Lethal combat and sex ratio evolution in a parasitoid wasp

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Sex allocation theory provides excellent opportunities for testing how behavior and life histories are adjusted in response to environmental variation. One of the most successful areas from this respect is Hamilton's local mate competition theory. As predicted by theory, a large number of animal species have been shown to adjust their offspring sex ratios (proportion male) conditionally, laying less female-biased sex ratios as the number of females that lay eggs on a patch increases. However, recent studies have shown that this predicted pattern is not followed by 2 parasitoid species in the genus *Melittobia*, which always produce extremely female-biased sex ratios. A possible explanation for this is that males fight fatally and that males produced by the first female to lay eggs on a patch have a competitive advantage over later emerging males. This scenario would negate the advantage of later females producing a less female-biased sex ratio. Here we examine fatal fighting and sex ratio evolution in another species, *Melittobia acasta*. We show that females of this species also fail to adjust their offspring sex ratio in response to the number of females laying eggs on a patch. We then show that although earlier emerging males do have an advantage in winning fights, this advantage 1) can be reduced by an interaction with body size, with larger males more likely to win fights and 2) only holds for a brief period around the time at which the younger males emerge from their pupae. This suggests that lethal male combat cannot fully explain the lack of sex ratio shift observed in *Melittobia* species. We discuss alternative explanations. *Key words:* body size, competition, contests, local mate competition, *Melittobia acasta*. *[Behav Ecol 18:709–715 (2007)]*

H amilton (1967) showed that when the offspring of one or a small number of females mate within the natal patch, prior to dispersal by females, a female-biased sex ratio is favored (local mate competition [LMC]). The explanation for this bias can be described equally well through an individual (inclusive fitness) or a hierarchical (group) selection approach (Colwell 1981; Taylor 1981; Harvey et al. 1985; Frank 1986a, 1998; West et al. 2007). The inclusive fitness approach suggests that the female bias is favored in order to 1) decrease competition between sons and 2) increase the number of mates available to them (Taylor 1981). In haplodiploids, an additional bias is favored because inbreeding increases the relative relatedness of mothers to their daughters (Hamilton 1972; Frank 1985; Herre 1985). LMC theory predicts that in haplodiploids, the evolutionary stable sex ratio (r; proportion male) is given by the equation $r = (N-1)(2N-1)/N(4N-1)$, where N is the number of foundress females laying on the patch. There has been widespread support for this prediction, with female-biased sex ratios being observed in a huge number of plant and animal species where LMC is likely to occur (Charnov 1982). Furthermore, individuals of over 60 species, from a range of taxa, have been shown to adjust their sex ratio conditionally in response to the number of females laying eggs on a patch (West et al. 2005).

In stark contrast, species of the parasitoid wasp genus *Melittobia* do not appear to shift sex ratio in accordance with LMC predictions (Abe, Kamimura, Kondo, et al. 2003; Cooperband et al. 2003; Abe et al. 2005). *Melittobia* species have a life history that meets the conditions where LMC is expected: competition for mates occurs between brothers, in order to gain mating opportunities with their sisters, and females disperse

after mating (see Methods for a more detailed life history description). As expected from this, *Melittobia* species have extremely female-biased average sex ratios and were given as an example by Hamilton (1967) in his original LMC paper. However, recent studies on *Melittobia australica* and *Melittobia digitata* have shown that females fail to shift their offspring sex ratio with increasing foundress number as predicted by LMC theory (Abe, Kamimura, Kondo, et al. 2003; Cooperband et al. 2003; Abe et al. 2005; see also Molumby 1996). It has been suggested that a possible explanation for this relative lack of a shift in sex ratio is that males fight fatally in order to gain mating opportunities (Abe, Kamimura, Ito, et al. 2003; Abe, Kamimura, Kondo, et al. 2003; Abe et al. 2005). Theory has shown that if 2 females lay eggs on a patch sequentially and males laid by the second female have a reduced mating success, then the second female can be selected to produce an extremely female-biased sex ratio, in contrast to the predictions of classic LMC (Abe, Kamimura, Ito, et al. 2003; see also Shuker et al. 2005). The idea here is that the fitness gain from later laid males is reduced because they may be killed without any mating success. Support for this idea has been obtained from the observation that later emerging males tend to be killed by older, previously emerged males (Dahms 1984; Gonzalez, Teran, and Matthews 2004; Abe et al. 2005).

