

Virginity and the clutch size behavior of a parasitoid wasp where mothers mate their sons

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Theoretical and empirical research on the evolution of clutch size has proved to be an extremely productive area of evolutionary biology. A general prediction is that individuals should produce a smaller number of offspring when resources are more limited, such as when multiple individuals compete for the same resources for their development. However, we expect that the opposite prediction arises with virgin females of haplodiploid species, which are subject to extreme local mate competition. We test the key assumption and predictions of this theory with the parasitoid wasp *Melittobia australica*. Our data demonstrate that there is a trade-off between the size of the first and subsequent clutches and that virgin females adjust their production of sons according to the mating status (mated or not) of cofounding females. We also found that mated females facultatively change their offspring sex ratio in response to the mating status of cofoundresses. We discuss the potential mechanisms used to recognize the mating status and the implications of our results in the context of the extremely female-biased sex ratios observed across *Melittobia* species. **Key words:** clutch size, constrained female, local mate competition, *Melittobia australica*, reproductive strategy, sex allocation. [*Behav Ecol* 21:730–738 (2010)]

Research on clutch size has played a key role in the development and testing of theory predicting how natural selection molds the behavior of individuals (Seger and Stubblefield 1996; Krebs and Davies 1997). When females reproduce, the number of offspring produced is mediated by a trade-off between number (clutch size) and quality of offspring (Darwin 1871, p. 317–320; Smith and Fretwell 1974; Wilson 1994). A general prediction that has gained considerable empirical support is that as resources become more limited, a lower clutch size is favored (Godfray et al. 1991; Mayhew and Glazier 2001). For example, birds lay smaller clutch sizes when they occupy poorer territories (Hogstedt 1980), and parasitoid wasps lay smaller clutches when multiple females are laying eggs on the same host (Parker and Courtney 1984; Skinner 1985; Godfray 1994).

Here, we examine a situation in which the opposite prediction arises for patterns of clutch size—when virgin females reproduce under conditions of extreme local mate competition (LMC). Hamilton (1967) showed that if mating occurs between the offspring of a small number of mothers and females disperse after mating on their natal patch, the sex ratio produced by each foundress is influenced by a process that he termed LMC. When a small number of foundress females oviposit on a patch, a female-biased sex ratio is favored because it reduces competition between sons (brothers) and increases the number of mates (daughters) available to those sons (Taylor 1981). LMC has been shown to explain female-biased sex ratios in a wide range of organisms, including parasitic protozoa, insects, mites, fish, snakes, and plants (West et al. 2005; West 2009).

Much work on sex ratio behavior has focused on organisms with haplodiploid sex determination, such as parasitoid wasps. With haplodiploidy, males develop from unfertilized (haploid) eggs, and females develop from fertilized (diploid) eggs (Cook 1993). As well as providing a mechanism for mothers to control the sex ratio of their offspring, haplodiploidy means that virgin females are able to reproduce, albeit only by producing sons. In species subject to LMC, the selection for female-biased sex ratios means that the fitness of virgins is severely reduced relative to mated females (Godfray 1990; Godfray and Hardy 1993; Heimpel 1994; West et al. 1997; West and Herre 1998). In the extreme, if only 1 female lays eggs per patch and this foundress is a virgin, then the production of sons by a virgin is essentially worthless because there will not be any female offspring available for the sons to mate. In some parasitoid wasps and scolytid beetles, this has been argued to have led to an egg-laying behavior in which virgin females lay a small number of male eggs, wait for these sons to mature, and then mate with one or more of them. This strategy could enable females to produce a subsequent mixed sex brood that brings substantially greater fitness returns than an all-male brood (Balfour Browne 1922; Godfray 1994; Ueda 1997; Hardy et al. 1999).

In this paper, we examine the egg-laying behavior of virgin females in the presence of cofounding mated females that lay mixed sex broods, providing potential mates for the sons of the virgin females. Briefly, when a virgin oviposits alone, she is predicted to lay only a small number of eggs to avoid wasting resources that could be saved for the future production of a mixed sex brood after mating with her own sons. In contrast, when ovipositing with another foundress, the virgin female is predicted to lay a larger clutch size because the female-biased sex ratio (due to LMC) produced by the mated foundress will result in mating opportunities for the virgin's sons, dramatically increasing the fitness returns from sons. Although this prediction may rely on the relative importance of the

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Received 26 October 2009; revised 14 March 2010; accepted 14 March 2010.

reproductive values of first (sons only) and subsequent (mixed sex) clutches, a game theoretical model shows that virgin females should always produce a larger number of male offspring when laying with a mated female than when laying alone (Appendix). We then test the main assumption and predictions of this model with the parasitoid wasp *Melittobia australica*. The mating system of this genus is characterized by LMC, where a single virgin female is able to produce a small number of male eggs, await their emergence on the host, and subsequently mate with her own son(s) (Balfour Browne 1922; Dahms 1984; Matthews et al. 2009). Specifically, we test whether 1) there is a trade-off between the number of offspring laid in sequential clutches and 2) virgin females lay a larger clutch size when ovipositing in the presence of a mated female than when ovipositing alone.

METHODS

Parasitoid wasps

Melittobia is a gregarious parasitoid wasp that parasitizes the (pre)pupae of various solitary wasp and bee species nesting above ground (Balfour Browne 1922; Dahms 1984; Matthews et al. 2009). Females mate on their natal patch and disperse to find hosts on which to feed (to mature their eggs; synovigeny) and then lay eggs. However, because of the extremely female-biased sex ratios and lethal combat among adult males in this genus, sometimes females cannot acquire males and must disperse as virgins (Hobbs and Kronic 1971). Accordingly, patches without males and all-male broods have been reported from field studies (Maeta and Yamane 1974; Iwata 1980). In the laboratory, the frequency of unmated females dispersing from mixed sex clutches is 1% (Abe J, unpublished data). Although data on the structure of natural populations of *Melittobia* are scarce, there are reports of multiple females laying eggs on the same host (Iwata and Tachikawa 1966; Freeman and Ittyeipe 1976, 1993; Freeman 1977; van den Assem et al. 1980; Cooperband et al. 2003; Matthews et al. 2009). Here, we used a strain of *M. australica* collected in Shiga, Japan, in 2000 (Abe et al. 2005, 2009). A microsatellite marker can be used to identify the mothers of offspring developing on the same host. The design of the primer set (MMS7-2) and detailed procedures to analyze the microsatellite are described elsewhere (Abe et al. 2005), and we used the ABI GeneMapper and a capillary sequencer (ABI 3730) for fragment analysis. We used the long-wing, dispersing female morph (Dahms 1984; Abe et al. 2005, 2009; Matthews et al. 2009; Innocent et al., unpublished data) and prepupae of the leaf cutter bee *Megachile sculpturalis* (Hymenoptera: Megachilidae) as hosts. We carried out all stock culturing and experiments at 25 °C and 16:8 h light:dark conditions.

Experiment 1: trade-off between present and future clutch sizes

Here, we tested the assumption that there is a trade-off between the sizes of current and future clutches. Specifically, we tested for a negative correlation between the size of brood produced by females during an initial period of oviposition (referred to as first clutch size) and the number of offspring subsequently produced during the rest of their lives (referred to as subsequently produced clutch size). To maximize our power to detect this trade-off, we created a broad range of brood sizes for the first clutches by manipulating both the mating status (mated or virgin) and the oviposition period (10 or 15 days) in 4 treatment combinations. We expected virgins to produce smaller clutches than mated females and fewer eggs to be laid in the shorter oviposition period. We set up 8 replicates for each of the treatment combinations.

