

Reproductive specialization in multiple-queen colonies of the ant *Formica exsecta*

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In polygynous (multiple queens per nest) colonies of social insects, queens can increase their reproductive share by laying more eggs or by increasing the proportion of eggs that develop into reproductive individuals instead of workers. We used polymorphic microsatellite loci to determine the genetically effective contribution of queens to the production of gynes (new queens), males, and 2 different cohorts of workers in a polygynous population of the ant *Formica exsecta*. For this purpose, we developed a new method that can be used for diploid and haplodiploid organisms to quantify the degree of reproductive specialization among breeders in societies where there are too many breeders to ascertain parentage. Using this method, we found a high degree of reproductive specialization among nest-mate queens in both female- and male-producing colonies (sex ratio is bimodally distributed in the study population). For example, a high effective proportion of queens (25% and 79%, respectively) were specialized in the production of males in female- and male-producing colonies. Our analyses further revealed that in female-producing colonies, significantly fewer queens contributed to gyne production than to worker production. Finally, we found significant changes in the identity of queens contributing to different cohorts of workers. Altogether, these data demonstrate that colonies of *F. exsecta*, and probably those of many other highly polygynous social insect species, are composed of reproductive individuals differing in their investment to gynes, males, and workers. These findings demonstrate a new aspect of the highly dynamic social organization of complex animal societies. **Key words:** ants, breeding system, polygyny, reproductive skew, reproductive specialization, social insects. [*Behav Ecol* 18:375–383 (2007)]

One of the major transitions in evolution is the shift from solitary organisms to societies with reproductive division of labor (Maynard Smith and Szathmary 1995; Szathmary and Maynard Smith 1995). A key feature differentiating animal societies is the apportionment of reproduction among individuals (Keller and Reeve 1994). Societies where all individuals reproduce equally versus societies where a single individual completely monopolizes reproduction represent end points of a continuum in the variance in the reproductive output among group members. In high-skew societies, reproduction is limited to one or a small subset of breeders whereas in low-skew societies, reproduction is more evenly distributed among group members (Keller and Reeve 1994).

In social Hymenoptera, reproductive skew is generally very high in colonies containing a single queen because queens typically monopolize most of the reproduction (Bourke 1988; Jeon and Choe 2003; Hammond and Keller 2004). However, in many species, colonies may contain 2 or more fertile queens (Keller 1993b, 1995; Bourke and Franks 1995). In such polygynous colonies, there is considerable variation between queens in their relative contribution to reproduction. Most studies conducted to date investigated the extent to which nest-mate queens differ in their overall reproductive output (reviewed in Reeve and Keller 2001). However, even if queens contribute similar numbers of eggs, there can be considerable variation in reproductive success (Keller 1993a) because the relative reproductive success of nest-mate queens does not depend only on their fecundity but also on the type of egg produced (haploid male or diploid female) and their

developmental fate (development of diploid eggs into queens or workers).

Only few studies have compared the relative contribution of queens to the production of gynes (new queens), males, and/or workers under natural conditions. In the ants *Solenopsis invicta* and *Formica sanguinea*, the effective number of queens contributing to gyne production is lower than the effective number of queens contributing to worker production (Ross 1993; Pamilo and Seppä 1994). In the ant *Pheidole pallidula*, a significant trade-off has been found between a queen's contribution to gyne and worker offspring but not between a queen's investment in gyne versus male production (Fournier et al. 2004). Finally in *Leptothorax acervorum*, there is evidence for a trade-off occurring between a queen's contribution to male and worker production (Hammond et al. 2006) as well as to male and gyne production (Bourke et al. 1997) but not between a queen's investment in gyne versus worker production.

A few more studies have investigated the differential contribution to castes in artificially composed colonies in the laboratory. In the fire ant *S. invicta*, reproductive skew is higher for gyne than for worker production (Ross 1988). A significant trade-off between a queen's contribution to male and gyne production has been demonstrated in *Linepithema humile* (Fournier and Keller 2001). In *Pachycondyla inversa*, reproductive skew was low but the data did not allow comparing the degree of reproductive skew for the production of all castes (Heinze et al. 2001). Finally, in *Leptothorax rugulatus*, queens were significantly specialized in the production of a particular class of individuals (gynes, males, or workers) or a combination of classes in 8 out of 23 colonies (Rüppell et al. 2002).

Altogether, these data show that queens may frequently contribute differently to the production of gynes, workers, and males. However, the large majority of these studies have been conducted on species that host only few queens per colony [2–7, but see Ross (1993) for an exception] and

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analyses were often limited to 2 classes of individuals. Obtaining data on reproductive specialization is particularly challenging for colonies containing many queens because it is very difficult to ascertain maternity when it is not possible to collect all queens. Examinations of such highly polygynous social systems, however, are of special interest because the range of possible variation in reproductive partitioning increases with the number of breeders, and detailed analyses of specialization among queens could provide important insights into the organization of these insect societies.

