



From inbreeding to social behaviour: exploring female-biased sex allocation in the silk wasp *Microstigmus rosae*

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Abstract

In the Hymenoptera, female-biased sex ratios are often attributed to Local Mate Competition (LMC), which occurs when related males compete for access to the same mates, reducing fitness returns for male offspring relative to females. In social species, advantageous interactions between cooperating females can also promote female bias via Local Resource Enhancement (LRE). Despite recent theory suggesting potential interactions between these phenomena, studies of LMC in social species remain scarce. This study investigates female-biased sex ratio adjustment in the silk wasp *Microstigmus rosae*, a species exhibiting both incipient sociality and likely localised mating. Our analyses reveal several genetic indicators of local mating, including heterozygote deficiency and high inbreeding coefficients. Moreover, pairwise relatedness values between mothers and their inferred mates indicate that 35% of mating pairs were siblings or parent-offspring. In further support of the role of LMC, we find that our observed sex investment ratio of 0.35 is statistically equivalent to the theoretically expected evolutionary stable sex ratio, as predicted by partial LMC. We also find a clearly defined male-first sequential sex allocation strategy (M-F-F), a pattern typically attributed to extreme LMC. In contrast, we find no perceivable effect of cooperative behaviour (LRE) on the brood sex ratio. This rare demonstration of inbreeding and LMC in a social aculeate hymenopteran highlights the potential role of these factors in driving the evolution of social group formation in this species.

Keywords Local mate competition (LMC) · Local resource enhancement (LRE) · Sex allocation · Social evolution · Inbreeding · Hymenoptera

Introduction

Within the Order Hymenoptera (bees, wasps, ants and sawflies), female-biased sex ratios are a common phenomenon. While early sex-ratio models emphasised the diminishing returns of producing an excess of one sex, through the effects of negative frequency-dependent selection (Fisher

1930), it is now understood that such sex ratio biases can be selectively advantageous. This can occur when interactions between related individuals cause fitness returns for sons and daughters to differ, resulting in selection on parents to favour production of the sex that offers higher reproductive value (Hamilton 1967; Charnov et al. 1981; Hardy 2002; West 2009). Hymenoptera are especially interesting for the study of such adaptive sex-ratio adjustment due to the phenomenon of haplodiploid sex determination, whereby fertilised eggs develop into female offspring and unfertilised eggs develop into males. In haplodiploid species, mothers can actively decide whether to fertilise eggs. Such maternal control of offspring sex has allowed for some of the most precise and elegant demonstrations of sex ratio theory in practice (Flanders 1939; West et al. 2005).

Possibly the most well-documented driver of sex ratio adjustment among Hymenoptera is “Local Mate Competition” (LMC). LMC is a specific type of resource competition that arises due to limited male dispersal leading to

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competition between related males for access to the same mates. Under these circumstances, any offspring sired by sons will diminish the potential reproductive output of their brothers, thereby generating selection for female-biased brood sex ratios as a result of reduced fitness returns for male offspring relative to females (Hamilton 1967; West 2009). In haplodiploid species, this effect is augmented as inbreeding also increases the relative relatedness of mothers to daughters (Herre 1985; Frank 1986). When mating between siblings takes place, the overproduction of daughters will also increase the number of mates available to sons - further reinforcing selection for female-biased sex ratios (Taylor 1981). However, LMC and inbreeding are not the only mechanism known to generate selection for female-bias in Hymenoptera. Cooperative species are also predicted to experience “Local Resource Enhancement” (LRE) owing to increased fitness benefits associated with individuals of the helping sex. As Hymenopteran helpers are almost always female, female-biased sex ratios are expected as a consequence of these advantageous cooperative interactions (Trivers and Willard 1973; Gowaty and Lennartz 1985; Emlen et al. 1986; Frank 1987; Frank and Crespi 1989; Pen and Weissing 2000; Gardner and Ross 2013).

Although studies of LMC in social taxa are rare, LMC and LRE are not mutually exclusive, with recent models emphasising how their interactions may lead to even more extraordinarily female-biased sex ratios (Iritani et al. 2021). Such female-bias has even been suggested to promote the evolution of cooperative helping behaviour itself, since the effective cost of helping may be reduced when there is increased competition between sisters for local resources (Gardner and Ross 2013; Quiñones and Pen 2017). The result is a hypothesised feedback between female bias and helping behaviour, which may work to increase selection for cooperative group formation. However, with much of the empirical research on LMC confined to the parasitoid wasps, which rarely exhibit social behaviour, opportunities for the exploration of these associations have been limited (although see: Abe et al. 2021; Tang et al. 2014). These studies are not only fundamental to our understanding of the ecological and evolutionary interactions that influence sex ratio adjustment but can also offer valuable insights into the broader implications of adaptive sex ratio bias.

This study uses population genetic analyses to explore the relationship between mating behaviour and sex allocation in *Microstigmus rosae*. *M. rosae* Field 2023 (Hymenoptera, Pemphredoninae) is an incipiently social silk wasp. Most nests contain just one adult female, but some contain between 2 and 4, usually daughters of the nest foundress. *M. rosae* nests are always founded by a single-female and social groups are formed when female offspring stay on the natal nest as helpers. Adult females mass provision each offspring

sequentially, remaining on the nest to produce subsequent broods. On average, eggs are laid every 10 days, and brood development takes > 35 days. Genetic analysis indicates a predominantly monogamous mating system (Bonifacii and Field 2023). Previous investigations into the nesting biology of *M. rosae* have also indicated that: (a) primary sex-ratios are female-biased and (b) mating behaviour may be consistent with ‘partial localised mating’, whereby, prior to dispersal, male offspring remain for some time on their natal nest possibly to mate with emerging sisters (Bonifacii and Field 2023). *Microstigmus rosae* therefore provides an ideal opportunity to examine the potential effects of LMC on sex allocation in an incipiently social species.

We aim to achieve two main objectives: (1) To establish the levels of localised mating, particularly mating between siblings (sib-mating), occurring in our wild populations; and (2) To identify the likely selective forces underlying the observed female-bias, by comparing our findings with theoretical predictions of sex allocation theory. We discuss our results in relation to the consequences of inbreeding and female bias, with particular focus on the likely implications for the evolution of social group formation.

Methods

Overview

We begin first by conducting a comprehensive analysis of various genetic indicators of localised mating, including: (a) estimating individual and population level inbreeding coefficients; (b) testing allele frequencies for departures from Hardy–Weinberg equilibrium; and (c) investigating the incidence of diploid male production (a scenario predicted under conditions of inbreeding) through detecting heterozygosity among morphologically male adults. We then estimate the occurrence of mating between siblings. To do this, we first infer the genotypes of each adult female’s mate by comparing her genotype to those of her female offspring. Using these inferred genotypes, we can then calculate the relatedness coefficients between all female-mate pairs and compare these with the distribution from known sister-brother relationships. We compare the observed sex ratios to theoretical predictions for the observed incidence of sib-mating according to partial LMC theory.

