COURTSHIP OF THE TWO FEMALE MORPHS OF *MELITTOBIA DIGITATA* (HYMENOPTERA: EULOPHIDAE)

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ABSTRACT

Courtship of sib-mating *Melittobia digitata* Dahms, a parasitoid of solitary wasps and bees, is reviewed, described, and quantified for 125 virgins of the non-dispersing brachypterous female (BF) morph paired with 24 experienced males, and for 158 virgins of the dispersing macropterous female (MF) morph paired with 21 males. Males performed 1-5 courtship bouts with both morphs; about half of all successful matings in both morphs occurred after a single bout. Depending on number of bouts performed, mean courtship durations ranged from 47-268 sec for MFs and 59-277 sec for BFs. Courtship success rates were greater for BF couples (80%) than for MF couples (57%). Compared to BF couples, MF couples were more apt to undergo multiple bouts. Results are interpreted in the context of the morphs' life history and the costs/benefits of alternatives. Failure to initiate any courtship during the 15-min observation period (22% for MF pairs, 21% for BF pairs) appeared to be due to apparent lack of interest or to occasional male violence toward females. Possible explanations for the latter, including mistaken identity, odor contamination, and nutritional stress are discussed.

Key Words: polymorphism, sexual selection theory, alternative reproductive strategies, reproductive isolation, aggression, sib mating

RESUMEN

El cortejo de *Melittobia digitata* Dahms, parasitoide de avispas y abejas solitarias que se aparea con sus hermanos, se revisa, se describe, y se cuantifica para 125 vírgenes de la forma hembra braquíptera que no dispersa (HB) apareadas con 24 machos experimentados, y para 158 vírgenes de la forma hembra macróptera que dispersa (HM) apareadas con 21 machos. Los machos ejecutaron 1-5 sesiones de cortejo con las dos formas femenias; aproximadamente la mitad de las uniones exitosas en las dos formas ocurrió después de una sola sesión. Dependiente del número de sesiones implementadas, las duraciones promedias para el cortejo duraron entre 47-268 segundos para HMs y 59-277 segundos para HBs. La tasa de cortejo exitoso fue más alta para parejas HB (80%) que para parejas HM (57%). Comparadas con las parejas HB, las parejas HM solían ejecutar sesiones múltiples. Los resultados se interpretan en el contexto de la historia vital de los morfos y los costos/beneficios de las alternativas. La falta de iniciar cortejo durante el período de observación de 15 minutos (22% para parejas HM, 21% para parejas HB) pareciera ser por falta de interés o por violencia ocasional de los machos hacia las hembras. Explicaciones posibles para éste, incluyendo identidad errónea, contaminación de olor y estrés nutricional se discuten.
Translation provided by the authors.

Melittobia are small (ca. 1 mm) eulophid wasps that are ectoparasitic upon prepupae or pupae of various larger insects, particularly solitary wasps and bees. Upon discovering a potential host, a female stings it, then feeds on host hemolymph emanating from the wound(s); this enables her to develop and then lay dozens to hundreds of eggs on that host (Dahms 1984b). Extreme inbreeding characterizes this genus; sib mating is the rule, and as a result of haplodiploid sex determination (arrhenotoky), virgin females produce sons with whom they can mate (Dahms 1984b).

Melittobia are unusual in having polymorphic female forms (Fig. 1), as first described by Schmieder (1933). Under certain conditions (apparently nutritional— see Cônsoli & Vinson 2002, 2004), a small number of females (<30) develop more quickly than the rest, and emerge as short-winged, stout-bodied individuals. Each of these "brachypterous" (BF) females (termed "second form" by Schmieder, 1933) is born with a clutch of about 30 mature eggs (Cônsoli & Vinson 2002) that they immediately lay on their natal host soon after mating with an early-emerging brother. All later-developing females on the same host possess functional wings. These "macropterous" (MF) females (termed "type form" by Schmieder, 1933) have incompletely developed eggs that mature only after they have fed on a new host after dispersing (Cônsoli & Vinson 2002).

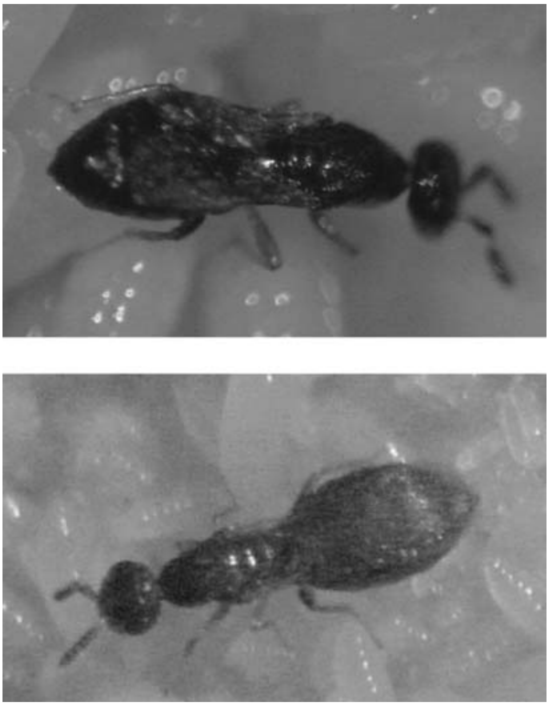

Fig. 1. Female morphs of *M. digitata*. Above: Brachypterous form [BF] (= second form of Schmieder 1933); Below: Macropterous form [MF] (= type form of Schmieder 1933). Body length of MF, 1.2mm.