The aim of this study was to develop a general method that quantifies the degree of reproductive specialization among breeders in societies where many individuals contribute to offspring production. Importantly, this method can be used for haplodiploid (males are haploid and females diploid) as well as diploid organisms. It is based on relatedness estimates within and between classes of offspring (sexes, castes, cohorts, etc.) and estimates the genetically effective proportion of breeders contributing to a single class or a combination of classes. We applied this method to polygynous colonies of the ant *Formica exsecta* in a population of the Swiss Jura Mountains. In this population, colonies contain many queens (Cherix et al. 1980; Brown and Keller 2000, 2002; Liautard and Keller 2001), and colony sex ratio is bimodally distributed (Brown and Keller 2000) with most colonies producing exclusively males and workers (referred to as male-producing colonies) and some colonies producing gynes, males, and workers (referred to as female-producing colonies). In *F. exsecta*, no brood overwinters and thus brood eclosing from July to October derives from eggs laid earlier that same year (Brown and Keller 2002). In July, sexuals (gynes and males) eclose along with the first cohort of workers (referred to as "summer workers"). Generally, the sexuals engage in mating activities that are followed by the recruitment of newly mated queens back to the parental colony (Brown and Keller 2002). After the release of sexuals in July, sexual production ceases, whereas worker production continues until late September/early October (Kümmerli R, Keller L, unpublished data) with these workers being referred to as "fall workers." We collected brood (gynes, males, summer, and fall workers) from male- and female-producing colonies and estimated the relatedness within and between classes of individuals using polymorphic microsatellite loci. We first used these relatedness estimates to compare the genetically effective number of queens contributing to each class of offspring and then applied our new method to estimate the degree of reproductive specialization among queens.

MATERIALS AND METHODS

Sampling

The study was carried out at "les Chenevières," a cattle pasture at 1220 m in the Swiss Jura Mountains. In this population, colony density is very high (Liautard et al. 2003), and colony sex ratio is strongly male biased with about 90% of the nests producing only males (Brown and Keller 2000). In 2002, we marked 411 colonies individually. On average, we collected 50 pupae per colony in July 2002 prior to the mating flight (summer brood). We determined the sex and the caste of each individual (gyne, male, or worker) by moistening pupae in ethanol and observing the size, eye shape, and genitalia of late-stage pupa (Brown and Keller 2000). Out of the 411 colonies, 20 male- and 16 female-producing colonies were randomly selected, and for each of these focal colonies, pupae of each class were prepared for genetic analysis and stored at -20°C (gyne, worker, and male pupae for female-producing colonies and worker and male pupae for male-producing col-

onies). At the end of the breeding season (late September/early October), when only workers are produced, we collected pupae from all focal nests still producing offspring (15 female- and 17 male-producing nests). Overall, we genotyped 9.8 ± 3.8 gyne pupae [mean \pm standard deviation (SD)], 16.4 ± 4.6 male pupae, 15.0 ± 5.2 summer worker pupae, and 15.6 ± 1.6 fall worker pupae per colony.

Laboratory methods

Genotypes of gyne, worker, and male pupae were determined at 8 microsatellite loci FL21 (Chapuisat 1996) and FE17, FE19, FE21, FE37, FE42, FE49, and FE51 (Gyllenstrand et al. 2002). DNA was extracted from the entire pupae in 500 μl of 5% Chelex and incubated for 10 min at 90°C , vortexed, and incubated for another 10 min at 90°C . Three multiplex polymerase chain reaction (PCR) were used: 1) multiplex PCR1 coamplified FE19, FE21, and FE51; 2) multiplex PCR2 coamplified FE17 and FE37; and 3) multiplex PCR3 coamplified FE42 and FE49. The locus FL21 was amplified in a simplex PCR. Amplification reactions were carried out in 10 μl reaction volumes. Full details of the amplification conditions for multiplex and simplex PCR are given in Kümmerli et al. (2005). PCR products were mixed and run on an automatic sequencer (ABI Prism 377XL). The number of alleles per locus ranged from 5 to 26 (11.6 ± 7.8 , mean \pm SD) with expected heterozygosities ranging between 0.44 and 0.84. Genotypes at the locus FE49 deviated significantly from Hardy-Weinberg equilibrium with the observed heterozygosity being much lower than the expected heterozygosity. This suggests that one or more null alleles were present at this locus. For this reason, we excluded FE49 from further analyses.

Comparing genetically effective number of breeders

A first test of reproductive specialization can be obtained by comparing whether the genetically effective number of breeders contributing to different offspring classes differs from one another. The genetically effective number of breeders, which corresponds to the number of equally reproducing breeders required to account for an observed relatedness pattern among offspring (Queller 1993; Ross 1993), can be calculated for diploid classes ($N_{e,dc}$) of offspring as

$$N_{e,dc} = \left(4r_{ds} - r_{fb} - \frac{2}{p_{mb}}r_{mb}\right) / \left(4r_{dc} - r_{fb} - \frac{2}{p_{mb}}r_{mb}\right), \quad (1)$$

where r_{ds} is the relatedness among diploid siblings, which equals $1/(2p_{mb}m_e) + 1/4$, with p_{mb} corresponding to the ploidy level of male breeders and m_e being the mean effective mating frequency of breeders in the group. r_{dc} is the relatedness among diploid offspring, r_{fb} and r_{mb} are the relatedness among female and male breeders in the group.

Similarly, the genetically effective number of breeders contributing to haploid offspring classes ($N_{e,hc}$) is given by

$$N_{e,hc} = (2\eta_{hs} - r_{fb}) / (2\eta_{hc} - r_{fb}), \quad (2)$$

where η_{hs} is the relatedness among haploid siblings, which equals $1/2$ and η_{hc} is the relatedness among haploid offspring. Equations 1 and 2 are derived and generalized versions of equations previously presented by Ross (1993, 2001) and Queller (1993).

We applied these equations to polygynous *F. exsecta* colonies by estimating r_{dc} for gyne, summer, and fall worker offspring and η_{hc} for male offspring with the program RELATEDNESS 5.0.8 (Queller and Goodnight 1989). We obtained standard errors (SEs) by jackknifing over nests. We further used

relatedness values for r_{fb} , r_{mb} , and m_e that have been previously estimated in our study population to be 0.084 ± 0.006 (mean \pm SE), 0.020 ± 0.003 (mean \pm SE) and 1.2, respectively (Haag-Liautard C, Pedersen JS, Ovaskainen O, Keller L, unpublished data).

