

Sex Ratios in a Socially Parasitic Bee and Implications for Host-Parasite Interactions

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Abstract Obligate social parasites of Hymenoptera, known as inquilines, have received enormous attention due to the elaborate adaptations they exhibit for exploiting their hosts, and because they have frequently been used to infer sympatric speciation. Their population biology can be difficult to infer as they are both rare and difficult to extract from host nests. Sex allocation has been studied for very few inquilines of social Hymenoptera. Here we report sex ratio patterns in the allodapine bee *Inquilina schwarzi*, which is an obligate social parasite of another allodapine, *Exoneura robusta*. We show that the sex ratio of this inquiline varies with its brood number, it is female-biased in the smallest broods, but becomes more even in larger broods, where the population-wide sex ratio is close to parity. We argue that this pattern of bias is consistent with local resource competition, where inquiline females compete to inherit their natal colony. We also argue that extremely female-biased sex ratios of the host species, combined with overall sex ratio parity in the parasite, may help ameliorate disparity in effective population sizes between these two species which are locked in an evolutionary arms race.

Keywords Bees · effective population size · inquilines · local resource competition · sex ratio · social parasites

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Introduction

Allodapine bees (family Apidae, tribe Allodapini) provide a model taxon for examining sex allocation bias. Nearly all species where sex allocation has been examined exhibit female biased allocation, with the proportion of males among maturing brood being as low as, or lower than 15% in several genera (Schwarz et al. 2007). This bias has largely been attributed to local resource enhancement (LRE, Schwarz 1988) and the production of ‘insurance daughters’ who play an alloparental role in the event of maternal death prior to brood maturation (Bull and Schwarz 2001; Aenmey et al. 2006; Thompson and Schwarz 2006). Both models assume a non-linear increase in fitness through investment in daughters, until an optimal number of daughters has been produced within a brood (West 2009).

Most detailed studies of allodapine sex allocation have indicated that the degree of bias depends strongly on colony size (Schwarz et al. 2007). For example, tropical allodapine bee, *Macrogalea Malawi* sp., has a very clear shift away from female-biased sex ratios once colonies contain more than six adult females, indicating the ability of females to assess the number of their nestmates (Thompson and Schwarz 2006). In another allodapine, *Exoneura nigrescens*, sex allocation varies quite precisely with the number of brood in a nest, indicating a remarkable ability of reproductive females to anticipate their final brood numbers (Bull and Schwarz 2001). The genus *Macrogalea* is a sister clade to all other allodapines and *Exoneura* is one of the most distal clades (Chenoweth and Schwarz 2011), suggesting that the ability to adjust sex ratios according to colony size is an ancestral trait for the Allodapini. The crown age of the allodapines is in the early Eocene (Rehan et al. 2012), so we might expect a very long period of selection on an ancestral ability of allodapines to adjust sex allocation to proximate conditions in ways that maximize fitness.

Many allodapine bees are attacked by other allodapine species that have evolved a socially parasitic strategy. Social parasitism has evolved at least 13 times in the Allodapini, more than all other bees and wasps combined (Smith et al. 2013). Most of these origins have resulted in obligate parasitism, referred to as ‘inquilinism’ (Smith et al. 2013). In these inquilines, parasitic species enter host colonies and replace some of the host brood with their own, which are then reared by the host species (Michener 1965; Smith and Schwarz 2006).

Sex allocation strategies in hymenopteran inquilines have rarely been explored because rates of infestation are very low (Shokri Bousjein et al. 2016) and determining inquiline sex allocation patterns requires the ability to sample sufficient parasitized colonies to characterize inquiline allocation strategies. In a study on two bumble bee social parasites (genus *Bombus*, subgenus *Psythiris*), Fisher (1992) argued that female biased sex allocation may be due to local mate competition (LMC). LMC arises when close male relatives compete with each other for access to females and could arise if males show strong philopatry, for example by mating within or close to their natal nest (Alexander and Sherman 1977; Werren 1980). In one inquiline parasitic ant *Plagiolepis xene*, a female biased sex ratio has also been attributed to local mate competition arising from sib-mating within nests (Aaron et al. 1999).

Inquilina schwarzi is an Australian allodapine inquiline that infests nests of *Exoneura robusta* (Smith and Schwarz 2006, 2008). Inquiline females delay oviposition until host egg-laying is near completion, and is potentially a strategy to allow the

inquiline's eggs acquire odour from the host's eggs to make them less detectable by the hosts (Smith and Schwarz 2006). Once inquiline egg production has commenced, some, but not all, host eggs are destroyed by the inquiline (Smith and Schwarz 2006). In infested colonies *Inquilina* brood development and maturation occur in synchrony with that of the host brood (Michener 1965) where they eclose as adults at the same time (Shokri Bousjein et al. 2016). Importantly, in most *Inquilina*-infested host nests, not all host brood are killed by the inquiline. This may be a strategy that allows one of the inquiline's daughters to 'inherit' a host colony (Shokri Bousjein et al. 2016), avoiding the costs of dispersal difficulties of infiltrating a new host colony.

