

Research article

Breeding system and reproductive skew in a highly polygynous ant populationC. Haag-Liautard^{1,2,4}, J.S. Pedersen^{1,3}, O. Ovaskainen² and L. Keller¹¹ Department of Ecology and Evolution, University of Lausanne, Biophore, CH-1015 Lausanne, Switzerland, e-mail: Cathy.Haag@haagliautard.net² Department of Biological and Environmental Sciences, University of Helsinki, PO Box 65, FI-00014 Helsinki, Finland³ Centre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark⁴ University of Fribourg, Institute of Ecology and Evolution, Chemin du Musée, CH-1700 Fribourg, Switzerland

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Abstract. Factors affecting relatedness among nest members in ant colonies with high queen number are still poorly understood. In order to identify the major determinants of nest kin structure, we conducted a detailed analysis of the breeding system of the ant *Formica exsecta*. We estimated the number of mature queens by mark-release-recapture in 29 nests and dissected a sub-sample of queens to assess their reproductive status. We also used microsatellites to estimate relatedness within and between all classes of nestmates (queens, their mates, worker brood, queen brood and male brood). Queen number was very high, with an arithmetic mean of 253 per nest. Most queens (90%) were reproductively active, consistent with the genetic analyses revealing that there was only a minimal reproductive skew among nestmate queens. Despite the high queen number and low reproductive skew, almost all classes of individuals were significantly related to each other. Interestingly, the number of resident queens was a poor predictor of kin structure at the nest level, consistent with the observation that new queens are produced in bursts leading to highly fluctuating queen number across years. Queen number also varied tremendously across nests, with estimates ranging from five to several hundred queens. Accordingly, the harmonic mean queen number (40.5) was six times lower than the arithmetic mean. The variation in queen number was the most important factor of the breeding system contributing to a significant relatedness between almost all classes of nestmates despite a high average number of queens per nest.

Keywords: *Formica exsecta*, relatedness, breeding system, polygyny, queen mating frequency.

Introduction

The evolution of complex social behaviour entails the integration of genetically non-identical individuals into cohesive groups (Keller and Reeve, 1999). A key component affecting the evolution and functioning of complex animal societies is their breeding system, that is the number, relationships and relative reproductive contributions of individuals that are in the same group (Ross, 2001). Over recent years, ants have emerged as a model system to study the evolution of complex social organization. Of particular interest is the fact that the number and identity of reproductive queens varies greatly within and between species (reviewed in Keller, 1993, 1995; Bourke and Franks, 1995; Ross and Keller, 1995; Crozier and Pamilo, 1996). Such variation is important because it affects the relatedness among colony members and thus the indirect benefits workers gain by helping to raise the brood. In ants and other social Hymenoptera variation in the breeding system is therefore predicted to alter the allocation of resources between growth and reproduction (Pamilo, 1991; Herbers et al., 2001; Reuter and Keller, 2001), the relative investment into male and female sexuals (Trivers and Hare, 1976; Pamilo, 1991; Chapuisat and Keller, 1999b) and the interplay of conflict and cooperation in social evolution (Queller and Strassmann, 1998; Chapuisat and Keller, 1999a; Keller and Reeve, 1999).

Molecular tools such as microsatellite markers have provided detailed information on the breeding system of several ant species (see Ross, 2001). So far, the majority of studies have focused on species and populations characterized by low queen numbers, yet high queen number occurs in a significant number of species (cf. Bourke and Franks, 1995; Keller, 1995; Crozier and Pamilo, 1996). Information on the breeding system of species with high

queen number is of particular interest (Ross, 2001) because they are characterized by low within-colony relatedness, a type of colony structure that cannot readily be explained by kin selection (Bourke and Franks, 1995; Queller and Strassmann, 1998).

One of the main problems faced by experimenters attempting to study the breeding system of ants with high nest queen numbers is to ascertain the maternity of male and female offspring. At the same time many factors jointly influence colony kin-structure. For example, the relatedness among offspring is influenced not only by the queen number but also by the degree of queen reproductive skew, queen-queen relatedness, the number of mates per queen, the relatedness between queens and their mates and the relatedness among mates of queens (Ross, 2001). As a result, there can be important differences in relatedness values between species containing similar queen number per nest. Such difference can be due to several factors, most importantly variation among nests in the turnover of queens (Queller et al., 1993) or high reproductive skew with one or a few queens monopolizing most of the colony reproduction (e.g. functional monogyny, Bourke and Franks, 1995).