We collected male and female individuals in late pupal stage from mass culture. We housed male pupae individually to prevent lethal combat between emerged adult males (Dahms 1984; Reece et al. 2007; Matthews et al. 2009) and kept female pupae in groups isolated from males. We collected females within 24 h of emergence and incubated them individually for 24 h either with an adult male (to produce mated females) or alone (to produce virgins). For all mating treatments, we placed virgin and <48-h-old males in small plastic cages (35 mm in diameter and 20 mm in height). After the mating treatment, we placed individual females in larger plastic cages (86 mm in diameter and 30 mm in height) containing a host and allowed them to lay their first clutch. After producing their first clutches (i.e., either on the 16th or 11th day), we allowed all females to mate by housing each individually with a male for 24 h. After 24 h, we removed males and placed females in new large plastic cages and provided a host to begin their second clutch. Every 16 days, we repeated the mating treatment and subsequently provided females with a new host until they died. By repeating this treatment, we measured the lifetime reproductive success of females, subsequent to their first clutches, and could also ensure that females were not constrained by either sperm or host limitation.

Once females had died, we dissected and measured their tibia length as a body size index using a digital microscope (Keyence VHX-200), recording the intermediate values of their 2 tibia lengths. Under our experimental conditions, the death rate of developing offspring is low (Abe, Kamimura, Kondo et al. 2003; Abe et al. 2009), so we counted the number of offspring produced on each host by undertaking daily checks and removing late stage pupae or newly emerged wasps. This also avoided the potential problem of the experimental foundress females being able to remate with their sons. Two females did not produce a clutch on their first host, and 1 female failed to mate, so we removed these replicates from the analysis.

Experiment 2: clutch size adjustment of virgin females

We then tested whether virgin females recognized the mating status of a cofoundress and adjusted their egg-laying behavior. In this experiment, we allowed virgin females to lay eggs with either a mated cofoundress, with another virgin, or alone. We specifically predicted that the virgin females would produce a larger number of (male) offspring when their cofoundress was a mated female compared with when ovipositing with another virgin or alone. We followed the procedures for the pre-treatment of foundress females and the oviposition period in Abe et al. (2005) in which 2 mated females were sequentially allowed to lay eggs on the same host, and their offspring were individually genotyped using the microsatellite DNA marker. Then, we conducted the following treatments in which each replicate consisted of a “first female,” allowed to oviposit on a host for 3 days, before a “second female” was added for a further 9 days: (i) mated first and virgin second, (ii) virgin first and mated second, and (iii) virgin first and virgin second. In addition, we set up control treatments with (iv) 1 virgin female ovipositing for 12 days and (v) 1 virgin female ovipositing for 9 days. We set up 8 replicates for each treatment.

To create females whose genotypes were known, we randomly collected female pupae from a mass culture, allowed them to mate with their sons, and genotyped them. We used the daughters of homozygous females as foundress females for this experiment. We created mated and virgin females by placing 8 females with 1 sibling male and without males, respectively, for a period of 3 days. We then randomly allocated the 8 females to each of the 5 different treatments (the remaining 3 females were not used for this experiment). In the

treatments in which 2 females laid eggs on the same host (treatments (i) to (iii)), we used females with different genotypes. We checked the parasitized hosts daily and removed and sexed all newly emerged offspring. In treatments (i) to (iii), we genotyped all the male offspring to identify their mothers. We discarded 3 replicates in which the females did not oviposit within 3 days.

Statistical analysis

Clutch size and male offspring numbers were analyzed using generalized linear models with the quasi-Poisson distribution and identity link because the data were overdispersed (Crawley 2007). We first constructed full models, including all main effects and the second order interactions, and deleted non-significant terms ($\alpha > 0.05$) to acquire minimal adequate models following backward stepwise deletion (Crawley 2007). Significance was tested using likelihood ratio tests to compare the change in model deviance after deleting each term. Along with the above analysis, we also analyzed the clutch size and offspring sex ratio produced by mated females using the data from the present experiment and Abe et al. (2005) in which the same oviposition treatments were applied to mated females. The sex ratio data (proportion of males) were analyzed using the quasi-binomial and logit link function. All analyses used R version 2.7.1 (The R Foundation for Statistical Computing; <http://www.R-project.org>).

RESULTS

Trade-off between present and future clutch sizes (Experiment 1)

Females produced between 3 and 5 clutches in their lifetime (Table 1). We found a significant negative relationship between the size of the first clutch and that produced in the subsequent second to fifth clutches ($F_{1,27} = 25.9$, $P < 0.001$, $R^2 = 0.47$; Figure 1). The subsequent clutch size was not significantly influenced by oviposition period ($F_{1,26} = 3.54$, $P = 0.07$), mating status ($F_{1,25} = 0.004$, $P = 0.95$), or tibia

length ($F_{1,24} = 0.0001$, $P = 0.99$). There were no significant interactions (all $P > 0.26$ except that the interaction between first clutch size and tibia length was marginal: $F_{1,23} = 3.73$, $P = 0.07$). We did not find a significant interaction between first clutch size and mating status ($F_{1,18} = 0.15$, $P = 0.70$), indicating that subsequent clutch sizes are not influenced by the sexes of offspring produced in the first clutch. No significant interaction between first clutch size and oviposition period ($F_{1,22} = 0.50$, $P = 0.49$) also indicates that there are no aging effects on subsequent clutch size between the 2 oviposition periods in the first clutch. In order to examine the clutch sizes produced by the females of each mating status, we also analyzed the data separately within each mating status treatment. For mated females, the same pattern was found, with a negative correlation between the size of the first and later clutches ($F_{1,13} = 11.7$, $P < 0.005$, $R^2 = 0.46$; Figure 1), and no significant relationship with oviposition period ($F_{1,11} = 0.80$, $P = 0.39$), tibia length ($F_{1,12} = 1.88$, $P = 0.20$), nor any significant interactions (all $P > 0.25$). In contrast, when we analyzed the clutch sizes produced by virgin females, there was no correlation between the size of the first brood and later clutches ($F_{1,10} = 0.058$, $P = 0.82$), oviposition period ($F_{1,12} = 1.19$, $P = 0.30$), tibia length ($F_{1,11} = 1.32$, $P = 0.27$), nor any significant interactions (all $P > 0.17$).

Clutch size adjustment of virgin females (Experiment 2)

As expected, virgin females produced much smaller clutch sizes than mated females ($F_{1,56} = 2643.5$, $P < 0.001$, $R^2 = 0.96$; Figure 2A). Although there was no direct significant correlation between clutch size and oviposition period ($F_{1,56} = 0.18$, $P = 0.67$), the interaction between the mating status of the focal female and oviposition period was significant ($F_{1,55} = 20.1$, $P < 0.001$, $R^2 = 0.007$). This is because although mated females produced larger clutches over 12 days than 9 days (Figure 2A), the virgin females produced a similar number of male offspring irrespective of oviposition period (Figure 2B). The other main effects and interactions were not significant

Table 1

The average longevity of experimental females, the numbers of replicates in which offspring emerged, average clutch sizes, and average sex ratios (proportion of males), when either a virgin or a mated female were allowed to lay eggs for either 10 or 15 days in the first clutch

	Virgin for 10 days	Virgin for 15 days	Mated for 10 days	Mated for 15 days
Mean longevity of females (days) (\pm SE) ^a	67.3 \pm 2.7	60.0 \pm 4.5	59.9 \pm 2.6	57.3 \pm 3.2
Number of replicates-produced offspring				
1st clutch	7	7	7	8
2nd clutch	7	7	7	8
3rd clutch	7	7	7	8
4th clutch	7	4	7	3
5th clutch	5	1	0	0
Mean clutch size (\pm SE) ^a				
1st clutch	2.0 \pm 0.4	2.1 \pm 0.3	426.7 \pm 38.6	735.4 \pm 49.5
2nd clutch	550.6 \pm 41.4	610.3 \pm 88.8	605.0 \pm 43.4	560.1 \pm 64.3
3rd clutch	515.9 \pm 81.6	458.9 \pm 49.0	374.7 \pm 60.3	177.0 \pm 49.4
4th clutch	228.0 \pm 37.4	91.7 \pm 41.9	111.4 \pm 40.2	2.6 \pm 2.1
5th clutch	10.4 \pm 7.5	0.1 \pm 0.1	0	0
Mean sex ratio (\pm SE) ^b				
1st clutch	1 \pm 0	1 \pm 0	0.014 \pm 0.001	0.01 \pm 0.001
2nd clutch	0.015 \pm 0.002	0.014 \pm 0.003	0.012 \pm 0.001	0.014 \pm 0.004
3rd clutch	0.013 \pm 0.002	0.025 \pm 0.014	0.043 \pm 0.031	0.141 \pm 0.123
4th clutch	0.041 \pm 0.009	0.03 \pm 0.008	0.227 \pm 0.14	0.353 \pm 0.324
5th clutch	0.407 \pm 0.242	1		

SE, standard error.

