



Sex ratio determination by queens and workers in the ant *Pheidole desertorum*

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Because workers in colonies of eusocial Hymenoptera are more closely related to sisters than to brothers, theory predicts workers should bias investment in reproductive broods to favour reproductive females over males. However, conflict between queens and workers is predicted. Queens are equally related to daughters and sons, and should act to prevent workers from biasing investment. Previous study of the ant *Pheidole desertorum* showed that workers are nearly three times more closely related to reproductive females than males; however, the investment sex ratio is very near equal, consistent with substantial queen control of workers. Near-equal investment is produced by an equal frequency of colonies whose reproductive broods consist of only females (female specialists) and colonies whose reproductive broods consist of only males or whose sex ratios are extremely male biased (male specialists). Because natural selection should act on *P. desertorum* workers to bias investment in favour of reproductive females, why do workers in male-specialist colonies rear only (or mostly) males? We tested the hypothesis that queens prevent workers from rearing reproductive females by experimentally providing workers with immature reproductive broods of both sexes. Workers reared available reproductive females, while failing to rear available males. Worker preference for rearing reproductive females is consistent with queens preventing their occurrence in colonies of male specialists. These results provide evidence that queens and workers will act in opposition to determine the sex ratio, a fundamental prediction of queen–worker conflict theory.

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Because of haplodiploid sex determination, workers in colonies of eusocial Hymenoptera (ants, bees and wasps) are more closely related to sisters than to brothers (Trivers & Hare 1976; Crozier 1979). Workers rear their colony's brood, and theory predicts that natural selection acts on workers to bias investment in reproductive broods to favour reproductive females (i.e. virgin queens, gynes) over males (Trivers & Hare 1976; Oster et al. 1977; Crozier & Pamilo 1996). This theory also predicts conflict between queens and workers over reproductive investment. Queens are equally related to daughters and sons, and natural selection should act on queens to prevent workers from biasing investment (Fisher 1930; Trivers & Hare 1976; Oster et al. 1977; Crozier & Pamilo 1996).

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Studies of the investment sex ratio in ant populations support this theory; estimates of investment sex ratios are often female biased, consistent with workers biasing sexual investment (Trivers & Hare 1976; Nonacs 1986; Boomsma 1989). In addition, workers in some ants are known to eliminate male brood and subsequently produce a reproductive brood biased in favour of females (Aron et al. 1994a; Keller et al. 1996; Sundström et al. 1996). Whether queens act to prevent or limit worker bias has seldom been addressed in sex ratio studies (but see Herbers 1984; Snyder & Herbers 1991; Herbers & Stuart 1998). As a result, there is little direct evidence that queens and workers act in opposition in sex ratio determination. Here we test experimentally whether such opposition occurs in the ant *Pheidole desertorum*.

Colonies of *P. desertorum* produce reproductive broods consisting of winged (alate) females and males. Following summer rains, the reproductives fly from nests and mate in aerial swarms (Helms 1995). Allozyme data are consistent with random mating within the study population (Helms 1999). After mating, new queens disperse and attempt independent colony foundation (Helms 1995).

Over 90% of established colonies sampled ($N=38$) appear to have a single reproductive queen inseminated by a single male (Helms 1999).

Similar to many other ants (e.g. Nonacs 1986), colonies of *P. desertorum* specialize in the sex ratio of reproductive broods. Some colonies produce exclusively females, while other colonies produce primarily or exclusively males (Helms & Rissing 1990; Helms 1999). Colonies of male and female specialists do not differ in size or other estimates of resource availability (Helms 1999). One possible reason for sex ratio specialization is that the degree to which workers are more closely related to reproductive females than males (relatedness asymmetry) varies between colonies. Workers are predicted to bias their colony sex ratio to favour reproductive females when relatedness asymmetry is high and bias their colony sex ratio to favour males when relatedness asymmetry is low (Boomsma & Grafen 1990, 1991). However, relatedness asymmetry does not differ between female- and male-specialist colonies (Helms 1999). Thus, worker control of investment according to variable intracolony relatedness (Boomsma & Grafen 1990, 1991) will not explain the occurrence of colonies of female and male specialists in *P. desertorum*.

