

# INEXPLICABLY FEMALE-BIASED SEX RATIOS IN *MELITTOBIA* WASPS

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The sex ratio behavior of parasitoid wasps in the genus *Melittobia* is scandalous. In contrast to the prediction of Hamilton's local mate competition theory, and the behavior of numerous other species, their extremely female-biased sex ratios (1–5% males) change little in response to the number of females that lay eggs on a patch. We examined the mating structure and fitness consequences of adjusting the sex ratio in *M. australica* and found that (1) the rate of inbreeding did not differ from that expected with random mating within each patch; (2) the fitness of females that produced less female-biased sex ratios (10 or 20% males) was greater than that of females who produced the sex ratio normally observed in *M. australica*. These results suggest that neither assortative mating nor asymmetrical competition between males can explain the extreme sex ratios. More generally, the finding that the sex ratios produced by females led to a decrease in their fitness suggests that the existing theory fails to capture a key aspect of the natural history of *Melittobia*, and emphasizes the importance of examining the fitness consequences of different sex ratio strategies, not only whether observed sex ratios correlate with theoretical predictions.

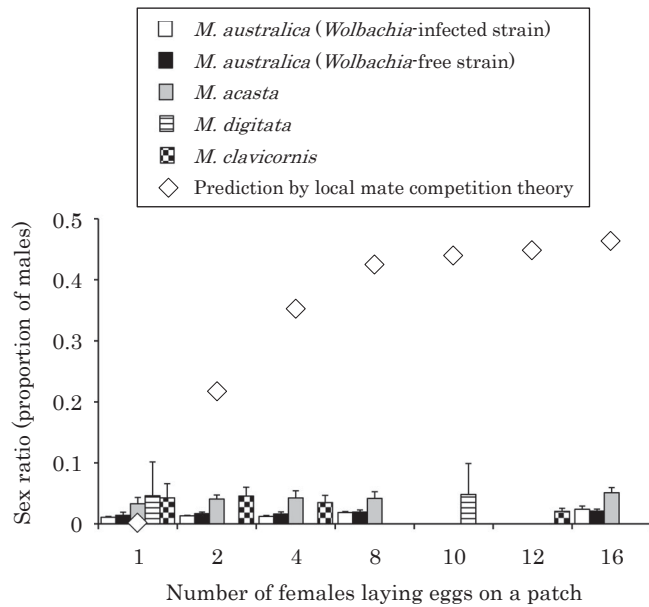
**KEY WORDS:** Adaptive behavior, evolutionarily stable strategy, game theory, lethal male combat, local mate competition, sex allocation.

The study of sex allocation is one of the most productive fields in evolutionary biology (Charnov 1982; Godfray 1994; Hamilton 1996; Frank 1998; Hardy 2002; West 2009). Various theories predict how offspring sex ratios should vary with environmental situations; there is a large empirical literature providing support for these predictions, with both observational and experimental data (Charnov 1982; West 2009). Possibly the most successful area of sex allocation is Hamilton's local mate competition (LMC) theory, which predicts that when the offspring of one or a few mothers mate among themselves in their natal patch before the daughters disperse, natural selection favors a female-biased sex ratio (Hamilton 1967). The female bias arises because it reduces competition among brothers for mates, and increases the number of mates for sons (Taylor 1981). Hamilton (1967) predicted that the evolutionarily stable sex ratio (proportion of males) is  $(n - 1)/2n$ , where  $n$  is the number of diploid females that lay eggs on the patch. Sex ratios thus decline from 0.5 for large  $n$  to 0 when  $n = 1$  (Fig. 1). The latter is interpreted as meaning that a female

should produce the minimum number of males required to fertilize all of her daughters. Numerous empirical studies have supported LMC theory by showing female-biased sex ratios in species where LMC is expected to occur, and facultative adjustment of offspring sex ratios in response to the number of females ( $n$ ) laying eggs on a patch (Charnov 1982; West et al. 2005; West 2009). Furthermore, in cases where the theory has been adjusted to consider biological details relevant to certain species, this has led to a closer fit between theory and data, for example, when allowing for haplo-diploid genetics (Frank 1985; Herre 1985), variation in the relative clutch size of females (Suzuki and Iwasa 1980; Werren 1980; Stubblefield and Seger 1990; Flanagan et al. 1998), the rate at which males disperse from patches (Frank 1986b; West and Herre 1998), and asymmetrical competition within patches (Abe et al. 2003a; Shuker et al. 2005).

However, a number of cases do not appear to be consistent with the existing theory (Orzack et al. 1991; Molbo and Parker 1996). Even when the predictions of modified models for





**Figure 1.** Observed sex ratios in *Melittobia* species (bars) and predicted sex ratios for haplo-diploid species by local mate competition theory (diamond; Hamilton 1979) depending on the number of females laying eggs on a patch. Observed data are from Abe et al. (2003b) for the *Wolbachia*-infected and *Wolbachia*-free strains of *M. australica*, Kamimura et al. (2008) for *M. acasta*, Cooperband et al. (2003) for *M. digitata*, and unpublished data (J.Abe) for *M. clavicornis*. Data from Innocent et al. (2007) are not shown because the clutch sizes are too small. Error bars indicate standard errors of the replicates.

different situations qualitatively fit the patterns of observed sex ratios, there remain unexplained variations with observed sex ratios being more female-biased in most cases (Herre et al. 1997; Reece et al. 2004; Shuker et al. 2004, 2005, 2006). The most striking of these is the extremely female-biased, and relatively invariant, sex ratios in the parasitoid wasp genus *Melittobia* (Werren 1987; Orzack 2002; Abe et al. 2003b, 2005, 2009, 2010; Cooperband et al. 2003; González et al. 2004; Innocent et al. 2007; Kamimura et al. 2008; Abe and Kamimura 2012). The life history of *Melittobia* wasps matches the key assumptions of the LMC theory, as mating and mate competition occurs within natal patches. In contrast to the prediction of LMC theory, the sex ratios of *Melittobia* species change very little with the number of females laying eggs on a patch, and remain at 1–5% males (Fig. 1). One of the potential explanations for this is that mate competition is asymmetrical, with later-emerging males more likely to be killed by males that emerge earlier (Abe et al. 2003b, 2005; Innocent et al. 2007, 2011; Reece et al. 2007), which could reduce the relative value of males and lead to a more female-biased sex ratio (Abe et al. 2003a, 2007, 2009). Another potential explanation is that there is a greater than expected rate of sibmating, which increases com-

petition for mates between closely related males, and, hence, also leads to more female-biased sex ratios (Nunney and Luck 1988).