Finally, we examine both precise and sequential offspring sex allocation by individual adult foundresses, comparing our observations with those expected under the two principal explanations for female-biased sex ratio adjustment in Hymenoptera (LMC and LRE).

Field methods

All nests used in these analyses were collected from two reserves located within the Pichincha province of North-central Ecuador: *Mashpi Biodiversity Reserve*. N 00°10.019' W 078°52.326' and *Santa Lucia Cloud Forest Reserve*. N 00°07.062' W 078°36.454'. Most were collected during two main field seasons: the first from 31-01-2016 to 01-05-2016 and the second from 12-01-2017 to 10-04-2017, but a smaller number were collected during three preliminary data collection periods from 23-08-2014 to 18-09-2014, from 15-08-2015 to 15-09-2015 and from 01-11-2015 to 21-12-2015.

Nests were found by searching the undersides of leaves and other structures. Our dataset includes two types of nests: (1) those for which we knew the approximate founding date (“newly founded”) and (2) those for which the founding dates were unknown (“pre-existing”). Nests of known founding date were used to examine the order of brood sex allocation by individual adult foundresses. These nests were identified by systematically monitoring 987 locations where *M. rosae* was commonly found nesting. Nesting locations primarily comprised *Xanthosoma sagittifolium* plants, but also included some man-made structures. Every five days, each nesting location was examined for the appearance of newly founded nests. Upon discovery, we assigned each nest a unique identification code and the location and date was recorded. This method enabled us to assign the date of nest founding to a five-day window. In contrast, pre-existing nests were those nests that were: (a) already established on the marked nesting locations at the start of either main field season; (b) found outside of our marked nesting locations or (c) found during the three preliminary data collection periods. These nests were used to examine population brood sex ratios. Since the founding dates of pre-existing nests were unknown, they represent a “snapshot” dataset comprising a range of nests at unknown stages of maturity.

Nests were collected by carefully detaching the nest petiole from the substrate and placing them into resealable bags. Collections took place in the evening, when all nest occupants, including foraging adults, were likely to be inside, thereby ensuring a complete sample. The same evening, we dissected nests using a Leica S6D binocular microscope and recorded the following data: number of prey ball-egg assemblages, larvae, prepupae, pupae including eye colour (yellow, brown, black) and pre-adults. After dissection, we stored all nest contents in 100% ethanol for subsequent genetic analysis.

DNA extraction, amplification and analysis

DNA was extracted using the ammonium acetate method for adults and larger brood (see Lucas et al. 2011). For eggs and small larvae, we used the “hot shot” method (Truett et al. 2000), which, although less refined, is more effective for small tissue samples. We then genotyped all individuals and brood of interest at 10 microsatellite loci originally developed for *M. nigrophthalmus* (Lucas et al. 2009) and later determined to be polymorphic for *M. rosae*: Mni002, Mni003, Mni009, Mni017, Mni019, Mni024, Mni028, Mni030, Mni034, Mni036 (Lucas et al. 2009; Bonifacii and Field 2023). Loci were amplified with multiplex polymerase chain reactions (PCR) and products were diluted and added to formamide before being sequenced using a MiSeq Benchtop Sequencer (Illumina) at the University of Exeter, Penryn. We scored alleles using the software Geneious 10.0.9. To calculate genotyping error rates, we repeated PCRs for 20% of DNA samples and compared the genotyping results using the software PENDANT (Johnson & Haydon, 2007). Marker data including error rates for the 10 microsatellite loci analysed can be found in Bonifacii and Field (2023).

Sex determination

We use brood sex ratio for our analyses as this should represent an accurate measure of population-level investment into each sex, unaffected by differential adult mortality. Brood sex was determined by observing the number of alleles present at each microsatellite locus; we considered offspring found to be homozygous at every locus to be males, whereas those that were heterozygous at any of the amplified loci were considered female. Based on the observed allele frequencies, the probability of a female being homozygous at every locus and thus wrongly assigned as a male was calculated as $3.4E-06$ (method of Boomsma and Ratnieks 1996).

Inbreeding estimates

To assess the level of inbreeding in our populations, we examined our microsatellite loci for departures from Hardy–Weinberg equilibrium using GENEPOP 4.7 (Raymond and Rousset 1995; Rousset 2008). GENEPOP calculates p-values using an exact test based on the Markov Chain Monte Carlo (MCMC) method, which is particularly robust for detecting deviations from equilibrium in smaller samples. We then conducted two independent one-tailed tests across all loci (one for each population), under the null hypothesis of no deficiency in heterozygotes following Hardy–Weinberg expectations (Louis and Dempster 1987). Parameters were set to 1,000 dememorization steps, 20 batches, and 1,000 iterations per batch.

We additionally estimated individual and population-level inbreeding coefficients using Wang's triadic maximum likelihood (TrioML) estimator, implemented in the software COANCESTRY (Wang 2007, 2011). TrioML is known to be more accurate than other estimators for data where both the reference allele frequencies and inbreeding coefficients must be calculated from the same sample, as is the case in this study (Wang 2014).

As an additional indicator of inbreeding, we examine the incidence of diploid male production using the genotypes of 38 of the 47 morphologically adult males which were present in nests upon collection (one per nest to avoid pseudoreplication). In Haplodiploid species inbreeding increases the likelihood of diploid male production due to the genetic mechanism of single-locus complementary sex determination (sl-CSD) (Whitehorn et al. 2009). Under this system, individuals that are heterozygous at this locus develop into females, while those that are hemizygous (haploid) or homozygous (diploid) develop into males (Whiting 1943). Using the same genetic samples, we repeated PCR amplifications three times for each adult male. Males were classified as confirmed diploids if they were consistently found to be heterozygous at one or more microsatellite loci on all repeat runs.

Genetic relationships

To estimate the frequency of mating between siblings, we start by statistically inferring within nests pedigree relationships using the software COLONY. This software uses a full-pedigree likelihood approach to simultaneously infer parentage and sibling relationships. We conducted three independent replicate analyses for each population, each initiated with a different random number seed to ensure the reliability and robustness of our results. These analyses used the following parameter settings: medium run length, full likelihood, specifying haplodiploid sex determination with female monogamy, male polygamy and error rates calculated from our repeat genotyping analysis. Relationship assignments were accepted as reliable if the same pedigree configuration between two individuals was consistently recovered with an assigned probability of over 90% in at least two out of the three runs.

