REPORT

Queen recruitment and split sex ratios in polygynous colonies of the ant *Formica exsecta*

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Abstract

Sex ratios in social insects have become a general model for tests of inclusive fitness theory, sex ratio theory and parent–offspring conflict. In populations of *Formica exsecta* with multiple queens per colony, sex ratios vary greatly among colonies and the dry-weight sex ratio is extremely male-biased, with 89% of the colonies producing males but no gynes (reproductive females). Here we test the queen-replenishment hypothesis, which was proposed to explain sex ratio specialization in this and other highly polygynous ants (i.e. those with many queens per nest). This hypothesis proposes that, in such ants, colonies produce gynes to recruit them back into the colony when the number of resident queens falls below a given threshold limiting colony productivity or survival. We tested predictions of the queen-replenishment hypothesis by following *F. exsecta* colonies across two breeding seasons and relating the change in effective queen number with changes in sex ratio, colony size and brood production. As predicted by the queen-replenishment hypothesis, we found that colonies that specialized in producing females increased their effective queen number and were significantly more likely to specialize in male production the following year. The switch to male production also coincided with a drop in productivity per queen as predicted. However, adoption of new queens did not result in a significant increase in total colony productivity the following year. We suggest that this is because queen production comes at the expense of worker production and thus queen production leads to resource limitation the following year, buffering the effect of greater queen number on total productivity.

Keywords

INTRODUCTION

Polygynous (multiple-queens per nest) populations of *Formica exsecta* Nyl. are characterized by a highly male-biased sex ratio and colony specialization on the production of male or female reproductive brood (Pamilo & Rosengren 1983; Brown & Keller 2000). In a previous study, we tested whether sex ratio specialization varied with differences in colony relatedness asymmetry (i.e. the differential relatedness of workers to males and gynes) (Brown & Keller 2000). Because of the haplodiploid system of sex determination, whereby diploid eggs develop into females and haploid eggs into males, workers are three times more related to their full sisters than to their brothers in ants and other social Hymenoptera. This relatedness asymmetry decreases when queens mate with several males or when colonies contain several related queens (Boomsma &

Grafen 1990, 1991; Boomsma 1993). Theory predicts that when relatedness asymmetry varies between colonies, workers benefit by producing the sex to which they are most related compared with the population average. Thus, gyne production should predominantly occur in colonies with high relatedness asymmetry and male production should occur in colonies with lower relatedness asymmetry (Boomsma & Grafen 1990, 1991; Boomsma 1993). In *F. exsecta*, we found no evidence of an association between relatedness asymmetry and colony sex ratios (Brown & Keller 2000). Instead, colony sex ratios were associated with the effective number of queens per colony. Colonies producing gynes had a lower effective queen number than colonies producing only males.

These results led us to propose a new hypothesis (the queen-replenishment hypothesis) for colony sex ratio specialization in this and other polygynous ants (Brown &

Keller 2000). In highly polygynous ants, queens frequently remain in their mother nest after mating and eventually disperse on foot with workers to initiate new colonies nearby (Bourke & Franks 1995; Keller 1995). This mode of reproduction may lead to intense local resource competition (Clark 1978) between queens within colonies and is often associated with dramatically male-biased sex ratios (reviewed in Bourke & Franks 1995; Crozier & Pamilo 1996). The queen replenishment hypothesis holds that, in such species, the production of new queens occurs preferentially or only in colonies that contain relatively few queens because these are the colonies that benefit most from recruiting new queens (Brown & Keller 2000). In polygynous ants, queen life-span is typically limited (Keller & Genoud 1997) and low queen number may cause egg and brood limitation in large colonies with abundant resources (Elmes & Keller 1993). Egg limitation in Hymenoptera may alter optimal sex ratios (Rosenheim *et al.* 1996; West & Rivero 2000). The queen-replenishment hypothesis suggests that as queen number decreases, local resource competition between queens is reduced and below a certain threshold there is a premium on recruiting new queens to enhance colony survival and productivity. This threshold and the value of new queens will depend on resource availability relative to the current number of queens.