^a The averages and SEs were calculated with all replicates analyzed.

^b The averages and SEs were calculated with replicates-produced offspring.

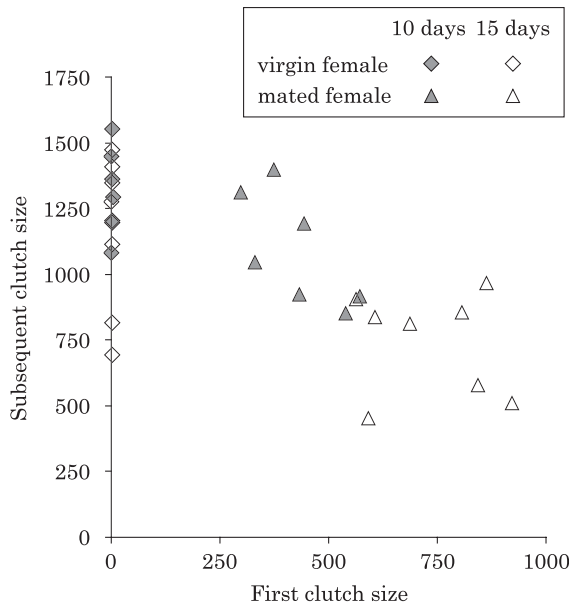


Figure 1

The relationship between the number of offspring produced in the first clutch and the sum of the numbers produced in the subsequent second to fifth clutches. The experimental females were allowed to lay eggs in the first clutch and were either virgin or mated females, given either for 10 or 15 days for oviposition. The minimal adequate model of the GLM with Poisson errors and identity link function was: (subsequent clutch size) = 1259.3 – 0.641 × (first clutch size).

(mating status of cofounding female: $F_{2,54} = 1.62$, $P = 0.21$; genotype of focal female: $F_{1,53} = 0.03$, $P = 0.86$; and other interactions: all $P > 0.37$).

To examine the response of virgin females to the mating status of a cofounding female, we analyzed the number of male offspring produced by virgin females and found a highly significant effect of cofounding female status (i.e., either mated, virgin, or none; $F_{2,40} = 6.62$, $P = 0.003$, $R^2 = 0.22$; Figure 2B). Although the effect of oviposition period was not significant ($F_{1,40} = 0.09$, $P = 0.77$) as a main effect, the interaction with mating status was significant ($F_{1,38} = 4.32$, $P = 0.020$, $R^2 = 0.15$; Figure 2B). When a virgin female was the first foundress to start laying eggs on a fresh host (12-day oviposition), she produced a similar clutch size regardless of whether or not another female later oviposited on the host (Figure 2B). In contrast, when a virgin female was the second female laying eggs on a host where another female had already started laying eggs (9-day oviposition), she changed her clutch size according to the mating status of the first females, producing a larger number of male offspring with a mated female than with another virgin female or alone (Figure 2B). Neither the effect of the genotype of the virgin females nor the interaction terms regarding the genotype were significant (genotype: $F_{1,39} = 0.08$, $P = 0.78$ and interactions: all $P > 0.93$).

Response of mated females to a virgin versus another mated female (Experiment 2)

We can compare the response of mated females to a virgin versus another mated female using the data from the present experiment and Abe et al. (2005). The clutch size of mated females had no significant relationship with the mating status of a cofounding female ($F_{1,28} = 1.64$, $P = 0.21$), although it increased with oviposition period ($F_{1,29} = 6.62$, $P < 0.001$, $R^2 = 0.52$). There were no significant effects of the genotype of the mated females nor any interactions (genotype: $F_{1,27} = 0.17$, $P = 0.68$ and interactions: all $P > 0.12$). However, the offspring sex ratio

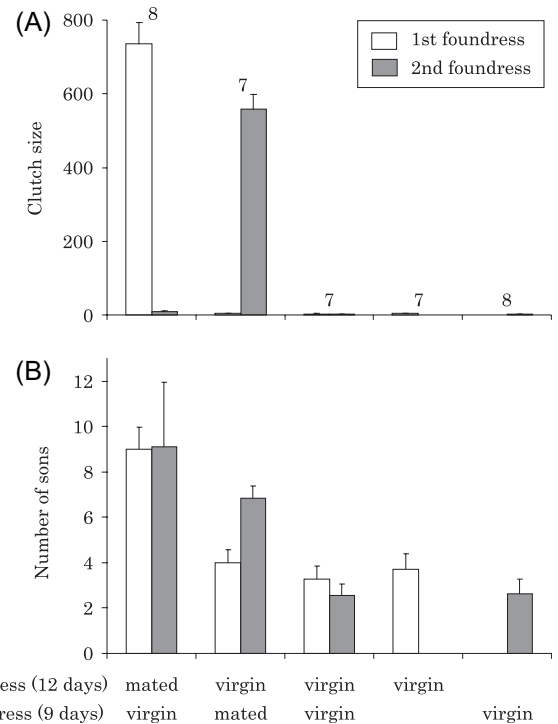


Figure 2

Mean clutch size (the number of sons and daughters; A) and the mean number of sons (B). A pair of females, comprised either a mated and a virgin female, a virgin and a mated female, or 2 virgin females or a single virgin female, were allowed to lay eggs on a host. The first female was allowed to lay eggs on a host for 12 days, and the second female was allowed to lay eggs on the same host starting 3 days later for 9 days (i.e., within a replicate, females were removed at the same time). Error bars indicate standard errors. Values above bars (A) indicate the number of replicates that were analyzed.

was significantly influenced both by the mating status of a cofounding female ($F_{1,28} = 4.71$, $P = 0.039$, $R^2 = 0.09$) and by the oviposition period ($F_{1,28} = 12.6$, $P = 0.001$, $R^2 = 0.25$), and the interaction between these 2 effects was also significant ($F_{1,27} = 9.20$, $P = 0.005$, $R^2 = 0.18$). This indicates that the mated females produced less female-biased sex ratios when ovipositing after another mated female than after a virgin female or before another female (Figure 3). There were no significant effects of the genotype of the mated females ($F_{1,27} = 1.07$, $P = 0.31$) or the other interactions (all $P > 0.73$).

DISCUSSION

We investigated the egg-laying behavior of virgin females in response to the presence of mated females in the same patch. We predicted that virgin females should produce a larger number of (male) offspring when laying with a mated female than when laying alone (Appendix). This prediction was based on the assumption of a trade-off between current and future reproduction, with the production of more offspring in the present clutch decreasing the number of offspring that could be produced in future clutches. We found empirical support for both this assumption (Figure 1) and prediction (Figure 2) in the parasitoid wasp *M. australica*.