For female broods where COLONY inconsistently assigned either a full-sibling or a mother-offspring relationship to a pair of individuals on different runs, we implemented an alternative relationship assignment method. Using the software KINGROUP v.2 (Konovalov et al. 2004), we examined whether inconsistently assigned pairs were significantly more likely to be (a) full sisters than mother-offspring, (b) mother-offspring than full sisters (c) mother-offspring than aunt-niece or (d) aunt-niece than

mother-daughter. Full sister relationships were assigned wherever such a relationship was found to be significantly more likely than a mother-offspring relationship, without the opposite being true. Those pairs for which a mother-offspring relationship was significantly more likely than a full-sister (without the opposite being true) were also tested against the likelihood of a maternal aunt-niece relationship. Thus, we assigned a pair as mother-offspring only if we found this relationship to be significantly more likely than both a full-sister and an aunt-niece relationship, without the opposite being true.

Due to the limited number of loci available, KINGROUP was not able to accurately determine relationships for haploid individuals. Therefore, male brood that could not be assigned to a mother using COLONY were assigned manually. We assigned relationships between adult females and male brood as mother-offspring if the male shared an allele at every locus with the focal female. More distant relationships between adult females and male brood were not assigned.

Inference of mate relatedness

We calculated female-mate relatedness for all adult females that could be confidently assigned at least one female offspring using the aforementioned methodology. To achieve this, we first inferred the putative genotypes of each female's mate using the software MATESOFT. This software is specifically designed for the analysis of mating systems in male-haploid organisms and can determine the genotypes of inferred mates from the genotypes of an adult foundress and her female offspring. Using the genotypes generated for these inferred mates, we then calculated relatedness coefficients for all female-mate pairs using the software RELATEDNESS (Queller and Goodnight 1989). In cases where the mother and her daughters have identical heterozygous genotypes at a locus, the exact male genotype cannot be conclusively determined and MATESOFT proposes several alternative genotypes for a given mate. Where this was the case, we calculated relatedness values for all proposed mate genotypes and used the average of these values for subsequent analyses. Separate analyses were conducted for both populations (Santa Lucia and Mashpi), nests were weighted equally, and data were jack-knifed over loci and nests to obtain standard error and 95% confidence intervals. For comparison, we also estimated the relatedness values for known sister-brother pairs (brood from newly founded nests) using the same methodology.

We plotted the probability density functions of the estimated relatedness coefficients using kernel density estimation and statistically examined the number of modes in both distributions using excess mass tests from the R-package

‘multimode’ (Ameijeiras-Alonso et al. 2021). This test estimates the number of modes for the density distribution underlying a sample by testing the null hypothesis of a unimodal distribution against an alternative hypothesis of specified multimodal distributions (bimodal, trimodal). P-values were calculated by bootstrapping 500 times over samples, a standard approach integrated within the ‘multimode’ package. Finally, as an approximation of the proportion of sib mating represented in the dataset, we calculated the number of pairwise female-mate relatedness values which were within one standard deviation from the mode of the distribution of known sister-brother relationships. Using the estimated proportion of sib-mating (k), we calculated the theoretically expected population sex ratio (sr), as predicted by partial LMC theory, using the following equation:

$$sr = \frac{(1 - k)(2 - k)}{4 - k}$$

(Taylor 1993; Greeff 2002).

Precise and sequential sex allocation

LMC theory predicts that, to maximise fitness, selection will favour precise sex allocation with the tendency to produce one male and $n-1$ females in a brood size of n . To investigate whether *M. rosae* females exhibit precise sex allocation, we implemented a binomial exact test. This test compares the observed number of broods with a single male to the expected number under a binomial distribution for each brood size. As we are interested the overall tendency towards precise sex allocation, we implemented a global binomial test by aggregating results across all brood sizes.

Local Mate Competition may also favour males being produced first, or early, in the oviposition sequence. Such a strategy reduces the likelihood that female-biased brood will contain no males and, for species which rely on inbreeding for female insemination, will minimise the likelihood of virgin females dispersing from the nest (Green et al. 1982; Waage and Ming 1984; Nagelkerke 1996; Kapranas et al. 2008). To test for the existence of sequential sex allocation, we ranked offspring within nests from eldest to youngest using the sequence of offspring development established by Bonifacii and Field (2023). We then implemented a logistical regression analysis using a binomial error structure to examine whether the conditional probability of an offspring being either male or female is related to its age-rank within the nest.

Statistical analyses & linear models

All statistical analyses were performed using R Version 3.5.3 (<http://www.r-project.org>) and RStudio Version 1.1.463 (<https://www.rstudio.com>). For our linear models we used the package ‘glmmTMB’ (version 0.2.3; Brooks et al. 2017, R Core Team 2019) which allows for the implementation of generalized linear mixed effect models with various extensions including zero-inflation.

Results

A total of 127 nests were analysed, collected across an altitudinal gradient ranging from 771 to 1950 m above sea level. Among these, 71 were newly founded, while 56 were pre-existing. A total of 145 adult females from 116 of these nests, 38 adult males from 32 nests, and 275 brood from 127 nests were successfully genotyped.

Inbreeding coefficients

The average estimated TrioML inbreeding coefficient (\pm SD) for pooled data was calculated to be 0.20 ± 0.2 . No significant difference in the level of inbreeding between years ($LRT_2 = 1.64$, $p = 0.44$) or between our two populations was detected (Santa Lucia: 0.17 ± 0.21 , Mashpi: 0.20 ± 0.20 , $LRT_1 = 0.982$, $p = 0.32$).

Tests for Hardy-Weinberg equilibrium

Numbers of heterozygotes observed and expected were calculated for 116 adult females (one female per nest to avoid pseudoreplication). Tests for departures from Hardy–Weinberg equilibrium revealed either significant or borderline significant heterozygote deficiency at all ten loci and within each population (Table 1. Supplementary Material). While such heterozygote deficiency is often indicative of non-random mating, it may also result from the presence of null alleles (alleles which cannot be detected by PCR amplification) resulting in the inaccurate assignment of homozygosity to null-allele heterozygotes. Duplicate genotyping analysis revealed low levels of allelic dropout for these loci (Bonifacii and Field 2023). Moreover, in species with haplodiploid sex determination, null alleles are easily detected in haploid males. As all genotyped *M. rosae* samples produced detectable peaks at every locus, it is unlikely that null alleles are contributing to the observed heterozygote deficiency in this analysis.

