

# Internest sex-ratio variation and male brood survival in the ant *Pheidole pallidula*

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Sex allocation in social insects has become a general model in tests of inclusive fitness theory, sex-ratio theory, and parent-offspring conflict. Several studies have shown that colony sex ratios are often bimodally distributed, with some colonies producing mainly females and others mainly males. Sex specialization may result from workers assessing their relatedness to male brood versus female brood, relative to the average worker-relatedness asymmetry in other colonies of their population. Workers then adjust the sex ratio in their own interest. This hypothesis assumes that workers can recognize the sex of the brood in their colony and selectively eliminate males. We compared the primary sex ratio (at the egg stage) and secondary sex ratio (reproductive pupae and adults) of colonies in the ant *Pheidole pallidula*. There was a strong bimodal distribution of secondary sex ratios, with most colonies producing mainly reproductives of one sex. In contrast, there was no evidence of a bimodal distribution of primary sex ratios. The proportion of haploid eggs produced by queens was 0.35 in early spring and decreased to about 0.1 in summer. Male eggs also were present in virtually all field colonies sampled in July, although eggs laid at this time of year never give rise to males. All male brood is, therefore, selectively eliminated beginning in July and continue to be eliminated through the rest of the year. Finally, the population sex-ratio investment was female-biased. Together, these results are consistent with the hypothesis that workers control the secondary sex ratio by selectively eliminating male brood in about half the colonies, perhaps those with high relatedness asymmetry. *Key words:* ant, haplodiploidy, *Pheidole pallidula*, sex allocation, sex ratio. [*Behav Ecol* 7:292–298 (1996)]

Important insights in sex-ratio theory (Fisher, 1930), inclusive fitness theory (Hamilton, 1964a,b), and parent-offspring conflict (Trivers, 1974) have come from studies of sex-ratio conflict between queens and workers in social Hymenoptera (ants, wasps, and bees). In Hymenoptera, females are diploid and males haploid, and this difference typically results in asymmetries of genetic relatedness among colony members. Asymmetries of within-colony relatedness cause colony members to have different inclusive fitness functions, leading to a parent-offspring conflict over relative allocation of resources in the two sexes (Charnov, 1978; Nonacs, 1986; Pamilo, 1991; Trivers and Hare, 1976). Because queens are equally related to both sexes of reproductives reared in their colony, their fitness is maximized by an even sex-ratio investment. In contrast, because workers are more closely related to their sisters than to their brothers, their inclusive fitness is maximized by an increased investment in female brood. Consequently, the population investment sex ratio is predicted to be even under queen control, and to be female-biased under worker control. Several contributions suggest that the population sex-ratio investment is female-biased in single-queen (monogynous) colonies, supporting at least partial worker control on sex-ratio investment (e.g., Boomsma, 1989; Bourke, 1989; Crozier and Pamilo 1993; Mueller, 1991, 1994; Nonacs, 1986; Trivers and Hare, 1976).

The prediction of female bias holds for the population-wide sex ratio. It follows that colony sex ratios might have a random, unimodal distribution about a population average (see Bourke and Chan, 1994). However, several studies showed that colony sex ratios are often bimodally distributed, with

some colonies producing mainly females and others mainly males (e.g., Elmes, 1987; Helms and Rissing, 1990; Nonacs, 1986; Pamilo and Rosengren, 1988; Vargo and Fletcher, 1987). Three hypotheses have been proposed to explain colony-level variation in sex ratios of social Hymenoptera: 1) The resource level hypothesis predicts that variation in resource levels or quality may shift colony sex ratios toward male or female bias (Boomsma, 1993; Crozier and Pamilo, 1993; Nonacs, 1986). When resources are scarce, females become workers rather than reproductives, while males still develop into adults; when resources are abundant, both males and females develop into reproductives. 2) Under the constant male hypothesis, competition among related males for access to mates (local mate competition; Hamilton, 1967) causes colonies to produce all males up to some threshold, and then only female reproductives if resources allow investment beyond the threshold (Frank, 1987; Yamaguchi, 1985). 3) The relative relatedness asymmetry hypothesis proposes that sex specialization results from workers assessing their relatedness to male brood versus female brood, relative to the average worker-relatedness asymmetry in their population. Workers then adjust the sex ratio in their own interests (Boomsma, 1991, 1993; Boomsma and Grafen, 1990, 1991; Bourke and Chan, 1994; Ratnieks, 1991). Colonies with relatedness asymmetries above average should specialize on production of female reproductives, whereas those with relatedness asymmetries below average are expected to specialize on males. Several factors may cause intercolony variation in relatedness asymmetry, like queen mating frequency (Boomsma and Grafen, 1991; Ratnieks, 1991), number of queens when queens are related (Boomsma, 1993; Frank, 1987; Nonacs, 1986), and extent of worker reproduction (Boomsma and Grafen, 1991).

The best evidence for the resource level hypothesis comes from a field study in which Deslippe and Savolainen (1995) provided additional food to colonies. These colonies allocated

Received 19 June 1995; revised 25 September 1995; accepted 1 October 1995.