The aim of this study was to examine the breeding system of a population of the ant *Formica (Coptoformica) exsecta* (Nylander 1846). This species is of particular interest because populations vary in the queen number per nest, with some populations containing a single queen (monogyny), whereas others contain numerous queens per nest (polygyny) (Pamilo and Rosengren, 1984; Sundström et al., 2005). Furthermore, many *Formica* ants exhibit extremely high queen numbers, even though relatedness among nestmates is significantly higher than zero (Rosengren and Pamilo, 1983). We selected a polygynous population located in Les Chenevières in the Swiss Jura mountains because this population has been monitored for over five years and many important aspects of its biology are well-described (Brown and Keller, 2000, 2002; Keller et al., 2001; Liautard and Keller, 2001; Brown et al., 2002, 2003; Liautard et al., 2003).

In this population, several factors potentially affecting the nest genetic structure have already been studied using indirect methods. A genetic analyses of the brood with microsatellites revealed that the effective queen number (i.e., estimated number of unrelated queens contributing to the production of workers) is moderate, with an average of 2.7 queens in female-producing nests and 6.7 queens in male-producing nests Brown and Keller (2000). The mitochondrial genotype of workers also revealed very restricted dispersal of queens both among populations and among nests within a population, with most queens remaining in their parental nest after mating (Liautard and Keller, 2001). Finally, the sex-ratio observed in the brood revealed that only a small proportion of the nests (about 10%) produce new queens in a given year and also suggested that queen number varies cyclically within a nest (Brown and Keller, 2000). These

queen-producing nests usually produce males as well (Brown and Keller, 2000; Liautard et al., 2003), potentially allowing intra-nest mating and inbreeding.

While these previous studies have provided valuable information on the general structure of the population, they give only indirect estimation of the breeding system, mainly because no reproductive queens were ever sampled and because the true queen number per nest is unknown. Therefore, to study breeding system, we first needed to estimate the actual queen number per nest. Because of the large number of individuals per nest and complex structure of nests it is impossible to sample all individuals. Moreover, we did not want to destroy nests. We therefore estimated the queen number by mark-release-recapture. We also used captured queens, in addition to pupae collected later in the season, to estimate the relatedness among and between all classes of individuals present in the nest. Those data were finally used to estimate the degree of reproductive skew among queens, as well as the relative influence of queen number and queen relatedness on nest genetic structure.

Materials and methods

Queen number

The population of *F. exsecta* studied, Les Chenevières, contains over 1200 nests (Liautard et al., 2003) over an area of about 300 × 300 m, from which a sample of 29 nests was selected. Because queens and workers move among nests we avoided to sample closely-located nests (average distance, 16.9 m, range 7.6–25.9 m). The number of queens per nest was estimated by mark-release-recapture after the first batch of eggs had been laid. This allowed us to minimize disturbance on the production of sexual eggs which are laid in early spring whereas workers eggs are mainly laid during the summer. Queens were collected from the uppermost layer of nest material that was inspected carefully in order to find all queens present, as they come close to the nest surface to warm up after the winter. More captures events were performed in nests exhibiting the lowest rates of recapture of marked queens. On average 5.4 successful recapture events per nest (with at least one queen captured) were carried out between April 25 and May 25, 2000 (see Table 1 for details). At each capture event per nest some queens were removed for genetic analyses and examination of their reproductive status. The remaining queens were marked with a thin metal wire around the petiole and released. A three-week experiment in the laboratory demonstrated that 10 queens marked in such a way did not lose the metal wire, and all survived.

The number of queens per nest was estimated with a Bayesian approach, in which we calculated the likelihood based on all capture events for each nest, including the unsuccessful ones, and accounted for the removal of individual queens (Supplementary online material). We included a random effect in the capture probability to account for possible variation among capture events due to weather conditions. We based the prior distribution for the queen number on independent data, and used otherwise uninformative prior distributions. We sampled from the posterior distribution using a Metropolis-Gibbs algorithm (Gelman et al., 2004), in which each parameter is updated in turn, conditional on the current values of the other parameter (Supplementary online material). The estimation procedure was programmed using Mathematica 5.1 (Wolfram Research, Inc. © 2004). We used the full posterior distributions to assess the arithmetic and harmonic queen numbers, but the mean estimates of the nest-specific queen numbers was used in our subsequent analyses (see below).