Female-mate relatedness

The average male to female relatedness coefficients (\pm SD) for the 54 inferred female-mate pairs was 0.23 ± 0.42 . GLM analyses reveal no significant difference between female-mate relatedness and the year that the nest was collected ($LRT_2=0.50$, $p=0.77$). Mean relatedness (\pm CI95) was higher at Santa Lucia (0.24 ± 0.35) than Mashpi (0.1 ± 0.43), but the difference was not significant ($LRT_2=0.98$, $p=0.32$).

Examination of the probability density function of individual relatedness values reveals a multimodal distribution with three distinct “peaks” (local maxima/ modes) of similar height (Fig. 1). These observations are statistically confirmed using excess mass tests which indicate that the true number of modes is greater than two (excess mass = 0.082, p -value = 0.04) but not greater than three (excess mass = 0.052, p -value = 0.45). The first of the three peaks falls at $r=-0.27$. These low relatedness values are indicative of outbreeding. The second mode is found at $r=0.2$ and is

suggestive of mating between an assortment of second and third-degree relatives. The third mode falls at $r=0.68$ and likely represents sister-brother pairs. For comparison, the distribution of relatedness values for 40 sister-brother pairs, from different nests, was also calculated. This distribution was found to be unimodal (excess mass = 0.072, $p=0.91$) with a single peak at $r=0.64 \pm 0.22$. Under a scenario of panmictic, monogamous mating, mean relatedness between a sister and her brother is expected to be $r=0.5$.

Females tend to exhibit precise sex allocation, with global binomial exact tests indicating a greater proportion of single-male broods than expected under binomial distribution at the expected sex ratio of 0.29 ($p < 0.001$). Furthermore, we also find a strong tendency towards precise, male-first sex allocation. Examination of offspring production in newly founded nests demonstrates that 80.4% of nests have a male as the first offspring produced. Statistical analyses confirm this, indicating that the position of an offspring in the oviposition sequence is a strong predictor of

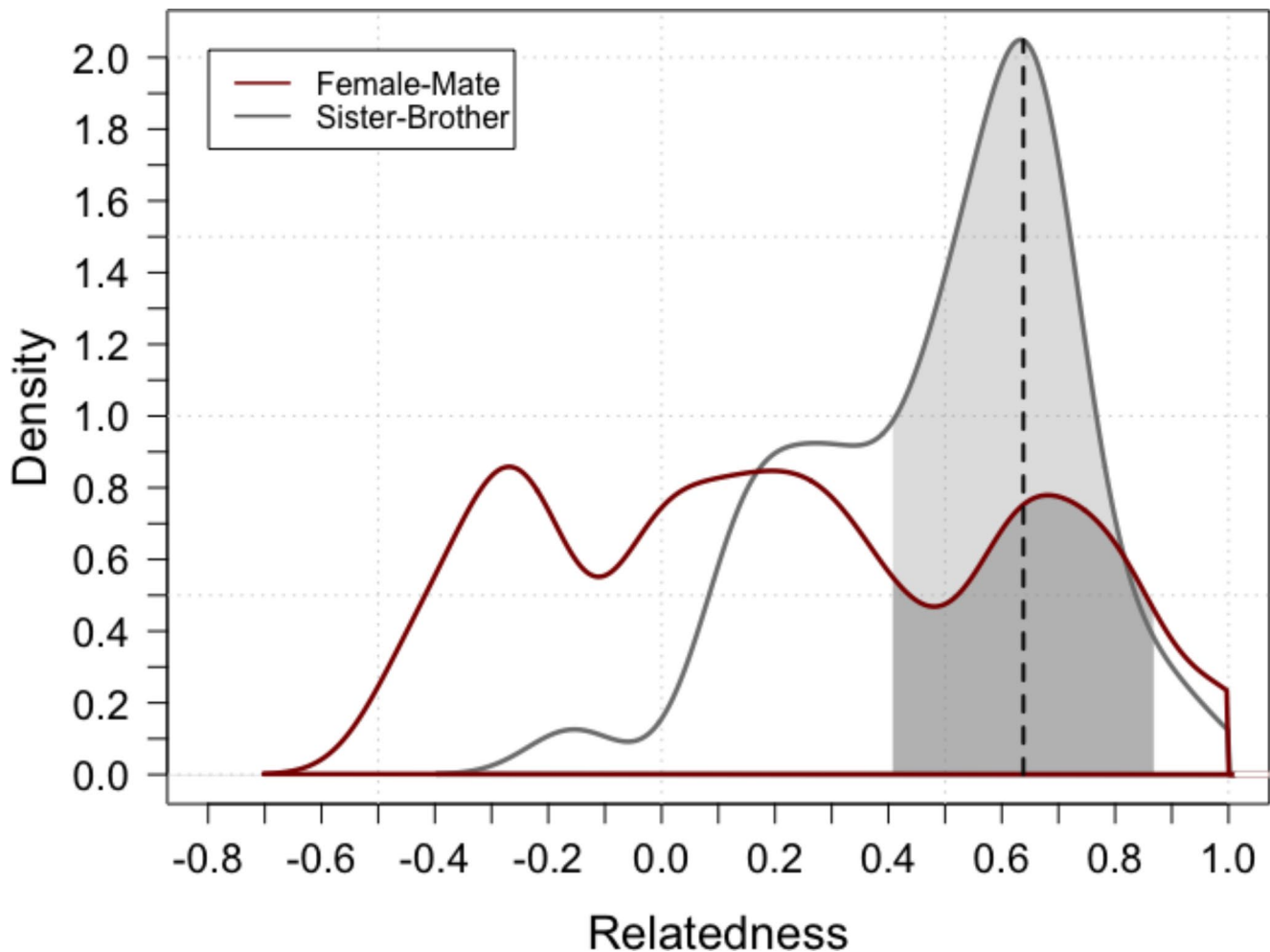


Fig. 1 Kernel density distribution of pairwise relatedness values between adult females and their inferred mates ($n=54$, red) and known brother sister pairs ($n=40$, grey). The dashed black line represents the

local maximum for known sister-brother relatedness values and the shaded area indicates one standard deviation from the mode of this distribution

sex in newly founded nests (Fig. 2b, $n=57$, $LRT=66.18$, $p<0.001$).

Discussion

This study investigates sex ratio adjustment in *Microstigmus rosae*, an incipiently social wasp known to exhibit female-biased brood sex ratios. Utilising microsatellite genotyping, we investigate the roles of LMC and LRE in generating selection for sex ratio adjustment. Significant heterozygote deficiency detected across both field sites combined with high inbreeding coefficients suggest that non-random mating

is occurring with some regularity in *M. rosae*. However, Hardy-Weinberg disequilibrium and high inbreeding coefficients can arise from either sib-mating within nests and/or limited dispersal causing population genetic structuring. While LMC will be generated via any form of non-random mating between relatives, examination of relatedness values between mothers and their inferred mates showed that 35% of foundress females had mated with a likely brother. These results indicate that mating between close relatives is common in this species and that the observed female-biased sex ratios are likely to have evolved due to the resulting competition between closely related males for mates (Hamilton 1967).

