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Colony sex ratios vary with queen number but not relatedness asymmetry in the ant *Formica exsecta*

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Split-sex-ratio theory assumes that conflict over whether to produce predominately males or female reproductives (gynes) is won by the workers in haplodiploid insect societies and the outcome is determined by colony kin structure. Tests of the theory have the potential to provide support for kin-selection theory and evidence of social conflict. We use natural variation in kinship among polygynous (multiple-queen) colonies of the ant *Formica exsecta* to study the associations between sex ratios and the relatedness of workers to female versus male brood (relatedness asymmetry). The population showed split sex ratios with about 89% of the colonies producing only males, resulting in an extremely male-biased investment ratio in the population. We make two important points with our data. First, we show that queen number may affect sex ratio independently of relatedness asymmetry. Colonies producing only males had greater genetic effective queen number but did not have greater relatedness asymmetry from the perspective of the adult workers that rear the brood. This lack of a difference in relatedness asymmetry between colonies producing females and those producing only males was associated with a generally low relatedness between workers and brood. Second, studies that suggest support for the relatedness-asymmetry hypothesis based on indirect measures of relatedness asymmetry (e.g. queen number estimated from relatedness data taken from the brood only) should be considered with caution. We propose a new hypothesis that explains split sex ratios in polygynous social insects based on the value of producing replacement queens.

Keywords: sex ratio; relatedness asymmetry; polygyny; social insects; Hymenoptera

1. INTRODUCTION

Social Hymenoptera have been key organisms for the study of Hamilton's inclusive-fitness theory (e.g. Bourke & Franks 1995; Crozier & Pamilo 1996; Bourke 1989; Queller & Strassmann 1998). The haplodiploid sex-determination system, by which females are diploid and males are haploid (arising by arrhenotokous parthenogenesis; Crozier 1971), results in asymmetries of genetic relatedness among colony members and a parent–offspring conflict over relative allocation of resources to the two sexes (Trivers & Hare 1976; Nonacs 1986*a*; Bourke & Franks 1995; Crozier & Pamilo 1996). 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, workers are more related to their sisters than to their brothers, and their inclusive fitness is maximized by an increased investment in female brood. Consequently, the population sex ratio is predicted to be even under queen control and 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. Trivers & Hare 1976; Nonacs 1986*a*; Boomsma 1989; Bourke 1989; Mueller 1991, 1994; Crozier & Pamilo 1992; Hasegawa 1994; Bourke & Franks 1995).

However, it has become clear that several factors may confound comparisons across species (Chapuisat & Keller 1999). For example, variation in relatedness asymmetry is typically associated with major changes in breeding system and life history that may also affect sex allocation. Moreover, the relative cost of females and males is difficult to estimate across sexually dimorphic taxa, such as ants. These shortcomings have lead to the conclusion that intraspecific studies of sex-ratio variation across colonies provide more powerful ways to assess the role that kin selection, relatedness asymmetry and within-colony conflicts play in sex allocation (Bourke & Chan 1994; Chapuisat & Keller 1999). Theory predicts that, under worker control, colonies with high or low relatedness asymmetry should specialize in producing females or males, respectively (Boomsma & Grafen 1990; Ratnieks & Boomsma 1997). This prediction can be tested within populations where colonies differ by a single factor altering their genetic structure (e.g. queen mating frequency, Boomsma & Grafen 1990; Ratnieks & Boomsma 1997; or the number of queens when queens are related, Nonacs 1986*a*,*b*; Boomsma 1993) but are similar in other characteristics.