Table 1. Estimation of queen number in colonies of *Formica exsecta* using mark-release-recapture with colonies ranked according to the number captured. Data are: the total number of different queens captured for each colony; the queen number that have been marked and released in total; total number of marked queens recaptured; the number of recapture events attempted; the number of successful recapture events, i.e. where at least one queen (marked or unmarked) was found; and the Bayesian estimate of colony queen number (N_Q ; median and 95 % highest posterior density interval).

Colony ID	Total captured	Released marked	Recapture marked	Recapture events	Informative recapture events	N_Q	(95 % CI)
100	1	1	2*	5	3	5	(1–17)
186	6	2	0	6	3	23	(8–62)
7	6	3	0	7	3	26	(9–76)
220	8	2	0	9	2	19	(9–42)
21	9	4	0	5	4	73	(15–296)
N	10	5	0	5	4	59	(17–181)
198	14	5	1	5	4	62	(20–219)
240	14	5	1	4	4	96	(20–302)
13	15	4	1	7	4	32	(17–66)
106	16	5	2	4	4	60	(20–193)
279	24	6	1	4	4	191	(48–543)
CM	25	6	1	4	4	209	(59–498)
40	28	8	1	5	4	148	(49–377)
93	28	8	0	5	4	213	(62–524)
111	32	8	1	6	4	105	(42–273)
129	36	10	1	6	4	145	(57–335)
319	38	9	2	4	4	135	(54–320)
242	41	10	1	5	4	197	(79–478)
205	46	11	0	7	3	168	(74–361)
R	48	11	0	8	4	176	(86–352)
BO	51	13	0	7	7	310	(129–602)
26	52	12	1	4	4	306	(126–694)
158	63	15	0	12	7	195	(112–337)
*4	106	24	0	7	7	550	(288–980)
283	137	29	2	8	7	481	(284–794)
EA	143	32	0	8	7	738	(417–1415)
45	164	36	0	7	7	899	(507–1553)
67	186	36	0	8	7	842	(480–1344)
70	204	44	1	7	7	859	(539–1393)
Mean	53.5					252.5	(202.6–318.4)

* The same queen was recaptured twice.

Genetic analyses

To determine the nest kin structure, we genotyped worker pupae, queen pupae and male pupae from all nests where they occurred at four microsatellite loci: Fe13, Fe17, Fe16 (Gyllenstrand et al., 2002), and Fl21 (Chapuisat, 1996). Samples sizes per nest were: 14.7 ± 5.7 for worker pupae (mean \pm SD; $N = 23$ nests), 11.4 ± 5.1 for queen pupae ($N = 4$) and 10.4 ± 5.7 for male pupae ($N = 24$; Table 2). We collected adult queens from all nests except one where a single queen was found. The sex of pupae was determined as in Liautard et al. (2003). To determine queen-mating frequency and the kin relationships of the queens' mates, we genotyped the content of the spermatheca of all mated queens (8.2 ± 2.7 per nest) at three microsatellites loci (Fe13, Fe17, and Fl21).

PCR amplifications were carried out in 10 μ l of reaction solution of 10 mM Qiagen® Taq Buffer (MgCl₂ at a final concentration of 1.5 mM, raised to 1.7 mM for Fe13 and Fe17), 2.5 μ M dATP, 25 μ M dTTP, 25 μ M dGTP, 25 μ M dCTP, 0.025 μ l of ³³P-dATP, 0.5 μ M of each primer, 0.5 unit of Taq Qiagen®, and 1 μ l of template DNA extracted applying a salt extraction protocol. PCR products were analyzed on standard denaturing sequencing gels (6% acryl-bisacrylamide, 8 M urea) and visualized by autoradiography.

Relatedness and queen-mating frequency

Genetic regression relatedness (r) was calculated based on genotypic data with the program RELATEDNESS 5.0.8 (Goodnight and Queller, 2001) which uses the algorithms developed by Queller and Goodnight

(1989). All individuals from the study were used to estimate population allele frequencies. For relatedness calculations the allele frequencies were bias-corrected by excluding the focal individuals as well as all other members of the nest. To estimate the average inbreeding coefficient (F_{IS}) of the female brood, we used the program FSTAT version 2.9.3 (available at <http://www.unil.ch/izea/software/fstat.html>; updated from Goudet (1995)). The inbreeding coefficient was computed 100 times by using a single diploid pupae, or a single queen, randomly selected in each nest using a program written by J. Goudet (personal communication) and the average of those value was taken.