However, another factor that can influence fights between males is body size. Larger males are more likely to win fights in *Melittobia* (Hartley and Matthews 2003; Reece SE, Innocent TM, West SA, forthcoming), as is the case in many animals, ranging from other insects (e.g., Murray 1987; Peterson and Hardy 1996; Greeff and Ferguson 1999; Bean and Cook 2001; Hartley and Matthews 2003; Taylor and Jackson 2003; Batchelor et al. 2005; Cook and Bean 2006) to mammals (Cluttonbrock and Albon 1979; Haley et al. 1994; McElligott et al. 2001; Preston et al. 2003; Lidgard et al. 2005). If this effect of size in *Melittobia* reduces or overrides the influence of emergence order, then fatal fighting will have a reduced influence on sex ratio evolution. In this situation, we would predict an interaction

between size and emergence order, for instance, emergence order may vary in importance as the asymmetry in the size of opponents changes. Furthermore, there may be an important distinction to be made between emergence order and age. Emerging early could be advantageous if males gained an advantage because males are relatively defenceless before or during emergence or if there is a period before cuticle sclerotization occurs (Abe et al. 2005). Consequently, any advantage of emergence order may only be transient, and age itself may not be important once all males have emerged.

Here, we aim to test the potential importance of fatal fighting in restricting sex ratio shifts in *Melittobia*. In Methods, we provide a more detailed description of the natural history of wasps from this genus, emphasizing similarities and differences relative to more familiar LMC model systems. The sex ratio behavior of the species (*Melittobia acasta*) we are working with has yet to be examined, so we first manipulated foundress number to show that females of this species also fail to adjust their offspring sex ratios as predicted by LMC theory. We also manipulated the time that females were allowed to spend ovipositing, as this influences the number of eggs that a female can lay, which has been shown to influence offspring sex ratios in several parasitoid wasps (Godfray 1994). We then carried out a series of experiments in which we examine the importance of both emergence order and age and how these factors interact with size to influence male fighting success. We first control size and focus on emergence order, placing pairs of males who have emerged from their pupal cases with males who have not, to test the idea that emerged males have an advantage in combat because they kill other males either before, during, or just after emergence. We then extend this scenario to include variation in size to examine whether or not size advantage can overcome the potential gain from early emergence. Finally, we investigate the effect of emergence order beyond the time of emergence (age) to assess how the relationship with size (competitive ability) changes through the period of combat.

METHODS

Natural history of the *Melittobia* genus

Melittobia acasta (Hymenoptera: Eulophid) is a gregarious ectoparasitoid wasp, with an exceptionally wide host range that includes Diptera and Lepidoptera, along with other Hymenoptera (Balfour Browne 1922; Freeman 1977; Van den Assem et al. 1980; Dahms 1984; Gonzalez, Teran, and Matthews 2004). *Melittobia* species exhibit pronounced sexual dimorphism. Males are blind with reduced wings and remain within the natal patch to compete for mating opportunities. Eclosing before females, males will fight to the death using their mandibles to attack and remove opponents' heads and limbs (e.g., Dahms 1984; Gonzalez, Teran, and Matthews 2004, see also Hamilton 1979). The males remaining alive will then mate females within the natal host pupa as they emerge. In contrast, females have fully functioning eyes and wings and will disperse after mating to find new patches of hosts. Females are typically able to lay large clutch sizes (~200 per host), and because *M. acasta* is haplodiploid, females can adjust (offspring) sex ratio, producing daughters from fertilized and sons from unfertilized eggs. Virgin females have been observed to lay a small first clutch of males, and mate with one of these sons upon emergence, in order to lay a larger second clutch containing females (Balfour Browne 1922; Dahms 1984).