Empirical data allowing predictions of the relatedness-induced split sex-ratio theory to be tested are available for about 20 species of social Hymenoptera and in most of these species colonies with higher relatedness asymmetry produced more females than colonies with lower relatedness asymmetry (Queller & Strassmann 1998). However, in many of these species the relatedness asymmetry has been measured indirectly from information on the breeding structure. For example, some studies have inferred relatedness asymmetry from estimates of queen number (either direct counts, Herbers 1984; Deslippe & Savolainen 1995; Vargo 1996; or the genetic effective queen number estimated from the relatedness between female brood, Evans 1995; or adults, Chan & Bourke 1994; Chan *et al.* 1999). Alternatively, other studies estimated relatedness asymmetry using relatedness between

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female and male brood or without distinguishing brood from adult workers (Evans 1998; Aron *et al.* 1999). These indirect measures require assumptions, such as adult workers being the progeny of the current queens, queen number not covarying with queen relatedness, or queen turnover being uncommon, that may not be met in many social Hymenoptera (Chapuisat & Keller 1999; Aron *et al.* 1999; J. Heinze and L. Keller, unpublished data; see § 4). The aim of the present study was to test the relationship between colony sex ratios and direct and indirect measures of relatedness asymmetry in a polygynous (multiple queen per colony) population of *Formica exsecta*.

We studied a *F. exsecta* population in the Swiss Jura Mountains. Populations of *F. exsecta* in this region are polygynous (Cherix *et al.* 1980) and sex ratios of polygynous populations in other areas have been shown to be bimodally distributed (Pamilo & Rosengren 1983; Fortelius *et al.* 1987). In contrast to some other ant species (Cook & Crozier 1995) there are no diploid males produced in this species (Pamilo & Rosengren 1984; Agosti & Hauschteck-Jungen 1987) and thus sex-ratio variation is not the passive consequence of differences in allelic variation at sex-determining loci. Previous studies of a monogynous population of *F. exsecta* in Finland demonstrated that bimodal sex ratios correspond with queen mating frequency, and give strong evidence that queen–worker conflict over sex ratio is manifest within colonies. Queens laid eggs with a sex ratio irrespective of mating frequency but, as predicted by the relatedness-asymmetry hypothesis, the workers biased the secondary sex ratio in favour of females when the queen was singly mated (Sundström *et al.* 1996; Chapuisat *et al.* 1997*b*).

We inferred relatedness asymmetry in three different ways, the first two of which have been used in previous studies. First, we measured the relatedness between female brood (both worker and gyne pupae) and inferred from these data the genetic effective queen number. Variation in queen number (either from direct counts or inferred from relatedness between female brood as we did) is commonly used as an indirect means of measuring relatedness asymmetry (e.g. Herbers 1984; Queller *et al.* 1993; Chan & Bourke 1994; Deslippe & Savolainen 1995; Evans 1995). Second, we measured the relatedness asymmetry in the brood (relatedness of female pupae to male and female pupae). This has also been used as an indirect means to assess the relatedness asymmetry of adult workers to male and female brood (Evans 1998; Aron *et al.* 1999) on the assumption that patterns of relatedness persist across generations. Finally, we measured relatedness asymmetry between adult workers and the male and female brood that they are raising. This direct measure accurately estimates the potential inclusive fitness benefits of sex-ratio manipulation to the adult workers (those individuals that alter the queen-laid primary sex ratio).

2. METHODS

We randomly chose 40 colonies from a population of over 300 located at Les Chenevières, a pasture at approximately 1200 m elevation in the commune of Le Vaud located in the Swiss Jura Mountains. Nineteen additional colonies with large pupae (presumably gynes) were chosen to increase the proportion of female-producing colonies (which are infrequent, see § 3). Then, to get an unbiased measure of population sex ratio, we sampled and sexed *ca.* 50 pupae from as many additional colonies as possible. Thus our estimate of population sex ratio is based on all nests from a defined area. A total of 191 additional colonies were sampled, which we added to our 59 focal samples for a total sample size of 250. In six of these colonies pupae were not sufficiently developed for sexing and another seven colonies produced only worker brood, yielding a net 237 colonies from which to calculate sex ratio. All colonies in the population were marked with numbered wooden stakes for identification. At least ten adult workers per focal colony were collected for genetic analysis prior to the eclosion of brood, and between 33 and 216 pupae per focal colony were collected for genetic analyses and to estimate sex ratio and worker production.