Aspects of the courtship behavior of 10 of the 14 known *Melittobia* species have been reported by Assem (1975, 1976); Assem et al. (1982); Assem & Maeta (1978, 1980); Dahms (1973); Doroshina (1989); Evans & Matthews (1976); González (1985, 1994); González et al. (1996); Lapp (1994); and Varanda et al. (1984). Guided largely by chemical and tactile cues, the blind males perform an elaborate sequence of leg, wing, and antennal movements that vary from one species to another but are always surprisingly complex. Males of several *Melittobia* species appear to release a pheromone to which virgin MFs are strongly attracted (Cônsoli et al. 2002; Hermann et al. 1974; González et al. 1985; González et al. 1996; Matthews et al. 1985), often to the extent of forming a queue around a courting couple (Assem 1976). The male pheromone of *M. digitata* has been identified as *trans*-bergamotene (Cônsoli et al. 2002).

Further knowledge of the nature of courtship in *Melittobia* is desirable for a number of reasons. With few exceptions (González 1985, 1994; González et al. 1996; Lapp 1994), previous studies have involved only MF. As a practical matter, study of *Melittobia* mating rituals might identify behavioral characters useful for understanding species relationships where morphological traits alone leave some uncertainty about species boundaries (Assem et al. 1982; González et al. 1996); in one case, such information already has been pivotal (Evans & Matthews 1976). Because *Melittobia* increasingly are being used as model organisms to teach various concepts in the life sciences curriculum (e.g., Guinan et al. 2000; Matthews 1997, 1982; Matthews & Matthews 2003; Matthews et al. 1996; Pyle et al. 1997), basic biological information such as that in this study also will help teachers and students by supporting and underpinning curriculum materials.

The objectives of this study were to quantify and compare the courtship interactions for both morphs (MF and BF) of *M. digitata,* and to relate findings to other aspects of the life history.

MATERIALS AND METHODS

Laboratory cultures of *Melittobia digitata* were started with individuals that were reared from field-parasitized nests of mud dauber wasps (*Trypoxylon politum* Say, Hymenoptera; Sphecidae). The parasitoids were maintained in continuous culture at 25°C and 75% RH on *T. politum* prepupae.

To obtain virgin females for the courtship trials, groups of female pupae (distinguished from males by the presence of eyes) were isolated. Because BFs develop somewhat faster than MFs (Cônsoli & Vinson 2002), to obtain them we isolated the first 20-30 pupae to develop on each parasitized mud dauber prepupa. All later-developing female pupae were of the macropterous morph.

At 24-48 h after eclosion, each female was placed with a randomly chosen male removed directly from laboratory stock cultures. Unlike females, males of *M. digitata* are not known to be polymorphic. By continually monitoring the stock cultures, we knew that the selected males were 1 to 3 days old; because males are well documented to mate readily and repeatedly (e.g., Assem et al. 1982; Dahms 1984), we presumed them all to be experienced.

Each male-female pair was placed in a deep well slide (8 mm diameter, 3 mm depth), capped with a glass cover slip, and illuminated with a fiber optic lamp. Interactions were observed at ambient temperatures (23°C ± 1°) and were recorded with a Sony digital video camera with an attached Macro-Zoom lens (18-108 mm). Data on pairing outcomes and durations of selected courtship components were subsequently transcribed from the video recordings. If no courtship activity occurred within 15 min, a trial was terminated. Between trials, slides were washed with 95% ethanol; new cover slips were used for each trial.

We recorded 158 pairings with MF and 125 pairings with BF. Individual males were used for 1-5 successive pairings; because females mate but once, every trial used a different female. Statistical comparisons of various parameters for the two morphs used the student's *t*-test.

RESULTS AND DISCUSSION

Overall, 124/158 (78%) of MF and 99/125 (79%) of BF began courting within 15 min after being placed with an experienced male. Among the 124 MF courting pairs, 71 females (57%) mated; among the 99 BF courting pairs, 79 females (80%) mated (Table 1). The difference in proportion of overall mating success was significant ($P < 0.001$). Bout-by-bout comparisons of mating success rates showed similar significantly greater success rates for BF pairs in bouts 1 ($P = 0.011$) and 2 ($P = 0.034$), but no differences between the proportions of each morph succeeding in bouts 3, 4, and 5. Every randomly assigned male mated at least once.

Initial Attraction

When a female responds to the male's odor by touching the side of his abdomen with her antennae, the male typically responds by turning toward her body and touching her side with his antennae. This is a female's first decision point. If she is not receptive, she attempts to move away, with her antennae held downward. If she is receptive, she becomes still. Approaching either posteriorly or laterally, the male then mounts her dorsum and usually turns first toward her posterior, then reverses and ultimately orients his body in a plane parallel to hers. He then moves forward and makes initial contact with his antennae. At this point, we defined a courtship bout as having begun.