Measuring reproductive specialization among queens

To quantify the reproductive specialization among breeders in their contribution to different classes of offspring, we used the equation developed by Pedersen and Boomsma (1999), which is analogous to the classic Jaccard index of similarity (Chao et al. 2005). This equation was initially used to estimate the genetically effective turnover of breeders in colonies of social insects across years, hence estimating the genetically effective proportion of breeders contributing to only one of 2 age cohorts. Thus, if this formula is applied to different classes of offspring that are produced simultaneously, it estimates the genetically effective proportion of breeders (τ_c) contributing to only one of 2 offspring classes. Hence, it provides a measure of reproductive specialization among breeders given by

$$\tau_c = 1 - \frac{N_{e,c_1 + c_2}}{N_{e,c_1} + N_{e,c_2} - N_{e,c_1 + c_2}}, \quad (3)$$

where N_{e,c_1} and N_{e,c_2} are the genetically effective number of breeders contributing to one of 2 offspring classes compared, and $N_{e,c_1 + c_2}$ is the genetically effective number of queens contributing to both offspring classes.

For comparisons of 2 classes of offspring having the same ploidy level, the genetically effective number of breeders can be substituted by the corresponding relatedness values and becomes

$$\tau_c = 1 - \frac{r_{c_1 \leftrightarrow c_2}}{r_{c_1} + r_{c_2} - r_{c_1 \leftrightarrow c_2}}, \quad (4)$$

where r_{c_1} is the relatedness among individuals of class 1, r_{c_2} is the relatedness among individuals of class 2, and $r_{c_1 \leftrightarrow c_2}$ is the symmetrical relatedness between individuals of class 1 and class 2. Equations 3 and 4 are generalized versions of equations previously presented by Pedersen and Boomsma (1999).

We calculated the relatedness among gynes, summer, and fall workers as well as the relatedness between gynes and summer workers, gynes and fall workers, and summer and fall workers. We then used these relatedness estimates to apply Equation 4 in 3 pairwise comparisons to calculate the genetically effective proportion of queens (τ_c) contributing to only one of 2 offspring classes under comparison. Finally, we calculated the SE for τ_c by jackknifing over colonies.

Because the calculation of τ_c involves 3 different relatedness values, each of them with some error, we tested the reliability of our estimates by conducting simulations. First, we obtained SEs for all relatedness values by jackknifing over loci. Second, we simulated the sensitivity of τ_c with respect to the SE in the relatedness estimates. To do so, we considered deviations within the range of the relatedness SE that would lead to lower τ_c values. We started from the observed τ_c assuming no error and then added errors for all 3 estimates in steps of 5% of their total SE until the lowest possible estimate of τ_c was reached (which was the case for $r_{c_1} - SE$, $r_{c_2} - SE$, and $r_{c_1 \leftrightarrow c_2} + SE$). For each of the 20 steps, we calculated τ_c and tested whether it was significantly greater than zero by applying 1-tailed *t*-tests and using the SE obtained by jackknifing over colonies (see above).

The estimates of τ_c can further be used to determine the genetically effective number of breeders being specialized in the production of offspring class 1 ($N_{e,c_1,only}$), offspring class

2 ($N_{e,c_2,only}$), or both offspring classes ($N_{e,c_1 + c_2}$) using the following formulas

$$N_{e,c_1 + c_2} = \frac{(N_{e,c_1} + N_{e,c_2})(1 - \tau_c)}{2 - \tau_c}, \quad (5)$$

and

$$N_{e,c_1,only} = N_{e,c_1} - N_{e,c_1 + c_2}, \quad (6)$$

and

$$N_{e,c_2,only} = N_{e,c_2} - N_{e,c_1 + c_2}, \quad (7)$$

where N_{e,c_1} and N_{e,c_2} were calculated from Equation 1. Equations 5–7 are directly derived from Equation 3.

We conducted 3 pairwise comparisons to estimate the genetically effective number of queens being specialized in gyne versus summer worker production, gyne versus fall worker production, and summer versus fall worker production.

When combining all 3 pairwise comparisons, there were 7 categories of queens, and we estimated their relative proportions under the assumption that they all had the same fecundity. The first 3 categories were those containing queens contributing to a single class of offspring (gynes, summer workers, or fall workers). The next 3 categories included queens contributing equally to the production of 2 offspring classes (i.e., gynes and summer workers, gynes and fall workers, or summer and fall workers). Finally, the last category of queens was composed of those contributing equally to all 3 offspring classes. The relative proportions of these 7 categories of queens provide a measure of reproductive specialization. A complete lack of specialization would be indicated by the presence of a single type of queen contributing equally to the production of all 3 offspring classes. At the other end of the continuum, a complete specialization would occur if there were 3 types of queen, each contributing to the production of a single offspring class. Intermediate levels of specialization would be reflected by different proportions of queens contributing to one or more classes.

For comparisons of 2 classes of offspring having different ploidy levels, Equation 4 can unfortunately not be used to estimate the genetically effective proportion of female breeders (only females can produce haploid offspring) being specialized in the production of diploid versus haploid offspring classes. This is because the relatedness between diploid and haploid offspring classes is asymmetric ($r_{dc \rightarrow hc} \neq r_{hc \rightarrow dc}$). We therefore developed an interpolative approach where we calculated the expected relatedness of diploid toward haploid offspring classes ($r_{dc \rightarrow hc}$) for given τ_c values. Next we estimated the τ_c value that led to the best match between the expected and observed relatedness values. The expected $r_{dc \rightarrow hc}$, assuming that all female breeders (N) in a group contribute equally to diploid and haploid offspring, can be broken down into the sum of 2 terms specifying how these individuals are related through their mothers (first term) and through the average relatedness among female breeders (second term):

$$r_{dc \rightarrow hc} = \frac{r_{ds \rightarrow hs}}{N} + \frac{r_{fb}}{4} \left(\frac{N-1}{N} \right), \quad (8)$$

where $r_{ds \rightarrow hs}$ is the relatedness of diploid siblings toward their haploid siblings and equals 1/4, while r_{fb} is the relatedness among female breeders. Equation 8 is analogous to equations previously presented by Ross (1993, 2001) and Queller (1993) for relatedness among female and among male brood. We further processed this formula by assuming that there is reproductive specialization among breeders such that only