1045-2249/96/\$5.00 © 1996 International Society for Behavioral Ecology

a greater proportion of their resources into female reproductives compared to untreated colonies. Evidence for relatedness-induced split sex ratios comes from several bee, wasp, and ant species in which colonies with high relatedness asymmetry produce predominantly females and colonies with low relatedness asymmetry males (Boomsma, 1991; Chan and Bourke, 1994; Mueller, 1991, 1994; Packer and Owen, 1994; Queller et al., 1993; Sundström, 1993, 1994, in press).

However, colony sex ratio specialization is not due to differences in relatedness structure in at least two ant species. In the monogynous form of *Solenopsis invicta* there is a strong bimodal distribution of sex ratios despite the lack of variation in genetic structure (Ross and Fletcher, 1985; Aron et al. 1995; Vargo, in press). Similarly, the bimodal sex-ratio distribution in *Formica sanguinea* apparently is not associated with the relatedness asymmetry, but it should be mentioned that this species is a facultative slave-making ant so that odor-cue diversity can be altered when colonies raid conspecific colonies (Pamilo and Seppä, 1994).

The three hypotheses make different predictions that can be tested experimentally. For example, the relative relatedness asymmetry hypothesis assumes that workers have partial or complete control over the sex ratio of the reproductives produced. Queens are in control of the proportion of haploid (male) and diploid (female) eggs they lay, but workers may possibly shift the sex ratio toward females by eliminating male brood or by increasing the resources invested per female reproductive.

The ability of workers to discriminate between the sex of the brood and to give preferential treatment to females is also a key assumption of the Trivers and Hare (1976) hypothesis and the presumed ability of workers to control investment ratios. Surprisingly, despite its theoretical interest and its consequences, few studies have investigated the potential ability of workers to distinguish the sex of brood in social Hymenoptera. Such studies are characterized by two major problems. First, it is extremely difficult to assess the primary sex ratio due to the difficulty of obtaining eggs without destroying the colony, and second it may be difficult to sex the eggs. Recently, Aron et al. (1994) developed a technique to sex eggs and they showed that the primary sex ratio (at the egg stage) was significantly more male-biased than the sex ratio at the pupal stage in the Argentine ant *Linepithema humile* (formerly *Iridomyrmex humilis*). Similar results were obtained in the fire ant *Solenopsis invicta* (Aron et al., 1995). Moreover, the latter study showed that males were selectively eliminated in some colonies.

In this study we compare the primary sex ratios (at the egg stage) and secondary sex ratios (adult reproductives) of the ant *Pheidole pallidula*. We selected this species for several reasons. First, workers are completely sterile (Passera, 1978), hence all sexuals develop from queen-laid eggs. Second, all sexuals develop from the eggs that queens lay during the first days after they resume egg laying in early spring (Passera, 1980). Third, there are both monogynous (single queen) and polygynous colonies. An earlier survey from the studied population showed that 6 out of 71 colonies (8.2%) were polygynous. Five colonies contained two queens and one contained three queens (Passera L, unpublished data). Because it is impossible to collect whole colonies, and queens may have been missed during collection (see Methods), this value represents a minimum estimate of the level of polygyny. Because queens are almost always related in species in which colonies contain low numbers of queens (see Keller, 1995), it is likely that intercast variation in queen number is associated with differences in relatedness asymmetry in *P. pallidula*. Finally, a preliminary survey suggested a bimodal distribution of colony secondary sex ratios. Therefore, our aim was to study whether

primary sex ratios of colonies were bimodal and whether male eggs were absent from some colonies. We also quantified the intercast variation in secondary sex ratio and the population sex-ratio investment to test predictions of the various hypotheses proposed to account for the bimodal distribution of sex ratios in ant colonies.

## METHODS

### Primary sex ratio

Estimating the primary sex ratio of individual colonies in the field is problematic for several reasons. First, there is variation among colonies in the date when queens resume egg laying so it is difficult to know whether eggs collected are the first eggs laid in the season. Second, it is impractical to collect a sufficient number of eggs from a colony to obtain a reliable estimate of the proportion of haploid eggs, particularly in early spring when colonies contain few eggs. Finally, it is easiest to determine the number of chromosomes for eggs 2–4 days old, but it is difficult to estimate the age of eggs collected from the field (Aron et al., 1994). Therefore, we set up experimental colonies to obtain sufficient numbers of eggs of known age. Our experimental procedure also allowed us to determine the date when queens resumed egg laying.