(a) *Sex ratio and worker allocation*

Numerical sex ratios were estimated from the proportions of males and gynes within a sample of 4197 pupae from our focal nests and another 9286 pupae from the additional nests. These pupae were sampled over two days just prior to the eclosion of most reproductive brood in mid-July. Additional samples taken at ten-day intervals confirmed that sex ratios were reasonably stable during the main period of the production of sexuals. Ratios of workers, gynes, large males and small males were estimated by moistening pupae in ethanol and observing the size, eye shape and genitalia of late-stage pupa (male *F. exsecta* are size dimorphic, Fortelius *et al.* 1987). Pupae fall into three nearly discrete size categories corresponding to workers, small males and the combination of large males and gynes. Male pupae were classified as large if they were grouped with gynes into the largest pupal size class prior to determining their sex. Otherwise they were counted as small males. Sex allocation ratios were estimated by weighting numerical ratios by the relative dry masses of large males, small males and gynes. Dry weight was determined in the following way. First, alate males and gynes were captured prior to flight as they emerged from their colonies. We avoided sampling alates prior to emergence from the colony to standardize their condition. Again, we made an *a priori* judgement of male size class based on the similarity of large males and gynes. All individuals were then frozen at $-20\,^{\circ}\mathrm{C}$, dried at $60\,^{\circ}\mathrm{C}$ for 24 h, and weighed on an MT5 Mettler (Mettler-Toledo GmbH, Greifensee, Switzerland) balance accurate to 0.01 mg. Male investment was adjusted for both the proportion of large versus small males and the relative weights of these classes of individual. We measured dry weights for as many focal colonies as possible but many estimates were missing due to the difficulty of collecting synchronously emerging brood. Therefore, missing values were substituted with the mean values (across colonies) for the appropriate sex-ratio category of the colony (see § 3(a)). Dry weights were not measured for the 191 non-focal colonies and thus we substitute mean values per sex-ratio category (see § 3(a)) from our focal samples.

Male allocation was the sum of dry-weight investment in large and small males. To correct for factors such as differential fat content and respiration rate that affect the female-to-male cost ratio, we also estimated sex allocation by applying the Boomsma *et al.* (1995) correction factor of $\mathrm{ADW}^{0.7}$, where ADW is the dry-weight ratio of females to males.

(b) *Relatedness estimates*

We determined genotypes at two microsatellite, FL20 and FL21, and two allozyme loci, leucine aminopeptidase (Lap) and phosphoglucomutase (Pgm). Allozymes were scored using

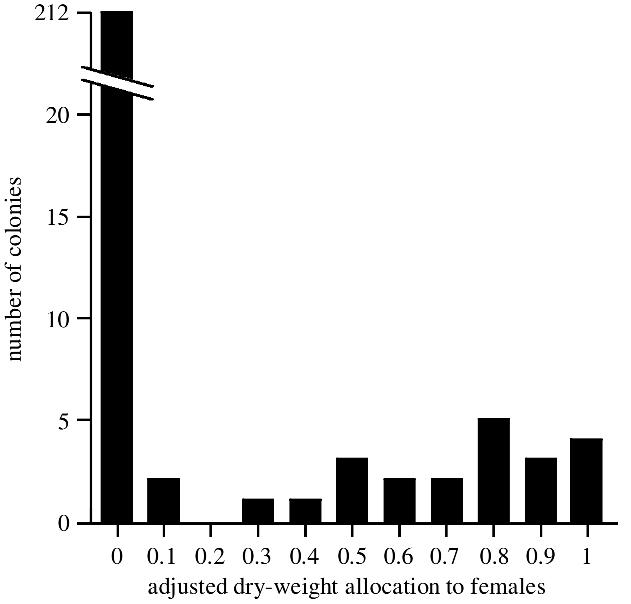

Figure 1. The frequency distribution of sex allocation in a polygynous population of *F. exsecta*. Sex allocation is measured as dry-weight allocation to the production of gynes and is adjusted according to Boomsma *et al.* (1995) to account for energetic differences of rearing male and female offspring.