Pheidole desertorum workers are estimated to be 2.8 times more closely related to females than males (Helms 1999). Under worker control of the sex ratio, the population investment sex ratio in *P. desertorum* should be near 2.8:1 (reproductive female:male) (Trivers & Hare 1976; Crozier & Pamilo 1996). However, the population investment sex ratio is very near equal (1.01:1), consistent with substantial queen control (Helms 1999). Equal investment results from an equal frequency of female- and male-specialist colonies that invest equal resources in their reproductive broods (Helms 1999). Helms (1999) proposed that queens in colonies of male specialists largely prevent the occurrence of reproductive females, perhaps by influencing female caste determination such that female eggs are destined to become workers. Frequency-dependent selection could act on such queens to result in an equal frequency of male- and female-specialist colonies and equal population investment in the reproductive sexes (Pamilo 1982; Helms 1999).

While the investment sex ratio in *P. desertorum* is consistent with queen control of workers, equal investment can also be predicted if selection does not act on workers to bias investment in favour of females and queen-worker conflict does not occur (Fisher 1930; Shaw & Mohler 1953; also see Kolman 1960). If queen-worker conflict is important, *P. desertorum* workers should increase investment in reproductive females when not prevented from doing so by queens. We tested this prediction by determining whether workers would preferentially rear reproductive females over males when given the opportunity. To do so, we provided workers with immature brood consisting of both reproductive females and males. If workers preferentially rear reproductive females over males, this would be consistent with queens of male-specialist colonies preventing workers from rearing reproductive females. Evidence that workers and queens act in opposition to determine colony sex ratios would provide

strong support for the theory of queen-worker conflict over the sex ratio.

METHODS

We collected queenless colony fragments consisting of workers and immature brood from 12 *P. desertorum* colonies by partial excavations of nests near Rodeo, New Mexico, in mid-May 1992. The study site and natural history of *P. desertorum* are described by Helms (1995). Six colonies were known from the previous year to be female specialists; the other six were known to be male specialists. *Pheidole desertorum* colonies show strong constancy in the sex of reproductive broods over time (Helms 1999) and could be reliably inferred to produce the same sex of reproductives as in the previous year.

In the laboratory, we separated workers from brood, then exchanged brood among worker groups to form control and experimental nests. We divided the workers from each colony into two groups of equal size and the brood from each colony into three groups. One brood group consisted of one half of the brood and the other two brood groups each consisted of one-quarter of the brood. Following the separation and division of workers and brood from four colonies, we reunited half of the workers from each colony with half of their brood to form control nests. We gave each remaining worker group one-fourth of the brood separated from the workers of two other colonies, one from a known female-specialist field colony and one from a known male-specialist field colony, to form experimental nests. Control and experimental nests were formed among three sets of four field colony collections to speed up the process and minimize brood exposure. The procedure is illustrated in Table 1.

Worker pupae and a small number of reproductive female and male pupae occurred in the brood we collected; most brood were larvae of various sizes. With the exception of reproductive females nearing pupation, we could not determine larval sex or caste by inspection; presence of reproductive brood was confirmed by their maturation during the experiment. In *P. desertorum*, reproductive females and males mature over a 2- to 3-month period (mid-May through mid-July, and sometimes into August; Helms 1995). As a result, reproductive larvae in collections probably varied from recently hatched to those nearing pupation. Because nests were only partially excavated, the numbers of individuals collected were small relative to the size of the field colony; however, the proportion of workers to brood in those samples were probably similar to those in the field.

Because the numbers of brood varied according to the source colony, for experimental nests we derived the numbers of reproductive females and males predicted to be reared in the absence of discrimination against brood from control nests. For example, we predicted that workers in experimental nest A would rear half the number of reproductive females reared by workers in control nest C and half the number of males (and any reproductive females) reared by workers in control nest B

Table 1. Procedure used to divide *Pheidole desertorum* workers and brood into control and experimental nests

Nest	Proportion of available workers included and the source colony	Proportion of available brood included and the source colony
Control A	1/2 Colony A (♂)	1/2 Colony A (♂)
Experimental A	1/2 Colony A (♂)	1/4 Colony B (♂), 1/4 Colony C (♀)
Control B	1/2 Colony B (♂)	1/2 Colony B (♂)
Experimental B	1/2 Colony B (♂)	1/4 Colony A (♂), 1/4 Colony D (♀)
Control C	1/2 Colony C (♀)	1/2 Colony C (♀)
Experimental C	1/2 Colony C (♀)	1/4 Colony B (♂), 1/4 Colony D (♀)
Control D	1/2 Colony D (♀)	1/2 Colony D (♀)
Experimental D	1/2 Colony D (♀)	1/4 Colony A (♂), 1/4 Colony C (♀)

Illustrated is one of three sets of four colonies where brood exchanges were made. ♂: Workers or brood collected from a known male-specialist field colony; ♀: workers or brood collected from a known female-specialist field colony.