Here, we tested these possible explanations for the greater than expected female bias in the species *Melittobia australica*. First, we determined the within-patch mating structure using two matrilineages that were fixed for different microsatellite alleles. A constructed mating structure within the natal patches (asymmetrical LMC) and a tendency for individuals to mate with brothers or sisters (assortative mating) selects for more female-biased sex ratios because these increase the frequency of sibling competition for mates (Nummery and Luck 1988; Abe et al. 2003a; Shuker et al. 2005). Second, we examined the fitness consequences of different sex ratios directly by manipulating the offspring sex ratios of each female on a patch, and measuring their fitness. There is a general lack of such data in the field of sex allocation; studies have focused on measuring sex ratio variation, and testing whether this correlates with the predictions of theory, rather than directly testing the fitness consequences of variations in the sex ratio (West 2009). In this experiment, we specifically examined whether the invariantly female-biased sex ratios in *Melittobia* are adaptive in a situation influenced by the effects of lethal male combat.

## Materials and Methods

### STUDY ORGANISM

*Melittobia* is a eulophid genus of gregarious ectoparasitoid wasps that attack the larvae and pupae of solitary bees and wasps (Matthews et al. 2009). Once females find host nests, which are generally built above ground, they make holes in the nest materials to reach the host. A single female can produce >500 eggs on the surface of a single host over several weeks. The eggs hatch and the larvae develop by sucking the host hemolymph from outside in an order almost corresponding to their oviposition order (Abe et al. 2009). Adult females are dimorphic in wing length (Matthews et al. 2009; Innocent et al. 2010), and short-winged morphs emerge earlier than long-winged morphs (Abe et al. 2005, 2009). Adult males are characterized by their reduced wing size, absence of compound eyes, and paler coloration, making it possible to distinguish them from females even in the late pupal stage. Males do not disperse from their natal patches (i.e., host cocoon or cells) and compete with each other to mate with females within the host patches before mated females disperse to search for new hosts, corresponding to an assumption of the LMC theory (Hamilton 1967).

We used wasps from a strain of *M. australica* that was collected in Shiga, Japan in 2000 for the experiments described below (Abe et al. 2005). We previously generated a microsatellite DNA marker from this strain that has two alleles (hereafter called S and R alleles) and can be analyzed with a primer set (MMS7–2; Abe

et al. 2005). We have described the detailed procedures for molecular analyses previously (Abe et al. 2005, 2009). In this study, we extracted DNA from the whole body of pupae and male adults, and the head and thorax of adult females to prevent contamination by sperm DNA. Similar to other Hymenopteran insects, *Melittobia* species have a haplo-diploid sex determination system, with diploid females developing from fertilized eggs and haploid males developing from unfertilized eggs. We used the prepupae of the leaf cutter bee, *Megachile sculpturalis* (Hymenoptera: Megachilidae), as the host unless otherwise described. A prepupae of *M. sculpturalis* can sustain the offspring of two *Melittobia* females with little reduction in offspring number by competition during development (Abe et al. 2003b). We carried out all pretreatments and experiments at 25°C and under 16L:8D light conditions.

### EXPERIMENT 1: MATING STRUCTURE WITHIN PATCHES

We determined the inbreeding rate and the relative reproductive success of each foundress' sons. We generated foundress females using our mating pretreatment described in Abe et al. (2005, 2009, 2010). Briefly, we allowed the females of the long-winged morph that emerged within 24 h to mate with a male for 5 days. We controlled the microsatellite genotypes of the females and their mates; homozygous females were allowed to mate with a male having the same allele. We introduced two mated foundresses with different alleles (SS and RR females) into a plastic case (86 mm in diameter and 20 mm in height) with a host inside its cocoon. Four replicates in which both foundresses entered the host cocoon within 3 days after the introductions were analyzed. We checked each replicate on a daily basis, and counted and collected all dispersing female offspring from the host cocoon. We also collected the foundresses dispersing from the cocoon, which could easily be distinguished from their daughters by their depleted small abdomens. We continued this procedure until day 31 after the foundress introductions to avoid possible contamination of the grandoffspring generation (the offspring generation emerges after ~16 days; under these condition; Abe et al. 2003b, 2005, 2009). On day 31, we dissected each host cocoon, and collected all emerged live and dead males from each replicate to determine the proportion of each foundress' sons. We identified the genotypes of the two foundresses for confirmation and all the males for analyses.

We randomly selected 20 short- and 20 long-winged mated females that dispersed from the cocoon in each replicate. We assigned one prepupae of *Coelioxys fenestrata* (Hymenoptera: Megachilidae) as a host for each female, and allowed them to reproduce. After the emergence of their offspring, we genotyped the females (the offspring of the foundresses) and their three daughters chosen randomly (the grandoffspring of the foundresses). We calculated the inbreeding rates of offspring as the proportion of ho-

mozygous females among the genotyped granddaughters [ $(20 + 20) \times 3$  individuals in each replicate] and obtained the relative reproductive success of S males from the genotyped grandoffspring. We also calculated the expected inbreeding rates of offspring and the expected relative reproductive success of S males when offspring mate randomly within their natal patches as described in Supporting Information. Although *Melittobia* is believed to be monoandrous (van den Assem et al. 1982; Matthews et al. 2009), offspring of at least 20% females showed multiple paternity (see Supporting Information). However, this affects neither the estimate of inbreeding rate nor the predictions of sex allocation.