The aim of this study is to measure reproductive parameters in individual colonies over two breeding seasons to test four predictions of the queen-replenishment hypothesis. First, we test whether gyne production is followed by an increase in queen number. Second, we determine whether brood production per queen decreases the year following gyne production. This decrease is predicted because colonies having produced gynes should no longer be limited by the rate at which eggs are laid and thus reproductive success of individual queens will be constrained by the availability of other resources. Third, we determine whether gyne production and recruitment result in an increase in overall colony productivity. Such an effect is predicted if gyne production is induced by a shortage of brood because of insufficient numbers of queens. Finally, we test whether colonies that produce gynes in a given year switch to male production the following year. Such a shift to male production should last until queen number again drops beneath the threshold for gyne production.

MATERIALS AND METHODS

We studied a population of *F. exsecta* located at Les Chenevières, a pasture at approximately 1200 m in the commune of Le Vaud in the Swiss Jura Mountains. Colonies in this population are known to be predominately polygynous (Cherix *et al.* 1980) and this population was the focus of our previous study on polygynous *F. exsecta* (Brown &

Keller 2000). We measured sex ratio in every colony for which we could collect sexual brood for both the 1996 and the 1997 breeding seasons, a total of 143 colonies. These colonies were marked in 1996 with a unique alphanumerical code written onto a wooden stake that was driven into the ground immediately adjacent to the nest. The location of the colonies was then mapped to ensure our ability to accurately relocate them in 1997.

As in previous studies (Brown & Keller 2000; Brown *et al.* in press) colonies were classified as female-producing colonies when producing gynes (generally in addition to males) and as male-producing colonies when producing only males. We randomly selected 22 male- and 22 female-producing colonies and measured total brood production, colony size and effective number of queens in 1996 and 1997. Two of these colonies, both female-producing in 1996, failed to produce sexual brood in 1997 and were excluded from the analyses. Patterns of sex allocation and effective queen number during the 1996 breeding season have been reported previously (Brown & Keller 2000).

Sex allocation and brood production

Our methods for measuring sex allocation, brood production and colony size are described elsewhere (Brown & Keller 2000). Briefly, a sample of the pupae during production of sexual brood was collected from each colony to estimate caste and sex ratios. In *F. exsecta*, larvae do not overwinter and thus adults eclosing in 1 year derive from eggs laid earlier that same year. Ratios of workers, gynes and males were estimated by moistening pupae in ethanol and observing the size, eye shape and genitalia of late-stage pupa. We estimated sex allocation as the fraction of sexual resources invested in gynes (i.e. $g/(g + m)$), with numerical ratios weighted by relative dry mass. We also applied the Boomsma *et al.* (1995) correction factor of $ADW^{0.7}$, where ADW is the dry-weight ratio of gynes to males. This attempts to correct for factors such as differential fat content and respiration rate that affect the gyne-to-male cost ratio. In no cases do the qualitative results differ depending on which measure we used. Therefore, for comparisons of colony-level sex ratio, we only report results from standard dry-weight estimates of productivity and investment.

We estimated brood production using mark-recapture methods of Sundström (1995). Between 20 and 3980 pupae per colony were collected from just beneath the surface of the nest. A total of 63 309 pupae in 1996 and 38 722 in 1997 were individually marked with a dot of ink from a nontoxic, permanent red marker (Sharpie®). Pupae were released back into the nest by gently opening the surface, returning the marked pupae and closing the nest. Two days later, we captured another sample of pupae and used the

proportion of marked to unmarked pupae to estimate the total number of pupae within the colony. We estimated total brood production as the sum of the number of males, gynes and workers times their respective dry mass. All measures of colony size and productivity were normalized (Kolmogorov-Smirnov test, Lilliefors $P > 0.05$) by ln transformation prior to analyses. Means were back transformed and are reported with back-transformed 95% confidence intervals.

Colony size and effective queen number

We estimated the size of the worker force using the protocol for worker mark-recapture outlined by Sundström (1995). Before brood emerged, 200–2432 workers per colony were collected. A total of 45 338 workers in 1996 and 108 437 in 1997 were counted, sprayed lightly with a leather and wood spray paint, and released back onto the nest. Approximately 24 h later, workers were recaptured on the nest and the total number of workers was estimated from the proportion of marked to unmarked ants.

