

Sex Allocation Conflict in Ants: When the Queen Rules

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Summary

Insect societies are paramount examples of cooperation, yet they also harbor internal conflicts whose resolution depends on the power of the opponents [1–4]. The male-haploid, female-diploid sex-determining system of ants causes workers to be more related to sisters than to brothers, whereas queens are equally related to daughters and sons [1]. Workers should thus allocate more resources to females than to males, while queens should favor an equal investment in each sex. Female-biased sex allocation and manipulation of the sex ratio during brood development suggest that workers prevail in many ant species [1, 2, 5]. Here, we show that queens of *Formica selysi* strongly influenced colony sex allocation by biasing the sex ratio of their eggs. Most colonies specialized in the production of a single sex. Queens in female-specialist colonies laid a high proportion of diploid eggs, whereas queens in male-specialist colonies laid almost exclusively haploid eggs, which constrains worker manipulation. However, the change in sex ratio between the egg and pupae stages suggests that workers eliminated some male brood, and the population sex-investment ratio was between the queens' and workers' equilibria. Altogether, these data provide evidence for an ongoing conflict between queens and workers, with a prominent influence of queens as a result of their control of egg sex ratio.

Results and Discussion

We investigated the outcome of the queen-worker conflict over sex allocation in *Formica selysi*, an ant species with a variable number of queens per nest [6]. In spring, queens lay a first cohort of eggs that develop into females (new winged queens) or males. Later in the season, queens lay a second cohort of eggs that develop into workers. We examined patterns of sex allocation over two consecutive years and assessed the relative power of queens and workers in manipulating colony sex allocation.

Colonies Specialize in the Production of One Sex

Sex allocation varied greatly among colonies. Most colonies specialized in the production of one sex, resulting in a bimodal distribution of colonies with respect to sex-

investment ratio (Figure 1). The split was particularly pronounced in colonies headed by a single queen. Indeed, the proportion of female- and male-specialist colonies (i.e., colonies investing more than 90% of the energy to one sex) was significantly higher in single- than in multiple-queen colonies (Fisher's exact test: $p < 0.001$ in both 2001 and 2002). Female- and male-specialist colonies did not differ significantly in productivity (median investment in queens or males: 1590 and 1364 energetic equivalent of one male, respectively; Wilcoxon rank-sum test, $n_1 = 20$, $n_2 = 23$, $Z = 0.74$, $p = 0.46$). Colony sex-investment ratio was stable over the two study years (correlation between years = 0.81; Spearman's rank correlation, $n = 52$, $Z = 5.24$, $p < 0.001$).

Colony Specialization is Not Due to Worker Control in Response to Variation in Relatedness Asymmetry

Colonies might produce single-sex brood if the workers manipulate sex allocation according to the relative relatedness asymmetry in their colony (the relatedness of workers to females divided by the relatedness of workers to males) [7]. Under this hypothesis, workers should produce the sex that is more valuable for transmitting copies of their own genes, which is females in colonies with relatively high relatedness asymmetry compared to the population average, and males in colonies with relatively low relatedness asymmetry [7]. Such sex-ratio specialization occurs in many species of eusocial Hymenoptera [5, 8] and can be achieved by workers culling males [9, 10], biasing female development toward queens rather than workers [11], or preventing adult males from feeding [12].

In our study population, we found no evidence that workers controlled colony sex allocation according to between-colony variation in relatedness asymmetry. First, we found no association between colony sex-investment ratio and direct measures of relatedness asymmetry with microsatellite markers (Spearman rank correlation, $n = 26$, $Z = -0.31$, $p = 0.75$). Second, relatedness asymmetry was significantly higher in single- than multiple-queen colonies (2.76:1 versus 1.39:1, respectively; Wilcoxon rank-sum test: $Z = 2.64$, $n_1 = 17$, $n_2 = 9$, $p = 0.008$), but the average colony sex-investment ratio was similar between the two types of colonies (Figure 1; 2001, Wilcoxon rank-sum test: $Z = -0.11$, $n_1 = 50$, $n_2 = 12$, $p = 0.91$; 2002, Wilcoxon rank-sum test: $Z = -0.01$, $n_1 = 51$, $n_2 = 17$, $p = 0.99$). Third, the relatedness asymmetry in male-specialist colonies was not significantly lower than the relatedness asymmetry in female-specialist colonies (2.61:1, versus 2.59:1, respectively; Wilcoxon rank-sum test: $Z = 0.74$, $n_1 = 13$, $n_2 = 4$, $p = 0.46$).