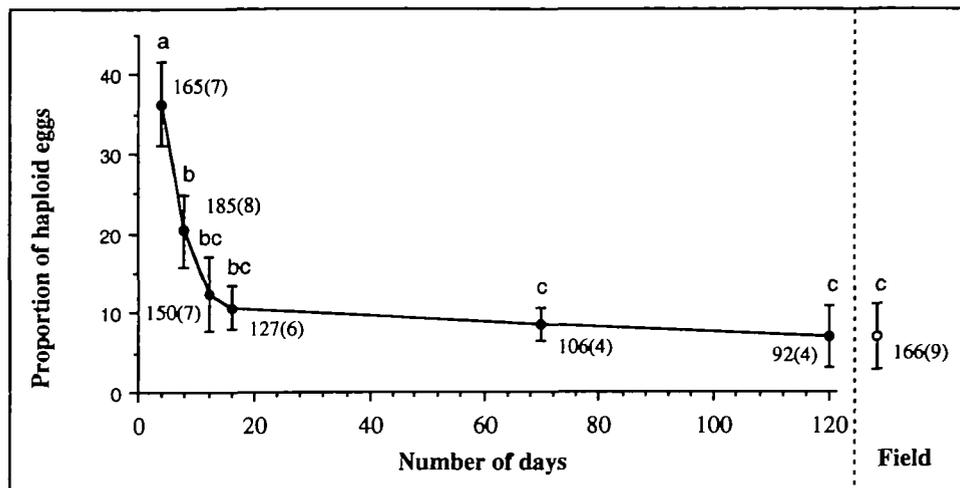
Colonies were collected with their queen on 22 March 1993 from La Frau, Tarn-and-Garonne, France. On this date only a few queens had resumed egg laying (no eggs were found in 13 of 15 excavated colonies). Eight queenright colonies containing no eggs were returned immediately to the laboratory where they were each split into four fragments of equal size. The proportion of soldiers was standardized to 5% in each of the 32 colony fragments and the queens were placed in one of the corresponding fragments. All colony fragments were maintained under "late winter" laboratory conditions ( $12 \pm 2^\circ\text{C}$ ). On 30 March (day 0), the sets of eight fragments with the queens were transferred to a room where they were fed on honey and crickets every other day and maintained under standard conditions at  $27^\circ\text{C}$  (Passera, 1980). After 4 days, all eggs were collected from these fragments to determine the proportion of haploid and diploid eggs. Another set of eight colony fragments was then transferred into the  $27^\circ\text{C}$  room and the queens transferred from the first to the second set of fragments. After another 4 days, all eggs were collected from these fragments and the queens transferred to a new set of colony fragments taken from the cold room. The same procedure was repeated with the last set of colony fragments after another 4 days. Four of the last eight fragments were then maintained with their queen under standard laboratory conditions until day 120. We collected eggs on days 70 and 120.

This experimental design allowed us to obtain eggs from queens on days 0–4 (referred to as day 4), 4–8 (day 8), 8–12 (day 12), 12–16 (day 16), 70, and 120 after they resumed egg laying. Eggs were 0–4 days old when collected from the colony fragments, but their age was unknown on days 70 and 120. Although egg survival was high during the 4 days, there may have been differential mortality between male and female eggs. All colony fragments were maintained under "late winter" condition until they received a queen. This procedure minimized any seasonal effect on the selective elimination of eggs for the first four censuses in which the primary sex ratio was estimated.

To sex the eggs, we used Aron et al.'s (1994) procedure of staining and counting the chromosomes ( $n = 12$ ,  $2n = 24$ ; Hauschteck, 1961). The mean number of eggs for which we could count the number of chromosomes varied between 13 and 36 (mean  $22.9 \pm 5.1$ ) per sample. An additional group of eggs was collected directly from nine field colonies on 7

**Figure 1**

Changes over time in the primary sex ratio (proportion of haploid eggs) in laboratory and field colonies. Primary sex ratio is given for six censuses over the first 120 days after queens resumed egg laying (30 March–28 July) and eggs collected from field colonies on 7 July. Means are given  $\pm$  SE. The total number of eggs and the number of colonies sampled (parentheses) are reported for each sample. Treatments with lowercase letters differed significantly (ANOVA and Scheffé  $F$  tests,  $p < .05$ ).



July 1993 from Bruniquel (approximately 4 km from La Frau). The primary and secondary sex ratios were determined for each of these nine colonies (8–25 eggs per colony, see below). At this time of the year colonies contain some trophic eggs that are laid by the female reproductives (the young winged unmated queens) (Passera, 1978). These eggs can be distinguished from the eggs laid by the functional queens because they are much smaller (Passera, 1978). These eggs were discarded from our samples.

### Secondary sex ratio

The secondary sex ratio was determined by the number of male and female reproductives (pupae and adults) sampled in field colonies. Colonies were sampled at three sites, from the same population in which experimental colonies were collected, to estimate the primary sex ratio. Six colonies were sampled at La Frau on 5 July 1993, 30 colonies at Montricoux (2 km from La Frau) on 5 July 1993, and 13 colonies at Bruniquel on 7 July 1993. Because the soil was extremely rocky we could not dig up whole colonies. However, the reproductive pupae and adults could be collected under stones covering the nest entrance on sunny days. Although we were able to collect most of the reproductives, some always escaped into the nest. However, male and female reproductives apparently did not differ in their tendency to escape collection. Thus, our collection procedure provides a relatively precise measure of the sex ratio of adults.

Few reproductive larvae were present when we sampled colonies, and the first mating flights took place after 20 July. Most of the yearly production of reproductives was thus present in the colonies either at the pupal or adult stages when we collected. Numerical secondary sex ratios of colonies were determined by the number of male and female reproductives (pupae and adults) found in each colony.