Table 1

The different types of genetically effective number of breeders used to estimate reproductive specialization among cobreeders in their contribution to 2 different offspring classes

Type of genetically effective number of breeders	Breeders contribute to	Reference to equation in the text
Comparisons between offspring classes having the same ploidy level		
N_{e,e_1}	Offspring class 1	1 or 2
N_{e,e_2}	Offspring class 2	1 or 2
N_{e,e_1+e_2}	Offspring classes 1 and 2	5
$N_{e,e_1,only}$	Offspring class 1 but not class 2	6
$N_{e,e_2,only}$	Offspring class 2 but not class 1	7
$N_{e,tot}$	Offspring class 1 and/or class 2	Table 4
Comparisons between offspring classes having different ploidy levels		
$N_{e,dc}$	Diploid offspring	1
$N_{e,hc}$	Haploid offspring	2
$N_{e,dc+hc}$	Diploid and haploid offspring	10

a given effective proportion of female breeders ($1 - \tau_c$) contribute equally to diploid and haploid offspring. Under this scenario, the contribution of the first term to $r_{dc \rightarrow hc}$ decreases, whereas the contribution of the second term to $r_{dc \rightarrow hc}$ remains unaffected such that the expected $r_{dc \rightarrow hc}$ becomes

$$r_{dc \rightarrow hc} = \frac{1}{4N}(1 - \tau_c) + \frac{r_{fb}}{4} \left(\frac{N-1}{N} \right), \quad (9)$$

with N given by

$$N = N_{e,dc} + N_{e,hc} - N_{e,dc+hc}, \quad (10)$$

where $N_{e,dc}$ and $N_{e,hc}$ are the genetically effective number of breeders contributing to diploid and haploid brood obtained from Equations 1 and 2, respectively. $N_{e,dc+hc}$ is the genetically effective number of breeders producing both classes of offspring in equal ratios and is dependent on τ_c . A summary of the different types of genetically effective breeder numbers used in this study is given in Table 1.

We used this method to conduct pairwise comparisons of reproductive specialization among queens for the production of males and gynes as well as for the production of males and summer workers. This method permits one to obtain an overall quantification of the differential investments of queens into the 2 offspring classes.

Statistical analyses

In colonies hosting many queens, the relatedness among brood is low and the estimated relatedness coefficients can sometimes be negative due to stochastic sampling errors arising from the limited number of loci and pupae genotyped. Such negative values lead to large negative estimates of effective queen number. To cope with this problem, we applied the reciprocal transformation $1/X$ to all estimates of effective queen number ($N_e = X$) prior to statistical analyses (Sokal and Rohlf 1995). This transformation yields a linear relationship between relatedness values and the reciprocals of the effective queen numbers, with the transformed values ranging from around zero (low or slightly negative relatedness values) to one (high relatedness values). Transformed values did not deviate from a normal distribution for summer brood

Table 2

Population level estimates of relatedness (r) among members of the same offspring class produced in female- ($n = 16$) and male-producing ($n = 20$) colonies in the ant *Formica exsecta*

	$r \pm SE$
Female-producing colonies	
Gynes	$0.25 \pm 0.06^{***}$
Males	$0.07 \pm 0.02^{***}$
Summer workers	$0.09 \pm 0.02^{***}$
Fall workers	$0.13 \pm 0.05^{**}$
Male-producing colonies	
Males	$0.06 \pm 0.01^{***}$
Summer workers	$0.12 \pm 0.04^{**}$
Fall workers	$0.09 \pm 0.04^*$

All relatedness values were significantly greater than zero (1-tailed t -tests: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$).

(Shapiro–Wilk normality tests: all $P > 0.2$) but did so for fall workers (Shapiro–Wilk normality tests: all $P < 0.05$). Hence, to test whether the effective queen number contributing to offspring classes produced in summer differed within colonies, we applied a parametric repeated measures analysis of variance (ANOVA) for female-producing colonies (followed by Tukey post hoc comparisons) and a paired t -test for male-producing colonies. In contrast, we conducted pairwise non-parametric Wilcoxon matched-pairs signed-rank tests for the comparison between summer offspring classes and fall workers. Because we conducted multiple comparisons on the same data set, we applied the Bonferroni correction with the nominal $\alpha = 5\%$ being 0.0125 and 0.017 for female- and male-producing colonies, respectively.

To test whether there was a significant reproductive specialization among queens within a colony in their contribution to 2 classes of diploid offspring, we tested whether $r_{e_1 \leftrightarrow e_2}$ (relatedness between classes) was significantly lower than $r_{e_1 + e_2}$ (relatedness within classes) using paired 1-tailed t -tests. The use of 1-tailed t -tests is justified because relatedness between classes can only be equal or smaller but never be greater than relatedness within classes. All relatedness values were normalized by applying logarithmic transformations prior to analyses. Due to multiple comparisons in female-producing colonies, the Bonferroni corrected probability for nominal $\alpha = 5\%$ was 0.017.