Here we explore sex ratios of the inquiline *I. schwarzi* based on samples obtained during brood maturation over a 3 year period and explore what kinds selective mechanisms might shape sex allocation patterns.

Methods

Nest Collection

Colonies of *E. robusta* were collected from the Dandenong Ranges, Victoria, Australia, between Kee Wee Rup North and Gembrook. This area comprises tall wet eucalyptus forest, and colonies were found in dead fronds of the tree fern *Cyathea australis*. Bee nests were collected whole in intact dead fronds of *C. australis* during early mornings and early evenings of late February in 2013, 2014 and 2015, when all colony occupants were present in the nest. Fronds were transferred to insulated boxes on ice and transported to Flinders University where they were kept at 4 °C until opening.

Estimating Sex Ratios

Our samples were taken at a time when host and inquiline brood were in the process of eclosing into adults, and therefore colonies comprised a mixture of parental generation females, newly eclosed brood, and late-stage immatures. Calculating *Inquilina* sex ratios at this time is not straightforward since it was not possible to confidentially decide whether any particular adult inquiline female was from the parental generation or a recently eclosed brood member. Wing wear as a function of foraging activity can be used to assign females to generational cohorts in the host species *E. robusta* (Schwarz 1986) but, being social parasites, *Inquilina* do not forage (Michener 1965; Smith and Schwarz 2006) and wing wear may not correspond to age. However, a recent study (Shokri Bousjein et al. 2016) indicated that approximately 98% of nests with maturing inquiline brood also had the mother of those brood still present. Therefore, in our study inquiline brood sex ratios would be better estimated by treating one of the adult inquiline females in each nest as a mother rather than a brood.

Given the above considerations, we estimated inquiline sex ratios in two ways: (i) by treating each inquiline adult or immature as a member of the brood; and (ii) by treating one adult inquiline female as a mother and calculating sex ratios from the remaining inquiline individuals. We subsequently refer to these sex ratios as 'raw' and 'adjusted' sex ratios respectively. Raw sex ratios are highly likely to over-estimate the proportion of inquiline females in a brood, because including a mother will falsely inflate estimates

of female allocation, especially where brood numbers are very small. On the other hand, ‘adjusted’ sex ratios may slightly underestimate female biased allocation if an inquiline mother was no longer present in a host nest.

Results

A total of 53 *E. robusta* colonies containing *I. schwarzi* adults or brood were collected from February 2013 ($N=6$ colonies), February 2014 ($N=30$) and February 2015 ($N=17$).

Before analyzing sex ratios we examined whether the total number of inquilines, hosts and adjusted and non-adjusted *Inquilina* sex ratio differed across our three samples (2013–2015) using non-parametric Kruskal Wallis tests, as sex ratio data is 0–1 truncated. These results indicated no significant yearly effects for any of the variables ($X^2_2 = 5.660$, $P = 0.059$; $X^2_2 = 1.133$, $P = 0.567$; $X^2_2 = 0.396$, $P = 0.820$; $X^2_2 = 2.022$, $P = 0.364$, respectively), so the yearly samples were pooled for subsequent analyses.

We then explored whether raw and adjusted *Inquilina* sex ratios were influenced by the number of hosts and inquilines, based on adults and post-feeding brood reared to adulthood, using generalized linear modeling (GLM). GLM was used because QQ plots (not shown here) indicated non-normality for both the dependent variable (inquiline sex ratio) and the independent variables (number of hosts and number of inquilines). When we examined the adjusted *Inquilina* sex ratio data, our GLM analysis indicated a highly significant effect of *Inquilina* numbers ($\beta = 0.094$, 95% likelihood ratio $X^2_1 = 7.262$, $P = 0.007$, 95% profile likelihood CI = 0.03–0.159) but no effect of the total number of *E. robusta* adults ($\beta = 0.002$, 95% likelihood ratio $X^2_1 = 0.039$, $P = 0.884$, 95% profile likelihood CI = -0.018–0.022). When we examined raw sex ratio data we found a highly significant effect of the number of *Inquilina* ($\beta = 0.072$, 95% likelihood ratio $X^2_1 = 23.714$, $P < 0.001$, 95% profile likelihood CI = 0.046–0.099) but no effect of the total number of *E. robusta* adults ($\beta = -0.003$, 95% likelihood ratio $X^2_1 = 0.0329$, $P = 0.566$, 95% profile likelihood CI = -0.012–0.007).