Single- and Multiple-Bout Courtship

Qualitatively, courtship was essentially the same for both morphs and our description thus applies to both. During a courtship bout, the male holds the female by placing his front tarsi just behind her head, his middle tarsi on the sides of her metathorax aligned with her middle and hind legs, and his hind tarsi on her dorsal anterior abdomen with wings and abdomen raised, he begins rhythmically opening and closing his antennae in a more or less lateral plane. As he does so, his antennae contact the clubs of the female's antennal flagellae. During each closing stroke, he maneuvers her clubs into the ventral grooves on his scapes, where they are briefly embraced by the modified pedicel and a finger-like scape projection (the "digit" that inspired this wasp's specific epithet). During each opening stroke, her antennal clubs are released, thereby completing an antennal stroking cycle.

Concurrently, the male begins a rhythmic kicking-lifting-swinging motion with his hind legs. At the end of each antennal cycle, the male's hind legs kick rapidly outwards, lift upward and forward, and slowly swing around return to their original position on the female's abdomen. Concurrently, the male also lowers his wings slightly and begins to flutter them, and rests the tip of his arched abdomen on the dorsum of the female's abdomen.

Initial alternations of antennal stroking and leg kicking appear leisurely, but the pace of the alternation soon accelerates. Kicking becomes less vigorous and more like continuous quivering, as antennal movements tighten in scope and increase in tempo. During each succeeding antennal phase, the male antennae open less widely, and ultimately do not appear to open at all. Concurrently, wing fluttering becomes more intense.

In a final convulsive motion, the male stretches his abdomen backward and straightens his hind legs, effectively lengthening his body in a plane parallel to the female's body axis, and swings his middle legs forward to hit the back of the female's head, concluding the bout.

First-bout conclusion represents a second distinct female decision point. The female's behavior at this time directs the courtship's subsequent direction. If she stretches lengthwise, flattens her abdomen into a wedge-like profile, and exposes her genital aperture, the male immediately undertakes a distinctive "backing-up" behavior with an easily measurable duration. He comes into position, bends his abdomen forward under hers, inserts his aedeagus, and copulation ensues.

TABLE 1. DURATION OF SUCCESSFUL COURTSHIPS BETWEEN *MELITTOBIA DIGITATA* MAKES AND FEMALES OF THE TOW MORPHOLOGICAL FORMS IN RELATION TO NUMBER OF BOUTS PERFORMED. TOTAL COURTSHIP DURATION IS SUM OF INDIVIDUAL BOUT LENGTHS PLUS BACKUP AND COPULATION TIME. VALUES ARE MEANS ± S.D.

	Total Courtship Duration (sec)					
Female Morphology	1 bout	2 bouts	3 bouts	4 bouts	5 bouts	All successful courtships
Macropterous	47.2 ± 18.3	85.6 ± 17.9	146.2 ± 33.2	201.5 ± 20.8	267.8	79.6 ± 50.2
(long winged)	($n = 36$)	($n = 24$)	($n = 7$)	($n = 3$)	($n = 1$)	($n = 71$)
Brachypterous	59.1 ± 24.2	106.8 ± 27.6	133.0 ± 24.1	169.2 ± 31.2	276.5	84.0 ± 43.6
(short winged)	($n = 45$)	($n = 29$)	($n = 2$)	($n = 2$)	($n = 1$)	($n = 79$)

If the female does not stretch and flatten (i.e., fails to signal receptivity), two possibilities arise. In the commonest outcome, a 'persistent' male does not back up nor dismount; instead, after a brief pause (<5 sec) he begins a second courtship bout (bout 2) with renewed slow and exaggerated antennal stroking. The other outcome occurs with a 'non-persistent' male. In this case, when the female fails to indicate receptivity, the male simply dismounts rather than beginning anew. In three cases of BF pairs a dismounted male immediately remounted the same female and began a new bout 1, but most often dismounted males moved away from the female without further interaction (compare flow charts at bout 1 in Figs. 2 and 3).

The fact that the females' behavior seems to determine the outcome of courtship is not surprising. However, the fact that *Melittobia* females wait until the conclusion of a complete bout sequence by the male (termed the "finale" by Assem et al. 1982) prior to indicating whether they are receptive or not is thought to be unique among the chalcidoid wasps. In other chalcidoid species so far studied, female receptivity may be indicated at varying points during the male's display, obviating the need for males to complete a full stereotyped display (Assem et al. 1982).

About half of all courtships that ultimately resulted in successful copulation occurred after only a single courtship bout (MF, 36/71; BF, 45/79); the other half required additional courtship, most commonly 1 more bout, rarely as many as 4 more (Figs. 2 and 3). With 2 bouts, the cumulative copulation success rate for both morphs increased dramatically (84.5% for MF pairings, 93.7% for BF pairings).