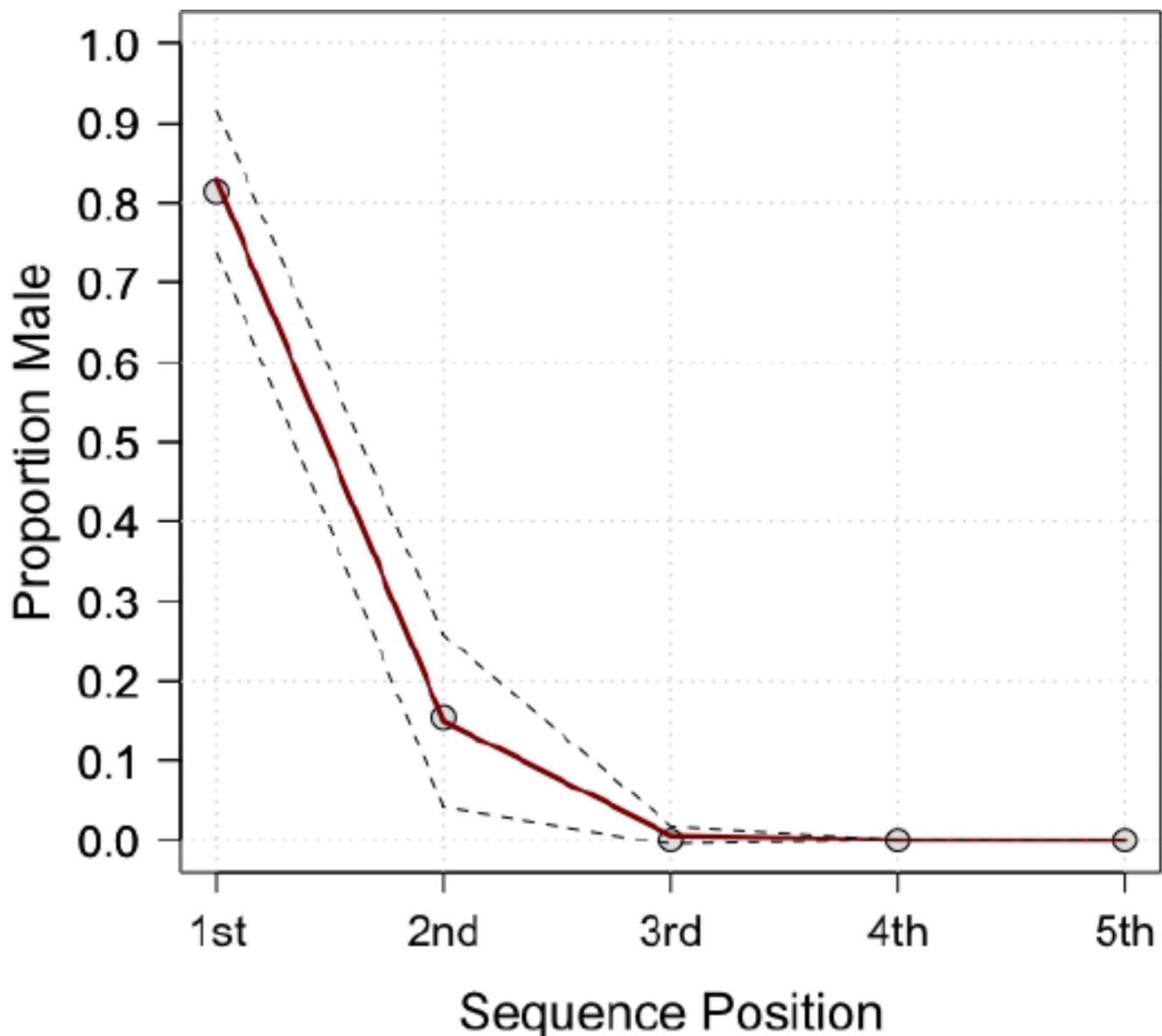


Fig. 2 The probability of an offspring being male is strongly related to the sequence of oviposition within nests. Points show the proportion of male offspring found at each sequence position and the red line shows

the fits of the binomial logistical regression analyses. The dashed lines show the 95% confidence intervals

Examination of newly founded nests has also shown that *M. rosae* exhibits a clearly defined male-first sequential sex allocation strategy (M-F-F), a pattern typically attributed to extreme LMC (Waage 1982; Wajnberg 1993; West 2009). LMC theory predicts that, to maximise fitness, selection will favour the production of males first in the oviposition sequence. Such a strategy reduces the likelihood that a brood will contain no males and, for species which rely on inbreeding for female insemination, will minimise the likelihood of virgin females dispersing from the nest (Green et al. 1982; Waage and Ming 1984; Nagelkerke 1996; Kapranas et al. 2008). In contrast, another possible explanation for female bias in this species, Local Resource Enhancement, predicts a female-first sex allocation strategy as early female offspring often contribute the most to maternal fitness (Packer 1990; Schwarz 1994; Neville et al. 1998; Hogendoorn et al. 2001; Bull and Schwarz 2001). As such, the male-first strategy observed suggests that LMC is the dominant force shaping sex allocation strategies in *M. rosae* and that the enhanced reproductive value of producing early males outweighs any potential advantages of an early female helper.

Due to the expected positive fitness effects of producing female offspring in this incipiently social species, we might also expect to see an effect of LRE creating additional selection for female bias in the brood sex ratio. As LMC and LRE are not mutually exclusive, their combined influence is hypothesised to give rise to even more female biased sex ratios than those predicted by LMC theory alone (Abe et al. 2021; Iritani et al. 2021). As the observed brood sex investment ratios in *M. rosae* are statistically equivalent to those predicted by partial LMC theory, we do not find evidence for the hypothesised additional effect of LRE in *M. rosae*. Because *M. rosae* is only incipiently social, the fitness benefits derived from cooperative behaviour may not exert a strong enough influence to drive further selection for female biased sex ratio allocation, but there are also other potential explanations:

- (1) As localised mating is high in our study population, using genetic heterozygosity to determine brood sex will underestimate female-bias. Although the likelihood of homozygous female brood being erroneously assigned as males is estimated to be very low, these calculations are made under the assumption of panmictic mating. Inbreeding, particularly sib mating, significantly increase the frequency of such errors, potentially obscuring any effects of LRC in increasing female bias.
- (2) Secondly, a mother's ability to achieve the optimal sex ratio is likely to be limited when brood sizes are small. The necessity of producing at least one male per brood, combined with the demonstrated male-first sequential sex allocation, inherently limits a mother's capacity to

achieve the optimal sex ratio within a nest. For example, if mothers are restricted to producing $n-1$ females in a brood size of n , the predicted optimal female bias for *M. rosae* of 0.29 (1:3 male to female) can be realised only in nests with brood sizes of four or more. Given the small brood sizes observed in *M. rosae*, the brood sex ratio is likely to be skewed towards males, further obscuring the detection of the full impact of LRE and LMC.