The mating frequency of queens was estimated from the maximum number of alleles observed in PCR-amplified sperm across the three loci studied. The estimated number of doubly-mated queens was corrected for undetected double matings (i.e., two males having by chance the same multilocus genotype) by using the program MATESOFT 1.0b (Moilanen et al., 2004) which uses the algorithms developed by Pedersen and Boomsma (1999). The relatedness of males that had mated with the same queen (r_{m2}) was estimated based on the observed male genotypes of queens which mated twice. However, this value is an underestimate because it does not take into account the cases where the males had identical genotypes (which results in undetected double matings). The underestimation of the observed average relatedness can be corrected if the probability of two males having the same genotype (which depends on the true relatedness among mates and the population allele frequencies) is known. The relationship between the observed relatedness among mates of multiply-mated queens (r_{m2}) and the true relatedness among mates of multiply-mated queens (r'_{m2}) is given by:

$$r'_{m2} = r_{m2} + (1 - r_{m2}) \prod_{k=1}^l \left(\left(\sum_{j=1}^m p_{kj}^2 \right) + \left(1 - \sum_{j=1}^m p_{kj}^2 \right) r'_{m2} \right) \quad (1)$$

where p_{kj} is the population allele frequency of the j th allele at the k th locus. This formula was used to obtain an estimate of r'_{m2} by an iterative procedure. In the exceptional cases (5/236) where queens had mated three or four times, we randomly selected two of the mates and applied the procedures above for the relatedness estimations. We may have failed to detect the sperm of males contributing only a small proportion to the queen's spermatheca (Gertsch and Fjerdingsstad, 1997), however such males would have only a limited impact on nest genetic structure.

Reproductive skew

Three approaches were used to estimate reproductive skew. First, we dissected 9.2 ± 3.3 (mean \pm SD) queens per nest to determine whether they had developed ovaries and sperm in the spermatheca. Second, we compared the population effective queen number which contributed to each caste (worker, male, queen) with the population harmonic mean queen number per nest contributing to this caste. And third, we calculated the expected relatedness among worker, queen and male pupae with the formulas of Ross (2001) and Bourke et al. (1997), respectively, assuming that queens shared reproduction equally (i.e., no reproductive skew). In these equations we used the population average F_{IS} value of diploids pupae and, for each nest, the mating frequency, the queen number in the nest, the relatedness among nestmate queens (r_Q), the relatedness among males that mated with the same queen (r_{m2}) and the relatedness among males that mated with different queens of the same nest (r_{m1}). For each nest we compared this expected value with the observed value to determine the degree of reproductive skew.

The association between nest genetic structure and variation across nests in queen number and queen-queen relatedness was analysed with a GLM where the significance of each variable and factor was tested with an ANOVA on type III residuals. The number of queens was log-transformed. All statistical analyses were performed using the software S-PLUS 6.1 (MathSoft Inc[©] 1988–2002).

Results

The number of queens per nest (N_Q) estimated by mark-recapture ranged from five to several hundreds (Table 1, arithmetic mean: 253; 95% CI: 203–318). The mean queen number was an order of magnitude larger than expected based on the study of Brown and Keller (2000; see Supplementary online material) and even the actual number of different queens captured exceeds the expected number by a factor 3 (Table 1). Because of the large variation in queen number across nests (Table 1), the harmonic mean queen number over nests (40.5, 95% CI: 18.9–69.5) was much lower than the arithmetic mean.

The dissections revealed that 89% (238/268) of the queens were mated. The majority of the 236 queens for which reliable data on sperm genotypes could be obtained had mated once (66.2% after correction for undetected double matings). Only five of the 65 multiply-mated queens (i.e., 2.1% of the total) had mated more than twice, with a maximum of four detected matings for one queen. The arithmetic and harmonic mean (\pm SE) mating frequencies of mated queens were 1.34 ± 0.03 and 1.20 ± 0.02 , respectively. Because the microsatellites were highly polymorphic (Table 2), the correction for undetected double mating had no significant effect on

these estimates. There was no significant correlation between queen-mating frequency (excluding unmated queens) and the proportion of mated queens per nest ($F_{1,25} = 2.8$, $P = 0.11$) or queen-queen relatedness ($F_{1,25} = 0.3$, $P = 0.60$).