Assem and colleagues (1982) are the only other researchers to have described the courtship of *M. digitata* (their *M.* species 4). They used only macropterous females and did not systematically track pairing outcomes, nor did they record frequencies or outcomes of unsuccessful courtship interactions. For comparisons with unsuccessful courtship, they paired males with previously mated females, because *Melittobia* females will usually allow only a single copulation. Their descriptive data for 25 MF pairs differ slightly in terminology, but agree with our observations in all essential respects. Their average of 76.6 ± 9.6 sec for courtship duration and average of 25 leg-kick-lift (swing) cycles are both similar to our MF findings. They do not present data for courtships having more than one bout, and although we regularly observed multiple-bout courtships (Figs. 2 and 3), it is not clear whether they ever saw any.

Morphological Effects

Although essentially the same proportion of both morphs began courting, virgins of the BF morph that courted were more initially receptive to mating than were courting MF virgins. As the numbers above indicate, BF were more likely to require only a single courtship bout, and had a higher copulation success rate overall. Understandably, as the number of courtship bouts required for inducing receptivity in *M. digitata* goes up, courtship duration does also (Table 1). Depending upon the number of courtship bouts that preceded female receptivity signaling, a successful courtship with MF required 47-268 sec. With BF, again depending upon number of bouts, successful courtship lengths ranged from 59-277 sec.

Comparison of duration of first bouts of successful (female displays receptivity posture) versus unsuccessful (female fails to display receptivity) courting couples revealed that unsuccessful courtship durations were significantly shorter in BF couples ($P = 0.035$, $t = -2.238$, 14 *df* versus $P = 0.657$, $t = 0.446$, 64 *df* for MF couples). Thus for BFs, decisions about whether to copulate may relate to male bout duration, but not for MFs. Assem et al. (1982) mention that there was no difference between successful and unsuccessful courtship durations in any species they studied except for *M. clavicornis,* however, they did not compare the two morphs. Interestingly, successful and unsuccessful couples of both morphs did not differ in leg-kick-lift-swings/min in their first courtship bouts ($P = 0.76$, $t = 0.313$, 10 *df* for BF couples; $P = 0.613$, $t = 0.658$, 60 *df* for MF couples). Thus differences in first bout duration of successful and unsuccessful couples of the two morphs was not related to the rate of leg-kick-lift-swings.

Overall, successful courtship durations averaged slightly longer for the BF pairings (Table 1), but the difference was not statistically significant ($P = 0.26$, $t = -0.572$, 140 *df*). However, bout-by-bout comparisons showed that for BF pairs average successful bout durations were significantly longer for the first two bouts (bout 1 $P = 0.007$; bout 2 $P = 0.001$), but considerably shorter for the third and forth bouts (Table 1).

The longer overall average courtship durations for successful BF pairs may simply reflect the fact that BFs are demonstrably thickset and slow-moving in comparison to their slimmer, livelier MF siblings (see Fig. 1). This difference in female shape and agility, and male compensation for it, may also account for the finding that average copulation time for all successful BF couples was longer than for MF couples (6.6 ± 2.5 sec versus 5.0 ± 1.4 sec, $P < 0.001$, $t = 4.668$, 121 *df*) and all back-up times were less for males with BFs (4.6 ± 2.9 sec versus 6.6 ± 1.6 sec, $P < 0.001$, $t = -5.051$, 124 *df*). The longer average duration of successful BF courtships may also reflect a disparity in female receptivity thresholds. Males successfully copulating with BFs used on average 3 more leg kick-lift-antennal stroke cycles per bout than successful males courting MFs (27.5 versus 24.5). In addition, the relative pace of the leg-kick-lift-swings/min was greater in successful MF couples (BF mean =36.3 ± 6.2, MF mean =39.5 ± 7.5; $P = 0.005$, $t = -2.889$, 148 *df*). Thus, males courting BFs used a greater number of leg-kick-lift cycles and performed them at a somewhat slower pace compared to males courting Mfs. This in combination with the longer average copulation time for BF couples resulted in the longer average courtship durations for BF couples compared to MF couples.

Courtship Success

In the course of their shared courtship reaction chain, both sexes have opportunities for choice. Females can signal decisions about male acceptance both at the initial encounter and after the male has mounted and completed a courtship display bout. Likewise, a male can decide whether to respond to a female's initial touch, and whether to persist or leave when a given bout does not result in female receptivity.

As noted above, the two morphs began courtship at about the same rate, but overall, BF couples had significantly higher courtship success rates over all bouts combined and for each of the first two bouts analyzed separately. Among all initial courtship pairings that failed to result in copulation, MF pairs quit about twice as often as BF pairs (43% versus 19%, Figs. 2 and 3). Of 124 MF pairs that began a first bout, only 36 successfully copulated at the conclusion of that bout (29%), compared to 45 of the 99 BF pairs (46%).

These differences may relate to the differing ecological roles of each female morph (Freeman & Ittyeipe 1976, 1982; González & Terán 2001). As the dispersive portion of the population, MFs emerge with undeveloped eggs. Their options include (1) to mate with their brothers (rarely with unrelated males, see below) and then disperse; (2) to disperse as a virgin to a new host and produce sons with which they can and do mate (e.g., Assem 1976; Dahms 1984; Schmieder 1933); or (3) to find both a new host and an unrelated male with which to mate. Options 2 and 3 likely are very uncommon in nature, since parasitized hosts normally always yield progeny of both sexes, and Abe et al. (2003a) has confirmed that all dispersing *M. australica* females are fertilized. Moreover, even if a virgin female somehow rejects one male's initial courtship attempt, she is likely to have other mating opportunities with the same or other sibling males in the same clutch.