RESULTS

Comparison of genetically effective queen number

Relatedness estimates among members of the same offspring class were generally low (range 0.06–0.25, Table 2) but all significantly greater than zero (1-tailed t -tests: all $P < 0.05$). We used these relatedness estimates to calculate the genetically effective queen number contributing to the different offspring classes. In female-producing colonies, there were significant differences in the genetically effective number of queens contributing to gynes, males, and summer workers (Figure 1a, repeated measures ANOVA: $F_{2,15} = 5.20$, $P = 0.012$). A posteriori multiple comparisons revealed that significantly fewer queens effectively contributed to gyne brood than to male (Tukey test: $P < 0.025$) and worker brood (Tukey test: $P < 0.05$), while the genetically effective queen number producing males and workers did not differ significantly from one another (Tukey test: $P > 0.05$). Furthermore, the genetically effective number of queens contributing to fall workers was not significantly different from the genetically effective number of queens contributing to each of the 3 offspring

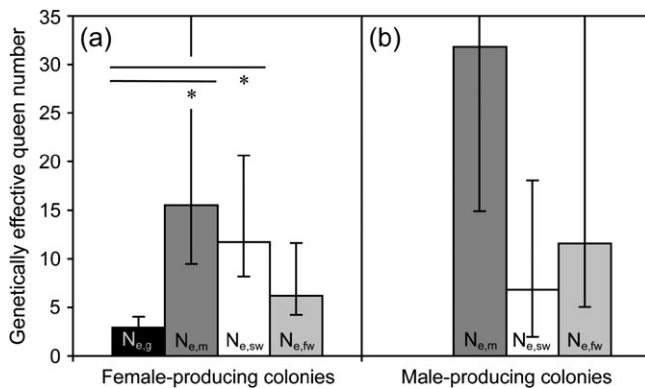


Figure 1

Genetically effective number of queens contributing to gyne ($N_{e,g}$), male ($N_{e,m}$), summer worker ($N_{e,sw}$), and fall worker ($N_{e,fw}$) brood in female-producing (a) and male-producing colonies (b) of the ant *Formica exsecta*. In female-producing colonies, significantly fewer queens contributed to gyne than to male and summer worker offspring ($*P < 0.05$). Values are transformations of raw relatedness estimates, yielding asymmetric SEs.

classes produced in summer [Wilcoxon matched-pairs signed-rank test ($n = 15$): gynes $P = 0.16$, males $P = 0.33$, workers $P = 0.91$, Figure 1a].

In male-producing colonies, the genetically effective number of queens contributing to male production did not differ from the genetically effective number of queens contributing to summer workers (paired t -test: $t_{19} = 1.90$, $P = 0.072$). These 2 values were also not significantly different from the genetically effective number of queens contributing to fall workers [Wilcoxon matched-pairs signed-rank test ($n = 17$): males $P = 0.52$, workers $P = 0.19$, Figure 1b].

Queen specialization pattern

In female-producing colonies, gyne and worker pupae were significantly more related to individuals of their own class than to individuals of the other class (1-tailed t -test: gyne–summer worker, $t_{15} = 2.73$, $P = 0.008$; gyne–fall worker, $t_{14} = 3.21$, $P = 0.003$, Table 3). In both, female- and male-producing colonies, summer and fall workers were more related to workers of their own class than those of the other class. This difference was however only significant in male-producing colonies (1-tailed t -test: $t_{16} = 3.31$, $P = 0.002$) but not in female-producing colonies (1-tailed t -test: $t_{14} = 1.38$, $P = 0.09$). In line with the finding that relatedness among female pupae tended to be higher within than between offspring classes, pairwise comparisons revealed that there was a high genetically effective proportion of queens (τ_c) contributing to a single female offspring class (Table 3). Our simulations show that an error accumulation of the 3 relatedness values lead to a decrease of τ_c estimates for all comparisons (Figure 2). Importantly, the τ_c estimates for the gynes–summer workers and the gynes–fall workers comparison in female-producing colonies remained significantly positive for all simulated steps (1-tailed t -tests: all $P < 0.05$). For the summer–fall workers comparison, t -tests for observed and simulated τ_c values also revealed consistent results by being all not significantly different from zero (one-tailed t -tests: all $P > 0.05$). In male-producing colonies, the simulated τ_c values for the summer–fall workers comparison remained significantly positive for 19 out of the 20 simulated steps and only became insignificant when accumulating the maximal error for all 3 relatedness estimates (Figure 2). Altogether, these simulations demonstrate that our method to calculate the genetically effective proportion

of queens produces reliable results and therefore provides a robust test to detect significant reproductive specialization among nest-mate queens.

For all pairwise comparisons, we calculated the genetically effective queen number that contributed to either one or both female offspring classes under comparison (Table 4). In female-producing colonies, the gyne–summer worker comparison revealed that a high percentage (75%) of the queens were effectively specialized in worker production, whereas only 25% of the queens effectively contributed to both gyne and worker production. The negative value of the estimated effective number of queens contributing to only gynes is probably a sampling error, and the data are compatible with no or only a few queens being effectively specialized in gyne production. The gyne–fall worker comparison in female-producing colonies was consistent with most queens effectively contributing to only fall workers (61%) and a lower proportion of queens effectively producing either gynes only (17%) or gynes and fall workers (22%). The comparison of the 2 worker cohorts indicated that most queens effectively contributed either to the summer workers (49%) or to both worker classes (47%) and that there were only few queens contributing exclusively to fall workers (4%). Finally, in male-producing colonies, the comparison between the 2 worker classes revealed that most queens effectively contributed to either fall workers only (49%) or both worker classes (37%). Only few queens effectively contributed to the production of only summer workers (14%).