We were interested in whether gyne production affected the number of resident queens the following breeding season. We opted for the use of effective queen number because it can be measured with greater ease and accuracy than actual queen number. We estimated the effective queen number per colony for both 1996 and 1997 using genotypic variation at two allozyme and two microsatellite DNA loci (see Brown & Keller 2000). The effective queen number was measured using the formula $n_{eg} = 0.75/r_f$, where r_f is the relatedness among nestmate females (workers and gynes) (Queller 1993; Ross 1993). It represents the idealized number of breeding queens responsible for a given amount of genetic variation if there were no inbreeding and queens were unrelated, singly mated and equally fecund. Mark-recapture analyses showed that the estimated number of queens per colony is correlated with the effective queen number measured with genetic markers (C. Liautard, unpublished data). However, the effective number of queens will differ from actual queen number because the effective mating frequency of queens is greater than 1 (1.22, C. Liautard, J. Pedersen and L. Keller, unpublished), queens are significantly related to each other ($r = 0.122 \pm 0.027$, C. Liautard, J. Pedersen and L. Keller, unpublished) and it is likely that queens do not contribute equally to brood production. However, there is no reason to expect that fecundity differences, queen relatedness or queen mating frequency will decrease following gyne production, except in ways that are caused by increases in actual queen number.

Statistical analyses

As in our previous study (Brown & Keller 2000), we classified colonies as producing males only (male-produc-

cing colonies) or producing some proportion of gynes (female-producing colonies). This classification is convenient for testing the queen-replenishment hypothesis because our aim is to determine the causes underlying gyne production, which can be accomplished by comparing colonies producing gynes to those producing only males.

We first analysed nonrandom occurrence of sex specialization between years using contingency-table analysis of all 143 colonies. The null hypothesis was no association within colonies between years, and the alternative hypothesis was that sex specialization in the second year is dependent upon sex specialization in the first year. This could be due to either consistency in sex specialization between years or propensity to switch specialization, as we predict from the queen-replenishment hypothesis.

We then performed paired analyses of the relationship between sex specialization in 1996 and 1997 and effective queen number in our focal colonies. Because of the general rarity of female-producing colonies, only one of our focal colonies was female producing in both years and none of our focal colonies switched from being male-producing in 1996 to being female-producing in 1997. Therefore, we are only afforded the comparison between colonies that switched from female- to male-producing and those that persisted in producing males in both years. Sex specialization in 1996 was coded as a factorial variable and transformed effective queen number in 1996 and 1997 as repeated measures in a repeated measures ANOVA. The queen-replenishment hypothesis predicts a statistical interaction between sex specialization and the repeated measure, caused by a greater increase in effective queen number in female-producing colonies the year after gyne production. One-tailed paired t-tests were used for univariate tests of the change in effective queen number between years, with the prediction that queen number will increase in female-producing colonies and decrease in male-producing colonies. We then used the same approach to analyse changes in productivity per queen and total brood production. Both overall colony productivity and productivity per queen were normalized by ln transformation prior to analyses. Assumptions of normality (Kolmogorov-Smirnov, Lilliefors $P > 0.05$) and homogeneity of variances (Bartlett's test $P > 0.10$) were satisfied for all analyses.

Female-producing colonies invest relatively less in worker production than do male-producing colonies, and this is especially so in relatively small female-producing colonies (Brown et al. in press). Thus, female-producing colonies are expected to show less growth between years than male-producing colonies. We analysed changes in colony size between years using ANCOVA with sex specialization in 1996 as a factor, colony size in 1996 as a covariate and colony size in 1997 as the dependent variable.

RESULTS

Variation in colony-level sex ratio

The distribution of colony sex ratios was remarkably similar between years (Fig. 1). In both 1996 and 1997, sex ratio was bimodally distributed and extremely male-biased with 89% of the colonies producing only male reproductives (Fig. 1). In 1997, the numerical population sex ratio was 6.3% female, with 196 of 221 colonies (88.7%) producing only males and 25 (11.3%) producing gynes along with males. (Results for 1996 are described in detail elsewhere (Brown & Keller 2000)). The average proportion of gynes within female-producing colonies was 61.3% ± 0.1%. Overall, dry-mass investment in females was 8.7%. Applying Boomsma's correction (Boomsma et al. 1995) had only a small effect, the female-investment ratio decreasing to 7.7% at the population level. Within female-producing colonies dry-mass investment in gynes was 66.7% ± 6.6% and 65.2% ± 6.6% after Boomsma's correction.