Available data suggest that the natural histories of species in the *Melittobia* genus are very similar (e.g., Van den Assem et al. 1980; Gonzalez, Abe, and Matthews 2004; Gonzalez, Teran, and Matthews 2004). Female-biased sex ratios have been re-

ported from field studies, suggesting that a lack of sex ratio shift in the lab is representative of natural conditions (Van den Assem et al. 1980; Gonzalez, Abe, and Matthews 2004). Data suggest that foundress number is variable in natural populations and is influenced by host size and density in a number of *Melittobia* species (*Melittobia hawaiiensis*: Freeman and Ittyeipe 1976; Freeman 1977; *Melittobia japonica* and *M. acasta*: Van den Assem et al. 1980; *Melittobia femorata*: Molumby 1996; Cooperband et al. 2003). Two female morphs are found, a long-winged dispersing and a short-winged nondispersing morph, of varying proportion between broods (*M. chalybii*: Schmeider 1933; *M. australica*: Freeman and Ittyeipe 1976, 1982; Dahms 1984; *M. digitata*: Consoli and Vinson 2002; Cooperband et al. 2003; *M. clavicornis*: Gonzalez, Abe, and Matthews 2004). The amount of dispersal relates to resource availability, which is known to vary across the host range, thus providing further (indirect) evidence of variation in foundress number.

Natural variation in male size has not yet been measured; however, all published data lie within the range we have found in our stock population (Balfour Browne 1922; Hartley and Matthews 2003; Gonzalez, Teran, and Matthews 2004). Furthermore, size is known to depend on clutch size and host quality (Consoli and Vinson 2002). Consequently, as *Melittobia* species have an extremely wide host range (Balfour Browne 1922; Freeman 1977; Van den Assem et al. 1980; Dahms 1984; Gonzalez, Teran, and Matthews 2004), male size is likely to vary in natural populations. The male emergence period can be relatively prolonged—16 days at 25 °C for *M. australica* (Abe et al. 2005). Furthermore, staggered emergence and overlapping generations of males are likely, given the possibility of multifoundress scenarios and the potential for nondispersing female offspring to superparasitize large hosts (Schmeider 1933; Freeman and Ittyeipe 1976; Cooperband et al. 2003). Males are highly aggressive and will start fighting almost immediately upon emergence, with the majority of fights occurring before the first females start to emerge (Buckell 1928; Van den Assem et al. 1980; Gonzalez, Teran, and Matthews 2004; Abe et al. 2005). Adult males will attack emerging pupal males, will almost always fight when encountering each other, and these violent encounters often lead to the death of at least one male (*M. acasta*: Balfour Browne 1922; Dahms 1984; *M. digitata* and *M. australica*: Buckell 1928; Abe, Kamimura, Kondo, et al. 2003; Gonzalez, Teran, and Matthews 2004; Abe et al. 2005).

General methods

For all experiments, we used wasps from our UK stock population, established from wasps collected in the field in 2004 (by Mark Shaw, Royal Museum of Scotland, Edinburgh) and subsequently (mass) cultured in the lab on *Calliphora vomita* pupae. All wasps were reared at 30 °C, with a 16:8 light:dark photoperiod. Under these conditions, generation time is 11–13 days for males, with females emerging 1–2 days later. For each generation, we placed groups of approximately 100 females in 70×25 mm glass vials stoppered with cotton wool containing ~20 host pupae for oviposition (0–200 offspring laid per host).

In order to produce the large numbers of males required for experimental work, we cultured virgin females taken from stock populations: thanks to the haplodiploid genetic system, virgins are limited to laying sons and thus produce all-male clutches (Cook 1993). We opened up hosts 10 days after oviposition and removed virgin female pupae, grouping and housing them with fresh hosts according to each experimental treatment (see below). After 6–8 days, we were able to dissect male pupae from these hosts, which we isolated in

gelatin capsules (volume = 0.21 ml, similar dimensions to *C. vomita* pupae) before emergence, to prevent fighting prior to the experiment.

Experiment 1: sex ratio adjustment and LMC

We first tested the predictions of LMC theory by manipulating the number of foundress females that are able to lay eggs (oviposit) on a single host and thus the degree of LMC experienced by offspring. Specifically, we set up 20 replicates within each of 5 levels of foundress number treatment: 1, 3, 10, 25, and 50 females (i.e., total sample size equals 100). We also manipulated the time females were allowed to oviposit upon hosts. Within each foundress treatment level, we allowed the females in 10 replicates to oviposit for 24 h and 10 replicates for 48 h. Mated adult females were (randomly) chosen from stock populations approximately 24–48 h after emergence and randomly assigned to treatment levels. All replicates were placed in stoppered glass vials with a single *C. vomita* host. After either 24 or 48 h, we removed females and returned the host pupae to the vials, which we kept at 30 °C until offspring emergence. We then opened each host pupa and sexed and counted all (adult) offspring to calculate mean brood size and sex ratio.