standard 12% horizontal starch gel electrophoresis. Each allozyme had two alleles, with expected heterozygosities of 0.49 (Lap) and 0.20 (Pgm). Microsatellites were analysed using primers and methods described in Chapuisat (1996). Pupae and old workers were stored at −20 °C. DNA was extracted by standard proteinase K/SDS digestion and phenol–chloroform purification (Sambrook *et al.* 1989). We replaced ^{35}S-dATP with 0.02 μl ^{33}P-dATP per PCR reaction, as recommended by Chapuisat *et al.* (1997*a*). FL20 had two alleles and FL21 had 26 alleles, with expected heterozygosities of 0.50 and 0.87, respectively, under the assumption of Hardy–Weinberg equilibrium. We determined the genotype of between 101 and 447 individuals of each caste, and sex of brood. In total, we determined the genotypes of 1025 individual brood and 408 old workers from 59 colonies.

We estimated genetic relatedness and inbreeding (F) using the computer program Relatedness 4.2c (Queller & Goodnight 1989; Goodnight & Queller 1994). Standard errors were obtained by jackknifing over colonies. Relatedness of adult workers to offspring gynes was inferred from relatedness to total female brood (i.e. gynes + workers), hence allowing us to estimate expected relatedness to gynes in colonies that produced no reproductive females. This procedure was justified because there was no significant difference in the relatedness of adult workers to gynes and new workers. Relatedness estimates were normalized by log transformation prior to analyses.

We quantified the genetic effective queen number ($n_{e,g}$) as $n_{e,g} = 0.75/r_f$, where r_f is the relatedness among nest-mate female brood (workers and gynes) (Queller 1993; Ross 1993). Genetic effective queen number represents the number of breeding queens responsible for a given amount of genetic variation assuming that there is no inbreeding and queens are unrelated, singly mated and equally fecund. It is likely that some of these assumptions are violated (e.g. because some of the queens mated multiply) in our population. However, our aim is not to determine exactly the effective number of queens per colony but to obtain a value correlated with the number of queens in order to compare the breeding structure of male- and female-producing nests.

Table 1. *Mean and 95% confidence intervals (CI) of dry weights of brood from female- and male-producing colonies*

		mean weight (mg)	95% CI[a]
female-producing colonies	workers	1.26[b]	1.19–1.33
	small males	1.92[c]	1.86–1.97
	large males	3.52	3.31–3.75
	gynes	4.24	4.07–4.42
male-producing colonies	workers	1.13[b]	1.07–1.19
	small males	1.71[c]	1.66–1.75

[a] Values are back-transformed from log-transformed means (except for small males) taken across colonies and this yields slightly asymmetrical confidence intervals.

[b] Dry weight of workers differed significantly between male- and female-producing colonies (nested ANOVA, $F_{1,444} = 17.75$, $p < 0.0001$) and among colonies within sex-ratio class ($F_{51,444} = 4.85$, $p < 0.0001$).

[c] Dry weight of small males differed significantly between male- and female-producing colonies (nested ANOVA, $F_{1,495} = 33.73$, $p < 0.0001$) and among colonies within sex-ratio class ($F_{39,495} = 3.14$, $p < 0.0001$).

3. RESULTS

(a) *Population sex ratio*

Assuming that colony sex ratio is not correlated with total reproductive investment, we estimate the average population sex ratio to be 5.8% female, or one female for each 17.2 males produced. Colony sex ratios were bimodally distributed and strongly male biased (figure 1), with 209 out of 237 colonies (88.2%) producing only small males, 24 colonies (10.1%) producing females along with large and/or small males, three colonies (1.3%) producing large males but no females, and one colony (0.4%) producing only females. All of the male-producing colonies also produced workers and thus the absence of reproductive females in the brood was never due to a lack of sperm for the production of diploid offspring. Average numerical sex ratio (proportion females) within female-producing colonies was 58.8 ± 5.8%.