Therefore, while we find no perceivable effect of LRE on the brood sex ratio in *M. rosae*, we cannot rule out LRE creating additional selection for female bias. It seems probable that the combined effects of increased homozygosity and the inherent limitations of small brood sizes may be obscuring any additional influence of LRE.

The empirical literature on Local Mate Competition (LMC) has primarily focused on the parasitoid wasps, a specific taxonomic lineage containing species exhibiting life history traits that align closely with theoretical expectations. This specific focus on certain taxa has constrained opportunities to investigate the relationships between sex ratio adjustments in the context of LMC and a broader array of life history characteristics. One such association is the hypothesised interplay between inbreeding, LMC, female biased sex allocation and social evolution. In inbreeding populations, mating among close relatives can lead to increased genetic relatedness between family members. Augmented relatedness will enhance indirect fitness advantages to helping, thereby strengthening selection for cooperation (Wade and Breden 1981; Breden and Wade 1981; Strassmann et al. 2011; Abbot et al. 2011). Although significant inbreeding has not been described for the majority of social Hymenoptera, local mating is common in other social taxa including the naked mole rats (Reeve et al. 1990; Faulkes et al. 1997), eusocial gall thrips (Chapman and Crespi 1998; Chapman 2003), bark and ambrosia beetles (Kirkendall 1983, 1993), spider mites (Saito 1997, p. 199, 2010), the social spiders (Riechert and Roeloffs 1993; Avilés 1997; Lubin and Bilde 2007; Avilés and Purcell 2012), myrmicine ants (Kinomura and Yamauchi 1987; Buschinger 1989; Cremer et al. 2012), and some psocids (Mockford 1957; New 1973).

Female-biased sex ratios generated via LMC may also result in increased competition between sisters, as the more common sex, for local resources. Increased kin competition may reduce the reproductive potential of females, thereby lowering the direct reproductive sacrifice made by individuals that engage in helping behaviour. This combination of decreased opportunities for direct reproduction with the potential for enhanced indirect fitness payoffs to helping (generated via inbreeding) may work synergistically to intensify selection for social behaviour (Gardner and Ross

2013). In addition, due to the genetic inheritance patterns associated with haplodiploid sex determination, inbreeding in haplodiploid organisms may increase the relative value of daughters versus sons which could act to promote the impact of haplodiploidy on eusociality via sex-ratio evolution (Tabadkani et al. 2012). Further investigations into these relationships may provide valuable insights into the broader mechanisms influencing the evolution of sociality in the animal kingdom.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00265-024-03533-9>.

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Author contributions Both authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Rosa Bonifacii. The first draft of the manuscript was written by Rosa Bonifacii with comments and editing by Jeremy Field. Both authors have read and approved the final manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available in the figshare repository, <https://figshare.com/s/1f53c7ac657f99f4ceec>.

Declarations

Competing interests The authors declare that they have no known competing interests that could influence the work reported in this paper.

Significance statement In species where inbreeding occurs, mothers may bias sex allocation towards females to reduce competition among sons for mates (Local Mate Competition/ LMC). Species with female helpers may also favour female bias due to cooperative interactions (Local Resource Enhancement/ LRE). This study uses genetic analysis to explore the causes and consequences of female bias in the silk wasp, *Microstigmus rosae*. We find that *M. rosae* exhibits a unique mating system characterised by frequent sibling mating. These results closely align with predictions of partial LMC theory and provide a rare example of LMC in a social Hymenopteran. Such unexpectedly high inbreeding coefficients parallel those found in other eusocial lineages such as gall-forming thrips and ambrosia beetles, prompting discus-

sion of the potentially significant role of inbreeding in the evolution of social group formation.

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References

- Abbot P, Abe J, Alcock J et al (2011) <ArticleTitle Language="En">Inclusive fitness theory and eusociality. *Nature* 471:E1–E4. <https://doi.org/10.1038/nature09831>
- Abe J, Iritani R, Tsuchida K et al (2021) A solution to a sex ratio puzzle in *Melittobia* wasps. *Proc Natl Acad Sci U S A* 118:e2024656118. <https://doi.org/10.1073/pnas.2024656118>
- Ameijeiras-Alonso J, Crujeiras RM, Rodriguez-Casal A (2021) multi-mode: An R Package for Mode Assessment. *J Stat Softw* 97:1–32. <https://doi.org/10.18637/jss.v097.i09>
- Avilés L (1997) Causes and consequences of cooperation and permanent-sociality in spiders. In: Crespi BJ, Choe JC (eds) *The Evolution of Social Behaviour in Insects and Arachnids*. Cambridge University Press, Cambridge, pp 476–498
- Avilés L, Purcell J (2012) Chap. 3 - The Evolution of Inbred Social Systems in Spiders and Other Organisms: From Short-Term Gains to Long-Term Evolutionary Dead Ends? In: Brockmann HJ, Roper TJ, Naguib M, (eds) *Advances in the Study of Behavior*. Academic Press, pp 99–133
- Bonifacii RL, Field J (2023) Nesting biology and social organisation of a silk wasp (*Microstigmus rosae*) from the North–West Ecuadorian Choco. *Insectes Sociaux* 70:167–179. <https://doi.org/10.1007/s00040-023-00914-7>
- Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. *Philos Trans R Soc Lond B Biol Sci* 351:947–975. <https://doi.org/10.1098/rstb.1996.0087>
- Breden F, Wade MJ (1981) Inbreeding and evolution by kin selection. *Ethol Sociobiol* 2:3–16. [https://doi.org/10.1016/0162-3095\(81\)90018-2](https://doi.org/10.1016/0162-3095(81)90018-2)
- Bull NJ, Schwarz MP (2001) Brood insurance via protogyny: a source of female-biased sex allocation. *Proc R Soc Lond B Biol Sci* 268:1869–1874. <https://doi.org/10.1098/rspb.2001.1687>
- Buschinger A (1989) Evolution, speciation, and inbreeding in the parasitic ant genus *Epimyrma* (Hymenoptera, Formicidae). *J Evol Biol* 2:265–283. <https://doi.org/10.1046/j.1420-9101.1989.2040265.x>
- Chapman TW (2003) An Inclusive Fitness-Based Exploration of the Origin of Soldiers: The Roles of Sex Ratio, Inbreeding, and Soldier Reproduction. *J Insect Behav* 4:481–501. <https://doi.org/10.1023/A:1027351206403>
- Chapman TW, Crespi B (1998) High relatedness and inbreeding in two species of haplodiploid eusocial thrips (Insecta: Thysanoptera) revealed by microsatellite analysis. *Behav Ecol Sociobiol* 43:301–306. <https://doi.org/10.1007/s002650050495>
- Charnov EL, Los-den Hartogh RL, Jones WT, van den Assem J (1981) Sex ratio evolution in a variable environment. *Nature* 289:27–33. <https://doi.org/10.1038/289027a0>

