Host Preference and Utilization by *Melittobia digitata* (Hymenoptera: Eulophidae) in Relation to Mating Status[1]

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Abstract Host preferences of virgin and mated females of *Melittobia digitata* Dahms were compared in the laboratory using pupae of the flesh fly *Neobellieria* (=*Sarcophaga*) *bullata* (Parker) as hosts. When simultaneously offered two hosts, virgin females used only one of the hosts more often than mated females did. However, the unused second host developed to adulthood significantly less often than did controls, suggesting that the female stung and paralyzed it. Because virgin females lay only a few eggs that always develop into males which utilize very little of the host resource, this behavior seems adaptive in that potential hosts remain available, but developmentally arrested, for later full exploitation by the same female (now mated by her offspring). An additional implication of these results is that females can discriminate one flesh fly host from the other, and choose to avoid oviposition on both.

Key Words Parasitism, parasitoid, host development, host recognition, venom, clutch size

Melittobia is a genus of small, cosmopolitan, gregarious ectoparasitoids of prepupae and pupae of many insect species ranging across different orders. *Melittobia* commonly parasitize solitary wasps and bees in nature (Maeta and Yamane 1974, Edwards and Pengelly 1966, Krombein 1967), and several species can be found parasitizing mud dauber wasps (Hymenoptera: Sphecidae) (Dahms 1984).

Insects can be valuable tools in science education (Matthews et al. 1997). Being easy to rear, handle and differentiate between the sexes, *M. digitata* Dahms has emerged as a particularly user-friendly classroom insect for exploring various concepts in biology such as life history strategies, orientation behavior, and population dynamics (Matthews et al. 1996). However, there is a paucity of basic knowledge about many aspects of the biology of this species.

Melittobia digitata has the ability to lay many eggs on a single host. Thus, a female could potentially lay so many eggs that all host food reserves would be used before her offspring fully developed, resulting in high brood mortality. However, it has been demonstrated that the number of eggs oviposited by some other species of *Melittobia* correlates with the amount of resources the host provides (Ittyeipe and Freeman, unpubl.). Our observations (unpubl.) indicate that *M. digitata* also does not overburden a host with offspring.

A normal clutch of *Melittobia* offspring produced by a single female on one host includes a disproportionate number of females as compared to males. For example,

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the offspring of unidentified species of *Melittobia* were shown to be, on average, about 95% females (Ittyeipe and Freeman, unpubl.), and *M. chalybii* Ashmead has been shown to produce 97% females (Schmieder 1938). *Melittobia digitata* also shares this skewed sex ratio favoring females (Dahms 1984). The disproportionate sex ratio seems to violate Fisher's principle, i.e., sex ratios should equilibrate over time (Hamilton 1967). However, in species where inbreeding is frequent, as occurs in *Melittobia,* high numbers of females to males are common (Hamilton 1967).

As in other Hymenoptera, *M. digitata* females are able to control offspring sex; unfertilized eggs are produced by withholding stored sperm, and such eggs always produce males. This mechanism allows some reproduction (yielding males only) by females that have failed to find mates (Hobbs and Krunic 1971), but its principal importance may be to allow resource allocation appropriate to the sex of the offspring. Among species that practice sibling mating, as is the case for *M. digitata,* resources can be devoted disproportionately to females, thus increasing the growth and spread of the family group, which is acting as a population.

The behavior of mated versus virgin *M. digitata* can be observed in the laboratory. Virgin *M. digitata* are easily obtained by isolating female pupae. Despite the ease of this procedure, little has been published about behavioral differences between virgin and mated females, and practically nothing has been done to investigate host use by virgin females. It is known that virgins initially oviposit only a few male eggs, and that the females survive long enough for their offspring to become reproductively mature. The virgins are then inseminated by their sons and proceed to oviposit a larger female-dominated brood on the same host (Dahms 1984, Balfour-Browne 1922).

Balfour-Browne (1922) reported a "paralyzation sting" by *Melittobia* females, but that observation could be interpreted in other ways (Dahms 1984). A non-feeding insertion of the stinger observed in *M. chalybii* was interpreted by Buckell (1928) as a paralyzing sting. It has been shown that hosts fed on by adult female *Melittobia* still matured and even emerged as long as eggs were not laid (Dahms 1984). It is unclear which of these attributes *M. digitata* might possess.

In attempting to duplicate production of a high proportion of male brood as reported by Whiting and Blouch (1948), we noticed that when virgin *M. digitata* females were given two hosts, males rarely developed from both hosts. Previously, Whiting (1947) noted that when unmated female *Melittobia* were placed with 132 blow flies, none emerged. Although they were identified only as *Melittobia,* based on a photo included in the paper some could have been *M. digitata* (Gonzalez, pers. comm.). Therefore, the objectives of the study reported here were to determine: (1) whether a virgin female is able to prevent potential hosts from completing their normal development, and (2) whether a virgin female, when presented with two hosts, will use only one for male production.

Materials and Methods

Two initial cultures were founded by placing a mated female *M. digitata* with three flesh fly, *Neobellieria* (=*Sarcophaga*) *bullata* Parker, puparia obtained from Carolina Biological Supply Co., Burlington, NC, into 1-dram glass shell vials stoppered with cotton to allow gas exchange. Cultures were maintained in an incubator at 26°C, and when the first females emerged, host puparia in each culture were opened with forceps to expose the remaining uneclosed pupae of *M. digitata.* These were sorted into two groups, one with males and females and the other with females only based on the presence of eyes (males lack compound eyes).