In contrast, as the nondispersive portion of the population, each BF lays her eggs upon the remnants of the natal host, a limited resource that is already declining in nutritional quality due to feeding pressure (Cônsoli & Vinson 2002). Despite attempts to facilitate BF dispersal on foot to neighbor hosts in the laboratory, we have never observed a BF female to leave her natal host (unpubl. observ.) and we doubt that it ever occurs in nature. Thus BFs are in resource- and time-driven competition with each other for the success of their own offspring. Without the option of dispersing to new hosts, readily mating with clutch mates and rapidly ovipositing on the natal host would be strongly favored. Thus, it is perhaps not surprising that BFs mate more readily than MFs.

The difference in sex ratios of the early and later emerging *M. digitata* may also have relevance for the higher receptivity of BF. The very first progeny to emerge from a single female *M. digitata*-parasitized mud dauber consist of an average of 26.7 BF and 12.1 males (R. W. Matthews, unpubl.). Thus the initial sex ratio is much less female-biased than the final sex ratio will be after all the MFs have emerged. From a lone male's standpoint, additional mating opportunities with virgin BFs are far fewer than for MFs and the competition from brothers is relatively greater. From a BF's perspective there are far more potential male mating partners than needed since she will only mate one time, and has a host immediately at hand. Taken together, these life history variables also may help to explain why males paired with BFs performed both longer duration bouts and displayed a higher level of persistence into the second bout than males paired with MFs. BFs under these circumstances may require more "proof" of a male's genetic worth.

As noted above, courtship durations for the two morphs were not statistically different. However, there was a trend for males to perform more leg-kick-lift-swing cycles with BFs but BFs were more likely to require only a single courtship bout. Conversely, males performed fewer leg-kick-lift-swing cycles but more bouts with MFs. A possible scenario to explain these differences assumes that originally females appeared as only the macropterous morph. (While no phylogenetic analyses exist for *Melittobia* species, this assumption seems reasonable since macroptery is the most prevalent condition in the Chalcidoidea.) Males attempting to mate with MFs were (and are) under intense time pressure. Not only are they in fierce competition with their brothers (see Abe et al. 2005), but they are also racing the clock because any unmated females will begin to disperse as virgins after they are a few days old (unpubl. observ.). Due to natural variation, some MFs likely would be willing to copulate sooner than the average; the problem for a male is that he has no way to know in advance which females these might be.

Male courtship behavior might also be expected to vary, with some males "cheating" by attempting to reduce the number of leg-kick-lift-swing cycles, or inserting a finale partway along the series of leg-kick-lift-swing cycles. If the reduced effort was genetically based and proved acceptable to the female, such males would gain additional time in which to court others; when it was not acceptable, they would lose only a few seconds, and could quickly resume the courtship. Such variation could lead to fewer leg-kick-lift-swing cycles per bout and an increase in the number of bouts required for success.

Compared to MF couples, time and performance pressures are reversed for BF couples. As noted above, for BFs time is of the essence. On the other hand, pressures to hurry along or "cheat" were/are much weaker for the males, due to a less extreme sex ratio and the lack of dispersal by BFs. Thus, in BF courtships, a gradual increase in the number of leg-kick-lift-swing cycles (resulting in an increased bout length) rather than fewer leg-kick-lift-swing cycle and more bouts might be favored.

Male Stability, Persistence, and Life Strategies

The world of the blind, flightless male of *M. digitata* is closed, violent, and highly competitive (see Abe et al. 2003a, 2003b, 2005; Hartley & Matthews 2003; and references therein), and he faces very real risks to life and limb from other males throughout his brief life. As a greatly outnumbered (average sex ratio, 3 males: 97 females [González & Matthews 2002]) male hurries to out-compete his brothers in the race to inseminate 500+ potential mates, success might be enhanced in many ways, but by any measure a male may gain an advantage through any reduction in courtship duration.

Assem et al. (1982) assert that successive courtships by a single male have very stable average durations (though they provide no data on the matter). Neither our study nor Assem's quantitatively compared virgin males with more experienced ones, but examination of our data subset for males with 2 successive single-bout courtships shows that for MF pairings (n = 9), the average duration of 42.7 sec with the first female was not different from the 43.4 sec duration with the second female (P = 0.835, t = -0.215, 8 *df*). Likewise, for BF pairings (n = 13), the average duration of 55.7 sec with the first female did not differ from the 68.1 sec duration with the second female (P = 0.133, t = -1.612, 12 *df*). These results suggest that experience does not increase the males' courtship efficiency.