Experiment 2: size, age, and fighting ability in males

We carried out 3 experiments to test how male fighting ability was influenced by male size, age, and their interaction. We have previously shown that relatively larger males have a higher probability of winning contests (Reece SE, Innocent TM, West SA, forthcoming). Here we examine a) the importance of emergence order (i.e., emerged males vs. unemerged males); b) the interaction between emergence order and size (a measure of fighting ability); and c) the effect of age beyond 24 h and its relative importance compared with variation in size.

Experiment 2a: importance of emergence order

In this experiment, we investigated the effect of emergence order on the fighting success of males using one-on-one arena experiments. We placed pairs of males from the same "size treatment" (see Experiment 2b) within gelatin capsules (volume = 0.21 ml). Each pair consisted of one adult male emerged within the preceding 24-h period and one pupal male due to emerge within the subsequent 24-h period. In order to generate males emerging at 24-h intervals, we set up groups of virgin females every day for 18 days, so that their offspring emerged at 1-day intervals for 18 days.

We generated 2 size classes of male, large and small. To create males of different sizes, we manipulated the number of hosts in a patch on which groups of females were laying eggs in order to vary the density of males developing within hosts. We manipulate host number rather than foundress number because virgin females are unlikely to oviposit if alone (Cooperband et al. 2003). We randomly assigned groups of 60 virgin females to treatments and gave them either 1 or 40 hosts—for large and small treatments, respectively. Males from the high male density treatment (high number of foundresses per host) had significantly larger body length ($F_{116,1}$ = 4.04, $P < 0.05$) and head width ($F_{116,1}$ = 8.74, $P = 0.004$). After 8 h, females were removed and hosts incubated for 6–8 days. At this stage, we opened hosts, dissected male pupae, and isolated them in gelatin capsules (see above). We then took a random sample of pupae from each size class, photographed them using a Leica dissecting microscope, and used Leica IM50 software to take measurements of head width and body length.

For the purpose of identification, we marked adult males on their abdomen with colored glass paint before placing them into capsules with pupae. We checked each pair every 24 h and recorded the date of pupal emergence, the identity of the winner and loser (where loser is defined as the first to die), and the longevity of both males. We discounted any replicates where there was no clear winner (i.e., neither male was dead) within 24 h after the date of pupal emergence, as contestants were no longer considered to be emerging beyond this point.

Experiment 2b: importance of emergence order and size

In this experiment, we wanted to examine the relative importance of emergence order and size on contest outcome. We created different sized males as described in experiment 2a but then incorporated the 2 size classes in a fully factored design to include the asymmetric pairings (i.e., large vs. small, small vs. large). All pairs were composed of one adult male and one pupal male, giving the following fight combinations: large adult versus large pupa; large adult versus small pupa; small adult versus small pupa; and small adult versus large pupa. Once again we checked each pair every 24 h and recorded the date of pupal emergence, the identity of the winner and loser (where loser is defined as the first to die), and the longevity of both males.

Experiment 2c: size versus age experiment

We experimentally manipulated both the age and the size of male opponents within contests to examine the relative importance of these factors on fighting success.

The key difference between this and the previous experiment is that in this case, we examined the effect of age beyond the 24-h window around emergence.

We used size treatments as described above in 2a and also created 3 age classes of male (young, middle aged, and old). To generate males of different age classes within size treatments, we staggered the hosting of groups of virgin females by 1-day intervals. We set up groups of virgin females for each size treatment as described above, every 24 h, for 18 days. We limited females to an 8-h oviposition period in order to minimize overlap in the age of males from successive days. We were able to collect male pupae after 6–8 days and then each day for an 18-day period, recording the date of emergence (and thus age) of every cohort. Experimental age classes were determined by preliminary work (Innocent TM, Savage J, Reece SE, West SA, unpublished data) based on the longevities of a sample of 50 virgin males isolated in gelatin capsules and incubated at 30° C. We categorized males as "young" on the day they emerged (0–24 h); "middle aged" 4 days after emergence (72–96 h); and "old" 7 days after emergence (144–168 h).