Year-to-year changes in sex ratio

Despite the similarity in distribution of colony-level sex ratios, the colonies that produced females were not consistent between years (Fig. 2). Twenty-eight of 29 colonies (97%) that produced females in 1996 switched to male production in 1997. Female-producing colonies were more likely than male-producing colonies to switch their sex specialization the following year ($\chi^2 = 57.32$, d.f. = 1, $P < 0.001$). This result could be explained by a random 10%–11% of colonies producing females each year but this was not the case. Colonies that produced females in 1996 were disproportionately less likely to produce females again the following year (1 of 29, i.e. 3%) than were male-producing colonies (24 of 114, i.e. 21%; $\chi^2 = 4.97$, d.f. = 1, $P < 0.05$).
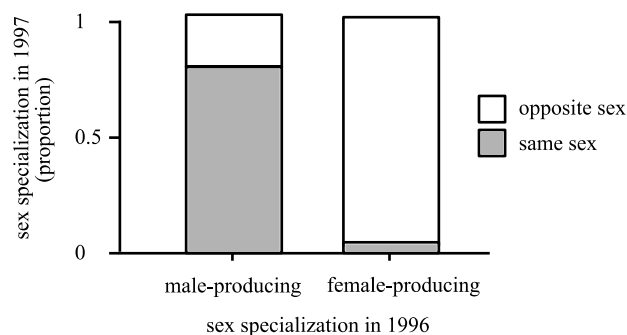

Figure 2 Proportion of colonies maintaining the same sex-specialization vs. switching specialization between 1996 and 1997 breeding seasons. Not only were female-producing colonies more likely to switch their sex specialization the following year, they were disproportionately more likely to produce only male reproductives.

Queen number and brood production

Of the 44 focal colonies, only one was judged to be monogynous in 1996 based on an effective queen number < 1. This colony failed to produce brood in 1997 and was excluded from subsequent analyses. All of the remaining focal colonies were polygynous in 1997 and thus polygyny appears to be almost obligate in this population.

Change in effective queen number between 1996 and 1997 was significantly associated with the category of colony sex allocation in 1996 (repeated measures ANOVA: $F_{1,29} = 4.87$, $P = 0.03$). Female-producing colonies had a significant increase in effective queen number from 1996 to 1997 (Fig. 3; one-tailed paired t-test, $t = 1.96$, d.f. = 11, $P = 0.04$), whereas male-producing colonies had a nonsignificant decrease in effective queen number (one-tailed paired t-test, $t = 1.48$, d.f. = 18, $P = 0.08$). The net result was that overall effective queen number did not significantly
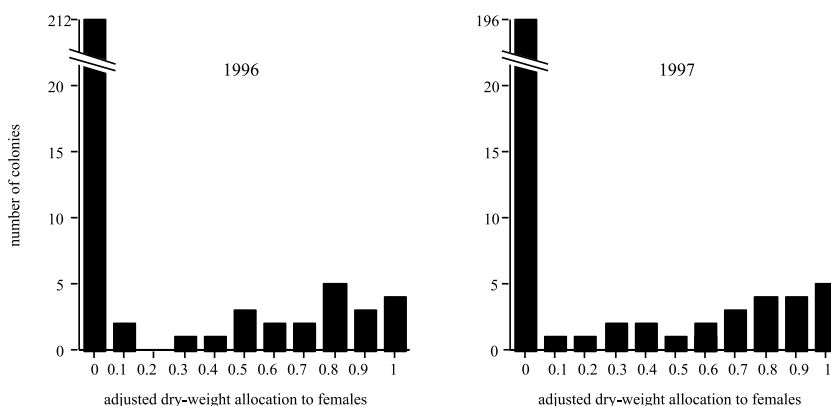

Figure 1 Sex ratio was similar in both years, with bimodal distributions of sex specialization and an equal proportion (89%) of colonies that produced only male reproductives.

change within the population between years ($F_{1,29} = 0.02$, $P = 0.88$). Across both years, effective queen number was higher, but nonsignificantly so in colonies that specialized in male production during both years compared with those that specialized on females in 1996 and males in 1997 ($F_{1,29} = 3.87$, $P = 0.06$).