The combination of the results of all pairwise comparisons in female-producing colonies allowed us to obtain an overall estimate of the relative contribution of queens to the summer and fall brood. Such a combination can be obtained by logical deduction, using the genetically effective queen numbers presented in Table 4, starting with the lowest estimate of N_{e,q_1+q_2} across all pairwise comparisons ($N_{e,q_1+q_2} = 1.7$ for the gynes–fall workers comparison, Table 4). This estimate shows that 1.7 queens effectively contributed to gynes and fall workers, although it is yet unknown whether these queens also produced summer workers. The gynes–summer workers comparison, however, reveals that no queens effectively contributed to gynes without also producing summer workers. Hence, the effective queen number that contributed to all 3 female offspring classes is equal to 1.7, whereas the effective queen number that contributed to gynes and summer workers but not to fall workers becomes 1.2 ($=2.9 - 1.7$). Accordingly, the genetically effective queen number contributing to summer and fall workers but not to gynes becomes 4.0 ($=5.7 - 1.7$), whereas the genetically effective queen number contributing to gynes and fall workers but not to summer workers becomes 0 ($=1.7 - 1.7$). Finally, the genetically effective queen number producing only a single offspring class are equal to 0 ($=2.9 - 1.7 - 1.2$) for gyne production, 4.8 ($=11.7 - 1.7 - 1.2 - 4.0$) for summer worker production, and 0.5 ($=6.2 - 1.7 - 4.0$) for fall worker production. Because there were no queens that effectively contributed only to gynes, our analysis provides one single solution of how the 3 pairwise comparisons can be combined. According to this combination, the best fit to our data was obtained when assuming that 39% of the queens contributed to summer workers only, 4% to fall workers only, 33% to both worker cohorts but not to gynes, 10% to gynes and summer workers but not to fall workers, and 14% to gynes and the 2 worker cohorts (Figure 3).

Our interpolative approach allowed us to estimate the extent to which queens were specialized in male versus female production. In female- and male-producing colonies, we found that the mean genetically effective number of queens contributing to male production ($N_{e,m}$) was higher than the genetically effective number of queens contributing to female

Table 3
Pairwise comparisons of queen specialization for diploid offspring classes produced in colonies of the ant *Formica exsecta*

Class 1 (c_1)	Class 2 (c_2)	$r_{c_1} + r_{c_2} - r_{c_1 \leftrightarrow c_2}$ (relatedness within classes)	$r_{c_1 \leftrightarrow c_2}$ (relatedness between classes)	P	$\tau_c \pm SE$
Female-producing colonies					
Gynes	Summer workers	0.25 ± 0.04	0.09 ± 0.03	0.008	0.66 ± 0.13
Gynes	Fall workers	0.31 ± 0.05	0.07 ± 0.05	0.003	0.78 ± 0.15
Summer workers	Fall workers	0.15 ± 0.04	0.07 ± 0.04	0.090	0.53 ± 0.33
Male-producing colonies					
Summer workers	Fall workers	0.15 ± 0.03	0.06 ± 0.02	0.002	0.63 ± 0.12

The genetically effective proportion of specialized queens (τ_c) is calculated following Equation 4. r_{c_1} and r_{c_2} are the relatedness among brood of the first and the second offspring class and $r_{c_1 \leftrightarrow c_2}$ is the relatedness between the 2 classes under comparison.

production ($N_{e,f}$). Accordingly, this sets a minimal degree of specialization ($\tau_{c-\text{minimal}}$) being equal to $1 - N_{e,f}/N_{e,m}$ (Table 5). Our analyses revealed that the best fit between the expected and observed relatedness values was obtained when τ_c was closest to $\tau_{c-\text{minimal}}$. However, in all comparisons, the observed relatedness estimates tended to be slightly higher than the expected relatedness values even for $\tau_{c-\text{minimal}}$ (Table 5).

DISCUSSION

Our genetic analyses revealed that, in female-producing nests, the effective number of queens (11.7) contributing to worker production in summer was about 4 times higher than the effective number of queens (2.9) contributing to gyne production (Table 4). Our analysis on the genetically effective proportion of queens (τ_c) contributing to the production of only workers or gynes showed that the observed pattern of genetic relatedness values was best explained by queens contributing to gyne production being a subset of those contributing to worker production (Table 4). Importantly, the estimate that about one-quarter of the queens contributed to the production of both gynes and workers is based on the assumption that all queens have the same fecundity and that, when contributing to several offspring classes, queens contribute equally to the production of these classes. If these assumptions were violated for this and all other analyses in our study, this would affect the estimated number of specialized queens (Table 4) but not alter the general conclusion of our study that a high proportion of queens are reproductively specialized. This is because all estimates of the proportion of effectively specialized queens (τ_c) are very high (Tables 3 and 5), a pattern that cannot simply arise from queens differing in their fecundity.

In female- and male-producing colonies, the effective number of queens (11.7 and 6.8, respectively) contributing to summer workers was not significantly different than the effective number of queens (6.2 and 11.6, respectively) contributing to fall workers (Figure 1). However, our analysis on the genetically effective proportion of specialized queens (τ_c) revealed that a high proportion of queens (0.53 and 0.63, respectively) contributed only to the production of summer or fall workers in female- and male-producing colonies (Table 3). This high degree of reproductive specialization can be explained either by some queens preferentially reproducing early or late in the reproductive season or by queen turnover (Pedersen and Boomsma 1999). In polygynous ants, queen life span is often low and queen turnover high (Keller and Genoud 1997). Hence, the pattern that different queens contributed to summer and fall workers can partly be explained

by some queens having died and/or some new queens being recruited. It is also possible that some queens changed nests during summer, further accounting for the lower relatedness between the workers produced in summer and fall compared with the relatedness among workers of the same cohort. Distinguishing between these different hypotheses would require marking queens and estimating queen mortality and internest movement between summer and fall.