We placed 2 adult males into a gelatin capsule (volume = 0.21 ml) marked with gold or pink glass paint for identification. We randomly allocated males to pairs using a fully factored design to set up all possible age–size combinations resulting from our 2 size classes and 3 age classes: large and small males considered young, middle-aged, or old against both large and small males from all age categories. We also cross-factored paint color marking (gold or pink) with all size and age treatment combinations. We checked all arenas every 24 h and recorded the identity and longevity of both the winning and losing male (as defined by order of death). We also set up controls from both size classes, placing individual males in capsules and recording longevity.

Statistical analysis

For maximum power when analyzing proportion data, analyses should assume binomial errors and use a logit link

function—as proportion data often have nonnormally distributed error variance and unequal sample sizes (Crawley 1993; Pickering et al. 2000). In this case, analyses using generalized linear models (GLMs) are simplified using analyses of deviance, in which changes in deviance are compared with a chi-squared distribution. However, proportion data can be overdispersed (residual deviance > residual degrees of freedom), and this can lead to overestimation of significance. If the dispersion parameter (heterogeneity factor [HF]) is <4, data can be scaled and F tests used to assess significance (Crawley 2002). We carried out our analyses using R (R: Copyright 2005, The R Foundation for Statistical Computing, version 2.1.0). We used binomial errors and chi-squared distributions as, in all cases, HFs were <1. Nonproportion data conformed to assumptions of GLM analyses using normal error distributions. We used the probability of the focal male winning the contest [P(winning)] as our GLM response variable, making it possible to fit size and age data as explanatory variables (Hardy and Field 1998). We tested whether the offspring sex ratios produced by females showed less than binomial variation using the regression method developed by Green et al. (1982) and described in further detail by West and Herre (1998a).

RESULTS

### Experiment 1: sex ratio adjustment and LMC

There was a significant increase in sex ratio with increasing foundress number ($\chi_4^2 = 13.60$, $P = 0.0001$; Figure 1). Treatments of 3, 10, 25, and 50 foundresses do not have significantly different sex ratios from one another but do have significantly less female-biased sex ratios than the single foundress treatment (treatment contrasts: $P > 0.05$ for all comparisons of 3, 10, 25, and 50 foundresses and $P < 0.05$ for single foundress compared with multifoundress treatments). However, this effect represents a very slight shift in sex ratio of 3% (treatments mean ± standard error for single foundresses = 0.036 +0.008, −0.007, and multifoundresses = 0.069 +0.015, −0.012). The sex ratio was significantly lower when females were given 48 h to oviposit compared with 24 h ($\chi_1^2 = 14.90$, $P = 0.009$; mean difference = 3.8 ± 0.3%; Figure 1). However, at 25 foundresses, the effect of time was not significant, resulting in a significant interaction ($\chi_4^2 = 10.62$; $P = 0.031$) between foundress number and oviposition duration.

The sex ratios produced by females when ovipositing alone showed significantly less than binomial variation (termed precise sex ratios). We calculated the ratio of observed variance in the sex ratio versus that compared with that expected given a binomial (random) distribution. If the ratio of these numbers, termed the green variance (GV), is significantly less than one, then this indicates that the sex ratios produced by females are precise and show less than binomial variation. We found that the variation in the offspring sex ratio was significantly less than binomial for both the 24-h (GV = 0.21; $\chi_7^2 = 1.49$; $P = 0.02$; $n = 9$) and 48-h (GV = 0.07; $\chi_7^2 = 0.52$; $P = 0.0006$; $n = 9$) oviposition periods.