The microsatellite data revealed that the relatedness asymmetry varied greatly among colonies. However, workers did not take advantage of this variation to increase their inclusive fitness by producing more females in colonies with high relatedness asymmetry and more males in colonies with low relatedness asymmetry. This

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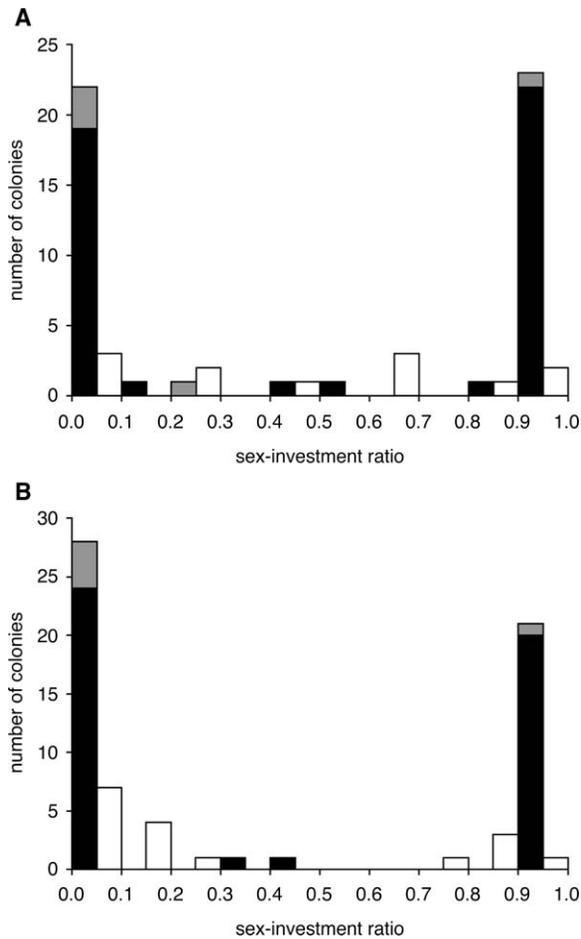


Figure 1. Colony Sex-Investment Ratio
Sex-investment ratio (expressed as the proportion of energy allocated to females) in colonies headed by one singly mated queen (black bars), one doubly mated queen (gray bars), and multiple queens (white bars), respectively. (A) Year 2001. (B) Year 2002.

lack of worker control contrasts with other studies of ants in which there were either signs of queen control but no variation among colonies in relatedness asymmetry [13–15] or evidence for worker control [5, 8, 11]. The absence of worker control despite strong variation in worker relatedness asymmetry among colonies suggests that *F. selysi* queens might be particularly effective in controlling sex allocation.

Queens Bias the Sex Ratio of their Eggs

Queens can influence colony sex allocation by controlling the fertilization of the eggs they lay [16, 17], which determines the primary proportion of haploid and diploid brood provided to workers. In some species, queens might prevent worker manipulation by limiting the number of diploid eggs, thus forcing workers to rear males [14, 15, 18]. Comparing the sex ratio of queen-laid eggs and worker-reared pupae is a powerful way to reveal ongoing conflicts and assess the relative power of each party in manipulating colony sex allocation [9].

Queens of *F. selysi* strongly influenced sex allocation by biasing the sex ratio of their eggs (Figure 2). Basically,

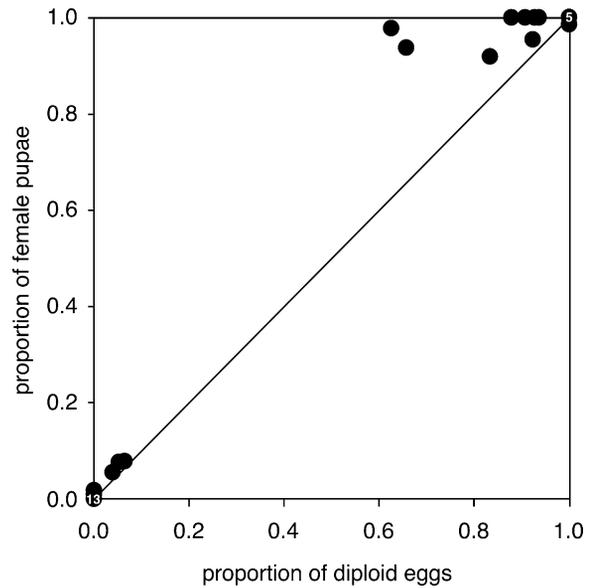


Figure 2. Sex Ratio of Eggs and Pupae
Relationship between the sex ratio of pupae (proportion of queens) and eggs (proportion of diploid eggs). $N = 31$ colonies; note that 13 colonies had only haploid eggs and male pupae, whereas five colonies had only diploid eggs and female pupae.

queens laid haploid eggs in some colonies and diploid ones in others. More precisely, queens from male-specialist colonies laid almost exclusively haploid eggs (the proportion of haploid eggs was 0.99 with a 95% confidence interval of 0.98 to 1.0). In sharp contrast, queens from female-specialist colonies laid mostly diploid eggs (the proportion of diploid eggs was 0.91 with a 95% confidence interval ranging from 0.83 to 0.98). The sex ratio of eggs differed significantly between the two groups of colonies (two-tailed t test, $t = 29.32$, $df = 29$, $p < 0.001$), and the correlation between egg and adult sex ratio was as high as 0.99 (Spearman rank correlation, $n = 31$, $Z = 5.19$, $p < 0.001$).

