylon politum* nests are very patchily distributed; usually one can find many nests in the same area, as they often occur under bridges close to mud and water sources. In general, human activity appears to have created extensive new habitats for mud dauber wasps. How this modified landscape affects *Melittobia* dispersal and host-finding remains unanswered.

In conclusion, host recognition by *Mel. digitata* seems to be an additive process, in which the hosts’ cocoon odors play an important role arresting the females. Odors from mud from the host nest and the parasitoid’s natal host had no significant effects on a female’s subsequent responses in an olfactometer assay. Overall, *Mel. digitata* females were more attracted to *Meg. rotundata*, a suitable laboratory host, and to its primary host, *T. politum*, than to the factitious host *N. bullata*. Further studies on parasitoid dispersal ability at long distances, including detailed genetic analysis to confirm or differentiate populations of *Melittobia* species could help clarify how these tiny insects have been so successful in parasitizing hosts worldwide.
ACKNOWLEDGMENTS

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Table 4.1. Comparison of mean time (seconds) spent by single mated inexperienced *Melittobia digitata* females in the olfactometer quadrant containing the odor of its host *Trypoxylon politum* and various associated nest materials during a 10 min trial

<table>
<thead>
<tr>
<th>Host treatment</th>
<th>Number of females responding out of 30 tested.</th>
<th>Mean Time ± SE&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. politum</em> (Cocoon + prepupa)</td>
<td>22</td>
<td>385.8 ± 39.6 a</td>
</tr>
<tr>
<td><em>T. politum</em> (Mud + cocoon + prepupa)</td>
<td>19</td>
<td>229.5 ± 34.00 b</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values followed by the same letter in a column are not significantly different by Wilcoxon test ($Z = -2.6541$, $P = 0.0114$).
Table 4.2. Percentages of *Melittobia digitata* females’ first and last choices in relation to options available in assays in a 4-arm olfactometer when exposed for 10 min to host odors simultaneously presented from *Trypoxylon politum*, *Megachile rotundata*, and *Neobellieria bullata*

<table>
<thead>
<tr>
<th>Mel. digitata natal host</th>
<th>Percentage of females choosing (n=30)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>T. politum</em></td>
<td><em>Meg. rotundata</em></td>
</tr>
<tr>
<td></td>
<td>First</td>
<td>Last</td>
</tr>
<tr>
<td><em>T. politum</em></td>
<td>29.63</td>
<td>22.22</td>
</tr>
<tr>
<td><em>Meg. rotundata</em></td>
<td>13.33</td>
<td>26.67</td>
</tr>
<tr>
<td><em>N. bullata</em></td>
<td>24.14</td>
<td>48.53</td>
</tr>
</tbody>
</table>
**Fig. 4.1.** Diagram showing the 4-arm olfactometer chamber.
**Fig. 4.2.** Diagram of 4-arm olfactometer set-up.
Fig. 4.3. Mean time (+SE) spent by a single mated inexperienced *Melittobia digitata* female in the olfactometer (each treatment + control bars correspond to an independent set of 30 females). Bars followed by an asterisk are significantly different from their respective controls by Wilcoxon test. The mean time spent on controls was the average of the three blank controls in each trial.

Note: *Megachile rotundata* prepupa + cocoon [MR (P+C)], *Meg. rotundata* prepupa [MR (P)], *Trypoxylon politum* prepupa + cocoon + mud [TP (P+C+M)], *T. politum* prepupa + cocoon [TP (P+C)], *Neobellieria bullata* pupa [NB].
Emerged from *Trypoxylon politum*  
\[ F = 10.03, P < 0.001 \]

<table>
<thead>
<tr>
<th>Olfactometer choice</th>
<th>Time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meg. rotundata</td>
<td>24/30</td>
</tr>
<tr>
<td>T. politum</td>
<td>18/30</td>
</tr>
<tr>
<td>N. bullata</td>
<td>15/30</td>
</tr>
<tr>
<td>Control</td>
<td>13/30</td>
</tr>
</tbody>
</table>

**Fig. 4.4.** Mean time (+SE) spent by single mated inexperienced *Melittobia digitata* females emerged from *Trypoxylon politum* in the respective areas of the olfactometer containing three hosts and a blank control. Bars followed by same letter are not significantly different by Waller-Duncan’s K-ratio t test. Inset: Number of *Mel. digitata* females responding per treatment.
Emerged from *Megachile rotundata*

\[ F = 12.07, P < 0.001 \]