Productivity per queen decreased in female-producing colonies (Fig. 4; paired t-test: $t = 3.47$, d.f. = 11, $P = 0.005$) but did not significantly change in male-producing colonies (paired t-test: $t = 0.48$, d.f. = 18, $P = 0.63$). Across both years, colonies that specialized in male production during both years did not differ significantly in productivity per queen from those that specialized on females in 1996 and males in 1997 ($F_{1,29} = 0.02$, $P = 0.88$).

Total colony productivity was significantly lower in 1997 than 1996 (Fig. 5; repeated measures ANOVA: $F_{1,32} = 15.24$, $P = 0.0005$) and lower in colonies that produced females in 1996 than in those that produced males during both breeding seasons ($F_{1,32} = 5.85$, $P = 0.02$). This latter result was probably due to lower production of worker brood by female-producing colonies in 1996, as reported in Brown *et al.* (in press). However, there was no statistical interaction between sex specialization and repeated measures of colony productivity ($F_{1,32} = 1.31$, $P = 0.26$). Thus, the change in colony productivity between years did not differ significantly between colonies that specialized on males or females in

1996, which runs contrary to our prediction that colonies will have a boost in reproduction following female production because they will no longer be egg-limited. This prediction, however, assumes that resource abundance and the size of the worker force remains relatively unaffected by female production. In contrast, we found that the slope of the regression of colony size in 1997 on colony size in 1996 was significantly greater for female-producing colonies (Fig. 6; ANCOVA: $F_{1,30} = 5.72$, $P = 0.02$) and y-intercept significantly lower for female-producing colonies ($F_{1,30} = 6.59$, $P = 0.016$) than those that specialized on males in 1996. This indicates that smaller female-producing colonies have a disproportionately extreme drop in colony size between years. In general, colony size covaried positively between years ($F_{1,30} = 53.17$, $P < 0.0001$).