In female-producing nests, the effective number of queens contributing to male production (15.5) was about 5 times higher than the effective number of queens (2.9) contributing to gyne production (Table 5). Similarly, in both female- and male-producing colonies, there was a tendency for the effective number of queens (15.5 and 31.8, respectively) contributing to males to be greater than the effective number of queens (11.7 and 6.8, respectively) contributing to summer

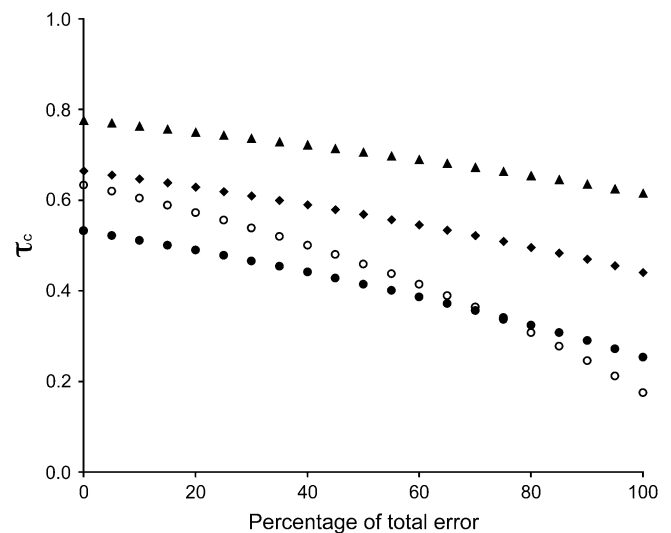


Figure 2
 Simulations on how SEs of the 3 relatedness estimates (r_{c_1} = relatedness among offspring class 1, r_{c_2} = relatedness among offspring class 2) affect estimates of τ_c (the genetically effective proportion of queens contributing to only offspring class 1 or class 2). Only simulations are presented that lead to lower than the observed τ_c estimates. The total error is obtained for $r_{c_1} - SE$, $r_{c_2} - SE$, and $r_{c_1 \leftrightarrow c_2} + SE$, and the percentage of total error therefore ranges between 0% (observed value) and 100% (total error). Filled and open symbols represent comparisons between female offspring classes in female- and male-producing colonies of the ant *Formica exsecta*, respectively. (diamond = gynes-summer workers comparison; triangle = gynes-fall workers comparison; circle = summer-fall workers comparison).

Table 4

Pairwise comparisons of the genetically effective number of queens being specialized in the production of the first ($N_{e,c_1,only}$), the second ($N_{e,c_2,only}$), or both offspring classes (N_{e,c_1+c_2}) under comparison in the ant *Formica exsecta*

Class 1 (c_1)	Class 2 (c_2)	N_{e,c_1}	N_{e,c_2}	$N_{e,c_1,only}$	$N_{e,c_2,only}$	N_{e,c_1+c_2}	$N_{e,tot}$
Female-producing colonies							
Gynes	Summer workers	2.9	11.7	-0.8 ^a (0.0)	8.0 (8.8)	3.7 (2.9)	11.7
Gynes	Fall workers	2.9	6.2	1.2	4.5	1.7	7.4
Summer workers	Fall workers	11.7	6.2	6.0	0.5	5.7	12.2
Male-producing colonies							
Summer workers	Fall workers	6.8	11.6	1.9	6.7	4.9	13.5

N_{e,c_1} and N_{e,c_2} are the genetically effective queen numbers contributing to offspring class 1 and class 2, respectively, whereas $N_{e,tot}$ is the total effective number of queens contributing to class 1 and/or class 2.

^a Negative estimate of the genetically effective queen number. This result most likely reflects a stochastic error in relatedness calculation and is best explained by the fact that no queens contributed exclusively to gyne brood (values given in brackets).

workers (Table 5). Our interpolative method estimating the genetically effective proportion of queens (τ_c) specialized in male or female (gynes of workers) production revealed that our data are best explained by queens contributing to gyne or worker production being a subset of those contributing to male production. The pattern of fewer queens contributing to gyne and worker than to male production cannot simply be explained by the presence of noninseminated queens. This is because a previous study in the same population revealed that the proportion of noninseminated queens is 11% (Haag-Liautard C, Pedersen JS, Ovaskainen O, Keller L, unpublished data), whereas our estimated effective proportion of queens being specialized in male production is much higher (25–81%, Table 5). This indicates that, as well as noninseminated queens that can only produce males, a considerable proportion of inseminated queens were specialized in male production. Moreover, the estimated effective number of queens contributing to male production is unlikely to be overestimated due to worker reproduction because both field data from a monogynous population of the same species (Sundström et al. 1996) and our own unpublished data from a laboratory brood rearing experiment of workers from the same Swiss Jura mountain population indicate that worker reproduction is absent in queenright colonies.

Because previously there were no methods that allowed the quantification of queen specialization in colonies with high queen number, there are no comparable studies conducted under natural conditions in other species of social insects. However, information from natural colonies on comparisons of genetically effective queen number is available from 4 other ant species. In *S. invicta* and *F. sanguinea*, the genetically effective number of queen contributing to gynes is also lower than the effective number of queens contributing to workers (Ross 1993; Pamilo and Seppä 1994). By contrast, there was no significant difference in the effective number of queens contributing to gyne and male production in *L. acervorum* (Bourke et al. 1997). The only study that compared the contribution of queens to gyne, male, and worker production in the field was conducted on the ant *P. pallidula* (Fournier et al. 2004), where significantly fewer queens contributed to male than to gyne and worker production. Furthermore, differential contribution of queens to gyne and male production has also been observed in the hover wasp *Liostenogaster flavolineata* where gyne production was generally monopolized by a single queen, while several queens contributed to male production (Sumner et al. 2002). Altogether, these data reveal that nest-mate queens commonly differ in their relative contribution to different offspring classes under natural conditions, indicating that reproductive specialization seems to be a general characteristic of polygynous insect societies.