Average dry masses of gynes, large and small males are listed in table 1. Using these values the overall investment ratio in females was 8.0%. Within female-producing colonies the investment in gynes was 70.7 ± 4.8%. Applying Boomsma's (Boomsma *et al.* 1995) correction factor had only a small effect, the female-investment ratio decreasing to 7.3% at the population level. Because of the clearly bimodal distribution of sex ratio, we classified colonies as producing males only (male-producing colonies) or producing some proportion of females (female-producing colonies) for further analyses.

(b) *Genetic data*

In our population inbreeding estimates (F) were low and not significantly positive based on jackknifed standard errors. For brood of female-producing colonies $F = 0.065 \pm 0.057$ ($t_{18} = 1.32$; $p > 0.2$) and for male-producing colonies

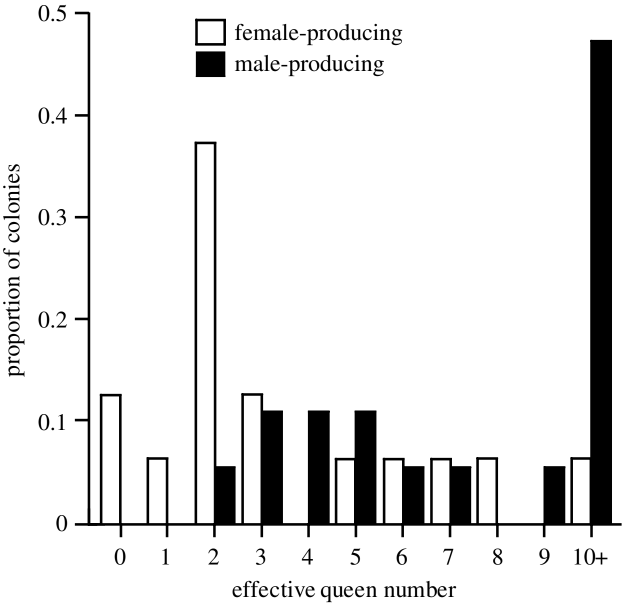

Figure 2. The distribution of effective queen number for female- and male-producing colonies. Female-producing colonies are those that reared gynes, usually in addition to males. Male-producing colonies reared males but no gynes. The distribution of effective queen number in male-producing colonies excludes 11 colonies for which we could not calculate effective queen number because our estimates of relatedness between female brood was less than or equal to zero. Values on the x-axis represent the lower value of the range of each category, e.g. the bar labelled 1 represents colonies with $1 \leqslant n_{e,g} < 2$.

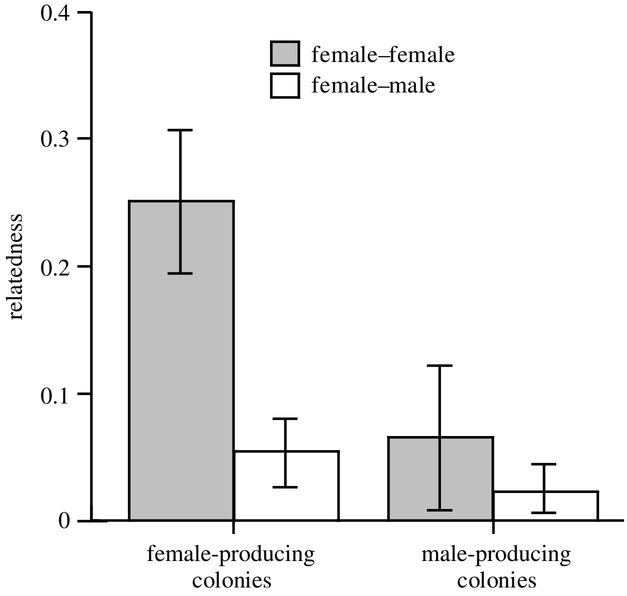

Figure 3. Relatedness asymmetry was significantly greater within the brood of female-producing colonies than within the brood of male-producing colonies, as indicated by the greater difference in the height of the bars (± s.e.m.) on the left (repeated-measures ANOVA, $F_{1,46} = 7.48$, $p = 0.009$). Relatedness between female brood was significantly greater than relatedness of female to male brood ($F_{1,46} = 14.94$, $p = 0.0004$) and overall relatedness was significantly greater in female-producing nests than in male-producing nests ($F_{1,46} = 18.56$, $p = 0.00009$).