### EXPERIMENT 2: FITNESS CONSEQUENCES OF ADJUSTING OFFSPRING SEX RATIOS

We determined whether the observed sex ratios were the best possible strategy. We simulated the situation where two virtual foundress females laid eggs on the same host. In this case, LMC theory predicts that each foundresses should produce 21.4% males (Hamilton 1979), whereas *M. australica* females produce 2% males (Abe et al. 2003b, 2005, 2009; Fig. 1). We assumed three types of foundresses that produced offspring with the sex ratio either of the LMC prediction (referred to as Hamiltonian or H), real *Melittobia* wasps (Melittobian, M), or an intermediate value between the two (Intermediate, I). We prepared six replicates for each of all six possible combinations of the two foundresses: two Melittobian (MM), two Intermediate (II), two Hamiltonian (HH), one Melittobian and one Intermediate (MI), one Intermediate and one Hamiltonian (IH), and one Melittobian and one Hamiltonian (MH).

We manipulated the offspring sex ratios of the virtual foundresses, which did not actually exist, by using male and female pupae that were produced by multiple surrogate females (Fig. S2). We obtained a sufficient number of males from the surrogate females using a sterile male technique with  $^{60}\text{Co}$  gamma ray irradiation, in which a female mated with a normal male and subsequently with an irradiated male produced approximately ~22% males with unchanged clutch size (Abe and Kamimura 2012). We confirmed that males produced by this technique could fertilize females, which subsequently produced offspring, with no differences from normal males (J. Abe, unpubl. data). The female pupae were collected from normal females that mated with two normal males. We controlled the genotypes of the male and female offspring of the virtual foundresses to enable identification of the mates of the female offspring (Fig. S2).

Because the outcome of male combat depends on the timing of emergence and the body sizes of the combatant males (Abe et al. 2003b, 2005; Innocent et al. 2007), we also controlled the timing of emergence and body sizes to reflect actual situations. The body sizes of emerging individuals gradually decrease with the lapse of their emergence period. To reflect this, we set the cultures

of the surrogate females within 3 days and used their offspring at the corresponding time. Two surrogate females were allowed to lay eggs on a host for 12 days corresponding to the procedure of Abe et al. (2009). We imitated the emergence schedules of male and female offspring by using the estimated relationships between oviposition and emergence order in *Melittobia* species (Abe et al. 2009) as described in Supporting Information. According to the simulated emergence schedules for each type of virtual foundress, we added lightly pigmented pupae, which generally emerge in ~2 days, on a daily basis. These pupae were introduced into a plastic case whose size mimicked the *M. sculpturalis* host cocoon (Fig. S2). The plastic case had a small hole, through which emerged females could disperse outside. The cocoon-mimicking case was placed inside a larger plastic container, allowing us to collect all dispersed females. In parallel with adding the offspring pupae into the cases, we also counted and collected dispersed females from the cases daily. The dispersed females were stocked according to their dispersed days which were divided into six terms (Fig. S2).

We randomly chose 16 females from each term of each replicate, of which eight were dissected to examine the presence of sperm in their spermatheca and to calculate the insemination rate of the dispersed females (Fig. S2). We allowed the other eight females to lay eggs on a host. After their offspring developed, we randomly collected 30 female pupae and analyzed their microsatellite genotypes to identify their paternity (Fig. S2). Using the information about the number of dispersed females, their insemination rate, and the genotypes of their daughters, we calculated the relative reproductive success of each virtual foundress' sons and the fitness of each virtual foundress as described in Supporting Information.

## STATISTICS

We analyzed all data using the statistical software R version 3.0.0 (R Core Team 2013; <http://www.R-project.org>). We tested inbreeding rates (experiment 1) and the relative reproductive success of males (experiments 1 and 2) using a nonparametric bootstrapping method (2000 iterations), in which a distribution of the observed values was produced and compared with the mean expected values. The bootstrapping consisted of two steps: the replicates were randomly resampled in the first step, following by random resampling of the genotyped grandoffspring from each selected replicate in the second step. The expected values were calculated for each replicate by assuming random mating among the males and females of given numbers according to a binomial distribution. The test statistics were the deviations of the observed (bootstrapped) values from the expected values, both of which were converted with a logit transformation before subtraction. The signed deviations (hereafter referred to as average) were used to determine whether an observed trend deviated in a specific direction (e.g., an excess of sibmating), whereas the

extent of variation (hereafter referred to as variance) was tested using the absolute values of the deviations (e.g., overdispersion from a binomial distribution). For the latter purpose, the expected mean deviations were calculated for each replicate by a parametric bootstrap method with 2000 iterations. We performed a power analysis to evaluate the statistical power of our tests for cases with no significant differences, using a parametric bootstrap method (2000 iterations) that was essentially identical to that of Abe and Kamimura (2012). We used a general linear model to analyze the fitness consequences of the foundresses assuming a normal distribution with the same variance. We included the terms for the types of virtual foundress as an explanatory variable, and tested significance with the *F*-statistic by comparing the change in deviance after removing the term. The cutoff point for rejecting of the null hypothesis was adjusted by the method of Benjamini and Hochberg (1995) for all the statistic comparisons described above to resolve the problem of multiple comparisons caused by repeated tests for different combinations.