In panmictic species, virgin females are predicted to produce all-male clutches, whereas mated females produce female-biased clutches to compensate for the excess of males in the population (Godfray 1990; Godfray and Hardy 1993)—a prediction that has been empirically examined in many haplodiploid species (Godfray 1994; West 2009).

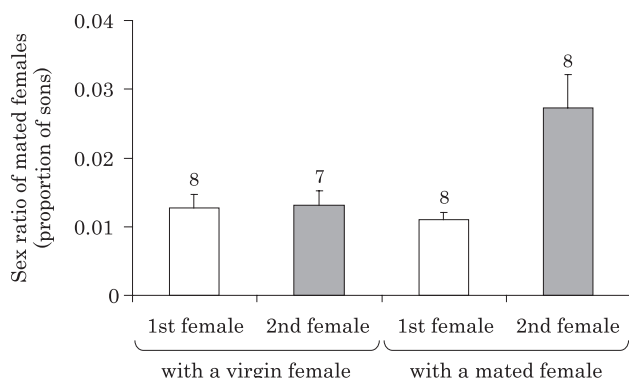


Figure 3

Offspring sex ratio produced by mated females that were allowed to lay eggs on the same host either with a virgin female or with another mated female. First females laid eggs for 12 days and second females did for 9 days by a 3-day interval. Error bars indicate standard errors. Data in which mated females oviposit with another mated female are derived from Abe et al. (2005). Values above bars indicate the number of replicates that were analyzed.

However, LMC alters this selective pressure because sons produced by virgin females have no mating opportunities unless mated females also produce offspring locally. We showed that the virgins of *Melittobia* produced larger clutches in the presence of mated females (Figure 2). These facultative adjustments are predicted when organisms encounter variable situations, and their fitness consequences largely depend on what strategy they choose in each situation (Herre 1987; West and Herre 1998). Currently, information about virgins in natural populations is lacking, but our data indicate that virgin and mated females lay eggs on the same host (see also below).

The direction of clutch size adjustment by virgin females is in contrast to the prediction of general clutch size models where smaller clutch sizes are predicted with increasing number of competitors (Parker and Courtney 1984; Skinner 1985; Mayhew and Glazot 2001). Another example where we expect to see this atypical direction of clutch size adjustment is in species with contest competition, such as solitary parasitoid wasps. In these species, females are likely to lay larger number of eggs to ensure their own parentage, when the probability that conspecific females add eggs on the same patch is higher (Ives 1989; Rosenheim and Hongkham 1996; Pexton and Mayhew 2005). More generally, our results stress that although there are many cases where sex ratios can be examined independently of clutch size (West 2009), this is not always the case. Other situations where the coevolution of sex ratio and clutch size must be considered include when there is asymmetric larval competition between the sexes for resources (Godfray 1986; Sykes et al. 2007), small clutch sizes (Williams 1979; Mayhew 1998), superparasitism with limited number of eggs (Nagelkerke 1994; Greeff 1997), and single sex broods (West et al. 1999, 2001).

The adjustment of clutch size by virgin females did not occur when the cofounding female was also a virgin (Figure 2B), which can be explained by the fact that virgin cofoundresses do not supply female mates for the focal virgin's sons. Although we do not know whether virgin females can directly recognize the mating status of cofoundresses, there are several possible indirect mechanisms that could be used as cues. One possibility involves estimating the number of eggs (or the attendant cues) laid by other females because by day 3, mated females produced much larger clutches than virgins (Figure 2A). This hypothesis fits with results from other parasitoid species in which the sex ratios of mated females were adjusted in response to the presence of eggs laid by other females

rather than the presence of other females (Shuker and West 2004). We also found that mated females of *Melittobia* adjusted their offspring sex ratios depending on whether they lay eggs after a virgin or another mated female (Figure 3). There have been no reports that mated females of other parasitoid species adjust their offspring sex ratios depending on the mating status of other females laying eggs on the same host (Werren 1984; King and D'Souza 2004; see also King 2002). Recently, Lebreton et al. (2010) showed that the females of a solitary parasitoid wasp adjusted their offspring sex ratios in response to the sexes of eggs previously laid by conspecific females.

Although the virgin females of *Melittobia* facultatively adjusted their clutch size in the direction as predicted, the change seems to be small. Our model provides quantitative predictions, suggesting that virgins should invest in a much larger number of males when they lay eggs with a mated female (Appendix). Why didn't the observed clutch size ratio quantitatively fit with the predictions of our model? First, the intensity of selection for clutch size adjustment is likely to be correlated with the frequency that virgin females lay eggs with a mated female in natural populations (Herre 1987; West and Herre 1998). Although our data indicate that the circumstances favoring facultative clutch size adjustment do occur in nature, this might happen at low frequency. There are 3 further potential explanations for the lack of quantitative fit between our model and data, which are linked to possible reasons why mated *Melittobia* females adjust their offspring sex ratios only slightly with increasing numbers of females laying eggs per patch, in contrast to the predictions of LMC models (Abe, Kamimura, Kondo et al. 2003; Abe et al. 2005, 2009; Innocent et al. 2007). Second, lethal male-male combat may select for ovipositing females to refrain from producing many males because later emerging males would just be killed by older adult males (Abe, Kamimura, Ito et al. 2003; Abe, Kamimura, Kondo et al. 2003; Abe et al. 2005, 2007, 2009; Shuker et al. 2005; Innocent et al. 2007). Third, if the virgin and mated females laying eggs on the same host are related, they may produce smaller numbers of male offspring to avoid competition among related males (Grafen 1984; Frank 1985, 1986; Shuker et al. 2004). Finally, females may avoid producing many males due to "mutual policing" (Kamimura et al. 2008), although this requires ovipositing females to recognize the sexes of eggs laid by other females. Data on the natural population structure of this genus would be extremely useful to distinguish between these possibilities.

Under LMC, ovipositing females face a trade-off between not wasting excess resources on sons and the need to produce enough sons to ensure against the possibility that they all die (Heimpel 1994; Nagelkerke and Hardy 1994; West et al. 1997; West and Herre 1998). The behavior of virgin females in *Melittobia* that mate with their own sons and facultatively adjust their clutch sizes in response to their cofoundresses mating status ensures their reproductive successes, even if they are virgins. Along with precise sex ratio adjustments over a prolonged oviposition period (Abe et al. 2009), this might be one of factors that facilitate the extremely female-biased sex ratios in this genus.

FUNDING

Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists (181102) to J.A. and Natural Environment Research Council, the Royal Society, and the Wellcome Trust to T.M.I., S.E.R., and S.A.W.

We are very grateful to Hiroshi Ito for discussion and providing the programs of numerical calculations and to Polly Fordyce and Momoko Ichinokawa for helpful discussions. We thank Hiroshi Ito and Yoshitaka

Kamimura and two anonymous referees for valuable comments on the manuscript.

APPENDIX

Here, we develop a model to predict the unbeatable clutch size ratio that virgin females should invest in their first clutch. We consider a situation where there are occasionally virgin females in a population that consists of numerous isolated patches (e.g., hosts). Virgin females only produce male eggs in their first clutch, then mate with one of their emerging sons; subsequently, mated virgin females (hereafter called ex-virgin females) can produce both sexes in their subsequent later clutches. For simplicity, we assume that virgin females produce at most 2 clutches. If an organism reproduces more than 2 clutches, we can regard the second clutch as an average of all later clutches.