$F = 0.041 \pm 0.031$ ($t_{33} = 1.14$; $p > 0.2$). Figure 2 shows that female-producing colonies had a relatively low genetic effective queen number, with a modal value of 2 and a harmonic mean (x_h) of 2.7. Despite this low value, all but two out of the 18 female-producing colonies (89%) had estimates of x_h greater than 1. Thus, the large majority of these colonies were apparently polygynous.

Genetic effective queen number was higher in male-producing colonies ($F_{1,39} = 11.43$; $p = 0.0017$) owing to a higher modal value (5) and a much greater skew towards higher values. Excluding the 11 out of 34 male-producing colonies with zero or negative estimates of r_f (for which x_h cannot be calculated) yields a conservatively low estimate of $x_h = 6.7$ in male-producing colonies. Within female-producing colonies, genetic effective queen number was not significantly correlated with the ratio of gynes to males (dry-weight sex ratio, $r = 0.25$, $n = 18$, $p = 0.32$; ADW$^{0.7}$ sex ratio, $r = 0.34$, $n = 18$, $p = 0.16$). Our estimates of genetic effective queen number are roughly consistent with direct observations of queen numbers equalling 2, 3 and 3 for three fully excavated colonies.

Relatedness between colony members within male- and female-producing colonies was generally low (ranging from 0.023 to 0.296 for various combinations of sexes and age cohorts). Within-brood relatedness differed systematically between male- and female-producing colonies. All estimates of relatedness within brood in female-producing colonies were significantly greater than zero (t-tests, all $p < 0.05$) and significantly greater than the comparable measure in male-producing colonies (figure 3). Male–male relatedness differs between female- (0.190 ± 0.043) and male-producing (0.031 ± 0.022) colonies ($t_{46} = 5.3$, $p < 0.0001$). Male–male relatedness is independent of queen mating frequency and therefore effective queen number does indeed vary with sex ratio. In contrast, none of the estimates of the relatedness of adult workers to other members of the colony (other adult workers, female brood or male brood) differed significantly between male- and female-producing colonies (figure 4; relatedness between adult workers = 0.087 ± 0.046 in female-producing nests, and $r = 0.062 \pm 0.025$ in male-producing nests, $t_{46} = 0.04$, $p = 0.97$).

To estimate relatedness asymmetry in the brood (pupae) we treated, first, relatedness between female brood (r_{ff}) and, second, relatedness of female to male brood (r_{fm}) as paired measures taken from each colony. We then used repeated-measures ANOVA to test whether the difference between r_{ff} and r_{fm} differed with the sex-ratio class of the colony. This relative difference in relatedness is indicated by a significant statistical interaction between sex-ratio class and the paired measures of relatedness. From figure 3, it is clear that relatedness among female brood relative to relatedness between female and male brood is much greater in female-producing colonies. The proportional difference in relatedness, measured as the ratio of r_{ff} to r_{fm}, also differed significantly with the sex-ratio class of the colony (Mann–Whitney $U = 338$; $p = 0.044$). This is exactly as we would predict based on the relatedness-asymmetry hypothesis.