The adjusted sex ratio of *I. schwarzi* as a function of the number of inquilines present in a nest is summarized as a boxplot in Fig. 1. This shows that when there is only one inquiline brood in a nest, the inquiline sex ratio is strongly female biased, and as brood number increases the sex ratio becomes less biased and maybe even male biased, though our samples for larger *Inquilina* brood numbers are very limited. A non-parametric correlation indicates that this pattern is significant (Spearman’s $\rho = 0.675$, $N = 15$, $P = 0.006$).

Lastly, we estimated population-wide inquiline sex ratios in two ways: (i) firstly we calculated the raw and adjusted sex ratio for each colony and then obtained a mean across these estimates and the 95% confidence interval for these means was obtained via bootstrapping with 1000 pseudoreplicates; (ii) secondly we pooled the total number of inquiline brood across all nests from all collections and used these to calculate a point estimate of sex ratio, where each inquiline individual was treated as an equally weighted data point. Mean raw sex ratio was estimated as $r = 0.223$ (95% CI = 0.094–0.385) and mean adjusted sex ratio was 0.280 (95% CI = 0.125–0.491). The population-wide sex ratio for the raw data (where every female is included) was

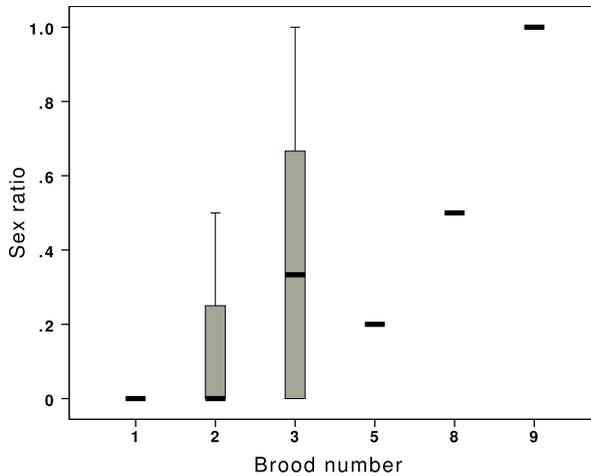


Fig. 1 Box plot with interquartile ranges of the sex ratio of *Inquilina schwarzi* as a function of inquiline brood number. Sex ratios and brood numbers were calculated by treating one adult female in each nest as mother of the remaining parasites

$r = 0.22$ and for the adjusted data (where one *Inquilina* in each nest is regarded as a mother and not used to calculate sex ratios) it was $r = 0.47$. It is not possible to calculate confidence intervals for this latter estimate.

Discussion

Our data indicate that sex ratios in *I. schwarzi* vary with inquiline brood number, regardless of whether one of the *I. schwarzi* adult females is treated as the mother or whether all adults and pupae are treated as brood. Furthermore, sex ratios in *I. schwarzi* are strongly female biased for the smallest brood numbers and become less biased, or even male-biased, in larger broods. However, the total number of males and females recovered across the 3 years of our study is close to parity when one adult female was regarded as the mother of the remaining adults and pupae, suggesting that at a population level sex allocation is unbiased. We now discuss possible evolutionary drivers for these patterns.

Fisher (1992) argued that female biased sex allocation in two socially parasitic bumble bees in the subgenus *Psithyrus* may be due to local mate competition (LMC). However, Bourke (1997) argued that this was unlikely because LMC predicts increasing female bias with larger brood numbers, whereas no effect of brood number sex ratios was found in those data. LMC arises when close male relatives compete with each other for access to females. For *I. schwarzi* LMC could occur if male inquilines show high levels of philopatry and if local populations are sufficiently small to make competition between close relatives likely. We are unable to exclude this possibility for *I. schwarzi*; however, LMC leads to declining rates of mean fitness returns through male investment and therefore predicts a decrease in male investment as brood number increases (West 2009), opposite to what we find. Therefore, our data are incompatible with LMC.

Local Resource Enhancement (LRE) arises when close female relatives cooperate in a way that leads to increasing per capita fitness returns through females as a function of

investment in daughters. Although LRE predicts greater female bias in small broods (Schwarz 1988), as we find for *I. schwarzi*, it is difficult to see how inquiline females would be able to cooperate in a way that increases their mean fitness. Although *I. schwarzi* females can remain in their natal nests during autumn and winter, this period does not involve any oviposition or brood rearing, and females disperse in spring so that there is only one inquiline female per host nest during brood rearing in spring and summer. We therefore believe that LRE is unlikely to operate in *I. schwarzi*.