- Cremer S, Suefuji M, Schrempf A, Heinze J (2012) The dynamics of male-male competition in *Cardiocondyla obscurior* ants. *BMC Ecol* 12:7. <https://doi.org/10.1186/1472-6785-12-7>
- Emlen ST, Emlen JM, Levin SA (1986) Sex-Ratio Selection in Species with Helpers-At-The-Nest. *Am Nat* 127:1–8. <https://doi.org/10.1086/284463>
- Faulkes CG, Abbott DH, O'Brien HP et al (1997) Micro- and macrogeographical genetic structure of colonies of naked mole-rats *Heterocephalus glaber*. *Mol Ecol* 6:615–628. <https://doi.org/10.1046/j.1365-294X.1997.00227.x>
- Field J (2023) Description and nesting biology of three new species of neotropical silk wasp (Hymenoptera: Apoidea: Pemphredoninae: *Microstigmus*). *J Nat Hist* 57:1–18. <https://doi.org/10.1080/0022933.2022.2157345>
- Fisher RA (1930) *The Genetical Theory of Natural Selection*. Clarendon Press Oxford
- Flanders SE (1939) Environmental Control of Sex in Hymenopterous Insects. *Ann Entomol Soc Am* 32:11–26. <https://doi.org/10.1093/aesa/32.1.11>
- Frank SA (1986) The genetic value of sons and daughters. *Heredity* 56(Pt 3):351–354. <https://doi.org/10.1038/hdy.1986.56>
- Frank SA (1987) Variable sex ratio among colonies of ants. *Behav Ecol Sociobiol* 20:195–201. <https://doi.org/10.1007/BF00299733>
- Frank SA, Crespi BJ (1989) Synergism between sib-rearing and sex ratio in Hymenoptera. *Behav Ecol Sociobiol* 24:155–162. <https://doi.org/10.1007/BF00292098>
- Gardner A, Ross L (2013) Haplodiploidy, Sex-Ratio Adjustment, and Eusociality. *Am Nat* 181:E60–E67. <https://doi.org/10.1086/669147>
- Gowaty PA, Lennartz MR (1985) Sex Ratios of Nestling and Fledgling Red-Cockaded Woodpeckers (*Picoides borealis*) Favor Males. *Am Nat* 126:347–353. <https://doi.org/10.2307/2461359>
- Greeff JM (2002) Mating system and sex ratios of a pollinating fig wasp with dispersing males. *Proc R Soc Lond B Biol Sci* 269:2317–2323. <https://doi.org/10.1098/rspb.2002.2160>
- Green RF, Gordh G, Hawkins BA (1982) Precise Sex Ratios in Highly Inbred Parasitic Wasps. *Am Nat* 120:653–665. <https://doi.org/10.1086/284018>
- Hamilton WD (1967) Extraordinary Sex Ratios. *Science* 156:477–488. <https://doi.org/10.1126/science.156.3774.477>
- Hardy IC (2002) *Sex ratios. Concepts and research methods*. Cambridge University Press, UK
- Herre EA (1985) Sex Ratio Adjustment in Fig Wasps. *Science* 228:896–898. <https://doi.org/10.1126/science.228.4701.896>
- Hogendoorn K, Watiniasih N, Schwarz M (2001) Extended alloparental care in the almost solitary bee *Exoneurella eremophila* (Hymenoptera: Apidae). *Behav Ecol Sociobiol* 50:275–282. <https://doi.org/10.1007/s002650100357>
- Iritani R, West SA, Abe J (2021) Cooperative interactions among females can lead to even more extraordinary sex ratios. *Evol Lett* 5:370–384. <https://doi.org/10.1002/evl3.217>
- Kapranas A, Pacheco P, Forster LD et al (2008) Precise sex ratios manifested by several encyrtid parasitoids (Hymenoptera: Encyrtidae) of brown soft scale, *Coccus hesperidum* L. (Hemiptera: Coccidae). *Behav Ecol Sociobiol* 62:901–912. <https://doi.org/10.1007/s00265-007-0514-z>
- Kinomura K, Yamauchi K (1987) Fighting and mating behaviors of dimorphic males in the ant: *Cardiocondyla wroughtoni*. *J Ethol* 5:75–81. <https://doi.org/10.1007/BF02347897>
- Kirkendall LR (1983) The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). *Zool J Linn Soc* 77:293–352. <https://doi.org/10.1111/j.1096-3642.1983.tb00858.x>
- Kirkendall LR (1993) Ecology and Evolution of Biased Sex Ratios in Bark and Ambrosia Beetles. In: Wrensch DL, Ebbert MA (eds) *Evolution and Diversity of Sex Ratio: in Insects and Mites*. Springer US, Boston, MA, pp 235–345
- Kononov DA, Manning C, Henshaw MT (2004) kingroup: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Mol Ecol Notes* 4:779–782. <https://doi.org/10.1111/j.1471-8286.2004.00796.x>
- Louis EJ, Dempster ER (1987) An exact test for Hardy-Weinberg and multiple alleles. *Biometrics* 43:805–811
- Lubin Y, Bilde T (2007) *The Evolution of Sociality in Spiders*. In: *Advances in the Study of Behavior*. Academic Press, pp 83–145
- Lucas ER, Horsburgh GJ, Dawson DA, Field J (2009) Characterization of microsatellite loci isolated from the wasp, *Microstigmus nigrophthalmus* (Hymenoptera). *Mol Ecol Resour* 9:1492–1497. <https://doi.org/10.1111/j.1755-0998.2009.02714.x>
- Mockford EL (1957) Life history studies on some Florida insects of the genus *Archipsocus* (Psocoptera). *Bulletin of the Florida State Museum (Biological Sciences)*. *Univ Fla* 1:253–274
- Nagelkerke CJ (1996) Discrete Clutch Sizes, Local Mate Competition, and the Evolution of Precise Sex Allocation. *Theor Popul Biol* 49:314–343. <https://doi.org/10.1006/tpbi.1996.0016>
- Neville T, Schwarz MP, Tierney SM (1998) Biology of a weakly social bee, *Exoneura* (*Exoneurella*) *setosa* (Hymenoptera: Apidae) and implications for social evolution in Australian allodapine bees. *Aust J Zool* 46:221. <https://doi.org/10.1071/ZO98002>
- New TR (1973) *The Archipsocidae of South America* (Psocoptera). *Trans R Entomol Soc Lond* 125:57–103. <https://doi.org/10.1111/j.1365-2311.1973.tb00538.x>
- Packer L (1990) Solitary and eusocial nests in a population of *Augochlorella striata* (Provancher) (Hymenoptera: Halictidae) at the northern edge of its range. *Behav Ecol Sociobiol* 27:339–344. <https://doi.org/10.1007/BF00164004>
- Pen I, Weissing FJ (2000) Sex-ratio optimization with helpers at the nest. *Proc R Soc Lond B Biol Sci* 267:539–543. <https://doi.org/10.1098/rspb.2000.1034>
- Queller DC, Goodnight KF (1989) Estimating Relatedness Using Genetic Markers. *Evolution* 43:258–275. <https://doi.org/10.2307/2409206>
- Quiñones AE, Pen I (2017) A unified model of Hymenopteran pre-adaptations that trigger the evolutionary transition to eusociality. *Nat Commun* 8:15920. <https://doi.org/10.1038/ncomms15920>
- Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and Ecumenicism. *J Hered* 86:248–249. <https://doi.org/10.1093/oxfordjournals.jhered.a111573>
- Reeve HK, Westneat DF, Noon WA et al (1990) DNA fingerprinting reveals high levels of inbreeding in colonies of the eusocial naked mole-rat. *Proc Natl Acad Sci U S A* 87:2496–2500. <https://doi.org/10.1073/pnas.87.7.2496>
- Riechert SE, Roeloffs RM (1993) Evidence for and consequences of inbreeding in the cooperative spiders. The natural history of inbreeding and outbreeding. The University of Chicago Press, Chicago, IL, pp 283–303
- Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour* 8:103–106. <https://doi.org/10.1111/j.1471-8286.2007.01931.x>
- Saito Y (1997) *Sociality and kin selection in Acari. The Evolution of Social Behaviour in Insects and Arachnids*. Cambridge University, Cambridge, United Kingdom, pp 443–457
- Saito Y (2010) Spider mites as study objects for evolutionary biology. In: Sabelis MW, Bruin J (eds) *Trends in Acarology*. Springer Netherlands, Dordrecht, pp 287–293
- Schwarz MP (1994) Female-Biased Sex Ratios in a Facultatively Social Bee and Their Implications for Social Evolution. *Evolution* 48:1684–1697. <https://doi.org/10.1111/j.1558-5646.1994.tb02205.x>