**Fig. 4.5.** Mean time (+SE) spent by single mated inexperienced *Melittobia digitata* females emerged from *Megachile rotundata* in the respective areas of the olfactometer containing three hosts and a blank control. Bars followed by same letter are not significantly different by Waller-Duncan’s K-ratio t test. Inset: Number of *Mel. digitata* females responding per treatment.
Fig. 4.6. Mean time (+SE) spent by single mated inexperienced *Melittobia digitata* females emerged from *Neobellieria bullata* in the respective areas of the olfactometer containing three hosts and a blank control. Bars followed by same letter are not significantly different by Waller-Duncan’s K-ratio t test. Inset: Number of *Mel. digitata* females responding per treatment.
CHAPTER 5
CONCLUSIONS

Because parasitoids’ survival and reproductive success depend on their behavior, and in the long run, natural selection only favors animals with efficient foraging strategies, this study investigated how females of the parasitoid Melittobia digitata locate and recognize their hosts. I focused on the following questions:

Is the initial location of hosts by Mel. digitata random or cue-driven?

Are Mel. digitata females able to discern and/or recognize the presence of a potential host through chemical cues/olfactory stimuli from its nest, cocoon, or body?

Does a female’s natal host species influence her later attraction to a potential host upon which to rear her offspring?

The results provide strong support for chemical cues mediating close range host seeking behavior by Mel. digitata. Parasitoid females successfully recognized and located host patches (T. politum, Meg. rotundata, and N. bullata) hidden under the filter paper in an experimental arena, and spent significantly more time on these patches than on non-host (blank and dummy), control patches.

Overall, Mel. digitata responded more intensely to chemicals released by Meg. rotundata and T. politum than to N. bullata puparia, T. politum nest mud, T. politum nude prepupae or their extracts, blank and dummy controls.

Female Mel. digitata also responded positively to nest mud compared to the controls; however this response was relatively low and similar to the response of females towards naked
prepupae or prepupal extracts, which were not significantly more attractive to them than the mud. Thus, these results suggest that chemicals from both mud and nude *T. politum* prepupae have a lesser role in *Mel. digitata* host finding and recognition. However, the combined chemical bouquet as found in the natural nest, (i.e. mud + cocoon + prepupa), appears to enhance host location and recognition.

In contrast, host cocoons and their extracts were significantly more attractive to *Mel. digitata* than the controls; however, response by *Mel. digitata* towards cocoon extracts was significantly lower than that elicited by the cocoon itself in case of *T. politum*.

Olfactometer trials confirmed that olfactory signals emanating from the hosts and their cocoons are important in helping *Mel. digitata* locate and recognize their hosts. Nevertheless, results also showed that the mud from a mud dauber nest does not increase the chances of host finding by the *Mel. digitata* females because the females responded significantly more to *T. politum* prepupae + cocoon than the same complex increased by nest mud.

Generally, natal host had no effect on the parasitoid females’ choice and appears not to play a role in adult chemosensory behavior. Regardless of natal host, females preferred *Meg. rotundata* and *T. politum* over *N. bullata* in the olfactometer. Our results could have been affected by the fact that *Mel. digitata* was reared on nude prepupae, except for *N. bullata*. By not having to chew their way out of the host’s cocoon and nest, they may have missed experiencing one or more critical cues important for later host recognition. It may also be that the relatively broad range of solitary Hymenoptera that are acceptable to *Melittobia* as hosts has resulted in little selective pressure to retain a “memory” of the natal host. Additionally, it is possible that if parasitoid females are pre-conditioned by one or more chemicals present in the natal host, and the same chemicals are present on the other hosts in a higher concentration, then parasitoid host
recognition and preference can be altered when they are offered the different hosts simultaneously.

The results provide evidence that host-related odors act as arrestments for *Mel. digitata* females. Since attraction should be indicated by a direct movement towards the host odor source, I expected that in an olfactometer, wasps would wander about until they encountered the host’s odor plume and then remain there. However, because a females’ first and last choice in most cases were different (and results of other studies have been similar) arrestment rather than attraction seems to be operating here. Initial movement of *Mel. digitata* in the assay chamber was apparently random and undirected, but once a host odor was encountered females tended to spend more time in that region.

Obviously, these conclusions are based on a limited number of experiments, carried out under standardized conditions. Further studies on parasitoid dispersal ability at long distances, involving detailed genetic analysis to confirm or differentiate populations of *Melittobia* species could clarify how this insect has been so successful in parasitizing hosts worldwide.