Table 2. Microsatellite loci used with information on the number of individuals analyzed given as diploid equivalents (i.e. males were counted as 0.5 n), the number of alleles detected (k), Nei's expected heterozygosity (H_{exp}), and the size range of alleles.

Locus	n	k	H_{exp}	Size range (bp)
Fe13	1133.5	11	0.84	211–247
Fe16	1094.5	20	0.87	113–157
Fe17	1150	16	0.83	177–194
Fl21	982.5	22	0.88	233–300

Almost all classes of individuals collected from the same nest were related (Table 3). The only exceptions were queens and their mates, which were not significantly related, and male pupae, which were not significantly related to female pupae (Table 3). There was no significant correlation between queen number and the relatedness among queens ($F_{1,26} = 0.053$, $P = 0.82$). The relatedness among queens was slightly lower, but not significantly so, from the relatedness among queen pupae (t -test: $t_{30} = 1.2$, $P = 0.25$). It should be noted, however, that this test had little power, since our sample contained only four queen-producing nests. The males that mated with the same queen were highly related (Table 3) and significantly more so than males that mated with different queens of the same nest ($t_{54} = 3.17$, $P = 0.003$). The relatedness among male pupae was intermediate between these two values, significantly higher than the relatedness of males that mated with different queens in a nest ($t_{51} = 5.45$, $P < 0.001$) and significantly lower than the relatedness of males that mated with the same queen ($t_{51} = 2.11$, $P = 0.04$). Importantly, the relatedness among male pupae was higher when considering only nests that produced queens ($r = 0.124 \pm 0.050$), and the relatedness among males in these nests was not significantly different from the relatedness among mates of a given queen ($t_{29} = 0.49$, $P = 0.63$).

The relatedness of worker pupae was significantly negatively correlated with the number of queens per nest (Table 4). In contrast, there was no significant correlation between queen number and the relatedness between male pupae or queen pupae (Table 4). The relatedness among nestmate queens was neither significantly correlated with the relatedness among worker pupae nor queen pupae (Table 4). By contrast, the relatedness of male pupae was significantly positively correlated with queen-queen relatedness (Table 4). The genetic analyses also revealed a significant deficit of heterozygotes among both the diploid pupae ($F_{IS} = 0.128$, $t_{99} = 26.6$, $P < 0.001$) and among adult queens ($F_{IS} = 0.114$, $t_{99} = 27.8$, $P < 0.001$).

Table 3. Estimates of genetic relatedness (r) within and between different classes of nestmates in *Formica exsecta*. SE is obtained by jackknifing over colony. N is the number of colonies (not all classes were present in all colonies studied) and n denotes the number of individuals for each estimate. Probabilities (P) in one-sided t -tests for deviation from zero are given.

Parameter	Group of nestmates	N	n	r	\pm SE	t	P
r_{wp}	Among worker pupae	22	263	0.103	0.019	5.39	<0.001
r_{Op}	Among queen pupae	4	59	0.141	0.040	3.55	0.019
r_{mp}	Among male pupae	24	280	0.086	0.013	6.71	<0.001
r_Q	Among adult queens	29	268	0.084	0.0055	15.29	<0.001
r_{m1}	Males mated with different queens	29	318	0.020	0.0025	8.00	<0.001
r'_{m2}	Among mates of the same multiply mated queen	27	142	0.274	0.083	3.30	0.001
r_{mQ}	Between adult queens and their mates	29	317, 236	0.017	0.011	1.53	0.07
r_{Qmp}	Between queen pupae and male pupae	4	59, 34	0.023	0.022	1.05	0.19

Ten percent (25/258) of the queens had undeveloped ovaries. The reproductive status of queens was not significantly associated with whether they were mated or not ($\chi^2_1 = 1.1, P = 0.30$). The proportion of queens with developed ovaries was not significantly associated with the queen number per nest or the relatedness among queens (Table 4). Similarly, there was no significant association between the proportion of mated queens

and either the nest queen number or queen-queen relatedness (Table 4).