## Results

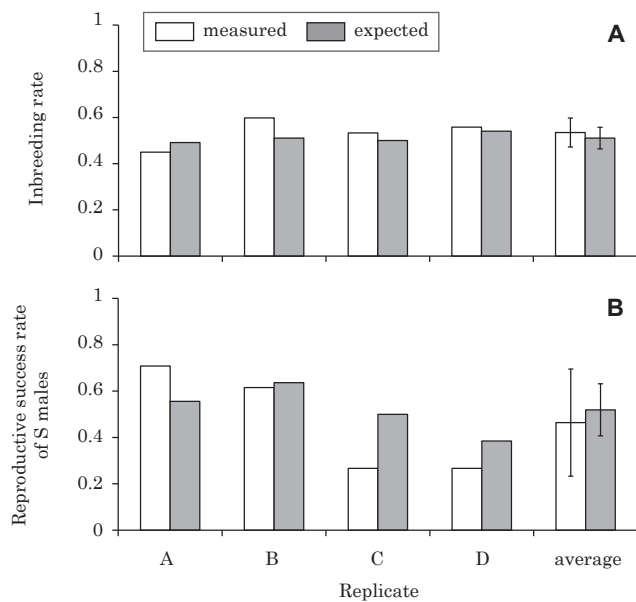
### EXPERIMENT 1: MATING STRUCTURE WITHIN PATCHES

In all four replicates, 12–22 males were emerged with more than 300 females (see Supporting Information for details). Genotyping of them and their offspring revealed that neither the average nor the variance of the inbreeding rate deviated significantly from expected values, which were calculated assuming that all males equally and randomly contributed to the matings within the patches ( $P = 0.44$  and  $0.35$ , respectively; Fig. 2A). A post hoc power analysis showed that our method could detect deviations of  $\pm 0.08$  for average and  $0.1$  for variance as significant (with  $\alpha = 0.05$  and  $1 - \beta = 0.8$ ). Although the relative reproductive success of S males was not significantly different from that expected from their numerical representations in the host ( $P = 0.49$ ; the statistical method applied could detect a difference of  $\pm 0.08$ ), the realized values were more dispersed than expected from a binomial distribution ( $P = 0.005$ ; Fig. 2B). This result indicates that sons derived from one foundress tended to dominate mating success regardless of their genotype.

### EXPERIMENT 2: FITNESS CONSEQUENCES OF ADJUSTING OFFSPRING SEX RATIOS

The usage of cocoon-mimicked case had no apparent effect on the development, emergence, and dispersal patterns of offspring (see Supporting Information for details). In all combinations of the different types of virtual foundresses (i.e., MI, IH, and MH combinations), the mean relative reproductive success of male offspring was not significantly different from the expected values





**Figure 2.** The inbreeding rate (A) and relative reproductive success of S males (B) of four replicates and the average in experiment 1. Unshaded and shaded bars represent measured and expected values, respectively. Error bars on the average bars indicate standard deviations of the replicates. Error bars on the shaded bars are expected standard deviations estimated by a parametric bootstrap method assuming binomial distributions (2000 iterations).

that assumed random mating within the patches, although the IH combination was marginally significant ( $P = 0.076$  cf.  $P > 0.43$  for the other cases; Fig. 3). The minimum significant difference that we could detect was  $\pm 0.07$ . Although the measured variances in relative reproductive success were larger than expected in the three combinations, the differences were marginal (all  $P < 0.072$ ), with the exception that the MI combination was significant after correcting for multiple comparisons (Fig. 3). The fitness consequences were significantly different among the three types of virtual foundresses in the three cases where the opponent virtual foundress was Melittobian, Intermediate, or Hamiltonian (Fig. 4; all  $F > 14.7$ , all  $P < 0.001$ , which were significant at  $P < 0.001$  after correcting for multiple comparisons). Post hoc pairwise comparisons revealed that Melittobian had significantly smaller fitness consequences than both Intermediate and Hamiltonian, but no significant differences were observed between Intermediate and Hamiltonian in all three cases of different opponent foundresses (Fig. 4).

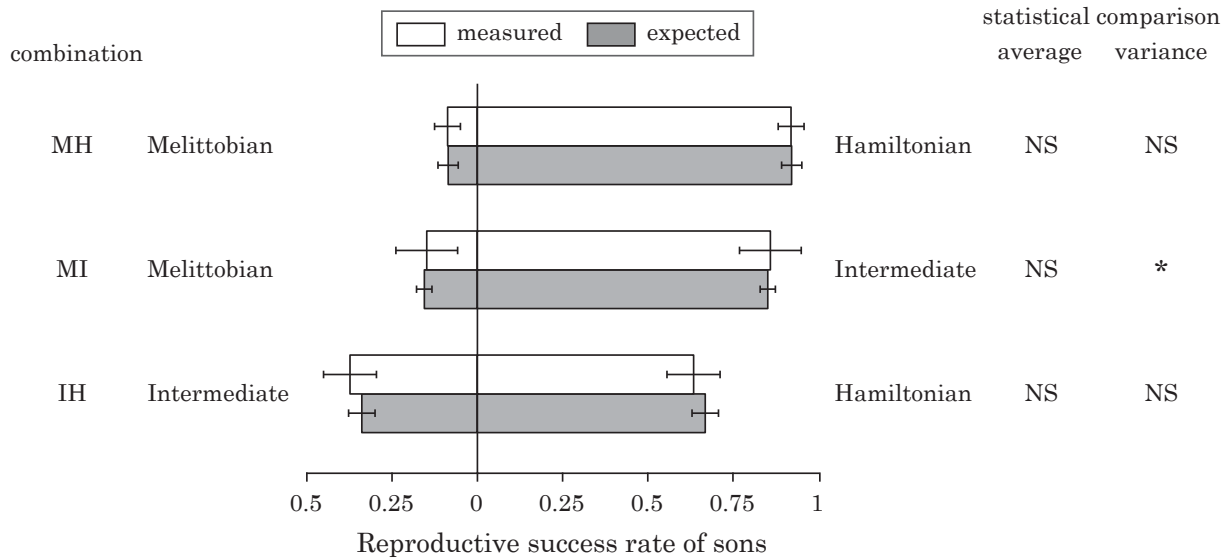
## Discussion

We examined the mating structure and fitness consequences of adjusting the sex ratio in *M. australica* when two females laid eggs on a patch. We found that (1) the rate of inbreeding did not differ from that expected with random mating within each patch (Fig. 2);

(2) the fitness of females who produced less female-biased sex ratios (10 or 20% males) was greater than that of females that produced the sex ratio normally observed in *M. australica* (2% males; Fig. 4). These results suggest that neither a tendency for individuals to mate with a brother or sister (assortative mating) nor asymmetrical competition between males that could be generated by lethal combat could explain the lack of sex ratio adjustment in *Melittobia* species. More generally, our results emphasize the usefulness of quantifying the actual fitness consequences of sex ratio manipulations (Komdeur 1998; Paul et al. 2000; Badyaev et al. 2001; Reece et al. 2008), rather than simply examining whether an observed sex ratio variation is consistent with the prediction of a particular theory.