The strong bias in egg sex ratio forced workers to rear females in some colonies and males in others, thus constraining workers' ability to manipulate colony sex allocation. This finding contrasts with the numerous studies in which workers played a major role in determining colony sex allocation [1, 2, 5, 8]. So far, evidence for partial queen control was limited to three ant and one bumblebee species [14, 15, 19–21]. In these studies, queens influenced sex allocation by laying a high proportion of haploid or worker-destined eggs in part of the colonies. In *F. selysi*, the bias in egg sex ratio is more pronounced and near bimodal, which suggests that queens have almost complete control over sex allocation in many of the colonies.

Signs of Worker Manipulation

Despite the strong queen control, we also detected signs of worker manipulation. The proportion of females was consistently higher in sexual pupae than in eggs (Figure 2, points above the diagonal). This shift in sex ratio was significant when considering all colonies (Wilcoxon signed rank test, $n = 31$, $Z = 3.29$, $p = 0.001$), male-specialist colonies (Wilcoxon signed rank test, $n = 17$,

$Z = 2.20$, $p = 0.028$), or female-specialist colonies (Wilcoxon signed rank test, $n = 14$, $Z = 2.49$, $p = 0.013$).

This change of sex ratio between eggs and pupae suggests that workers destroyed some male brood in order to rear more females. On the basis of other studies of ant sex ratio, a higher intrinsic mortality of male brood is unlikely [9, 18, 22, 23]. Policing of worker-produced eggs [24, 25] is also unlikely, because no sign of worker reproduction was detected in a large sample of eggs from the study colonies (see **Experimental Procedures**).

The population sex-investment ratio was slightly female biased and fell between the equilibria that are expected if either queens or workers have full, cost-free control of sex allocation. The population sex-investment ratio was 1.36:1 ($n = 49$), which is between the theoretical relatedness asymmetry for queens (1:1) and the average relatedness asymmetry for workers measured with microsatellite markers (1.87:1, $n = 26$). This intermediate sex-investment ratio indicates that workers have some impact on sex allocation, which results in either mixed control or control by one party with a high cost of sex ratio manipulation [26, 27]. Costs of sex-ratio manipulation are difficult to evaluate, but the similar productivity of female- and male-specialist colonies is consistent with the idea that sex-ratio biasing toward males is not particularly costly in this species.

The Balance of Power

Together, the data suggest an ongoing conflict between queens and workers over colony sex allocation, with a strong influence of queens that gain control by biasing the sex ratio of their eggs. The factors and constraints affecting the outcome of the queen-worker conflict over sex allocation remain poorly understood. If the workers can influence whether diploid eggs develop into queens or workers [11], the ecological constraints acting on the time at which each caste is produced will strongly affect the balance of power. When new queens, males, and workers are produced at the same time, queens will have little power to manipulate egg sex ratio, because they will have to provide diploid eggs for worker production. In contrast, when the production of sexual and worker broods occurs at different times, as in *F. selysi*, queens will gain more power because they can limit the number of diploid eggs provided to the workers when queens and males are being produced [28]. Queen power also increases if the queens can determine the caste fate of diploid eggs, as suggested in some species of ants [15, 21, 28]. Small idiosyncratic differences in a species' s life cycle and biology can thus have a large impact on conflict resolution [4].

Queen number might also affect the balance of power. When there is one queen per colony, she can control the sex ratio of all eggs provided to the workers. However, when there are multiple queens, competition among queens is likely to limit the degree of queen control over egg sex ratio, because queens laying eggs of the sex preferred by the workers will have a competitive advantage [13]. In agreement with this hypothesis, the adult sex ratio was more split in single- than in multiple-queen colonies of *F. selysi*. Whether queens adaptively modify the sex ratio of their eggs in response to a change in queen number remains to be experimentally tested.

In conclusion, this study shows that queens can gain control over colony sex allocation by strongly biasing the sex ratio of their eggs. More generally, it confirms that relatedness-induced worker control of sex allocation is far from universal. Small differences in social structure, ecology, and life history are likely to affect the balance of power between queens and workers, which helps to explain the diverse outcome and evolutionary persistence of sex allocation conflicts in insect societies.