(see Table 1; as in *P. desertorum* colonies in nature, male-specialist nests in our experiment sometimes reared a minority of reproductive females; Helms & Rissing 1990; Helms 1999).

We housed control and experimental groups in fluon-lined plastic boxes (35 × 17 cm and 9 cm high) containing a damp slab of dental plaster covered with soil. When brood were placed into the boxes, workers retrieved them in both control and experimental nests. Although worker ants rarely tolerate non-nestmate adults, they will adopt immature brood (Hölldobler & Wilson 1990). Workers excavated chambers in the soil and under the plaster, where they reared the brood. We provided nests with water, crickets, mealworms and honey until all reproductive brood had pupated (early July), at which time we counted the numbers of reproductive females and males in each nest.

We analysed whether experimental nests reared the number of reproductive females and males predicted using paired *t* tests, where pairs were the actual number of reproductive females and males reared versus the number of each predicted among nests. Distributions of data did not differ significantly from normal (Kolmogorov–Smirnov goodness-of-fit tests) and statistical tests were performed on the untransformed data.

RESULTS

The sex ratios produced by control nests were consistent with our prediction; control colonies produced the same sex-ratio bias as in the previous year. Control nests from female-specialist field colonies ($N=6$) that produced reproductive brood ($N=5$) produced only reproductive females ($\bar{X} \pm SE=12.40 \pm 4.74$). Control nests from male-specialist field colonies ($N=6$) that produced reproductive brood ($N=5$) produced only males, or an extremely male-biased sex ratio (89.4–100% males; $\bar{X} \pm SE$: males: 55.80 ± 23.85 ; reproductive females: 3.40 ± 2.91). Because not all control nests produced reproductive brood, three of the 12 experimental nests were not predicted to receive reproductive brood of both sexes. In the nine remaining experimental nests, the percentage of reproductive brood

predicted to be available for rearing that was female brood ranged from 10.3 to 88.9 (Table 2) and all of these nests reared significantly fewer males than predicted (paired *t* test: $t_8 = -4.18$, $P=0.003$; Table 2), while there was no significant difference between the number of females reared and predicted available (paired *t* test: $t_8 = -0.20$, $P=0.846$; Table 2). The results were independent of whether nests were initiated with workers from colonies of female or male specialists; all nests reared fewer males than the number predicted to be available for rearing (Table 2).

DISCUSSION

Our experiment shows that *P. desertorum* workers failed to rear a substantial proportion of available males, while rearing available reproductive females. This is consistent with workers discriminating against males in favour of reproductive females. The results were independent of whether workers were from female-specialist or male-specialist field colonies. Because male-specialist *P. desertorum* colonies in nature produce only males or a sex ratio that is extremely male biased (Helms 1999), the results are consistent with queens of those colonies substantially preventing the production of reproductive females. To our knowledge, this study is the first providing experimental evidence that queens and workers of social Hymenoptera will act in opposition in determining the reproductive sex ratio. The ability of queens to prevent the production of reproductive females is an important prerequisite for the evolution of colony sex-ratio specialization under queen control (Pamilo 1982; Helms 1999). Our experiment provides evidence that it occurs in *P. desertorum*. In addition, queen control over sexual investment has never been shown conclusively in social insects (Keller & Nonacs 1993). Evidence from this study (also see Helms 1999) provides strong evidence that it occurs.

Discrimination against males in our experiment was often incomplete. In some of our experimental nests, a large proportion of males predicted to be available for rearing were reared (mean 31.7%, range 0–63.9%; Table 2), resulting in reproductive sex ratios that were less sex

Table 2. Numbers of reproductive females and males reared and predicted to be available for rearing in *Pheidole desertorum* experimental nests predicted to rear both sexes