We also used repeated-measures ANOVA to estimate the relatedness asymmetry of adult workers to female and male brood (the relevant measure to determine potential inclusive fitness benefits to adult workers). In this case,

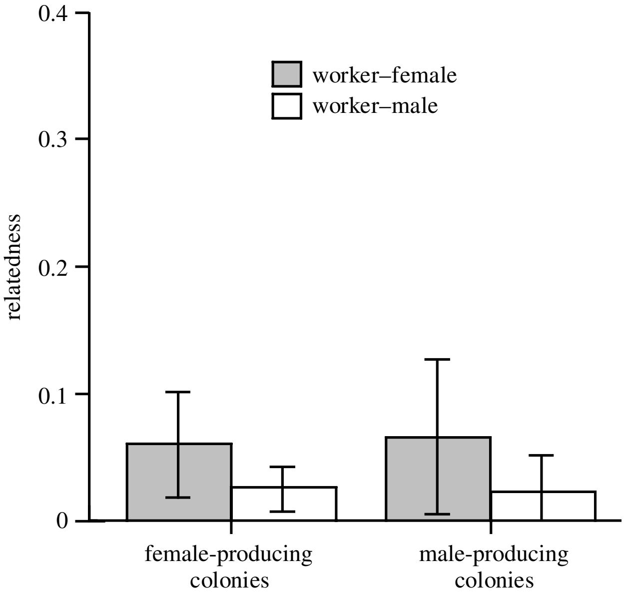

Figure 4. There was no significant relatedness asymmetry from the perspective of adult workers in either female- or male-producing colonies ($F_{1,37} = 0.83, p = 0.37$) and thus no significant difference in relatedness asymmetry between sex-ratio classes ($F_{1,37} = 0.001, p = 0.97$). Overall relatedness (± s.e.m.) between adult workers and brood did not differ significantly between male- and female-producing nests ($F_{1,37} = 0.14, p = 0.71$).

there was no significant difference in relatedness asymmetry between male- and female-producing colonies (i.e. no significant interaction and no significant difference in the ratio of worker–female to worker–male relatedness: Mann–Whitney $U = 169$; $p = 0.83$; figure 4). Moreover, relatedness asymmetry within brood was significantly greater than that between brood and adult workers in female- (Wilcoxon signed-ranks test, $Z = 2.04$, $p = 0.04$) but not male-producing colonies ($Z = 0.55$, $p = 0.58$). There was also no significant difference between male- and female-producing colonies in either worker–female or worker–male relatedness. Finally, worker–female relatedness did not differ significantly from worker–male relatedness within either sex-ratio category. Thus, despite having a lower genetic effective queen number and higher relatedness within the brood of female-producing colonies, there appears to be no difference in relatedness asymmetry between sex-ratio classes and no evidence of significant queen–worker conflict over sex ratio.

4. DISCUSSION

The population investment ratio was highly male biased, which is not uncommon for polygynous ants (Crozier & Pamilo 1996). This is probably due to colony budding creating local resource competition (Clark 1978; Frank 1987) and the need to count workers that form each daughter colony as female investment (Bourke & Franks 1995). Colony sex ratios were also strongly bimodally distributed as predicted by split-sex-ratio theory.

When using estimates of queen number and relatedness between female brood as indirect measures of relatedness asymmetry, we found that sex ratios in *F. exsecta* are split as predicted by split-sex-ratio theory. Colony sex ratios were split depending on the genetic effective queen number, as has been found previously in several other ant species (Chan & Bourke 1994; Deslippe & Savolainen 1995; Evans 1995). Moreover, we also found that a lower effective queen number caused a significant increase in relative relatedness asymmetry within brood. That is, not only was relatedness among female brood generally higher in female-producing colonies, but the difference between female–female relatedness and female–male relatedness was significantly greater. This difference in relatedness asymmetry was driven by the genetic effective queen number. This is similar to Evans' (1998) report that the difference in relatedness of workers to males and gynes was greater in colonies of *Myrmica tahoensis* that produce gynes than in those rearing only males.

However, when we directly measured the relatedness between adult workers and male and female brood there was no difference in relatedness asymmetry between male- and female-producing colonies. This points out the hazard of using indirect measures of relatedness asymmetry. Interestingly, our data show that worker relatedness to brood was uniformly low (but positive) and did not differ significantly between the sexes, regardless of genetic effective queen number. Low relatedness between cohorts may stem from worker movement between colonies, queen movement or high queen turnover, and this is probably why there was a positive relationship between colony sex ratios and indirect measures of relatedness asymmetry but not with the actual relatedness asymmetry of adult workers to male and female brood.