Persistence may play an important role in the eventual success of a male. According to investment theory we can imagine that at the end of each unsuccessful bout, a male must choose between two alternatives: an investment in persistence in which he continues additional bouts with a so-far unreceptive female, or an investment in "playing the field" in which he always moves on after one bout. As noted earlier, although about half of all ultimately successful courtships with both morphs were consummated after a single bout, males paired with MFs succeeded in a single bout at a considerably lower rate than males paired with BFs. Males that continued through a second bout with initially unreceptive females dramatically increased their success rate, by 60% for males paired with MFs and 41% for males paired with BFs.

Pursuing the two alternatives, we assume a situation where virgin females are already queued up, so that searching time is minimized and courtship with a new individual can begin almost immediately after leaving the previous one. A successful single bout courtship with a MF takes about 47 s (Table 1), but in an initial encounter, a "field-playing" male has only a 51% chance of ultimately copulating. Since such a male cannot know in advance which "first date" will ultimately give rise to success, he would need to court 2 females (requiring an average of 94 s) to achieve an average of 1 copulation. On the other hand, a persistent male who does not leave after a single bout more than doubles his chances, and successful 2-bout courtship only requires an average of 86 s, including copulation. Thus for a male courting a MF, persistence is superior to playing the field. In addition, if a male stays in a "committed relationship" with a MF no matter how long it takes rather than leaving, his cumulative chance of ultimate success rises steadily. In contrast, the non-persistent field-player's chances of success remain at the initial 51%. Moreover, by persisting with a single female, a male's probability of encountering another aggressive male is nil, compared to what could ensue if he dismounted to play the field.

With BF pairs, a different picture emerges. A single-bout courtship takes somewhat longer than for a MF pair, about 59 s, but carries a higher potential success rate, so that a field-playing male on average will need to court fewer than two BF per copulation, at most 108 s. A persistent BF-courting male with success after 2 bouts requires 107 s, and increases his chances of success by less than 50%. Thus, time investment for the two alternative strategies is more similar in BF pairings than in MF pairings, relaxing selection for male persistence with the former. On the other hand, because BFs do not queue around males (unpubl. observ.), a male may require extra time and encounter increased risks from competing males in a search for another receptive female, factors that might favor persistence. It would be interesting to manipulate such factors experimentally to gain further insight into the evolution of alternative strategies.

When Courtship Goes Awry

All previous work on *Melittobia* courtship (including our own earlier work) ignores data for unsuccessful courtship pairings. However, it may be instructive to examine the cases in which courtship goes wrong. As is evident from our data and the flow charts for both female morphs (Figs. 2 and 3), most instances of failure to court appeared to be a matter of non-attraction, at least during our relatively short observation period.

Among those 34 MF pairs that failed to court, 29 showed no sign of interest or receptivity on the part of one or the other sex during the trial (Fig. 2). Similarly, of those 26 BF pairs that did not court, 23 displayed no apparent interest (Fig. 3). The proportion of pairings in which females actively rebuffed the male's attempt to mount and appeared to refuse to cooperate was about the same for each morph, 26/158 (17%) for MF and 19/125 (15%) for BF.

Reasons for apparent lack of interest by the virgin females are unknown. Although it is possible that a deficiency in male pheromone production might have accounted for some of the female disinterest, we feel this is unlikely. Our males were 1-3 days old, and Cônsoli et al. (2002) found that male pheromone production peaked at 2 days post-emergence. Furthermore, each male used in our pairings successfully attracted and copulated with a virgin female on at least one occasion.

Alternatively, these cases might simply have been an artifact of the experimental situation. In our study, as in those before us, single individuals of each sex were confined together within a comparatively large, lighted space, whereas in their natural context, the sexes would emerge inside a crowded and dark host cocoon and have essentially unlimited time to get together. The relatively brief time allotted in our trials, plus possible physiological stress as a result of handling and manipulation, may have been contributing factors.

The remaining failures to court in our experiments involved cases of overt male aggression toward females (5/34 for MF pairs and 3/26 for BF pairs). In 2 of the trials with MFs, males killed their partner. With BFs, males also killed twice.