Experiment 2: size, age, and fighting ability in males

Experiment 2a: importance of emergence order

Out of a total of 80 fights, 37 had a clear winner after 24 h; therefore, only these fights were considered in the analysis. Amongst these, emergence order had a highly significant effect on the likelihood of winning a fight, with 92% (34 of 37) of fights won by the male that was already emerged ($\chi_1^2 = 26.00$, $P < 0.001$; Figure 2a).
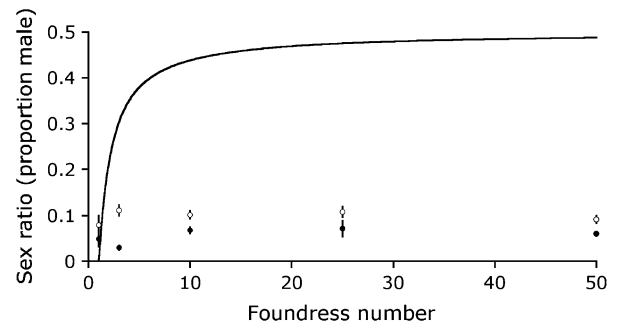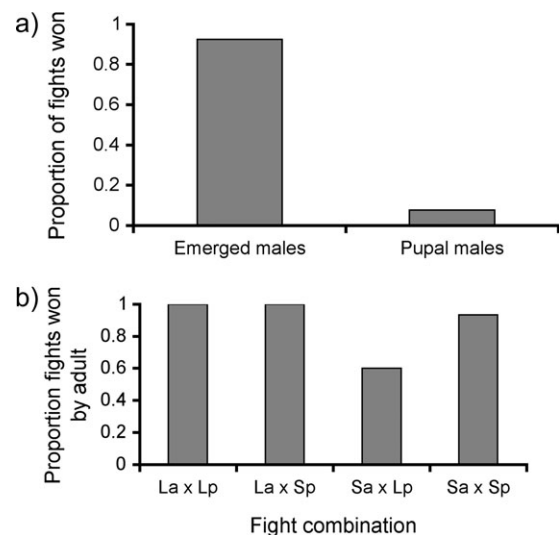

Figure 1

Mean brood sex ratios (proportion male) for each foundress number treatment when females are given either 24 h (open symbols) or 48 h (closed symbols) to oviposit. Note that in all cases of multifoundress treatments, observed sex ratios are lower than that predicted by LMC theory (solid line). The bars show asymmetric standard errors.

Experiment 2b: importance of emergence order and size

Out of a total of 144 fights, 52 had a clear winner after 24 h; therefore, only these were considered in the analysis. Once again, emergence order had a significant effect on the likelihood of the emerged adult male winning when fighting a smaller or size-matched opponent (93–100% of fights won by the adult male in large adult vs. small pupa; large adult vs. large pupa; and small adult vs. small pupa; Figure 2b). In the fourth group, where the size asymmetry favored the pupal male, the effect of emergence order on the probability of winning was significantly less than expected. The probability of the emerged adult male winning in the small adult versus large pupa treatment was significantly lower than all other treatments ($\chi_3^2 = 12.96$, $P = 0.005$; Figure 2b). This shows an interaction between size and emergence, where emerged males have a clear competitive advantage over emerging males, but only when fighting same-sized or smaller opponents.

Figure 2

(a) Proportion of fights won by emerged adult and emerging pupal males, when fighting a size-matched opponent. (b) Proportion of fights won by the already emerged adult male in each of the 4 fight combinations, where La = large adult, Lp = large pupa, Sa = small adult, and Sp = small pupa.
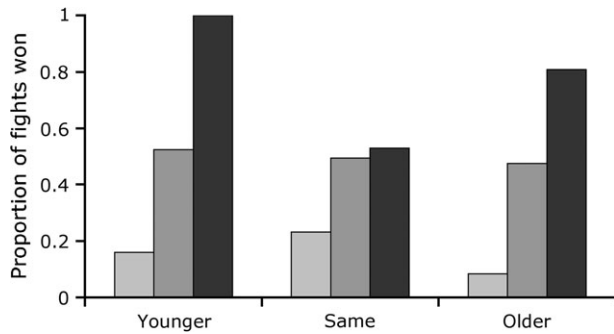
Figure 3
Proportion of fights won by focal males plotted against the age of focal males relative to their opponents. Shading represents the size of focal males relative to their opponents: smaller (unshaded bars); same size class (light gray); larger (dark gray).

Experiment 2c: size versus age experiment
Experimental males had significantly shorter longevity than control males ($F_{1,367} = 379.80$, $P < 0.0001$), suggesting a cost of fighting. Identification color had no effect on either the longevity ($F_{1,317} = 0.09$, $P > 0.05$) or the probability of winning ($\chi^2_1 = 0.53$, $P > 0.05$); we therefore chose to use gold males as our focal males for further analyses.

When fitting the size and age of the focal male, irrespective of their opponent's characteristics, large males are significantly more likely to win contests ($\chi^2_{1,317} = 23.88$, $P < 0.0001$), but there was no effect of the absolute age of the focal male. Large focal males won 27% more contests than small focal males.