Experimental Procedures

Study Population

Our study population of *F. selysi* is situated along the river Rhône between Sierre and Susten in Switzerland [6]. We assessed the social structure of 112 colonies by genotyping eight to 100 workers from each colony at nine microsatellite markers [6, 29]. The majority of the colonies (57%) were headed by one singly mated queen, a few (6%) had one doubly mated queen, and the remaining (37%) had multiple queens, with a genetically effective queen number of 4.3. Single- and multiple-queen colonies had similar mating systems and showed little genetic differentiation above the nest level [6].

Sex Allocation

We estimated colony sex allocation in 2001 and 2002. In each colony, we sampled pupae three to four times between mid-May and the end of June, collecting on average 59 female and male pupae. Colony sex-investment ratio was estimated as the proportion of energy allocated to females. The number of females was multiplied by the female-to-male energetic cost ratio C , which corrects for sex-specific cost variation due to differences in fat content and respiration rate [$C = (\text{female dry weight}/\text{male dry weight})^{0.7}$] [30]. The population sex-investment ratio was obtained by multiplying colony sex-investment ratio by colony productivity.

In 2001, we estimated colony productivity with a mark-recapture method. For each colony, we performed three to four sessions of mark-recapture between the end of May and mid-June, after larvae had pupated but prior to the first emergence of winged queens and males. All female and male pupae found under a flat stone were marked with a nontoxic pen and returned to the nest. Recapture was made 48 hr later, so that workers had time to mix marked and unmarked pupae. On average, 29% of the marked pupae were recaptured. We estimated the number of pupae produced by each colony with the Lincoln index, averaging over all mark-recapture sessions.

Relatedness Asymmetry

We tested whether the sex-investment ratio was positively correlated with relatedness asymmetry, as predicted by the relative relatedness asymmetry hypothesis [7]. When the number of queens per colony varies, the relatedness asymmetry can range between 1:1 and 3:1 depending on the number and relatedness of queens [31]. Therefore, we measured the colony relatedness asymmetry with microsatellite markers. We selected a subsample of 26 colonies that had a proportion of single- and multiple-queen colonies very close to the one of the larger population sample (17 and 9 versus 71 and 41, respectively; Fisher' s exact test, $p > 0.99$). In each colony, we genotyped at least eight adult workers, eight male pupae, and eight female pupae at nine microsatellite markers [6, 32, 33] and estimated the relatedness of workers to female and male pupae with the computer program RELATEDNESS 5.0.8 (available at <http://www.gsoftnet.us/GSoft.html>). In ten colonies that only produced males, we used worker pupae to estimate the relatedness toward females. Colony relatedness asymmetry was estimated as the ratio between the life-for-life relatedness of workers to females and males, respectively.

Egg Sex Ratio

Eggs were sampled in March and April of 2002 from 17 colonies producing mostly males (male-specialist colonies, with sex-investment ratio below 0.1) and 14 colonies producing mostly females (female-

specialist colonies, with sex-investment ratio above 0.9), respectively. All the colonies were headed by a single queen, except for four of the male-specialist colonies, which had multiple queens. The ploidy of eggs was determined with microsatellite markers as previously described [6], except that the DNA was purified with a standard phenol-chloroform extraction protocol before being resuspended in 50 μ l of distilled water, and PCR reactions were carried out in a final volume of 25 μ l. In single-queen colonies, eggs were genotyped at one or two microsatellite loci for which the queen and her mate(s) had different alleles, so that diploid eggs were heterozygote. In multiple-queen colonies, eggs were genotyped at all variable microsatellite loci, and eggs were scored as diploid when at least one of the loci was heterozygous. The reliability of this sexing method was high because in this population less than 1% of the genotyped queens or workers (12 out of 1335) were homozygous at all loci, and all genotyped males (88 in total) were homozygous at all markers. Overall, 76% of all eggs yielded reliable amplification products, and we determined the sex of 18 ± 7 eggs per colony, on average.

No sign of worker reproduction was detected in the colonies included in the analysis of relatedness asymmetry and in the comparison of egg and adult sex ratios. In particular, all 341 male eggs from the 27 single-queen colonies had microsatellite genotypes consistent with their being the sons of the queens, with no worker-derived paternal allele. Four additional colonies showed signs of worker reproduction. The 59 male eggs from these colonies showed queen and paternal alleles at similar frequencies. Moreover, three of these colonies were probably orphaned, because they stopped producing workers and died out in the following year. These four colonies with worker reproduction were included in the estimation of the population sex-investment ratio, but were excluded from all other analyses. In the results and statistical tests, the sample sizes (n) refer to the number of colonies.

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