To estimate investment ratios from numerical sex ratio we used dry weight of male and female reproductives. Male and female reproductives were collected on 22 July 1993 at Bruniquel from two and five colonies, respectively. After drying in an oven at 70°C for 24 h, each ant was weighed to the nearest 0.001 mg on a Mettler electronic balance. Several lines of evidence suggest that reproductives were sexually mature at this time. First, founding queens were found under stones at the same site. Second, no more reproductive pupae were present in the colonies. Finally, the average weight of female reproductives (see Results) was more than 2.3 times higher than the average weight of the young female reproductives

collected on 5 and 7 July 1993 ( $2.66 \pm 0.76$  mg;  $N = 41$ ). This increase is typical for independent founding queens during the period of sexual maturation (see Boomsma and Isaaks, 1985; Keller and Passera, 1989; Keller and Ross, 1993).

Investment ratios were calculated as

$$P_m = \frac{N_m W_m}{N_m W_m + N_f W_f}$$

where  $P_m$  is the proportional investment in males,  $N_m$  is the number of males,  $W_m$  is the dry weight of males,  $N_f$  is the number of females, and  $W_f$  is the dry weight of females (Trivers and Hare, 1976).

### Statistical analyses

Proportions of haploid eggs were square root arcsin transformed to give normal distributions (Zar, 1974). ANOVAS were performed on these transformed values. Multiple comparisons among pairs of means were carried out using Scheffé  $F$  tests.

There was a strong bimodal distribution of the proportion of both male and female pupae and adults (see Results). Therefore we used nonparametric tests (Spearman rank correlation, Mann-Whitney  $U$  and Kruskal-Wallis tests) to analyze data on secondary sex ratios.

## RESULTS

### Primary sex ratio

There was significant temporal variation in the primary sex ratio (proportion of haploid eggs;  $F_{6,44} = 25.38$ ,  $p < .001$ ; Figure 1). Whereas the average proportion of haploid eggs laid by the queens was .35 when queens resumed egg laying, it decreased to about .1 during the following 2 weeks and remained stable thereafter. The proportion of haploid eggs among the eggs collected directly from the field on 7 July did not differ significantly from the proportion of haploid eggs laid by queens in the laboratory on days 12, 16, 70, and 120, suggesting that the continuous production of haploid eggs by queens was not an artifact of laboratory conditions. The queens laid similar proportions of haploid eggs and each of them laid some male eggs during the six censuses of the primary sex ratio. In each of the six censuses there was no significant difference among queens in the proportion of haploid eggs they laid ( $\chi^2_{(2)}$  tests: day 4: 1.33,  $p = .97$ ; day 8: 2.18,

Table 1

Primary sex ratio (proportion of haploid eggs) and secondary sex ratio (proportion of males out of the reproductive brood) in nine field colonies from Bruniquel sampled on 7 July

Colony	Primary sex ratio	No. eggs sexed	Secondary sex ratio
2	0.13	8	0.99
3	0.10	20	0.00
5	0.05	21	0.88
6	0.10	20	0.98
7	0.00	17	0.98
8	0.04	25	0.66
11	0.06	17	0.00
12	0.05	20	0.10
13	0.11	18	0.00

$p = .95$ ; day 12: 2.40,  $p = .88$ ; day 16: 0.58,  $p = .99$ ; day 70: 0.44,  $p = .93$ ; day 120: 1.59,  $p = .66$ ).

An average of 18.4 eggs (range 8–25) could be sexed in the nine colonies sampled directly from the field on 7 July (Table 1). Because of the relatively small number of eggs per colony and the low proportion of haploid eggs (10%) at this time of year, the probability of sampling no haploid eggs from colonies was high. However, haploid eggs were found in eight of the nine (89%) colonies. Moreover, the only colony in which haploid eggs were not found produced predominantly males (colony 7, Table 1), indicating that haploid eggs must also have been present in this colony.

#### Secondary sex ratio

Detailed information on the number and gender of reproductive pupae and adults for each of the 49 colonies is given in Table 2. There was no difference among sites in the numerical sex ratio for either reproductive pupae (Kruskal-Wallis,  $H = 0.64$ ,  $df = 2$ ,  $p > .05$ ) or adults (Kruskal-Wallis,  $H = 4.49$ ,  $df = 2$ ,  $p > .05$ ). Therefore we pooled data from the three sites for subsequent analyses.

There was a clear bimodal distribution of colony sex ratios, with one mode of colonies producing exclusively or predominantly males and another mode producing mostly females (Figure 2). Forty-five out of the 49 colonies (92%) produced more than 80% individuals of the same sex. Seventeen colonies (35%) produced only females and five colonies (10%) only males. Sex specialization of colonies remained relatively stable during the reproductive season as demonstrated by the high correlation between pupal and adult sex ratios (Spearman rank correlation  $\rho = 0.80$ ,  $z = 4.86$ ,  $N = 38$ ,  $p < .001$ ).

The population-wide numerical sex ratio (given as the numerical fraction of males) was 0.66 for pupae and 0.71 for adults. The slightly more male-biased numerical sex ratio for adults compared to pupae possibly reflects a faster development rate of males due to their smaller body size (adult dry weight:  $0.88 \pm 0.07$  mg,  $N = 25$ ) compared to females (the dry weight of young immature female reproductives collected on 5 and 7 July 1993 was  $2.66 \pm 0.76$  mg,  $N = 41$ ). Differences in developmental time associated with the magnitude of sexual dimorphism have been documented in various social Hymenoptera species (Boomsma and Eickwort, 1993; Boomsma and Isaaks, 1985; Passera and Keller, 1987).