Our data showed that the genetically effective queen number across offspring classes and colonies was higher for male- (31.8) than for female-producing colonies (15.5, Table 5). The difference in queen number between male- and female-producing colonies is best explained by the queen-replenishment hypothesis positing that colonies containing many queens should only produce males, whereas colonies with few queens benefit from also producing and recruiting new queens to enhance colony survival and productivity (Brown and Keller 2000; Kümmerli et al. 2005). In relation to this, our results revealed that the pattern of reproductive specialization among queens was more extreme in male- than in female-producing colonies. First, the genetically effective proportion of queens being specialized in male production was higher in male- (79%) than in female-producing (25%) colonies. Second, a significant effective proportion of queens were specialized in the production of summer or fall workers in male- but not in female-producing colonies. This positive association between queen number and the degree of reproductive specialization among queens supports the view that reproductive partitioning gets more extreme with increasing number of breeders, a relationship that has already been suggested for the fire ant *S. invicta* (Ross 1993).

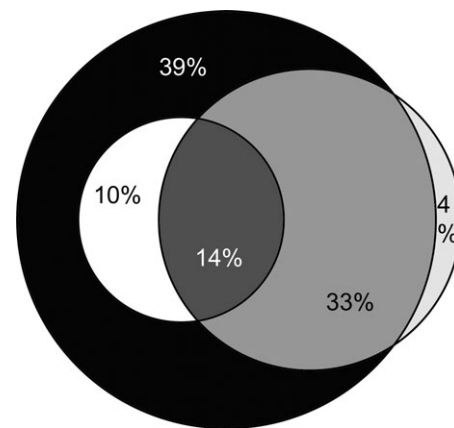


Figure 3

Percentage of queens that contributed to 1, 2, or all female offspring classes in female-producing colonies in the ant *Formica exsecta*. The areas of the circles correspond to mean genetically effective queen numbers that contributed to worker brood in summer (large circle), worker brood in fall (medium circle), and gyne brood (small circle) with the total effective queen number contributing to female offspring classes being 12.2. The overlap area between 2 circles corresponds to the genetically effective number of queens that contributed to both offspring classes under comparison.

Table 5

Interpolative estimation of the genetically effective proportion of queens (τ_c) being specialized in female (diploid) versus male (haploid) production in the ant *Formica exsecta*

Female class	Male class	$N_{e,f}$	$N_{e,m}$	$\tau_c - \text{minimal} = \frac{1 - N_{e,f}/N_{e,m}}$	$r_{f \rightarrow m}$ expected ($\tau_c - \text{minimal}$)	$r_{f \rightarrow m} \pm \text{SE}$ observed
Female-producing colonies						
Gynes	Males	2.9	15.5	0.81	0.023	0.033 ± 0.013
Summer workers	Males	11.7	15.5	0.25	0.032	0.039 ± 0.017
Male-producing colonies						
Summer workers	Males	6.8	31.8	0.79	0.022	0.052 ± 0.008

$N_{e,f}$ and $N_{e,m}$ are the effective queen numbers contributing to female and male brood and $r_{f \rightarrow m}$ is the asymmetric relatedness of female toward male brood.

By combining data from this study and information on the biology of *F. exsecta*, we propose the following scenario to account for the pattern of queen specialization described in this study. Field observations revealed that in the studied population, the first adult workers, males, and gynes produced within a breeding season emerge simultaneously in July (Brown and Keller 2000; Liautard et al. 2003). Because workers are 1.5–3.4 times lighter than males or gynes (Brown and Keller 2000), they presumably need less time to develop from the egg to the adult stage. Thus, because no brood overwinters (Brown and Keller 2002), the first eggs produced end of March/beginning of April (personal observations) probably develop into gynes and males. Accordingly, the best explanation for our data is that all queens contributed to male production in early spring and that, in female-producing nests, a subset of these queens (19%) also produced diploid brood developing into queens. The summer workers most likely developed from the second batch of eggs laid in late April and May. These eggs were apparently laid by a subset of the queens that contributed to male production. This would be consistent with our findings that there were no queens contributing to summer workers but not to male production and that the effective number of queens contributing to summer workers (11.7 in female-producing nests and 6.8 in male-producing nests) was lower than the effective number of queens contributing to males (15.5 in female-producing nests and 31.8 in male-producing nests). Several factors may account for the lower number of queens contributing to workers than to males. The first is that 11% of the queens are unmated. Second, some mated queens may also specialize in the production of male eggs. Third, queen mortality in early spring may account for a decrease in the effective number of queens contributing to the second batch of eggs compared with the first batch of eggs. The fall workers developed from eggs that were laid after the emergence of the summer brood. Our data show that about half the queens contributing to summer workers were not the same as those contributing to fall workers. As mentioned earlier, this pattern could be explained by queens being reproductively specialized in the production of summer and/or fall brood or by an important queen turnover during the reproductive season.

In conclusion, this study presents a new method that quantifies the reproductive specialization among breeders in their contribution to different offspring classes in societies where there are too many breeders to assign parentage. We applied this method to highly polygynous field colonies of *F. exsecta* and could show that a high proportion of queens were apparently specialized in the production of only a subset of offspring classes present in colonies. Our data also revealed considerable changes in the identity of queens contributing to summer and fall workers. These data demonstrate that *F. exsecta* colonies, and probably those of many other highly polygynous social insect species, are composed of reproduc-

tive individuals which exhibit significant differences in their relative contributions to the different types of individuals composing the colonies. Moreover, the identity of these individuals, and perhaps their reproductive roles, vary considerably over time. Hence, our new method to quantify the degree of reproductive specialization should prove useful in future studies aiming at understanding the dynamic structure and complex social organizations characterizing many ants and other social organisms.

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