Nest	Number of males		Number of females		Percentage of brood predicted to be female
	Reared	Predicted	Reared	Predicted	
A ♂	39	63.5	5	9.0	12.4
B ♂	31	48.5	6	7.0	12.6
E ♂	0	13.5	14	14.0	50.9
G ♂	0	13.0	1	1.5	10.3
H ♂	0	1.0	9	7.5	88.2
C ♀	33	63.5	26	21.5	25.3
D ♀	27	48.5	4	7.0	12.6
F ♀	7	13.5	4	3.0	18.2
I ♀	0	1.0	8	8.0	88.9
Mean	15.2	29.6	8.6	8.7	35.5

♂: Workers collected from a known male-specialist field colony; ♀: workers collected from a known female-specialist field colony.

biased than generally occur in nature (Helms & Rissing 1990; Helms 1999). Of 24 *P. desertorum* colonies collected in the field, 19 produced unisexual reproductive broods and all produced reproductive broods consisting of more than 98% of one sex (Helms 1999). However, to discriminate adaptively against males, workers must discriminate against them early in male development, prior to substantial investment in their production (Nonacs & Carlin 1990). Male pupae appear in male-specialist colonies in the field from mid-May into July, and sometimes into August (Helms 1995). Because we collected broods in mid-May, a small number of male pupae occurred in our collections from male-specialist colonies; males in those broods would vary from recently hatched larvae to pupae. Workers in our study may have eliminated young male larvae while rearing older male larvae and pupae.

The failure of workers to rear some proportion of males in our experimental nests substantially reduced total reproductive output (Table 2). This result suggests that elimination of males is costly in *P. desertorum*. However, our experiment may greatly overestimate that cost. In nature, female- and male-specialist colonies appear (on average) to invest equal resources in reproduction (Helms 1999). In natural colonies, a largely mixed sex reproductive brood may only occur in cases where there are sufficient reproductive females to eliminate males without substantially decreasing reproductive output. If so, the cost of eliminating males is largely restricted to resources invested in males prior to their elimination. If recognition of males occurs early in male development, the cost of eliminating them may be small (e.g. Nonacs & Carlin 1990).

In our experiment, control nests reared related brood while experimental nests reared unrelated brood (Table 1). One potential interpretation of our results is that *P. desertorum* workers discriminate against males (but not reproductive females) on the basis of relatedness. This might occur under worker policing, a phenomenon predicted for species where queens are inseminated by multiple males (polyandry) and workers are capable of producing viable male eggs (Ratnieks 1988). Because of

polyandry, males produced by workers would be destroyed by other workers because workers are, on average, more closely related to males produced by the queen (Ratnieks 1988). Worker policing is unlikely to apply to *P. desertorum*, however. In *P. desertorum*, polyandry is very rare, if it occurs. In a previous study, enzyme genotypes in 35 of 38 colonies analysed were consistent with singly inseminated queens (Helms 1999). Genotypes in three remaining colonies could have resulted from either multiple reproductive queens (polygyny) or polyandry; the data did not allow for discrimination between these possibilities (Helms 1999). No data were consistent with worker reproduction (Helms 1999), and *Pheidole* workers lack functional ovaries and are unable to produce male eggs (Hölldobler & Wilson 1990).

In addition to our experiment, other evidence suggests *P. desertorum* workers will discriminate against males in favour of reproductive females. In nature, nearly half of male-specialist colonies produce a small number of reproductive females, while female specialist colonies never produce any males (Helms 1999). Recent studies of *Linepithema humile*, *Solenopsis invicta*, *Pheidole pallidula* and *Lasius niger* show that ant queens are probably always producing male eggs, whether or not males are being reared (Aron et al. 1994a, b; Keller et al. 1996; Passera & Aron 1996; Aron & Passera 1999; also see Sundström et al. 1996). In *P. pallidula*, male eggs occur in colonies at initiation of the reproductive season (and afterwards), even though many colonies produce only female reproductive broods (Keller et al. 1996). It is likely that queens of female-specialist *P. desertorum* colonies produce male eggs during the reproductive season, and yet workers never rear them.

Although the population investment sex ratio in *P. desertorum* is consistent with substantial queen control (Helms 1999), our results are also consistent with workers acting to influence the sex ratio. If, as we propose, mostly or only male reproductives are available for rearing in male-specialist colonies, while both reproductive females and males are available to rear in female-specialist colonies, it is possible that workers use a conditional 'rule of

thumb': destroy or neglect males when reproductive females are encountered frequently, but rear males (and any available reproductive females) when reproductive females are absent or encountered rarely. This may be the best fitness option available to workers if queens largely control the occurrence of reproductive females.

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