Using the total number of male and female reproductives (pupae and adults) found in the 49 colonies and their dry weights (mature female sexuals =  $6.14 \pm 1.44$  mg,  $N = 36$ ) we estimated the proportional sex-ratio investment in males to be 0.24, that is, a 3.2:1 female to male investment. Sex investment ratio based only on dry weight differences tend to

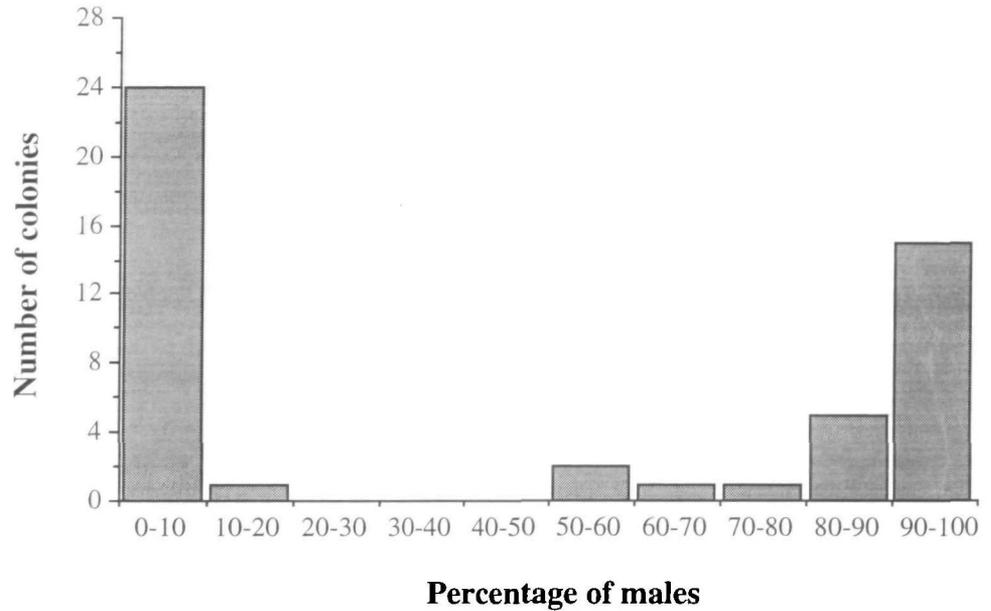
Table 2

Composition of 49 colonies of *Pheidole pallidula* collected at three different sites

Sites	Colony	Male pupae	Male adults	Queen pupae	Queen adults	Proportion of males	
La Frau	1	59	38	0	0	1.00	
	2	39	73	24	1	0.82	
	3	0	1	15	10	0.04	
	4	0	1	55	28	0.01	
	5	0	3	13	0	0.19	
	6	0	0	13	0	0.00	
	Montricoux	1	92	170	37	6	0.86
		2	34	116	1	4	0.97
		3	0	26	0	1	0.96
		4	18	42	0	0	1.00
		5	7	159	6	18	0.87
		6	34	27	34	21	0.53
		7	156	45	1	0	1.00
8		275	101	1	12	0.97	
9		253	120	0	0	1.00	
10		17	33	1	1	0.96	
11		0	0	4	0	0.00	
12		0	0	1	30	0.00	
13		0	0	42	23	0.00	
14		0	1	0	20	0.05	
15		16	31	0	4	0.92	
16		0	0	1	34	0.00	
17		27	64	17	2	0.83	
18	0	1	0	38	0.03		
19	0	0	0	9	0.00		
20	0	0	73	24	0.00		
21	1	0	61	19	0.01		
22	0	0	32	37	0.00		
23	0	0	0	19	0.00		
24	21	37	10	5	0.79		
25	0	0	45	22	0.00		
26	0	0	24	61	0.00		
27	0	32	0	22	0.59		
28	0	0	22	23	0.00		
29	0	1	0	13	0.07		
30	0	0	13	13	0.00		
Bruniquel	1	0	61	0	0	1.00	
	2	33	64	0	1	0.99	
	3	0	0	4	127	0.00	
	4	0	1	59	77	0.01	
	5	167	220	36	16	0.88	
	6	172	263	10	0	0.98	
	7	156	73	3	2	0.98	
	8	42	93	69	1	0.66	
	9	9	96	0	1	0.99	
	10	0	26	0	0	1.00	
	11	0	0	20	38	0.00	
	12	0	0	63	27	0.00	
	13	0	0	41	21	0.00	
Total		1628	2019	851	831		

be biased in favor of females. Because males generally have a higher metabolic rate they cost more on a per weight basis to produce and maintain than females (Boomsma, 1989; Boomsma et al., 1995). To correct for differences in metabolic rates it has been suggested that female:male dry weight ratios (D) should be converted to energetic costs (C) by the formula  $C = D^{0.7}$  (Boomsma, 1989; Boomsma et al., 1995). This conversion gives a somewhat less female-biased investment ratio of 0.36 (i.e., 1.8:1 female to male investment).