- Strassmann JE, Page RE, Robinson GE, Seeley TD (2011) Kin selection and eusociality. *Nature* 471:E5–E6. <https://doi.org/10.1038/nature09833>
- Tabadkani SM, Nozari J, Lihoreau M (2012) Inbreeding and the evolution of sociality in arthropods. *Naturwissenschaften* 99:779–788. <https://doi.org/10.1007/s00114-012-0961-x>
- Tang X, Meng L, Kapranas A et al (2014) Mutually beneficial host exploitation and ultra-biased sex ratios in quasisocial parasitoids. *Nat Commun* 5:4942. <https://doi.org/10.1038/ncomms5942>
- Taylor PD (1981) Intra-sex and inter-sex sibling interactions as sex ratio determinants. *Nature* 291:64–66. <https://doi.org/10.1038/291064a0>
- Taylor PD (1993) Female-biased sex ratios under local mate competition: An experimental confirmation. *Evol Ecol* 7:306–308. <https://doi.org/10.1007/BF01237747>
- Trivers RL, Willard DE (1973) Natural Selection of Parental Ability to Vary the Sex Ratio of Offspring. *Science* 179:90–92. <https://doi.org/10.1126/science.179.4068.90>
- Truett GE, Heeger P, Mynatt RL et al (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* 29:52. <https://doi.org/10.2144/00291bm09>
- Waage JK (1982) Sib-mating and sex ratio strategies in scelionid wasps. *Ecol Entomol* 7:103–112. <https://doi.org/10.1111/j.1365-2311.1982.tb00648.x>
- Waage JK, Ming NS (1984) The Reproductive Strategy of a Parasitic Wasp: I. Optimal Progeny and Sex Allocation in *Trichogramma evanescens*. *J Anim Ecol* 53:401–415. <https://doi.org/10.2307/4524>
- Wade MJ, Breden F (1981) Effect of inbreeding on the evolution of altruistic behavior by kin selection. *Evolution* 844–858. <https://doi.org/10.2307/2407855>
- Wajnberg E (1993) Genetic variation in sex allocation in a parasitic wasp: variation in sex pattern within sequences of oviposition. *Entomol Exp Appl* 69:221–229. <https://doi.org/10.1111/j.1570-7458.1993.tb01745.x>
- Wang J (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genet Res* 89:135–153. <https://doi.org/10.1017/S0016672307008798>
- Wang J (2011) coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Mol Ecol Resour* 11:141–145. <https://doi.org/10.1111/j.1755-0998.2010.02885.x>
- Wang J (2014) Marker-based estimates of relatedness and inbreeding coefficients: an assessment of current methods. *J Evol Biol* 27:518–530. <https://doi.org/10.1111/jeb.12315>
- West SA (2009) *Sex Allocation*. Princeton Univ. Press, Princeton, NJ
- West SA, Shuker DM, Sheldon BC (2005) Sex-Ratio Adjustment When Relatives Interact: A Test of Constraints on Adaptation. *Evolution* 59:1211–1228. <https://doi.org/10.1111/j.0014-3820.2005.tb01772.x>
- Whitehorn PR, Tinsley MC, Brown MJ et al (2009) Impacts of inbreeding on bumblebee colony fitness under field conditions. *BMC Evol Biol* 9:152. <https://doi.org/10.1186/1471-2148-9-152>
- Whiting PW (1943) Multiple Alleles in Complementary Sex Determination of *Habrobracon*. *Genetics* 28:365–382. <https://doi.org/10.1093/genetics/28.5.365>

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