DISCUSSION

The queen-replenishment hypothesis holds that highly polygynous ant colonies subject to intense local resource competition produce gynes when the number of resident queens is low. Below some threshold of queen number, gynes are produced and recruited within the colony to increase colony survival or ensure an optimal rate of brood production (Brown & Keller 2000). Three fundamental predictions of the hypothesis were upheld. First, female
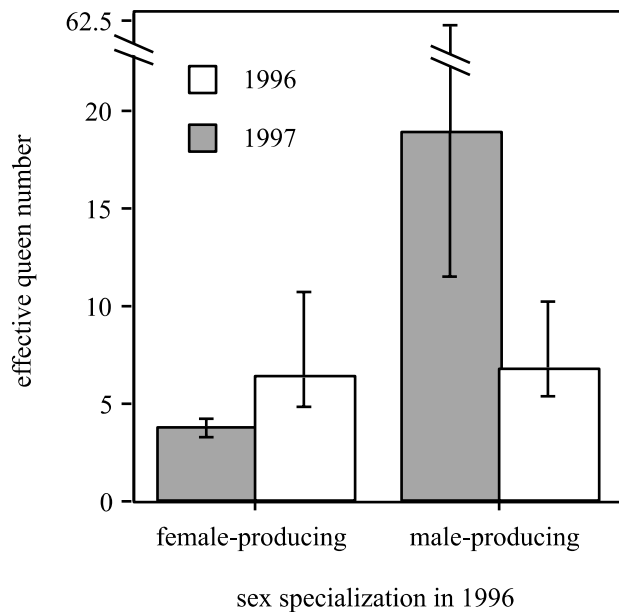
Figure 3 Changes in effective queen number between years for colonies that produced only males (male-producing) or both males and females (female-producing) in 1996. There was a significant interaction between sex specialization and repeated measures of effective queen number. Values are transformations of raw relatedness values, yielding asymmetric SE.
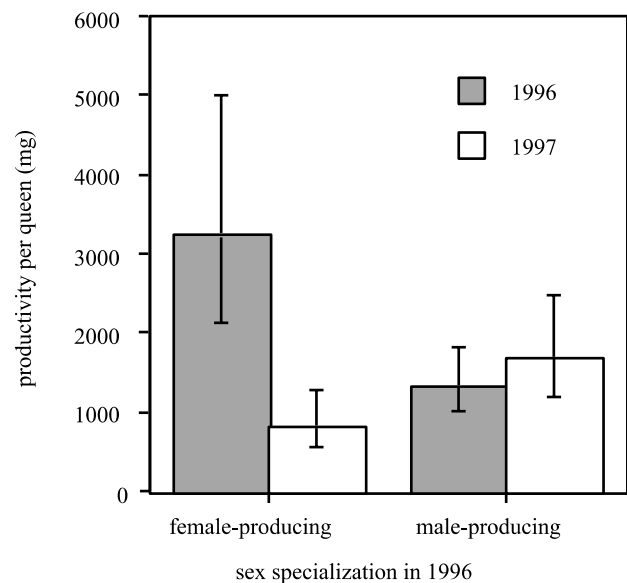
Figure 4 Change between years in productivity per queen differed between colonies that specialized in females in 1996 and those that specialized in males in 1996 (repeated-measures ANOVA: $F_{1,29} = 5.40$, $P = 0.027$). Overall productivity did not differ between years ($F_{1,29} = 2.70$, $P = 0.11$). Values are back-transformations from ln-transformed biomass, yielding asymmetric standard errors.
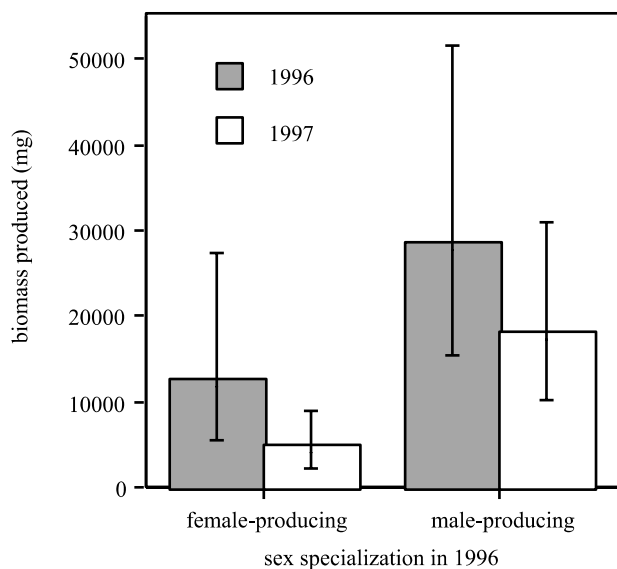
Figure 5 Colony productivity was significantly lower in 1997 and lower in colonies that produced females in 1996. Values are back-transformations from ln-transformed biomass, yielding asymmetric 95% confidence intervals.

Figure 6 Relationship between log-transformed colony size (worker number) in 1996 and 1997.

production was followed by an increase in queen number, consistent with the adoption of daughter queens. Second, productivity per queen decreased following the production and adoption of additional queens, as expected if queen adoption leads to a shift from egg limitation to a limitation of other resources. Finally, increased queen number was correlated with a switch to rearing only males and workers the following year.