When the relative difference in size and age between the focal male and his opponent is considered, relatively larger males have a significantly higher probability of winning contests ($\chi^2_{2,312} = 52.97$, $P < 0.0001$; Figure 3). Fight outcome was only influenced by relative age when the opponents were the same age: in this case, the advantage of being large is reduced, but there is still a disadvantage of being smaller ($\chi^2_{8,304} = 18.87$, $P = 0.02$).

DISCUSSION

We have shown that average sex ratios in *M. acasta* are extremely female biased (9% male) and shift only marginally in response to foundress number (Figure 1). In addition, when ovipositing alone, females produce precise sex ratios with less than binomial variation, as is expected and observed in species with LMC (Green et al. 1982; Hardy 1992; Morgan and Cook 1994; Hardy and Cook 1995; West and Herre 1998a; Lopez-Vaamonde et al. 2005). Amongst fighting males, we have shown that asymmetry in emergence order of opponent's leads to a significant competitive advantage for early emerging adult males: these males are better able to attack emerging males during or immediately after emergence (Figure 2a). Nonetheless, this advantage is mediated by the relative size of opponents—if the later emerging male is larger, then this can override their opponent's advantage from early emergence (Figure 2b). Furthermore, beyond a critical window of emergence (~24 h), age is less important, and size is the major determinant of fighting success (Figure 3). Abe, Kamimura, Ito, et al. (2003) suggest that fatal fighting can explain female-biased sex ratios if we assume that the early emerging male has a very large advantage compared with later emerging males, such that early emerging males have approximately 100% survival and later males have approximately 5% survival relative to early emerging males. Our experimental results suggest that the advantage to earlier laid males in fatal fights may not be sufficiently strong to explain the lack of sex ratio shift in *M. acasta* (Figure 1).

Our contest data suggest that the probability of winning a fight can be divided into 2 parts: that resulting from emergence order and that concerning the time beyond the emergence period. Emerged adult males are able to kill emerging males (Figure 2a,b), and behavioral observations suggest that this advantage may be due to attacking before emergence is complete. After this critical period, any advantage of emergence order is reduced, and size becomes the key factor: large males have a considerable competitive advantage (Figure 3). If slower development allows larger body size, this raises a series of questions about how the success associated with earlier emergence is traded off against body size. More generally, the importance of size can be context dependant as, for instance, competitor density is known to influence both the frequency and severity of contests (Murray and Gerrard 1984, 1985; Murray 1987; West et al. 2001; Griffin and West 2002; Reinhold 2003; Reece SE, Innocent TM, West SA, forthcoming), and opponent assessment is predicted to decrease contest frequency (Enquist and Leimar 1983; Leimar and Enquist 1984; Gammell and Hardy 2003; Reinhold 2003). However, in *M. acasta*, there is no evidence of opponent assessment, suggesting that all males will fight in any contest regardless of opponent because of the high stakes involved (Reece SE, Innocent TM, West SA, forthcoming). This is likely to be due to the large current value of the reward in comparison with its future value (Maynard Smith and Price 1973; Enquist and Leimar 1983, 1987, 1990). For *Melittobia*, this explanation applies to both competitors in a fight, as all males are expected to emerge (and fight) before any of them get to mate (Balfour Browne 1922; Dahms 1984): this is not simply an explanation for early male advantage but a potential reason why any male should ever fight. Consequently, size is likely to be considered very important in determining male fitness.

Although we found a statistically significant shift in sex ratio with the number of females laying eggs on a patch, this shift is much smaller than predicted by LMC theory. For example, the predicted sex ratio for the 50 foundress treatment = 0.49, whereas the observed sex ratio is 0.08 (Hamilton 1967, 1972). In addition, we observed that the 24-h treatment had a significantly higher sex ratio than the 48-h treatment for all foundress numbers. A possible explanation for this may be the laying order of sons and daughters: this sex ratio pattern would occur if females chose to lay sons at the beginning of the oviposition period and then daughters as time went on (Van den Assem et al. 1980). Because we have shown that earlier emerging *M. acasta* males have a greater chance of winning fights, laying sons first would maximize their chance of success. Moreover, the fact that we have observed significantly less than binomial variation in offspring sex ratios shows that females can choose with precision when to produce sons.