The female investment bias increased significantly with colony productivity (measured by the total investment in reproductives) when dry weights were used to estimate cost of pro-



**Figure 2**

Frequency distribution of sex ratio (numerical fraction of males) in the 49 colonies sampled. Number of males and females is determined by the sum of male and female pupae and adults.

duction of reproductives (Spearman rank correlation  $\rho = 0.41$ ,  $z = 2.82$ ,  $N = 49$ ,  $p = .005$ ). However, this correlation was not significant when Boomsma's conversion was used to calculate the relative cost of production of males and females (Spearman rank correlation  $\rho = 0.18$ ,  $z = 1.33$ ,  $N = 49$ ,  $p > .05$ ).

## DISCUSSION

The present study demonstrates a clear bimodal distribution of the secondary colony sex ratios with most nests producing mostly or exclusively males or females. In contrast, there was no evidence of a similar bimodal distribution in the primary sex ratio. All queens laid about 30–40% haploid eggs in early spring and gradually fewer later in the season. There are two possible explanations for this difference. First, bimodal distribution of secondary colony sex ratios is due to differences among colonies in survival of male brood with only few or no males reaching the pupal stage in some colonies and some other colonies preferentially rearing males over females. Second, all eight colonies we sampled were male-producing colonies (thus the reason why all queens laid a high proportion of haploid eggs). However, this possibility is very unlikely. The proportion of colonies rearing predominantly (more than 50%) males was 49%. The probability of sampling eight such colonies out of a sample of eight is .49<sup>8</sup>, or less than .004. The probability remains small even when considering the conservative likelihood of sampling eight colonies all producing at least some adult males. The proportion of colonies producing only female brood being 35%, this probability is .65<sup>8</sup>, that is, less than .04.

The conclusion that the bimodal distribution of colony secondary sex ratios is due primarily to differences among colonies in survival of male brood is further supported by the complete absence of males in 17 (35%) of the 49 colonies where secondary sex ratio was determined, whereas haploid eggs were found in 16 (94%) of the 17 colonies (the eight laboratory and the nine field colonies). This difference is significant ( $\chi_1^2 = 17.8$ ,  $p < .001$ ), and the only colony where no haploid eggs were found most likely also contained haploid eggs (see above). Moreover, three of the nine field colonies where both the primary and secondary sex ratio were quantified produced no males, although they all contained male eggs (Table 2).

Inter-nest variation in male brood survival is inconsistent with two of the three hypotheses proposed to account for the frequent occurrence of bimodal sex ratios within colonies of ants (see Introduction). The resource level hypothesis predicts that colonies with high food abundance will channel a higher proportion of the female brood into becoming reproductives (Nonacs, 1986). However, if food is abundant there is no reason to eliminate males. Yet, 35% of the colonies produced female reproductives but no males, a pattern that cannot be explained by the resource level hypothesis. The high proportion of colonies producing females only also is inconsistent with the constant male hypothesis, because the hypothesis predicts that colonies should produce all males up to some threshold, and then if resources permit, only females (Frank, 1987). Thus, queen-worker conflict over sex ratio is the most likely explanation for the selective elimination of males in some colonies. More than one queen was found in 8.2% of the nests and the proportion of polygynous nests is probably substantially higher due to the difficulty of finding queens in this species (see Methods). Differences in queen number cause variation among colonies in relatedness asymmetry. Moreover, differences in relatedness asymmetry may arise also if some queens mate multiply, as commonly occurs in ants (Keller and Reeve, 1994; Page, 1986). Whether males are selectively eliminated in colonies with high relatedness asymmetry remains to be tested.

Thirty-one percent of colonies produced males but only few or no female reproductives. These colonies, as other colonies, produced workers. Thus female brood was probably channeled into becoming workers rather than reproductives in these colonies, as seems to occur in other ant species when conditions are not favorable for the production of female reproductives (e.g., Hölldobler and Wilson, 1990; Keller and Nonacs, 1993; Passera, 1984). Relative investment in females increased significantly with colony productivity (measured by the total investment in reproductives) when dry weights were used to estimate cost of production of reproductives. Such an association has been shown to occur in many ant species (Nonacs, 1986). However, the correlation was not significant in *P. pallidula* when Boomsma's conversion was used. This raises the question of whether the positive association between the relative investment in females and productivity might stem from an overestimation of the cost of production of female

reproductives compared to male reproductives when using dry weights as a measure of their cost of production.

Queens apparently laid male eggs all year round, even at times when all eggs develop into workers. Similar results were obtained in two other species. In the Argentine ant *Linepithema humile* (= *Iridomyrmex humilis*) the average proportion of haploid eggs laid by queens is always higher than 25% (Aron et al., 1994; Keller et al., 1996). Similarly, in the ant *Solenopsis invicta* the 12 queens studied by Aron et al. (1995) all produced about 10% or more haploid eggs. Together these data suggest that the continuous production of some male eggs by queens may reflect inefficiency in egg fertilization. Ant queens have a limited lifetime supply of sperm and they use only few sperm to fertilize each egg (Tschinkel and Porter, 1988), possibly resulting in some eggs not being fertilized.