We assume that a virgin female invests a proportion s ($0 \leq s \leq 1$) of her total resources in her first clutch and the remainder $1 - s$ of her resources in her second clutch (i.e., that there is a trade-off between clutch sizes). Our main aim here is to seek the unbeatable value of s that cannot be outcompeted by any other strategies. When a virgin female is the only foundress in a patch, the unbeatable strategy for the first clutch is to produce the minimum number of sons required for insemination ($s^* = 0$). Hereafter, we seek the unbeatable clutch size ratio (s^*) when virgin females lay eggs with a cofounding mated female. Whereas virgin females need to wait until their second clutch to produce both sexes, we suppose that mated females invest all their resources in 1 clutch. We also assume that each patch is visited by at most 2 females and define $r_{i,j}$ as the sex ratio (the proportion of male offspring) of i female laying eggs with j female in the same patch, where i and j are either $v1$ (a virgin female in her first clutch), $v2$ (an ex-virgin female in her second clutch), m (a mated female), or o (no females). Hereafter, to simplify, we use r_{v1} , r_{v2} , r_{m1} , and r_{m2} in place of $r_{v1,m}$, $r_{v2,m}$, $r_{m,v1}$, and $r_{m,v2}$, respectively. In this case, r_{v1} is always equal to 1 because virgin females are constrained in their sex allocation in their first clutches.

After producing her first clutch of sons and mating, the ex-virgin female is assumed to disperse to look for a new patch and to find a new patch with a probability P ($0 \leq P \leq 1$, $1 - P$ is the cost of dispersal). When producing her second clutch, the ex-virgin female lays eggs either alone or with another mated female with the probability $1 - q$ or q ($0 \leq q \leq 1$), respectively. To allow for offspring suffering from increasing resource competition with the number of eggs laid in the patch, we employ exponential competition function of Waage and Godfray (1985) for the developmental survival probability of their offspring ($f(b) = \exp(-kb)$, b is clutch size and k is a constant). Although we also applied quadratic form of Parker and Courtney (1984), the results were qualitatively identical.

We define W_v as the fitness of a virgin female and W_{m1} and W_{m2} as the fitness of cofounding mated females laying eggs during the first and second clutches of virgin females, respectively. The fitness consists of the number of daughters and the number of females that sons mate with, weighted by the relatedness and reproductive values. Although the sons mate both the daughters and also the virgin females themselves, the effect of the virgin females is negligible unless their clutch size is very small. Then,

$$W_v = sf(c(1 + s))R_M v_M \frac{1 - r_{m1}}{s + r_{m1}} + P(1 - s) \left[qf(c(2 - s)) \left\{ R_F v_F (1 - r_{v2}) + R_M v_M r_{v2} \frac{(1 - r_{v2})(1 - s) + 1 - r_{m2}}{r_{v2}(1 - s) + r_{m2}} \right\} + (1 - q)f(c(1 - s))(R_F v_F + R_M v_M) \right], \tag{A1a}$$

$$W_{m1} = f(c(1 + s)) \left\{ R_F v_F (1 - r_{m1}) + R_M v_M r_{m1} \frac{1 - r_{m1}}{s + r_{m1}} \right\}, \tag{A1b}$$

$$W_{m2} = f(c(2 - s)) \left\{ R_F v_F (1 - r_{m2}) + R_M v_M r_{m2} \frac{(1 - r_{v2})(1 - s) + 1 - r_{m2}}{r_{v2}(1 - s) + r_{m2}} \right\}, \tag{A1c}$$

where R_F (R_M) is the relatedness coefficient of the female to her daughters (sons) and v_F (v_M) is the class reproductive value for daughters (sons) (Taylor, 1996). We assume that species are haplodiploid here, hence $v_F = 2/3$ and $v_M = 1/3$ (Taylor 1996), $R_F = 1/(2 - k)$, where k is the proportion of sibmating in the population, and $R_M = 1$ (Taylor 1993). Because virgin females are rare in this population, therefore,

$$k = \frac{1 - q + q(3/2)J \cdot 1/2}{1 - q + q(3/2)J} = \frac{4 - (4 - 3J)q}{4 - 2(2 - 3J)q}, \tag{A2}$$

where J is an inbreeding factor (Suzuki and Iwasa 1980):

$$J = \frac{2R_M v_M}{R_F v_F + R_M v_M}. \tag{A3}$$

The unbeatable clutch size ratios and sex ratios depend on the information available to ovipositing females. We prove the 2 derivations of these ratios according to whether or not the females can distinguish the mating status of a cofounding female and facultatively alter their strategy.

In the case that females cannot recognize a virgin

In this case, mated females are assumed to lay eggs with fixed sex ratios, as if they were laying eggs with a mated cofounding female (note that virgin females are rare in this population). We now seek the optimum strategies (s^* and r_{v2}^*) that maximize the fitness of the virgin females when the strategies of mated females (r_{m1} and r_{m2}) are fixed. The optimum strategies are found by simultaneously solving the following 2 equations,

$$\begin{cases} \partial W_v / \partial s |_{s=s^*} = 0, & \text{(A4a)} \\ \partial W_v / \partial r_{v2} |_{r_{v2}=r_{v2}^*} = 0. & \text{(A4b)} \end{cases}$$

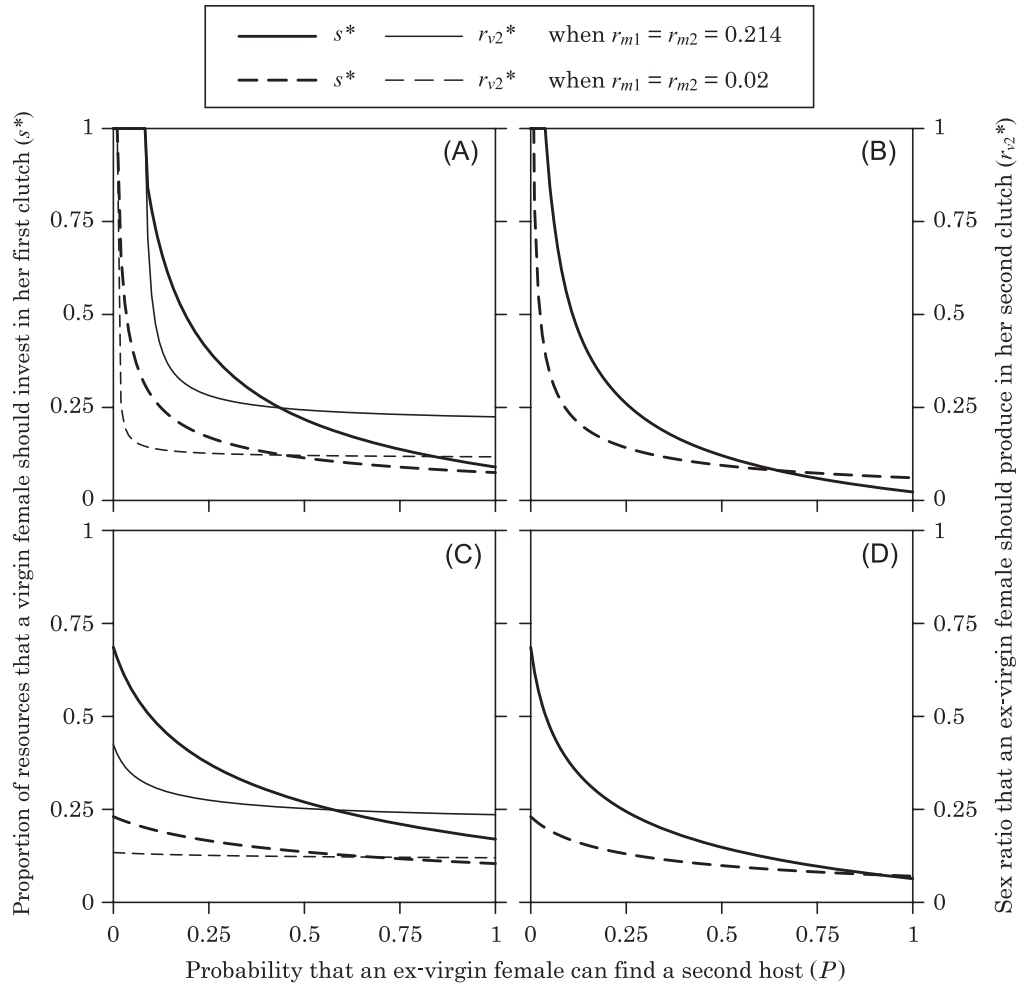
These were numerically solved using a bisection method for a given parameter set after they were analytically reduced to 1 equation. We verified local evolutionary stability for all given parameter sets. We numerically (Frane and Bairstow methods) checked that the real parts of all eigenvalues of the following matrix were negative:

$$\begin{pmatrix} \frac{\partial^2 W_v}{\partial s^2} & \frac{\partial^2 W_v}{\partial s \partial r_{v2}} \\ \frac{\partial^2 W_v}{\partial r_{v2} \partial s} & \frac{\partial^2 W_v}{\partial r_{v2}^2} \end{pmatrix}.$$