Does lethal male combat provide a reasonable explanation for the relative lack of sex ratio adjustment observed in *M. acasta* and other *Melittobia* species (Abe, Kamimura, Ito, et al. 2003; Abe, Kamimura, Kondo, et al. 2003; Abe et al. 2005)? We have shown that earlier emerging males are able to kill later emerging males (Figure 2), as was also shown previously for *M. australica* (Abe et al. 2005). However, in order to completely explain the lack of conditional sex ratio, the advantage to early emerging males has to be very large (see above). Our results suggest 2 reasons why this may not be the case: 1) this age advantage only exists for a brief critical window around the time that the later emerging male is emerging (Figure 3) and 2) variation in male size can reduce this advantage (Figure 2b). Our data therefore suggest that although there is a possible role for lethal male combat in sex allocation (Abe, Kamimura, Ito, et al. 2003; Abe et al. 2005), it cannot fully explain the sex ratio pattern in *M. acasta*. Furthermore, the

life history of this species suggests that brood emergence can take place over a relatively lengthy time, which is longer than male longevity (Buckell 1928; Dahms 1984; Gonzalez, Teran, and Matthews 2004; Abe et al. 2005). This means that even when females arrive sequentially on a patch, there can still be considerable overlap in the timing of when their broods emerge, blurring the distinction between early and late emerging males (Van den Assem et al. 1980; Gonzalez, Teran, and Matthews 2004; Abe et al. 2005). In this case, the pattern of emergence and fighting may show some similarity to fig wasps, where conditional sex ratio adjustment has been documented numerous times (Frank 1985; Herre 1985, 1987; Herre et al. 1997, 2001; West and Herre 1998b; Kinoshita et al. 2002; Moore et al. 2002; Molbo et al. 2003). In order to further assess the importance of male combat for sex ratio adjustment, it would be extremely useful to obtain data on natural populations to complement the available data from lab cultures (see Methods for summary of natural history), examining variation in male size, synchrony of male emergence period, and the temporal distribution of fighting, in conjunction with data on the simultaneous variation in host size/species/quality.

For example, if it was found that competition for resources led later emerging males to be smaller, then this would select against producing later males, and hence could potentially increase the likelihood of lethal combat providing an explanation for the relative lack of sex ratio adjustment. We conclude by discussing 2 alternative possibilities for the lack of sex ratio adjustment in *Melittobia* species (Abe, Kamimura, Ito, et al. 2003; Abe, Kamimura, Kondo, et al. 2003; Cooperband et al. 2003; Abe et al. 2005). One possibility is that selection for sex ratio adjustment has been too weak. If females in a natural environment only ever encounter single foundress situations, then there will be weak selection for altering sex ratio behavior in multifoundress situations (Herre 1987; Herre et al. 1997, 2001). However, multiple *Melittobia* females often appear to lay eggs on a single host in nature (Freeman and Ittyeipe 1976; Freeman 1977; Molumby 1996; Cooperband et al. 2003). A second possibility is that cofounding females are usually highly related, in which case much smaller sex ratio shifts are predicted by LMC theory (Frank 1985; Frank 1986b; Shuker et al. 2004). When females are related, they are also related to each other's sons; therefore, a more female-biased sex ratio is favored to reduce competition between males and provide more mates for them (Frank 1986b). The natural history of *Melittobia* species and the presence of a nondispersing female morph suggest that cofoundresses will often be highly related (Schmeider 1933; Freeman and Ittyeipe 1976, 1982; Van den Assem et al. 1980; Dahms 1984; Consoli and Vinson 2002; Cooperband et al. 2003; Gonzalez, Abe, and Matthews 2004). Study of natural populations will be necessary to fully understand the frequency of multifoundress scenarios, the relatedness between these foundress females, and the natural variation in the occurrence of the 2 female morphs. Furthermore, competition between related females could select for less female-biased sex ratios, highlighting the need for specific theory that takes life history details into account (Frank 1985, 1986b; Bulmer 1986; Taylor and Crespi 1994; Courteau and Lessard 2000; West et al. 2002). This also raises the possibility that we would expect different patterns of sex ratio adjustment between dispersing and nondispersing females (Taylor and Crespi 1994).

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