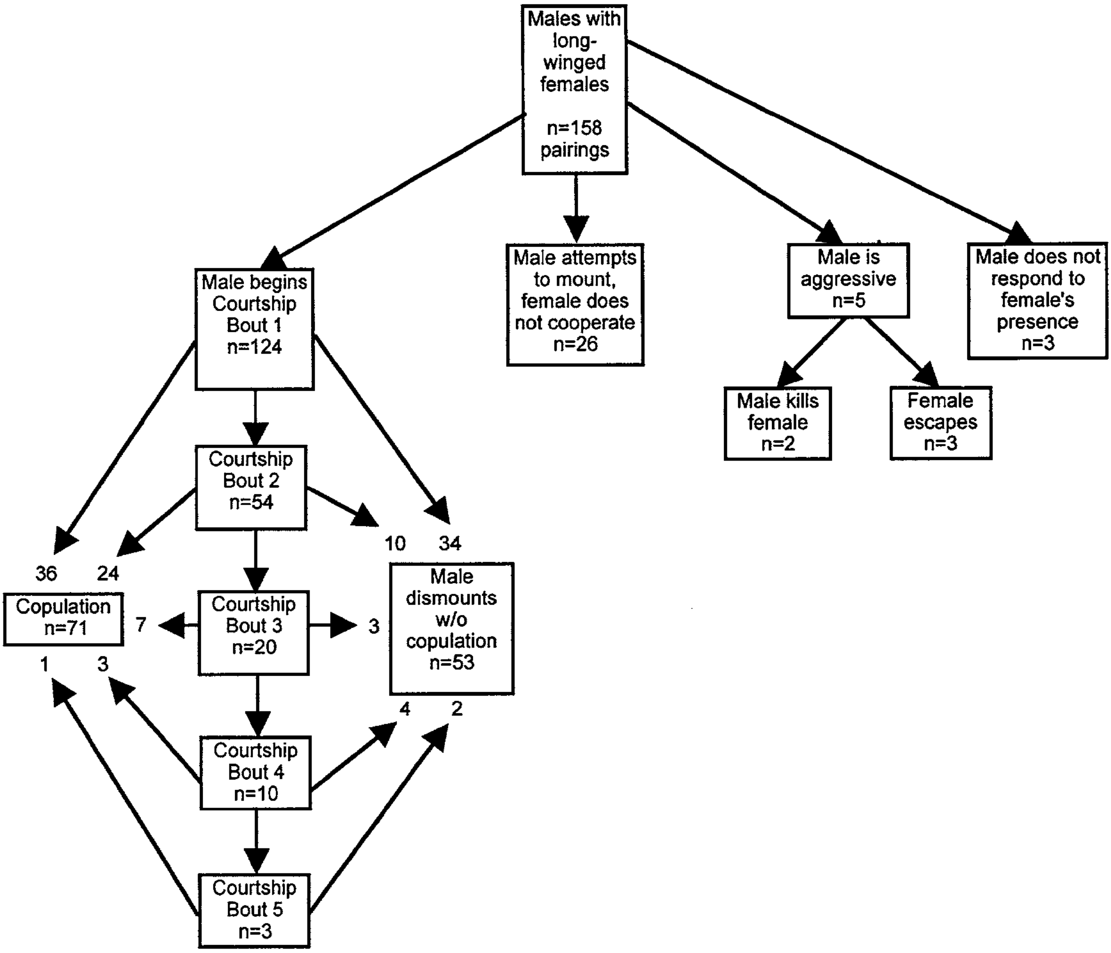

Fig. 2. Flow chart of the outcomes of 158 pairings of MF of *M. digitata*.