Reproductives of *P. pallidula* engage in large mating flights and queens initiate new colonies independently (Bontpart, 1964; Emery, 1911; Passera L, personal observation). Therefore it is unlikely that local resource competition or local mate competition are important factors affecting preferred investment ratios of queens and workers. When there is no local resource competition and local mate competition, the preferred investment ratio of workers lies somewhere between 1:1 and 3:1 female investment, whereas the preferred investment of queens is 1:1 (see Boomsma, 1988; the lack of data on the genetic structure of colonies of this species do not allow more precise estimation of the worker optimal investment ratio). The female-biased population investment ratio therefore suggests that workers have at least partial control over investment ratios.

Finally, the results of this study suggest that workers can distinguish the sex of the brood, and that they exert their control by selectively eliminating males. Similar results have been reported for the Argentine ant *L. humile* (Aron et al., 1994) and the fire ant *S. invicta* (Aron et al., 1995). Together these studies suggest that workers' ability to distinguish between male and female offspring, an implicit assumption of hypotheses and empirical studies on the conflict between queens and workers in highly eusocial species is fulfilled. The stage of development at which workers distinguish males from females remains unknown.

We thank Giorgina Bernasconi, Koos Boomsma, Andrew Bourke, Michel Chapuisat, Philippe Christe, Richard Deslippe, Ulrich Mueller, Peter Nonacs, Paul Schmid-Hempel, Liselotte Sundström, and Edward Vargo for helpful comments on the manuscript. This work was supported by the Swiss National Science Foundation (grants no. 31-35584.92, 31-36907.93, and 3100-040828.94 to L.K.), the Ministère français de l'Environnement (L.P.), and the Société Entomologique de France (S.A.).

## REFERENCES

- Aron S, Passera L, Keller L, 1994. Queen worker conflict over sex ratio: a comparison of the primary and the secondary sex ratios in the Argentine ant, *Iridomyrmex humilis*. *J Evol Biol* 7:403–418.
- Aron S, Vargo EL, Passera L, 1995. Primary and secondary sex ratios in monogyne colonies of the fire ant *Solenopsis invicta*. *Anim Behav* 49:749–757.
- Bontpart H, 1964. Recherches préliminaires sur la biologie de *Pheidole pallidula* Nyl. (Hymenoptère, Formicoïde, Myrmicidae). Toulouse: Thèse.
- Boomsma JJ, 1988. Empirical analysis of sex allocation in ants: from descriptive surveys to population genetics. In: Population genetics and evolution (De Jong G, ed). Berlin: Springer Verlag; 42–51.
- Boomsma JJ, 1989. Sex-investment ratios in ants: has female bias been systematically overestimated? *Am Nat* 133:517–532.
- Boomsma JJ, 1991. Adaptive colony sex ratios in primitively eusocial bees. *Trends Ecol Evol* 6:92–95.
- Boomsma JJ, 1993. Sex ratio variation in polygynous ants. In: Queen number and sociality in insects (Keller L, ed). Oxford: Oxford University Press; 86–109.
- Boomsma JJ, Eickwort GC, 1993. Colony structure, provisioning and sex allocation in the sweat bee *Halictus ligatus* (Hymenoptera: Halictidae). *Biol J Linn Soc* 48:355–377.
- Boomsma J, Grafen A, 1990. Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. *Evolution* 44:1026–1034.
- Boomsma J, Grafen A, 1991. Colony-level sex ratio selection in the eusocial Hymenoptera. *J Evol Biol* 3:383–407.
- Boomsma JJ, Isaaks JA, 1985. Energy investment and respiration in queens and males of *Lasius niger* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 18:19–27.
- Boomsma JJ, Keller L, Nielsen MC, 1995. Comparative analysis of sex ratio investment parameters in ants. *Funct Ecol* 9:743–753.
- Bourke AFG, 1989. Comparative analysis of sex-investment ratios in slave-making ants. *Evolution* 43:913–918.
- Bourke AFG, Chan GL, 1994. Split sex ratios in ants with multiple mating. *Trends Ecol Evol* 9:120–122.
- Chan GL, Bourke AFG, 1994. Split sex ratio in a multiple-queen ant population. *Proc R Soc Lond B* 258:261–266.
- Charnov EL, 1978. Evolution of eusocial behavior: offspring choice or parental parasitism. *J Theor Biol* 75:451–465.
- Crozier RH, Pamilo P, 1993. Sex allocation in social insects: problems in prediction and estimation. In: Evolution and diversity of sex ratio in insects and mites (Wrensch DL, Ebberts M, eds). New York: Chapman and Hall; 369–383.
- Deslippe RJ, Savolainen R, 1995. Sex investment in a social insect: the proximate role of food. *Ecology* 76:375–382.
- Elmes GW, 1987. Temporal variation in colony populations of the ant *Myrmica sulanodis*. 2. Sexual production and sex ratios. *J Anim Ecol* 56:573–583.
- Emery C, 1911. La fondazione di formicai da femmine fecondate di *Pheidole pallidula* e di *Tetramorium caespitum* sulla intolleranza o fratellanza fra le formiche di formicai differenti. *Boll Lab Zool Gen Agr Port* 5:184–189.
- Fisher RA, 1930. The genetical theory of natural selection. Oxford: Clarendon.
- Frank SA, 1987. Variable sex ratio among colonies of ants. *Behav Ecol Sociobiol* 20:195–201.
- Hamilton WD, 1964a. The genetical evolution of social behavior. 1. *J Theor Biol* 7:1–16.
- Hamilton WD, 1964b. The genetical evolution of social behavior. 2. *J Theor Biol* 7:17–52.
- Hamilton WD, 1967. Extraordinary sex ratios. *Science* 156:477–488.
- Hauschteck E, 1961. Die Chromosomen von fünf Ameisenarten. *Rev Suisse Zool* 68:218–223.
- Helms KR, Rissing SW, 1990. Single sex alate production by colonies of *Pheidole desertorum* and *Pheidole xerophila tussonica* (Hymenoptera: Formicidae). *Psyche* 97:203–218.
- Hölldobler B, Wilson EO, 1990. The ants. Berlin: Springer-Verlag.
- Keller L, 1995. Social life: the paradox of multiple-queen colonies. *Trends Ecol Evol* 10:355–359.
- Keller L, L'Hoste G, Balloux F, and Plumey O, 1996. Queen number influences the primary sex ratio in the Argentine ant, *Linepithema humile* (= *Iridomyrmex humilis*). *Anim Behav* 51:445–449.
- Keller L, Nonacs P, 1993. The role of queen pheromones in social insects: queen control or queen signal? *Anim Behav* 45:787–794.
- Keller L, Passera L, 1989. Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). *Oecologia* 80:236–240.
- Keller L, Reeve HK, 1994. Genetic variability, queen number, and polyandry in social Hymenoptera. *Evolution* 48:694–704.
- Keller L, Ross KC, 1993. Phenotypic plasticity and cultural transmission of alternative reproductive strategies in the fire ant *Solenopsis invicta*. *Behav Ecol Sociobiol* 33:121–129.
- Mueller UG, 1991. Haplodiploidy and the evolution of facultative sex ratios in a primitively eusocial bee. *Science* 254:442–444.
- Mueller, UG, 1994. DNA fingerprinting analysis of parent-offspring conflict in a bee. *Proc Nation Acad Sci USA* 91:5143–5147.
- Nonacs P, 1986. Ant reproductive strategies and sex allocation theory. *Q Rev Biol* 61:1–21.
- Packer L, Owen RE, 1994. Relatedness and sex ratio in a primitively eusocial halictine bee. *Behav Ecol Sociobiol* 34:1–10.
- Page RE, 1986. Sperm utilization in social insects. *Ann Rev Entomol* 31:297–320.