Previous work on LMC has generally assumed that mating occurs as random within patches (Hamilton 1967). If mating opportunities are asymmetrical among the sons produced by different females, then the females are predicted to adjust their offspring sex ratios accordingly (Abe et al. 2003a; Shuker et al. 2005). Shuker et al. (2005, 2006) found that females of the parasitoid wasp *Nasonia vitripennis* achieved this by laying a less female-biased sex ratio in cases where their offspring would encounter mate competition from males previously laid on the patch. In contrast, we found that males and females of *M. australica* mated randomly within their cocoon (Fig. 2), although paternity tended to be biased toward the sons of one foundress (Figs. 2B and 3), which would be expected if male combat skewed mating success toward a particular male or males. One plausible cause of this skew is difference in the timing of the two foundresses starting to lay eggs. However, the sex ratios of *Melittobia* change little even when two foundresses are asynchronously allowed to parasitize (Abe et al. 2005).

Even if mating opportunities are equal among sons produced by different females, evolutionarily stable sex ratios could be biased by asymmetrical competition among sons produced by the same female (Abe et al. 2007). Lethal male combat in *Melittobia* could bias the sex ratio toward females, because later-emerging males are often killed by males that have already emerged, even if they are brothers (Abe et al. 2005; Innocent et al. 2011). As predicted by a dynamic game model with the effects of the asymmetrical lethal combat (Abe et al. 2007), a small number of males are produced intermittently throughout the emergence period in *Melittobia* species (Abe et al. 2009). However, our manipulation of offspring sex ratio experiment showed that the excessively female-biased sex ratios were not beneficial for ovipositing females under conditions that mimicked a real situation. The relative reproductive success of males did not deviate from random regardless of the sex ratios produced by their mothers (Fig. 3), and the measured fitness consequences suggested that the real sex ratio of *Melittobia* (2% males) could be outcompeted by females producing less female-biased sex ratios (10–20% males; Fig. 4).



**Figure 3.** The mean relative reproductive success of males in MH, MI, and IH combinations in experiment 2. Unshaded and shaded bars represent measured and expected values, respectively. Error bars are the standard deviations of six replicates. Error bars on the shaded bars are expected standard deviations estimated by a parametric bootstrap method assuming a binomial distribution (2000 iterations). The result of comparison between the measured and expected values after correcting for multiple comparisons is shown; \* $P < 0.05$ .

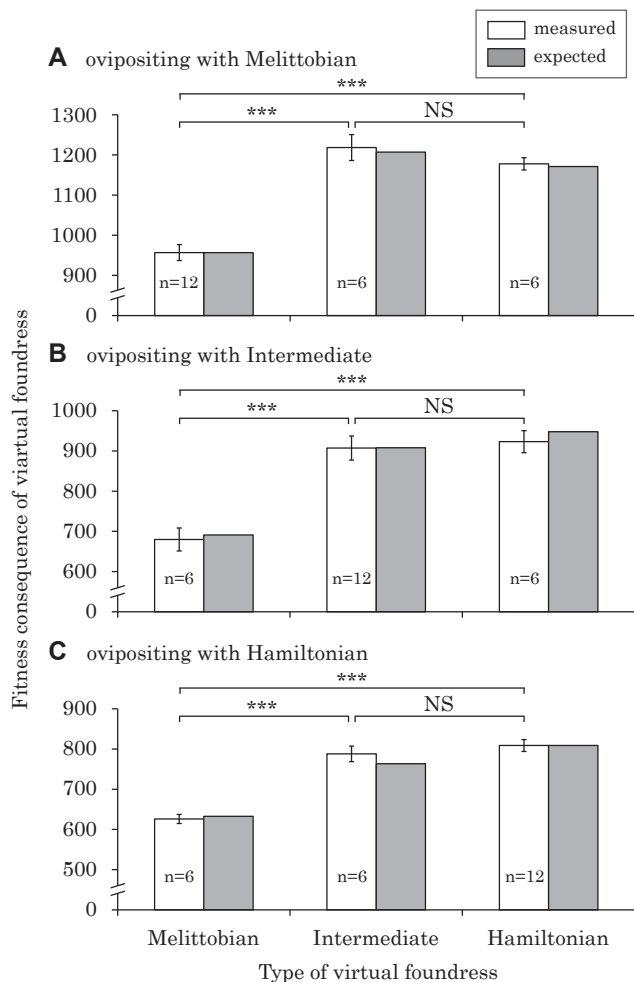
One possible explanation for the observed maladaptiveness of Melittobians is that our artificial manipulation procedure caused unnatural behavior. First, individuals in the offspring generation that we supposed were derived from a single foundress were actually a mixture from multiple surrogate females. However, males kill other males independently of their relatedness (Abe et al. 2005), and the results of experiment 1 showed that mating was random with respect to kinship (Fig. 2A). Second, because we manipulated offspring sex ratios at the late pupal stage, we could not consider events that occurred at earlier stages. Although *Melittobia* males have been reported to attack other males of adult or late pupae stages (Abe et al. 2005; Dayrup et al. 2006), there is no information regarding behavioral interactions between larvae or the influence of emerged males on younger juveniles. However, sex ratios at oviposition are not different from those at emergence in *Melittobia* (Abe et al. 2009), indicating that such events are unlikely to be important. Moreover, adult *Melittobia* females appear to be unable to perceive the offspring sex ratios produced by other females (Abe and Kamimura 2012). Adult males are also unlikely to discriminate male juveniles and attack them. Third, our experimental set up might have lacked some key feature associated with natural hosts.