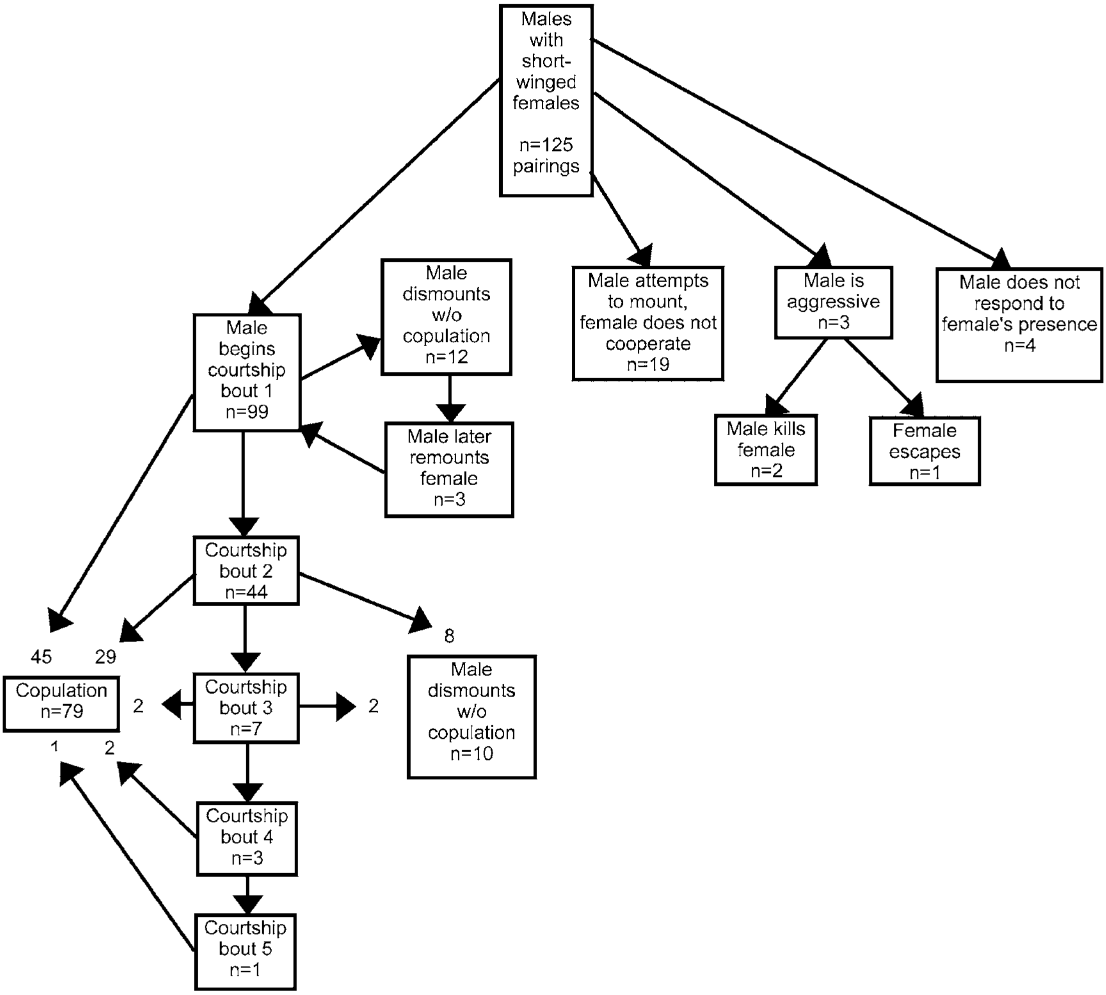

Fig. 3. Flow chart of the outcomes of 125 pairings of BF of *M. digitata*.

These attacks typically occurred after the male had mounted and appeared ready to begin a courtship bout. However, instead of moving forward to contact the female's antennae, the male instead would begin to chew at her neck or anterior thorax region. Our "killer males" mated normally on other occasions, but because each male in our study was used for only a limited number of trials, the question is left open as to whether these aggressive incidents represent an isolated (and perhaps environmentally influenced) fluke. Longer-term studies involving larger numbers of pairings with males that show such behavior would be valuable.

As noted above, male-male aggression often resulting in death is a characteristic of the genus *Melittobia*. However, the occurrence of aggressive actions toward conspecific virgin females is seldom mentioned in the literature and appears to vary between species. Balfour-Browne (1922) reported that males of *M. acasta* and *M. chalybii* (=*M. australica*) commonly killed females, but attributed this behavior to experimental conditions. Dahms (1984b) noted similar female killing behavior occasionally in his observations on *M. australica*. Neither previous study quantified the incidence of male violence to females.

The basis for such seemingly maladaptive behavior is unclear. Dahms (1984b) postulated that remnants of male odor might remain in the courtship chamber, stimulating male aggression that mistakenly became directed toward females. However, such a "laboratory artifact" is in fact the natural situation inside a host cocoon where, because males continually fight with one another, male odor is likely to be a constant part of the olfactory milieu.

Mistaken identity and/or inappropriate signaling might also be a factor. On occasion we have observed females behaving atypically. For example, sometimes upon being antennated by a male, a female will retract her legs and assume an inert pupa-like form (unpubl. observ.). Such visually apparent weirdness would have little impact on sightless males within a dark host cocoon, but it might be accompanied by relevant (but as yet unknown) chemical, auditory, or tactile cues.

Nutritional stress provides a third not mutually exclusive explanation. Hermann (1971) mentioned that males of *M. chalybii* (=*M. australica*) 8 or more days old would "grasp and feed on" a receptive female. Matthews (1975) also noted males of this species chewing on the female victim during or after attack. Whether any nutritional benefit accrues to the male in these cases awaits further study.

Why Such Complex Courtship?

The elaborate courtship rituals observed in *Melittobia* parasitoids are reportedly some of the most intricate known in this large group of insects (Assem 1975). Since it is generally believed that *Melittobia* males never leave their natal host, and that all females are fertilized by their brothers (Dahms 1984b), the existence and maintenance of such complexity is somewhat perplexing.

Assem et al. (1982) hypothesized that the courtship might serve to prevent sperm depletion in males by spacing out copulations. However, this would not seem particularly relevant for males courting BFs, since there are rarely more than 30 females in a clutch of BF offspring. Additionally, Assem et al. (1982) raised the possibility that the leg-raising component may have arisen as a result of male-male competition and the need to fend off intruders, particularly other females attracted to the queue surrounding courting couples. Their argument would not apply particularly well to courtships with BFs, which behave sluggishly and show little tendency to queue around males. However, if, as suggested above, ancestral females only existed as the macropterous morph, the competitive nature of males, once evolved, may have persisted even after the BF morph appeared.

The role of courtship behavior in the maintenance of reproductive isolation may be of more importance to *Melittobia* than researchers have previously appreciated. Superparasitism in some *Melittobia* has been reported. Molumby (1996) discovered up to 5 *M. femorata* females (mean, 1.84) per host cell in a sample of 53 parasitized cells from 28 *Trypoxylon politum* hosts nests in Mississippi. Whether multiple species colonize a single host in nature is not known. In part this may reflect the fact that prior to 1984 it was believed that only one species (*M. chalybii*) existed in North America and another in the Old World (*M. acasta*). However, Schmeider (1933) mentioned possibly having more than one species in his studies and Dahms (1984a) recognized 8 species from North America. Indeed, on one occasion 3 different *Melittobia* species were found within a single field-collected host cell of *Trypoxylon politum* in Georgia (J. M. González, unpubl.). Furthermore, Matthews et al. (1985) note that *Melittobia* females are sometimes attracted to odors of males of other species. Thus the potential exists for multiple species to occur and interact in some localities. Even if relatively rare, cases of inter- and intraspecific host settling could provide a selective context favoring development and maintenance of elaborate courtship.

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