We derive the following results (Figure A1). The virgin female is predicted to invest fewer resources in her first clutch when there is a greater probability that 1) she can find a new host on which to lay a second clutch (larger P ; Figure A1) and 2) she will be the only foundress on a patch for her second clutch (smaller q ; Figure A1). These predictions arise because the virgin female is favored to save a greater proportion of resources for her second clutch because she is likely to gain greater reproductive success from it. However, if her offspring suffer from competition for resources in their developmental stage ($k > 0$), the virgin female should 3) distribute her resources more evenly between her first and second clutches (Figure A1C,D). Finally, when a cofounding mated female produces a less female-biased sex ratio, the virgin female 4) produces a larger number of sons due to competition between males for mates (Figure A1), but this relationship is reversed in an extreme case when the virgin female is expected to acquire a relatively large reproductive success in her second clutch (large P and small q ; Figure A1B,D).

Figure A1

Optimal proportion of resources that a virgin female should invest in her first clutch (s^*) and optimal sex ratio that the ex-virgin female should produce in her second clutch (r_{v2}^*), depending on the probability that the ex-virgin female can find her second host (P), when females cannot distinguish the mating status of cofounding females. In this case, the sex ratios of mated females (r_{m1} and r_{m2}) are given and either 0.214 (the prediction of an LMC model with 2 foundresses; Hamilton 1979) or 0.02 (the average of observed sex ratios when 2 mated females laid eggs on a patch; Abe, Kamimura, Kondo et al. 2003; Abe et al. 2005, 2009) are used. The probability that the ex-virgin female lays eggs with a cofounding mated female in the second clutch (q) is 1 (A and C) or 0 (B and D). Developmental competition between offspring either does not occur ($a = 0$; A and B) or does occur ($c = 500$ and $k = 7 \times 10^{-4}$; this is the case if the 2 females lay all their eggs in the same clutch, where half of their offspring can survive; C and D).



In the case that females can recognize a virgin

In this case, the mated females also adjust their strategies of sex ratios (r_{m1} and r_{m2}) depending on the mating status of a cofounding female. The unbeatable strategies are characterized by evolutionary stable strategies (ESSs; s^* , r_{m1}^* , r_{m2}^* , and r_{m2}^*) that simultaneously maximize their own fitness values. We solved the following simultaneous equations to obtain the ESSs:

$$\begin{cases} \frac{\partial W_v}{\partial s}|_{s=s^*} = 0, & (A5a) \\ \frac{\partial W_v}{\partial r_{v2}}|_{r_{v2}=r_{v2}^*} = 0, & (A5b) \\ \frac{\partial W_{m1}}{\partial r_{m1}}|_{r_{m1}=r_{m1}^*} = 0, & (A5c) \\ \frac{\partial W_{m2}}{\partial r_{m2}}|_{r_{m2}=r_{m2}^*} = 0. & (A5d) \end{cases}$$

These equations could be reduced to 1 equation by solving analytically, and it was numerically (bisection method) solved for a given parameter set. We verified local evolutionary stability by numerically checking (Frane and Bairstow methods) that the real part of the eigenvalue of the following matrix was negative:

$$\begin{pmatrix} \frac{\partial^2 W_v}{\partial s^2} & \frac{\partial^2 W_v}{\partial s \partial r_{v2}} & \frac{\partial^2 W_v}{\partial s \partial r_{m1}} & \frac{\partial^2 W_v}{\partial s \partial r_{m2}} \\ \frac{\partial^2 W_v}{\partial r_{v2} \partial s} & \frac{\partial^2 W_v}{\partial r_{v2}^2} & \frac{\partial^2 W_v}{\partial r_{v2} \partial r_{m1}} & \frac{\partial^2 W_v}{\partial r_{v2} \partial r_{m2}} \\ \frac{\partial^2 W_{m1}}{\partial r_{m1} \partial s} & \frac{\partial^2 W_{m1}}{\partial r_{m1} \partial r_{v2}} & \frac{\partial^2 W_{m1}}{\partial r_{m1}^2} & \frac{\partial^2 W_{m1}}{\partial r_{m1} \partial r_{m2}} \\ \frac{\partial^2 W_{m2}}{\partial r_{m2} \partial s} & \frac{\partial^2 W_{m2}}{\partial r_{m2} \partial r_{v2}} & \frac{\partial^2 W_{m2}}{\partial r_{m2} \partial r_{m1}} & \frac{\partial^2 W_{m2}}{\partial r_{m2}^2} \end{pmatrix}.$$

We derive the following results (Figure A2) along with the same qualitative results (1–3) described above. 5) The mated female is predicted to compensate for the excess of males produced by the virgin female by producing a more female-biased sex ratio in her clutch (Figure A2). This contrasts to the situation in which females cannot recognize the mating status of cofoundresses under LMC conditions in which the effect of compensation by mated females is negligible (Godfray 1990). 6) When females contribute clutches to the same patch, the ex-virgin and mated females are predicted to produce less female-biased sex ratios as their contributions to the clutch decrease (Suzuki and Iwasa 1980; Werren 1980; Figure A2A,C).

Overall, the unbeatable first clutch size ratios of virgin females (s^*) were larger than zero for any combination of parameters examined (Figures A1 and A2), indicating that virgin females should always produce larger number of sons when they lay eggs with mated females than when alone. Along with qualitative predictions, our models also provide a number of quantitative predictions that could be tested with our experimental results (Figures A1 and A2), although it should be noted that our models are largely heuristic and included effects regarding population structure (P and q) that are difficult to parameterize. In the case of *Melittobia*, we can estimate the level of developmental competition (k) using the data in Figure 2A in Abe, Kamimura, Kondo et al. (2003) where variable numbers of foundress females parasitized the same host species as in the experiments of the main text. We used a maximum likelihood method assuming that all the foundresses produced constant clutch sizes and that the errors of the clutch sizes are represented by the Poisson distribution. The resulting parameters are estimated as $k = 1.2 \times 10^{-4}$ and $c = 561.7$. Given this and the observed sex ratios of mated females, our model predicts that virgin females should invest at least 6% of their resources in their first clutch ($s^* > 0.06$). Although our model predicts smaller clutch size ratios in the cases of larger P and smaller q , these cases are