Another potential explanation for the extremely female-biased sex ratio of *Melittobia* is that it represents a cooperative behavior (West et al. 2007a, b) that increases the overall reproductive productivity of a patch (compare the results of MM with those of II and HH in Fig. 4). Theory predicts that reciprocity could lead to this, if a restricted number of females continue to

lay eggs for a prolonged period, they can monitor the sex ratios produced by other females, and they can adjust their own sex ratios accordingly (Kamimura et al. 2008). However, *Melittobia* females do not change their offspring sex ratios even when their artificially manipulated counterparts lay eggs in a less female-biased sex ratio, suggesting that reciprocity is not a factor in the extremely female-biased sex ratio (Abe and Kamimura 2012).

Could our results be explained by alternative frameworks such as multilevel selection (van Veelen 2009; Simon 2014)? Selection for female-biased sex ratios due to LMC has been examined with various frameworks (Frank 1998). However, although these different methodologies use different notational conventions and tools, they do not make different predictions (Frank 1986b, 1998, 2013; Gardner et al. 2009; West 2009). These theories are just alternate ways of conceptualizing the dynamics of natural selection, and still predict that organisms will be selected to maximize their inclusive fitness (Hamilton 1975; Wade 1985; Frank 1986b; Queller 1992; reviewed by West and Gardner 2013). Consequently, we suggest that possible explanations for the sex ratios of *Melittobia* must come from determining what aspects of the biology in the natural environment the existing theory has not included.

We suggest two potential explanations for the extremely female-biased and relatively invariant sex ratios of *Melittobia* wasps. First, if only one female usually lays eggs on a patch in the natural environment, then there will be weak selection for altering sex ratio behavior in multi-female situations (Herre 1987). This is a specific case of the more general point that organisms can



**Figure 4.** The mean fitness consequence of a virtual foundress that was supposed to lay eggs either with a Melittobian (A), Intermediate (B), or Hamiltonian (C) in experiment 2. Unshaded and shaded bars represent measured and expected values, respectively. Error bars are the standard errors of the replicates. Symbols between two unshaded bars indicate the result of a pairwise comparison between the measured fitness consequences after correcting for multiple comparisons;  $***P < 0.001$ .

be constrained from being “perfectly adapted.” However, multiple *Melittobia* females often appear to lay eggs on a single host in nature (Freeman and Ittyeipe 1976; Freeman 1977; van den Assem et al. 1980; Iwata and Tachikawa 1966; Matthews et al. 2009). Field studies have found that variable number of females lays eggs on a single host, with an average foundress number of 1.47 (Freeman and Ittyeipe 1993) and 1.8 (Molumby 1996). This suggests that weak selection is unlikely to provide the answer.

Second, if multiple females lay eggs on the patch and these females are highly related, then this selects for more female-biased sex ratios. The reason for this is that a female-biased sex ratio would reduce mate competition and increase mating opportunities not only for each female’s own sons but also for the

male offspring of their relatives (Grafen 1984; Frank 1985; Shuker et al. 2004; Gardner et al. 2009). Although females cannot directly recognize the relatedness of other ovipositing females in *Melittobia* (J. Abe, unpubl. data) or other parasitoid species (Shuker et al. 2004), relatedness could increase due to limited dispersal in structured population (Hamilton 1964; Nagelkerke and Sabelis 1996; Gardner et al. 2009). The exact predictions for sex ratio evolution in structured populations depend upon biological details, such as the extent to which the benefits of increased relatedness are negated by increased competition for hosts by relatives (Bulmer 1986; Frank 1986a; Taylor 1988; Nagelkerke and Sabelis 1996; West et al. 2002; Grafen 2007; Gardner et al. 2009). Consequently, a key future step is to identify the population structure and pattern of dispersal in natural populations.

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#### LITERATURE CITED

- Abe, J., and Y. Kamimura. 2012. Do female parasitoid wasps recognize and adjust sex ratios to build cooperative relationships? *J. Evol. Biol.* 25:1427–1437.
- Abe, J., Y. Kamimura, H. Ito, H. Matsuda, and M. Shimada. 2003a. Local mate competition with lethal male combat: effects of competitive asymmetry and information availability on a sex ratio game. *J. Evol. Biol.* 16:607–613.
- Abe, J., Y. Kamimura, N. Kondo, and M. Shimada. 2003b. Extremely female-biased sex ratio and lethal male-male combat in a parasitoid wasp, *Melittobia australica* (Eulophidae). *Behav. Ecol.* 14:34–39.
- Abe, J., Y. Kamimura, and M. Shimada. 2005. Individual sex ratios and offspring emergence patterns in a parasitoid wasp, *Melittobia australica* (Eulophidae), with superparasitism and lethal combat among sons. *Behav. Ecol. Sociobiol.* 57:366–373.
- . 2007. Sex ratio schedules in a dynamic game: the effect of competitive asymmetry by male emergence order. *Behav. Ecol.* 18:1106–1115.
- Abe, J., Y. Kamimura, M. Shimada, and S. A. West. 2009. Extremely female-biased primary sex ratio and precisely constant male production in a parasitoid wasp *Melittobia*. *Anim. Behav.* 78:515–523.
- Abe, J., T. M. Innocent, S. E. Reece, and S. A. West. 2010. Virginity and the clutch size behaviour of a parasitoid wasp where mothers mate their sons. *Behav. Ecol.* 21:730–738.
- van den Assem, J., M. J. Gijswijt, and B. K. Nubel. 1980. Observation of courtship and mating strategies in a few species of parasitoid wasps (Chalcidoidea). *Neth. J. Zool.* 30:208–227.
- van den Assem, J., H. A. J. in den Bosch, and E. Prooy. 1982. *Melittobia* courtship behavior: a comparative study of the evolution of a display. *Neth. J. Zool.* 32:427–471.
- Badyaev, A. V., G. E. Hill, M. L. Beck, A. A. Dervan, R. A. Duckworth, K. J. McGraw, P. M. Nolan, and L. A. Whittingham. 2001. Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science* 295:316–318.

- Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* 57:289–300.
- Bulmer, M. G. 1986. Sex-ratio theory in geographically structured populations. *Heredity* 56:69–73.
- Charnov, E. L. 1982. *The theory of sex allocation*. Princeton Univ. Press, Princeton, NJ.
- Cooperband, M. F., R. W. Matthews, and S. B. Vinson. 2003. Factors affecting the reproductive biology of *Melittobia digitata* and failure to meet the sex ratio predictions of Hamilton's local mate competition theory. *Entomol. Exp. Appl.* 109:1–12.
- Deyrup, L. D., R. W. Matthews, and M. Deyrup. 2006. Feeding and siblicidal cannibalism in a male parasitic wasp (Hymenoptera: Eulophidae). *Fla. Entom.* 89:135–140.
- Flanagan, K. E., S. A. West, and H. C. Godfray. 1998. Local mate competition, variable fecundity and information use in a parasitoid. *Anim. Behav.* 56:191–198.
- Frank, S. A. 1985. Hierarchical selection theory and sex ratios II. on applying the theory, and a test with fig wasps. *Evolution* 39:949–964.
- . 1986a. The genetic value of sons and daughters. *Heredity* 56:351–354.
- . 1986b. Hierarchical selection theory and sex ratios I. general solutions for structured populations. *Theor. Popul. Biol.* 29:312–342.
- . 1998. *Foundations of social evolution*. Princeton Univ. Press, Princeton, NJ.
- . 2013. Natural selection. VII. History and interpretation of kin selection theory. *J. Evol. Biol.* 26:1151–1184.
- Freeman, B. E. 1977. Aspects of regulation of size of Jamaican population of *Sceliphron Assimile* Dahlbom (Hymenoptera Sphecidae). *J. Anim. Ecol.* 46:231–247.
- Freeman, B. E., and K. Ittyeipe. 1976. Field studies on cumulative response of *Melittobia* sp (Hawaiiensis complex) (Eulophidae) to varying host densities. *J. Anim. Ecol.* 45:415–423.
- . 1982. Morph determination in *Melittobia*, a eulophid wasp. *Ecol. Entomol.* 7:355–363.
- . 1993. The natural dynamics of the eulopid parasitoid *Melittobia australica*. *Ecol. Entomol.* 18:129–140.
- Gardner, A., A. Arce, and J. Alpedrinha. 2009. Budding dispersal and the sex ratio. *J. Evol. Biol.* 22:1036–1045.
- Godfray, H. C. J. 1994. *Parasitoids: behavioral and evolutionary ecology*. Princeton Univ. Press, Princeton, NJ.
- González, J. M., J. Abe, and R. W. Matthews. 2004. Offspring production and development in the parasitoid wasp *Melittobia clavicornis* (Cameron) (Hymenoptera: Eulophidae) from Japan. *Entomol. Sci.* 7:15–19.
- Grafen, A. 1984. Natural selection, kin selection and group selection. Pp. 62–84 in J. R. Krebs and N. B. Davies, eds. *Behavioural ecology: an evolutionary approach*. 2nd ed. Blackwell Scientific Publications, Oxford, U.K.
- . 2007. Detecting kin selection at work using inclusive fitness. *Proc. R. Soc. Lond. B* 274:713–719.
- Hamilton, W. D. 1964. The genetical evolution of social behavior I & II. *J. Theor. Biol.* 7:1–52.
- . 1967. Extraordinary sex ratios. *Science* 156:477–488.
- . 1975. Innate social aptitudes of man: an approach from evolutionary genetics. Pp. 133–155 in R. Fox, ed. *Biosocial anthropology*. Wiley, New York.
- . 1979. Wingless and fighting males in fig wasps and other insects. Pp. 167–220 in M. S. Blum and N. A. Blum, eds. *Sexual selection and reproductive competition in insects*. Academic Press, New York.
- . 1996. *Narrow roads of geneland volume I: evolution of social behaviour*. Freeman, New York.
- Hardy, I. C. W. 2002. *Sex ratios: concepts and research methods*. Cambridge Univ. Press, Cambridge, U.K.
- Herre, E. A. 1985. Sex ratio adjustment in fig wasps. *Science* 228:896–898.
- . 1987. Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature* 329:627–629.
- Herre, E. A., S. A. West, J. M. Cook, S. G. Compton, and F. Kjellberg. 1997. Fig wasp mating systems: pollinators and parasites, sex ratio adjustment and male polymorphism, population structure and its consequences. Pp. 226–239 in J. C. Choe and B. J. Crespi, eds. *The evolution of mating systems in insects and arachnids*. Cambridge Univ. Press, Cambridge, U.K.
- Innocent, T. M., J. Savage, S. A. West, and S. E. Reece. 2007. Lethal combat and sex ratio evolution in a parasitoid wasp. *Behav. Ecol.* 18:709–715.
- Innocent, T. M., J. Abe, S. A. West, and S. E. Reece. 2010. Competition between relatives and the evolution of dispersal in a parasitoid wasp. *J. Evol. Biol.* 23:1374–1385.
- Innocent, T. M., S. A. West, J. L. Sanderson, N. Hyrkanen, and S. E. Reece. 2011. Lethal combat over limited resources: testing the importance of competitors and kin. *Behav. Ecol.* 22:923–931.
- Iwata, K., and T. Tachikawa. 1966. Biological observations on 53 species of the superfamilies, Chalcioidea and Prototruiudeam from Japan (Hymenoptera: Apocria). *Trans. Shikoku. Entomol. Soc.* 9:1–26.
- Kamimura, Y., J. Abe, and H. Ito. 2008. The continuous public goods game and the evolution of cooperative sex ratios. *J. Theor. Biol.* 252:277–287.
- Komdeur, J. 1998. Long-term fitness benefits of egg sex modification by the Seychelles warbler. *Ecol. Lett.* 1:56–62.
- Matthews, R. W., J. M. Gonzalez, J. R. Matthews, and L. D. Deyrup. 2009. Biology of the parasitoid *Melittobia* (Hymenoptera: Eulophidae). *Annu. Rev. Entomol.* 54:251–266.
- Molbo, D., and E. D. Parker. 1996. Mating structure and sex ratio variation in a natural population of *Nasonia vitripennis*. *Proc. R. Soc. Lond. B* 263:1703–1709.
- Molunby, A. 1996. *The evolutionary ecology of a gregariously nesting wasp, Trypoxylon politum*. Ph.D. dissertation, University of Chicago, Chicago.
- Nagelkerke, C. J., and M. W. Sabelis. 1996. Hierarchical levels of spatial structure and their consequences for the evolution of sex allocation in mites and other arthropods. *Am. Nat.* 148:16–39.
- Nunney, L., and R. F. Luck. 1988. Factors influencing the optimal sex ratio in structured populations. *Theor. Popul. Biol.* 33:1–33.
- Orzack, S. H. 2002. Using sex ratios: the past and the future. Pp. 383–398 in I. C. W. Hardy, ed. *Sex ratios: concepts and research methods*. Cambridge Univ. Press, Cambridge, U.K.
- Orzack, S. H., E. D. Parker, and J. Gladstone. 1991. The comparative biology of genetic variation for conditional sex ratio behavior in a parasitic wasp, *Nasonia vitripennis*. *Genetics* 127:583–599.
- Paul, R. E. L., T. N. Coulson, A. Raibaud, and P. T. Brey. 2000. Sex determination in malaria parasites. *Science* 287:128–131.
- Queller, D. C. 1992. Quantitative genetics, inclusive fitness and group selection. *Am. Nat.* 139:540–558.
- R Development Core Team. 2013. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Reece, S. E., D. M. Shuker, I. Pen, A. B. Duncan, A. Choudhary, C. M. Batchelor, and S. A. West. 2004. Kin discrimination and sex ratios in a parasitoid wasp. *J. Evol. Biol.* 17:208–216.
- Reece, S. E., T. M. Innocent, and S. A. West. 2007. Lethal male-male combat in the parasitoid *Melittobia acasta*: are size and competitive environment important? *Anim. Behav.* 74:1163–1169.
- Reece, S. E., D. R. Drew, and A. Gardner. 2008. Sex ratio adjustment and kin discrimination in malaria parasites. *Nature* 453:609–614.