Recent studies indicate that worker movement between colonies, queen movement or high queen turnover are probably also more common than previously assumed in other polygynous ants (Evans 1996; Pedersen & Boomsma 1999). For example, queen turnover is common in several *Leptothorax* species, leading to colony kin structures differing strongly from those expected if adult workers and the brood had been produced by the reproductive queens present in the colony (e.g. Bourke *et al.* 1997; Foitzik & Heinze 1998). Problems arising from using indirect means to assess relatedness asymmetry are also illustrated by another recent study. In the ant *Pheidole pallidula*, female bias increases with queen number, a pattern seemingly in conflict with the relatedness-asymmetry hypothesis (Aron *et al.* 1999). However, Aron *et al.* (1999) also found that relatedness asymmetry increases with queen number rather than decreasing as expected. Thus, by not assuming a particular relationship between queen number and relatedness asymmetry, the authors suggest that their unusual results were nevertheless potentially consistent with the relatedness-asymmetry hypothesis. They do not give an explanation for this unusual pattern of relatedness. Perhaps polygyny is associated with strong inbreeding in *P. pallidula* or queen number covaries with other social parameters affecting relatedness asymmetry.

Our results raise the question of why gynes are predominantly produced by colonies with fewer queens in *F. exsecta*. One possibility is a selective lag: recent increases in worker migration or queen turnover render sex-ratio manipulation in response to queen number to be no longer adaptive, but selection has yet to eliminate the ancestral condition whereby workers alter sex ratio in response to queen number. This seems unlikely, given the selective costs and wasted resources that colonies appear to pay for sex-ratio manipulation (Chapuisat *et al*. 1997*b*) and the seeming ease with which workers could evolve a simple lack of response to cues of genetic variation. Another possibility is that low adult worker to brood relatedness is the consequence of the migration of older foragers, while the younger nurse workers (who are more likely to adjust sex ratios) remain. This dilutes relatedness between brood and adult workers as a whole but selection for sex-ratio adjustment may be operating only on the resident workers. However, the migration of truly unrelated workers will by no means affect relatedness asymmetry. This is because unrelated workers will decrease relatedness to male and female brood in equal proportions, therefore not affecting the actual relatedness asymmetry.

We think a more likely alternative is that queen number influences the relative reproductive value of the sexes for a different reason. We propose a new hypothesis that explains split sex ratios in polygynous species of social insects based on local resource competition (Clark 1978) and the value of producing replacement queens. Polygynous ant colonies that reproduce by budding are subject to intense local resource competition, resulting in dramatically male-biased sex ratios, as has been observed in this study. However, queens may suffer high rates of mortality, making adoption of daughter queens advantageous for continued colony survival (Nonacs 1988) and increased ability to send dispersing queens on foot to neighbouring colonies. Benefits of gyne production will decrease with the number of currently existing queens and thus above some threshold of queen number no replacement gynes are produced.

This hypothesis begs the question of why female-producing colonies produce so many gynes, rather than just the few necessary to ensure colony survivorship. A possible explanation comes from the known mechanisms used by highly polygynous ants with poor capacity for nest-mate recognition to reject foreign gynes. In the highly polygynous ant *Formica paralugubris*, gynes are much more likely to be accepted in foreign colonies producing gynes than in colonies producing no gynes (Fortelius *et al*. 1993). Selective destruction of all gynes in colonies producing no gynes may have evolved as a simple rule of thumb to decrease the probability of acceptance of foreign unrelated gynes. Hence, production of more gynes than necessary may be adaptive because it dilutes the effect of accepting foreign gynes during the time window where selective elimination of foreign gynes does not occur. This hypothesis remains to be tested, but it is interesting to note that the studied population of *F. exsecta* has indeed been found to exhibit poor nest-mate recognition capacity (W. D. Brown and L. Keller, unpublished data).

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