Lastly, Local Resource Competition (LRC; Clark 1978) arises when females compete for limited resources, such that increasing investment in daughters leads to declining returns in mean daughter fitness. LRC predicts female bias in the smallest brood numbers, with increasing investment in males as brood numbers become larger, which is consistent with our data on *I. schwarzi*. LRC could arise if inquiline sisters compete to inherit their natal host nests and where successful inheritors have greater mean fitness than females who are forced to disperse and invade new hosts. We believe that this scenario is highly plausible for two reasons. Firstly, any odour cues of inquiline brood raised within a host colony would be familiar to their co-developing host brood, therefore if any ability to recognize non-nestmates is based on familiarity of semiochemicals, such as inquilines should be less easily detected as enemies. Secondly, the very low rate of inquiline infestation, 5.8% of all host *E. robusta* nests (Shokri Bousjein et al. 2016), suggests that the likelihood of being able to successfully invade a new host colony is very low. Consequently, it is plausible that one female in an inquiline brood could ‘inherit’ her host colony, but that subsequent inquiline sisters would have lower fitnesses because of the need to successfully invade new host colonies.

Our results are important for several reasons. Firstly, they suggest that the ancestral ability to adjust sex ratios according to benefits of cooperation (LRE) in allodapines can be co-opted to adjust sex ratios in line with the costs of competition (LRC). Secondly, our GLM results suggest that *I. schwarzi* is able to adjust sex ratios according to its anticipated brood number, rather than just its host colony size.

Lastly, our data have some implications for understanding evolutionary arms races between *Exoneura* and *Inquilina*, which we now discuss. Because *I. schwarzi* is an obligate social parasite of *E. robusta* we expect that selection will operate on *E. robusta* to evolve strategies for minimizing rates of parasitism, but will also operate on *I. schwarzi* to overcome host defences, leading to an evolutionary ‘arms race’. However, rates of adaptive evolution are curtailed by effective population size, N_e (e.g. Kimura and Ohta 1971; Woolfit 2009; Lanfear et al. 2014). Shokri Bousjein et al. (2016) have shown that infestation rates of *I. schwarzi* are approximately 5.8% of potential host colonies, and that relative N_e may be as much as two orders of magnitude greater for the host than its inquiline species. However, for haplodiploids, such as ants, bees and wasps, N_e is a function of sex ratios, viz. $N_e \sim 9 N_f N_m / (2N_f + 4N_m)$ where N_f and N_m refer to the number of females and males respectively (Wright 1933; Hedrick and Parker 1997). *E. robusta* has extremely female biased sex allocation at a population-wide level, $r \approx 0.15$, due to local resource enhancement (Schwarz 1988). However, our data indicate that *I. schwarzi* has a population-wide sex ratio close to parity, and this may serve to decrease the disparity in N_e that is otherwise suggested purely by infestation rates, which we now discuss.

Figure 2 shows the relationship between N_e and sex ratio for haplodiploids with panmixis and where arrows indicate the population-wide sex ratios estimated for both *E. robusta* (Schwarz 1988) and *I. schwarzi* (data presented here). This figure

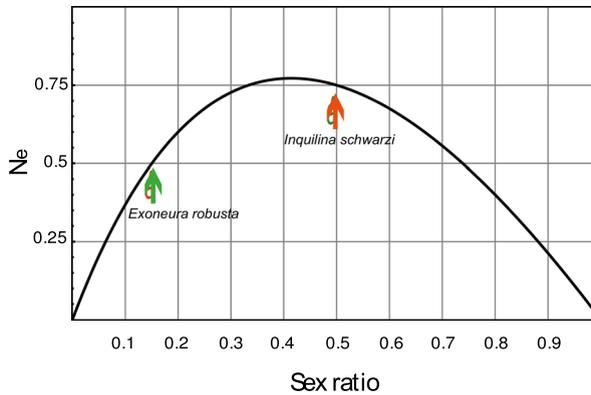


Fig. 2 Graph of effective population size, N_e , as a function of sex ratio for panmictic hapodiploids. The population-wide sex ratio of the host *Exoneura robusta* is indicated by a green arrow, and the sex ratio of its inquiline *Inquilina schwarzi* by a red arrow

suggests that the relative disparity in N_e between hosts and parasites is much less than predicted by infestation rates alone, due to the extremely female biased allocation in *E. robusta*. In fact, the relative N_e of *I. schwarzi* is approximately 50% higher than if it had the same population-wide sex ratio as its host.

Sex ratio patterns in inquilines have largely been ignored in previous studies, despite the attention given to origins of inquilinism and mechanisms that inquilines use to exploit their hosts. Ability to adjust sex ratio from a multitude of inter and intra specific parameters suggests that we often may underestimate the level of complication within social insects and their parasites. These systems require attention as the evolution of adjusted sex ratios and the associated evolutionary arms race repercussions will allow better predictions and models of host/parasite interactions on an ancestral and global scale.

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