- Shuker, D. M., S. E. Reece, J. A. L. Taylor, and S. A. West. 2004. Wasp sex ratios when females on a patch are related. *Anim. Behav.* 68:331–336.
- Shuker, D. M., I. Pen, A. B. Duncan, S. E. Reece, and S. A. West. 2005. Sex ratios under asymmetrical local mate competition: theory and a test with parasitoid wasps. *Am. Nat.* 166:301–316.
- Shuker, D. M., I. Pen, and S. A. West. 2006. Sex ratios under asymmetrical local mate competition in the parasitoid wasp *Nasonia vitripennis*. *Behav. Ecol.* 17:345–352.
- Simon, B. 2014. Continuous-time models of group selection, and the dynamical insufficiency of kin selection models. *J. Theor. Biol.* 349: 22–31.
- Stubblefield, J. W., and J. Seger. 1990. Local mate competition with variable fecundity: dependence of offspring sex ratios on information utilization and mode of male production. *Behav. Ecol.* 1:68–80.
- Suzuki, Y., and Y. Iwasa. 1980. A sex ratio theory of gregarious parasitoids. *Res. Popul. Ecol.* 22:366–382.
- Taylor, P. D. 1981. Intra-sex and inter-sex sibling interactions as sex ratio determinants. *Nature* 291:64–66.
- . 1988. Inclusive fitness models with two sexes. *Theor. Popul. Biol.* 34:145–168.
- van Veelen, M. 2009. Group selection, kin selection, altruism and cooperation: when inclusive fitness is right and when it can be wrong. *J. Theor. Biol.* 259:589–600.
- Wade, M. J. 1985. Soft selection, hard selection, kin selection, and group selection. *Am. Nat.* 125:61–73.
- Werren, J. H. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. *Science* 208:1157–1159.
- . 1987. Labile sex ratios in wasps and bees: life history influences the ratio of male and female offspring. *Bioscience* 37:498–506.
- West, S. A. 2009. *Sex allocation*. Princeton Univ. Press, Princeton, NJ.
- West, S. A., and A. Gardner. 2013. Adaptation and inclusive fitness. *Curr. Biol.* 23:R577–R584.
- West, S. A., and E. A. Herre. 1998. Stabilizing selection and variance in fig wasp sex ratios. *Evolution* 52:475–485.
- West, S. A., I. Pen, and A. S. Griffin. 2002. Cooperation and competition between relatives. *Science* 296:72–75.
- West, S. A., D. M. Shuker, and B. C. Sheldon. 2005. Sex ratio adjustment when relatives interact: A test of constraints on adaptation. *Evolution* 59:1211–1228.
- West, S. A., A. S. Griffin, and A. Gardner. 2007a. Evolutionary explanations for cooperation. *Curr. Biol.* 17:R661–R672.
- . 2007b. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *J. Evol. Biol.* 20:415–432.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

#### Supporting Methods and Result in Experiment 1

#### Supporting Methods and Result in Experiment 2

**Table S1.** Number of adult males and females in the host cocoon and adult females dispersed from the cocoon in experiment 1.

**Table S2.** Mean proportion ( $\pm$ standard error) of mated individuals among dispersed females depending on foundress combination and dispersed timing.

**Figure S1.** The number of female offspring dispersed from a host cocoon on each day in experiment 1.

**Figure S2.** The supposed situation (left side) and actual manipulation procedures (right side) in experiment 2, in which a host was assumed to be parasitized by two foundresses with different offspring sex ratios either of M (Melittobian), I (Intermediate), or H (Hamiltonian; see text for details).

**Figure S3.** The number of male and female pupae supposed to be produced by a Melittobian female (A), an Intermediate female (B), or a Hamiltonian female (C) on each day in experiment 2.

**Figure S4.** The number of female offspring dispersed from a cocoon mimicked case on each day in experiment 2.