- Pamilo P, 1991. Evolution of colony characteristics in social insects. 1. Sex allocation. *Am Nat* 137:83–107.
- Pamilo P, Rosengren R, 1983. Sex ratio strategies in *Formica* ants. *Oikos* 40:24–35.
- Pamilo P, Seppä P, 1994. Reproductive competition and conflicts in colonies of the ant *Formica sanguinea*. *Anim Behav* 48:1201–1206.
- Passera L, 1978. Une nouvelle catégorie d'oeufs alimentaires: Les oeufs alimentaires émis par les reines vierges de *Pheidole pallidula* (Nyl.). *Insect Soc* 25:117–126.
- Passera L, 1980. La ponte d'oeufs préorientés chez la fourmi *Pheidole pallidula* (Nyl.). *Insect Soc* 27:79–95.
- Passera L, 1984. L'organisation sociale chez les fourmis. Toulouse: Privat.
- Passera L, Keller L, 1987. Energy investment during the differentiation of sexuals in the Argentine ant, *Iridomyrmex humilis* (Mayr). *Mitt Schweiz Entomol Gesells* 60:249–260.
- Queller DC, Strassmann JE, Solis CR, Hughes CR, Deloack DM, 1993. A selfish strategy of social insect workers that promotes social cohesion. *Nature* 365:639–641.
- Ratnieks FLW, 1991. Facultative sex allocation biasing by workers in social Hymenoptera. *Evolution* 45:281–292.
- Ross KG, Fletcher DJC, 1985. Comparative study of genetic and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance. *Behav Ecol Sociobiol* 17:349–356.
- Sundström L, 1993. Genetical population structure and sociogenetic organisation in *Formica*. *Behav Ecol Sociobiol* 33:345–354.
- Sundström L, 1994. Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. *Nature* 367:266–268.
- Sundström L, 1996. Sex allocation and colony maintenance in monogyne and polygyne colonies of *Formica truncorum* (Hymenoptera: Formicidae): the impact of kinship and mating structure. *Am Nat* 146:182–201.
- Trivers RL, 1974. Parent-offspring conflict. *Am Zool* 14:249–264.
- Trivers RL, Hare H, 1976. Haplodiploidy and the evolution of the social insects. *Science* 191:249–263.
- Tschinkel WR, Porter SD, 1988. Efficiency of sperm use in queens of the fire ant, *Solenopsis invicta* (Hymenoptera, Formicidae). *Ann Entomol Soc Am* 81:777–781.
- Vargo EL, in press. Sex investment ratios in monogyne and polygyne populations of the fire ant *Solenopsis invicta*. *J Evol Biol*.
- Vargo EL, Fletcher DJC, 1987. Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta*. *Physiol Entomol* 12:109–116.
- Yamaguchi Y, 1985. Sex ratios of an aphid subject to local mate competition with variable maternal condition. *Nature* 318:460–462.
- Zar JH, 1974. *Biostatistical analysis*. New York: Prentice-Hall.