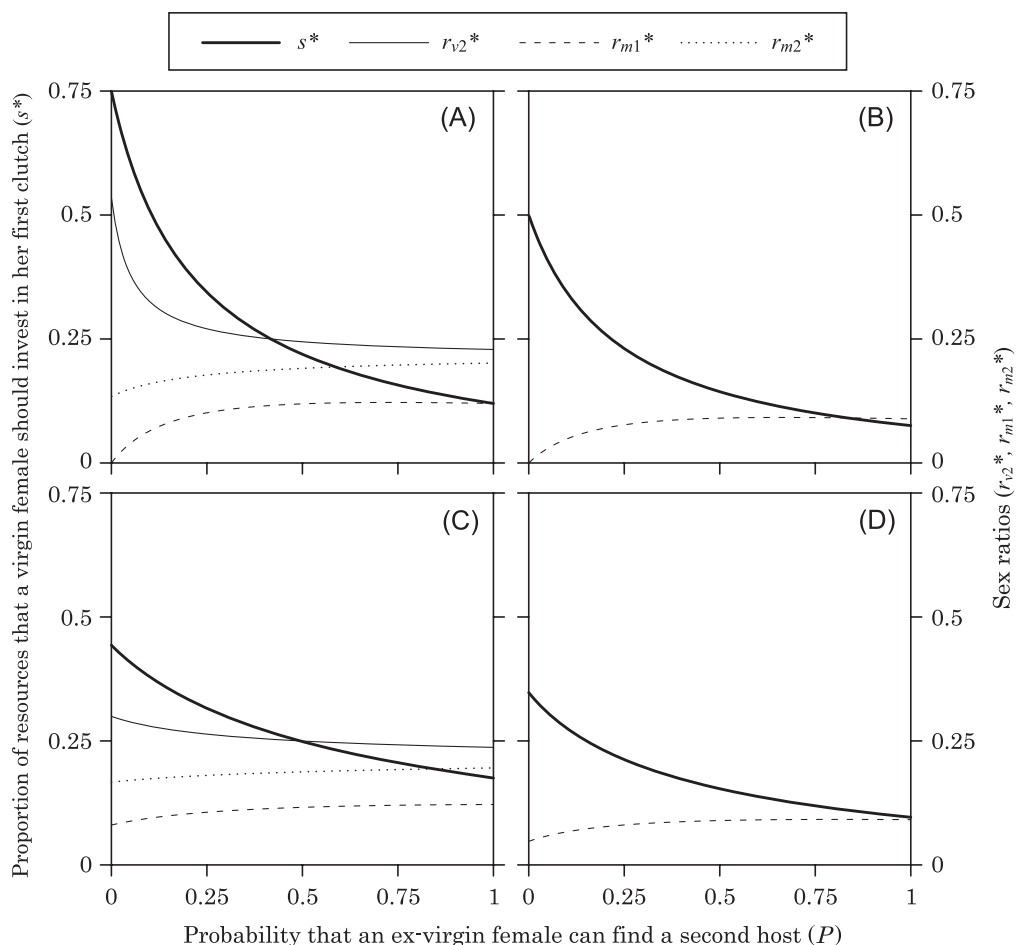


Figure A2
Evolutionary stable (ES) proportion of resources that a virgin female should invest in her first clutch (s^*) and ES sex ratios that the ex-virgin female should produce in her second clutch (r_{v2}^*) and that the mated females should produce in the first clutch (r_{m1}^*) and in the second clutch (r_{m2}^*), depending on the probability that the ex-virgin female can find her second host (P), when females can distinguish the mating status of cofounding females. The probability that the ex-virgin female lays eggs with a cofounding mated female in the second clutch (q) is 1 (A and C) or 0 (B and D). Developmental competition of offspring either does not occur ($a = 0$; A and B) or does occur ($a = 500$ and $k = 7 \times 10^{-4}$, see the Figure 1 legend for details; C and D).

not likely to occur in *Melittobia*. Because their common host species are distributed in clumps, dispersing females will find hosts within the same clump with a high probability (high P), but this means that they need to compete for host resources with other dispersing females (high q) and more than 2 foundresses are expected. Therefore, we predict much higher clutch size ratio ($s^* \gg 0.06$).

REFERENCES

- Abe J, Kamimura Y, Ito H, Matsuda H, Shimada M. 2003. 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, Kamimura Y, Kondo N, Shimada M. 2003. Extremely female-biased sex ratio and lethal male-male combat in a parasitoid wasp, *Melittobia australica* (Eulophidae). *Behav Ecol.* 14:34–39.
- Abe J, Kamimura Y, Shimada M. 2005. Individual sex ratios and offspring emergence patterns in a parasitoid wasp, *Melittobia australica* (Eulophidae), in relation to superparasitism and lethal combat between sons. *Behav Ecol Sociobiol.* 57:366–373.
- Abe J, Kamimura Y, Shimada M. 2007. Sex ratio schedules in a dynamic game: the effect of competitive asymmetry by male emergence order. *Behav Ecol.* 18:1106–1115.
- Abe J, Kamimura Y, Shimada M, West SA. 2009. Extremely female biased primary sex ratio and precisely constant male production in a parasitoid wasp *Melittobia*. *Anim Behav.* 78:515–523.
- van den Assem J, Gijswijt MJ, Nübel BK. 1980. Observation of courtship and mating strategies in a few species of parasitic wasps (Chalcidoidea). *Neth J Zool.* 30:208–227.
- Balfour Browne F. 1922. On the life-history of *Melittobia acasta*, Walker; a chalcid parasite of bees and wasps. *Parasitology.* 14: 349–370.
- Cook JM. 1993. Sex determination in the Hymenoptera: a review of models and evidence. *Heredity.* 71:421–435.
- Cooperband MF, Matthews RW, Vinson SB. 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.
- Crawley MJ. 2007. *The R book*. West Sussex (UK): John Wiley and Sons Ltd.
- Dahms EC. 1984. A review of the biology of species in the genus *Melittobia* (Hymenoptera: Eulophidae) with interpretations and additions using observations on *Melittobia australica*. *Mem Queensl Mus.* 21:337–360.
- Darwin C. 1871. *The descent of man and selection in relation to sex*. London: John Murray.
- Frank SA. 1985. Hierarchical selection theory and sex ratios. II. On applying the theory, and a test with fig wasps. *Evolution.* 39: 949–964.
- Frank SA. 1986. The genetic value of sons and daughters. *Heredity.* 56:351–354.
- Freeman BE. 1977. Aspects of the regulation of size of the Jamaican population of *Sceliphron assimile* Dahlbom (Hymenoptera: Sphecidae). *J Anim Ecol.* 46:231–247.
- Freeman BE, Ittayeipe K. 1976. Field studies on the cumulative response of *Melittobia* sp. (*Hawaiiensis* complex) (Eulophidae) to varying host densities. *J Anim Ecol.* 45:415–423.
- Freeman BE, Ittayeipe K. 1993. The natural dynamics of the eulophid parasitoid *Melittobia australica*. *Ecol Entomol.* 18:129–140.
- Godfray HCJ. 1986. Models for clutch size and sex ratio with sibling interactions. *Theor Popul Biol.* 30:215–231.
- Godfray HCJ. 1990. The causes and consequences of constrained sex allocation in haplodiploid animals. *J Evol Biol.* 3:3–17.
- Godfray HCJ. 1994. *Parasitoids: behavioral and evolutionary ecology*. Princeton (NJ): Princeton University Press.