The only prediction that was not supported by our data was that female production should lead to greater colony productivity the following year because adoption of daughter queens releases colonies from being egg limited. Both male- and female-producing colonies showed an overall decrease in total brood production between years. This decrease was probably due to environmental differences that affected the entire population. But, our study did not support the prediction that female-producing colonies increased colony productivity relative to male-producing colonies. A possible explanation is that gyne production does not foster increased productivity because of a trade-off between gyne and worker production. We have demonstrated that female-production comes at a significant cost to the production of new workers (Brown *et al*. in press). Insufficient replacement of workers may cause substantial resource limitation that counters the increase in egg availability the year following female production. If lower investment in workers does incur a cost in terms of colony size, relatively small female-producing colonies will have a greater drop in colony size because smaller female-produ-
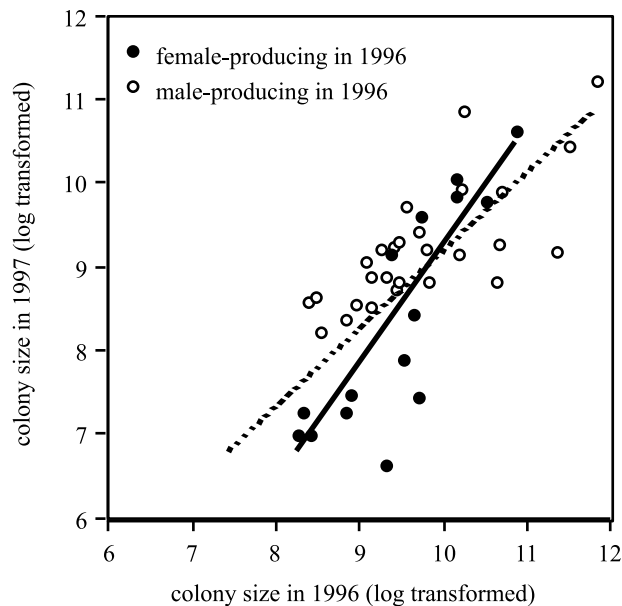
cing colonies tend to make the lowest proportional investment in workers (Brown *et al*. in press). This appeared to occur, with smaller female-producing colonies showing the greatest drop in size between years. It may thus take two or more breeding seasons after gyne production to achieve measurable productivity increase. Alternatively, the supply of eggs may not be factor limiting colony productivity and queen replenishment may function to reduce the probability of orphaning the colony. Further experiments are needed to determine more precisely the effect of gyne production on colony productivity and colony survival. This will require following colonies during several breeding seasons. Such studies will also allow us to determine whether the costs and benefits of producing gynes vary in function of colony size.

The pattern of gyne production in *F. exsecta* is similar to the phenomenon of cyclic oligogyny in epiponine wasps (Queller *et al*. 1993) but the causes are different. Nests of four species of epiponine wasps follow an extended period of decline in queen number, often ending with a single queen, with the production of new queens and a subsequent increase in queen number. Similarly, Elmes (1987) found that the average number of queens varied in a regular manner over 6 years in the ant *Myrmica sulcinodis*, presumably due to cycles of queen mortality and recruitment. A change in colony sex ratio between years has also been reported in some other polygynous ants (e.g. *Formica obscuripes*) (Herbers 1979), but it has not been tested whether such changes are associated with differences in colony queen number. In the epiponine wasps, gyne production is apparently due to the

inclusive fitness benefits for workers of rearing females when queen number drops to one and worker-gyne relatedness is elevated (Queller *et al*. 1993). Thus, the cycling of gyne production is not directed at maintaining queen number per se, but a consequence of cycling in relatedness asymmetry.

Cycling of relatedness asymmetry, however, cannot explain sex ratio specialization in *F. exsecta*. We previously found that the relatedness asymmetry of workers to male and female brood is not correlated with queen number or with whether or not colonies produce gynes (Brown & Keller 2000). The correlation between queen number and relatedness asymmetry may be low for a number of reasons. For example, if nestmate queens are only distantly related, variation in queen number will have a lower effect on relatedness asymmetry. In addition, gene flow by queens and migration of workers between nests will erode the relationship between queen number and relatedness asymmetry. We suggest that the reason why queen replenishment is a better explanation of sex allocation in this population, rather than variation in relatedness asymmetry, is that the *F. exsecta* population has a high frequency of polygynous colonies, relatively many queens per polygynous colony, and low queen–queen relatedness. This contrasts with other polygynous species (*M. sulcinodis*, *Leptothorax acervorum* (Chan *et al*. 1999)) in which polygyny is less frequent (usually facultative), queen number is lower and relatedness is higher, and in which a link of split sex allocation with relatedness asymmetry has been demonstrated. The reason for the difference is that, in this second class of species, relatedness asymmetry is likely to vary more strongly, workers are more likely to find themselves in a colony with high relatedness asymmetry, and colony budding is likely to be one, but not the main mode of reproduction (so local resource competition is less intense).

Overall, the results of the present study provide additional support for the queen-replenishment hypothesis, with the important exception that relative productivity of colonies did not increase in the year immediately following gyne production. There are now two avenues of support for the queen-replenishment hypothesis – (1) support for predicted relationships between sex allocation and productivity per queen between colonies during a single breeding season (Brown *et al*. in press) and (2) predicted shifts in these variables within the same colonies on subsequent years.

ACKNOWLEDGEMENTS

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