One day after the *M. digitata* adults eclosed, equal numbers of females from each initial culture were combined to form one group of virgins and another group of mated females (in *M. digitata* mating normally occurs soon after eclosion when males are present). These groups were used to set up the experimental cultures using the same procedures and conditions as for the initial cultures, except that only two randomly chosen host fly puparia were provided in each culture vial. From prior observations, we expected that some individuals of the virgin female group would die without producing offspring. Therefore, more replicates were set up in the virgin group. In all, 100 mated female cultures, 127 virgin female cultures, and 25 controls (hosts without females) were simultaneously established.

Thirteen days later, by which time any *M. digitata* progeny would be beginning to pupate or unparasitized fly puparia would have eclosed as adults, all host puparia were opened. For the virgin group we recorded number of hosts with developing *M. digitata,* number of males and developmental stage of each, and number of unemerged adult flesh flies. The mated group was scored for number of hosts containing *M. digitata* progeny and the developmental stages of the offspring on each host. For the controls we recorded the number of emerged adult flesh flies.

1. Prevention of host development. For virgin female cultures in which only one of the two hosts was used (no matter how many males were laid on that one host) we counted the number of unused flesh flies that matured to adulthood. Using a Chi-square test (Zar 1974), we compared this number to the expected value derived from the control group.

2. A Chi-square test (Zar 1974) was used to determine whether the virgin female group restricted oviposition to a single host. Observed results were determined by assessing how many of the hosts had offspring at different developmental stages (adult, pupae, or larvae), and the number of hosts used in each culture (one or both). Cases in which multiple offspring were at the same developmental stage were omitted because those might have resulted from a single oviposition session. The expected value was deduced by taking the number of mated females that used both hosts (to take into account the previously observed proclivity of mated females to use just one host), multiplied by 0.5, the probability that if only two eggs were randomly oviposited that they would be on the same host.

Periodic observations of the virgin female cultures showed that during the first several days of the experiment these females would leave the host pupae and be found crawling on the vial walls. After 13 days most females were observed resting on one of the host pupae. This validates introduction of the probability factor, because if the female remained on the initially chosen host for the entire 13 days, then no opportunity for making choices would exist. Because females did leave the hosts periodically, a host choice had to be made each time the female returned. If she were able to recognize previously visited hosts (an ability possessed by other species of parasitic wasps), then she could bias her choice.

Results and Discussion

In 85 of the 127 virgin female cultures male offspring were produced on only one of the two available hosts. In these, significantly fewer flesh fly adults eclosed compared to the control group (Table 1). Although stinging and host feeding were not

Table 1. Number of flesh flies that emerged as adults in the virgin group compared to controls with no female present. The data include only those cultures where only one of the two hosts was used

Group	Emerged	No emergence
Virgins	5	80
Expected	78.2	6.8
Controls	46	4

$P < 0.001$, $\chi^2 = 856.496$, 1 df.

directly observed, the simplest explanation is that these unused and uneclosed hosts were stung by the virgin females, and the sting inhibited further development. However, the chemical nature of *M. digitata* venom is unknown. In addition to venom, other compounds affecting development potentially could be injected during stinging. In *Dendrocerus carpenteri* Curtis (Hymenoptera: Megaspilidae), an aphid parasite, juvenile hormone injected at the time of attack has been shown to stop development (Holler et al. 1993).

In 50 of 85 cases where males at distinctly different developmental stages were found, virgin *M. digitata* oviposited on only one of the two hosts (Table 2). This suggests that although they typically did not remain on their original host (particularly during the first week), the female wasps were significantly more likely to preferentially return to the same host to lay their next egg.

Because *M. digitata* lays its eggs through the puparial "shell" of the flesh fly, previously laid eggs provide no visual cues, yet the female can clearly tell which host has received them. Several explanations are possible, though not mutually exclusive. For example, the mother might chemically mark the host. This occurs in other Hymenoptera such as *D. carpenteri* (Holler et al. 1993). Alternatively, she might obtain and remember the physical dimensions of the host on which she previously laid her eggs during her initial inspections of the available host resources. An additional possibility is that the female may simply repeat the assessment process by which she

Table 2. Cases in the virgin female group where one or two hosts were used compared to the expected values. Observed is the number of virgin cultures having males of distinctly different developmental stages. The expected numbers come from the observed total (71) multiplied by 0.5, the probability of a random choice multiplied by the percentage of mated females who used both hosts (0.94) for "2 Hosts Used" and (1.06) for "1 Host Used"

	1 Host Used	2 Hosts Used
Observed	50	21
Expected	37.6	33.4

$P = 0.003$, $\chi^2 = 8.693$, 1 df.

made her initial egg host choice. Many species of parasitoid wasps are capable of assessing host suitability (Vinson and Iwantsch 1980); they might be expected to consistently make the same decision again.

It seems likely that a mated female *Melittobia* is capable of laying eggs continuously until she exhausts either hosts or sperm. If a female fails to be mated or exhausts her sperm but still has hosts available, she might be expected to select one host, lay a few eggs, then wait quietly nearby until her sons mature, mate with one of them, and commence egg laying again, as was shown by Balfour-Browne (1922).

The skewed sex ratio in *Melittobia* seems adaptive for both virgin and inseminated females. For a mated female, producing either just males or more males than necessary for insemination of her female brood is not adaptive, because males are not equipped for dispersal and the vast majority of dispersing females that might find them would likely have already mated [like most parasitoid wasps, female *Melittobia* mate only once (Assem et al. 1982)]. In contrast, a virgin female that produces just a few males ultimately ensures that she will become mated (to a son) and thereby be able to produce a predominantly female brood often on the same host used to produce her clutch of sons. When more than one host is available, a virgin is well served by the host utilization behavior we observed. By preventing both hosts from developing past the stage where they would be suitable for her offspring, then using only one for male production, a virgin female can "save" the other for her full, predominantly female brood.

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