- Godfray HCJ, Hardy ICW. 1993. Sex ratio and virginity in haplodiploid insects. In: Wrensch DL, Ebbert MA, editors. Evolution and diversity of sex ratio in insects and mites. New York: Chapman and Hall. p. 402–417.
- Godfray HCJ, Partridge L, Harvey PH. 1991. Clutch size. *Annu Rev Ecol Syst.* 22:409–429.
- Grafen A. 1984. Natural selection, kin selection and group selection. In: Krebs JR, Davies NB, editors. Behavioural ecology: an evolutionary approach. 2nd ed. Oxford: Blackwell Scientific Publications. p. 62–84.
- Greiff JM. 1997. Offspring sex allocation in externally ovipositing fig wasps with varying clutch size and sex ratio. *Behav Ecol.* 8:500–505.
- Hamilton WD. 1967. Extraordinary sex ratios. *Science.* 156:477–488.
- Hamilton WD. 1979. Wingless and fighting males in fig wasps and other insects. In: Blum MS, Blum NA, editors. Sexual selection and reproductive competition in insects. New York: Academic Press. p. 167–220.
- Hardy ICW, Pedersen JB, Sejr MK, Linderoth UH. 1999. Local mating, dispersal and sex ratio in a gregarious parasitoid wasp. *Ethology.* 105:57–72.
- Heimpel GE. 1994. Virginity and the cost of insurance in highly inbred Hymenoptera. *Ecol Entomol.* 19:299–302.
- Herre EA. 1987. Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature.* 329:627–629.
- Hobbs GA, Krunic MD. 1971. Comparative behavior of three Chalcidoid (Hymenoptera) parasites of the alfalfa leafcutter bee, *Megachile rotundata*, in the laboratory. *Can Entomol.* 103:674–685.
- Hogstedt G. 1980. Evolution of clutch size in birds: a comparative analysis. *Am Nat.* 134:72–87.
- Innocent TM, Savage J, West SA, Reece SE. 2007. Lethal combat and sex ratio evolution in a parasitoid wasp. *Behav Ecol.* 18:709–715.
- Ives AR. 1989. The optimal clutch size of insects when many females oviposit per patch. *Am Nat.* 199:671–687.
- Iwata K. 1980. Field notes of a naturalist 2. Tokyo (Japan): Asahi-Shinbunsha. in Japanese.
- Iwata K, Tachikawa T. 1966. Biological observations on 53 species of the superfamilies, Chalcioidea and Proctotruoideam from Japan (Hymenoptera: Apocria). *Trans Shikoku Entomol Soc.* 9:1–26.
- Kamimura Y, Abe J, Ito H. 2008. The continuous public goods game and the evolution of cooperative sex ratios. *J Theor Biol.* 252:277–287.
- King BH. 2002. Sex ratio response to conspecifics in a parasitoid wasp: test of a prediction of local mate competition theory and alternative hypotheses. *Behav Ecol Sociobiol.* 52:17–24.
- King BH, D'Souza JA. 2004. Effects of constrained females on offspring sex ratios of *Nasonia vitripennis* in relation to local mate competition theory. *Can J Zool.* 82:1969–1974.
- Krebs JR, Davies NB. 1997. Behavioural ecology. An evolutionary approach. 4th ed. Oxford: Blackwell Scientific.
- Lebreton S, Chevrier C, Darrouzet E. 2010. Sex allocation strategies in response to conspecifics' offspring sex ratio in solitary parasitoids. *Behav Ecol.* 21:107–112.
- Maeta Y, Yamane S. 1974. Host records and bionomics of *Melittobia japonica* Masi (Hymenoptera, Eulophidae). *Bull Tohoku Nat Agric Exp Stn.* 47:115–131.
- Matthews RW, González JM, Matthews JR, Deyrup LD. 2009. Biology of the parasitoid *Melittobia* (Hymenoptera: Eulophidae). *Annu Rev Entomol.* 54:251–266.
- Mayhew PJ. 1998. Offspring size-number strategy in the bethylid parasitoid *Laelius pedatus*. *Behav Ecol.* 9:54–59.
- Mayhew PJ, Glaizot O. 2001. Integrating theory of clutch size and body size evolution for parasitoids. *Oikos.* 92:372–376.
- Nagelkerke CJ. 1994. Simultaneous optimization of egg distribution and sex allocation in a patch structured population. *Am Nat.* 144:262–284.
- Nagelkerke CJ, Hardy ICW. 1994. The influence of developmental mortality on optimal sex allocation under local mate competition. *Behav Ecol.* 5:401–411.
- Parker GA, Courtney SP. 1984. Models of clutch size in insect oviposition. *Theor Popul Biol.* 26:27–48.
- Pexton JJ, Mayhew PJ. 2005. Clutch size adjustment, information use and the evolution of gregarious development in parasitoid wasps. *Behav Ecol Sociobiol.* 58:99–110.
- Reece SE, Innocent TM, West SA. 2007. Lethal male-male combat in the parasitoid *Melittobia acasta*: are size and competitive environment important? *Anim Behav.* 74:1163–1169.
- Rosenheim JA, Hongkham D. 1996. Clutch size in an obligately siblicidal parasitoid wasp. *Anim Behav.* 51:841–852.
- Seeger J, Stubblefield JW. 1996. Optimization and adaptation. In: Rose MR, Lauder GV, editors. Adaptation. San Diego (CA): Academic Press. p. 93–123.
- Shuker DM, Pen I, Duncan AB, Reece SE, West SA. 2005. Sex ratios under asymmetrical local mate competition: theory and a test with parasitoid wasps. *Am Nat.* 166:301–316.
- Shuker DM, Reece SE, Taylor JAL, West SA. 2004. Wasp sex ratios when females on a patch are related. *Anim Behav.* 68:331–336.
- Shuker DM, West SA. 2004. Information constraints and the precision of adaptation: sex ratio manipulation in wasps. *Proc Natl Acad Sci U S A.* 101:10363–10367.
- Skinner SW. 1985. Clutch size as an optimal foraging problem for insects. *Behav Ecol Sociobiol.* 17:231–238.
- Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. *Am Nat.* 108:499–506.
- Suzuki Y, Iwasa Y. 1980. A sex ratio theory of gregarious parasitoids. *Res Popul Ecol.* 22:366–382.
- Sykes EM, Innocent TM, Pen I, Shuker DM, West SA. 2007. Asymmetric larval competition in the parasitoid wasp *Nasonia vitripennis*: a role in sex allocation? *Behav Ecol Sociobiol.* 61:1751–1758.
- Taylor PD. 1981. Intra-sex and inter-sex sibling interactions as sex determinants. *Nature.* 291:64–66.
- Taylor PD. 1993. Female-biased sex ratios under local mate competition: an experimental confirmation. *Evol Ecol.* 7:306–308.
- Taylor PD. 1996. Inclusive fitness arguments in genetic models of behaviour. *J Math Biol.* 34:654–674.
- Ueda A. 1997. Brood development of an inbreeding spermatophagous scolytid beetle, *Coccotrypes graniceps* (Eichhoff) (Coleoptera: Scolytidae). *Jpn J Entomol.* 65:677–687.
- Waage JK, Godfray HCJ. 1985. Reproductive strategies and population ecology of insect parasitoids. In: Sibly R, Smith RH, editors. Behavioural ecology. Oxford: Blackwell Scientific. p. 449–470.
- Werren JH. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. *Science.* 208:1157–1159.
- Werren JH. 1984. Brood size and sex ratio regulation in the parasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Neth J Zool.* 34:123–143.
- West SA. 2009. Sex allocation. Princeton (NJ): Princeton University Press.
- West SA, Flanagan KE, Godfray HCJ. 1999. Sex allocation and clutch size in parasitoid wasps that produce single sex broods. *Anim Behav.* 57:265–275.
- West SA, Flanagan KE, Godfray HCJ. 2001. Variable host quality, life-history invariants and the reproductive strategy of a parasitoid wasp that produces single sex broods. *Behav Ecol.* 12:577–583.
- West SA, Herre EA. 1998. Stabilizing selection and variance in fig wasp sex ratios. *Evolution.* 52:475–485.
- West SA, Herre EA, Compton SG, Godfray HCJ, Cook JM. 1997. A comparative study of virginity in fig wasps. *Anim Behav.* 54:437–450.
- West SA, Shuker DM, Sheldon BC. 2005. Sex-ratio adjustment when relatives interact: a test of constraints on adaptation. *Evolution.* 59:1211–1228.
- Williams GC. 1979. The question of adaptive variation in sex ratio in out-crossed vertebrates. *Proc R Soc Lond B Biol Sci.* 205:567–580.
- Wilson K. 1994. Evolution of clutch size in insects. I. A review of static optimality models. *J Evol Biol.* 7:339–363.