Table 4. Summary table of the GLM performed. Independent variables are the log of queen number (N_Q) and the relatedness among queens (r_Q). Dependent variables are: relatedness among worker pupae (r_{wp}); relatedness among queen pupae (r_{Op}); relatedness among male pupae (r_{mp}); proportion of queens with developed ovaries; proportion of mated queens; and the difference between observed and expected relatedness (skew) among worker pupae, queen pupae, and male pupae, respectively. Significant P values are in bold.

Dependent variable	Independent variable	$\log N_Q$	r_Q	residuals
relatedness worker	df	1	1	18
	F	6.3	2.2	
	P	0.022	0.15	
relatedness queen	df	1	1	1
	F	0.9	0.07	
	P	0.55	0.83	
relatedness male	df	1	1	20
	F	1.6	10.4	
	P	0.22	0.004	
developed ovaries	df	1	1	25
	F	1.5	0.18	
	P	0.22	0.67	
mated	df	1	1	25
	F	3.0	0.29	
	P	0.094	0.59	
skew worker	df	1	1	18
	F	4.9	0.71	
	P	0.04	0.41	
skew queen	df	1	1	1
	F	0.09	1.74	
	P	0.82	0.41	
skew male	df	1	1	20
	F	0.67	0.14	
	P	0.42	0.71	

In line with the findings that only a minority of queens had undeveloped ovaries, the combined genetic data and mark-recapture census revealed a low reproductive skew for the production of worker, male, and queen pupae (Fig. 1). At the population level, the mean effective queen number based on brood relatedness (which represents the number of equally contributing mothers) was 9.6 for worker pupae and 10.5 for male pupae. These values fall below the 95% CI of the harmonic mean queen number (18.9–69.5). Similarly, the mean effective queen number for production of queen pupae (6.8) was significantly lower than the harmonic mean queen number in the four nests producing queens (harmonic mean: 32.1; 95% CI: 18.5–55.3). Analyses at the nest level had lower power and the difference between the observed and expected relatedness values under the hypothesis of no reproductive skew was significant only for male pupae (worker pupae: paired t -test: $t_{20} = 0.18, P = 0.86$; male pupae: paired t -test: $t_{23} = 2.3, P = 0.031$; queen pupae: paired t -test: $t_3 = 1.6, P = 0.21$).

Further analyses at the nest level revealed that the estimated skew for worker production was significantly negatively correlated with nest queen number, whereas no significant association was found with the relatedness among queens (Table 4). For both queen and male brood, the degree of reproductive skew was neither significantly associated with nest queen number nor with the relatedness among queens (Table 4). None of the interaction terms of the GLM presented in Table 4 were statistically significant ($P > 0.05$ for all; interactions with skew and relatedness of queen pupae could not be tested due to low sample sizes).

Discussion

This study provides a detailed analysis of the breeding system and of the effect of queen number, queen-queen relatedness and reproductive skew on the genetic structure of a highly polygynous ant. The repeated mark-

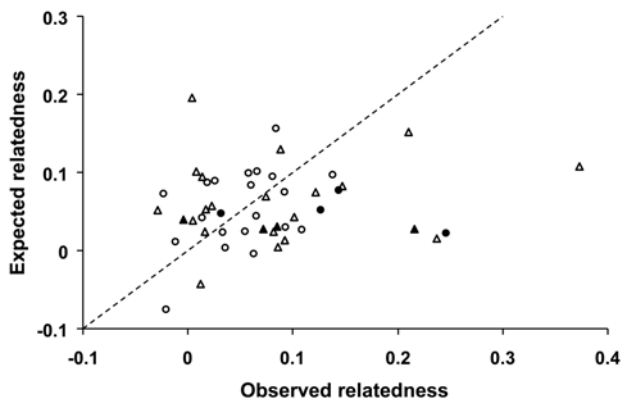


Figure 1. The association between observed relatedness and expected relatedness assuming equally reproducing queens of female brood (workers and queens; circles) and male brood (triangles) in colonies of *Formica exsecta*. Closed symbols show colonies producing queen pupae. Skew is indicated by points below the line of slope one.

release-recapture study revealed considerable variation across nests in queen number, with some nests containing only a few queens while others containing over several hundred queens. As a result of this high variation in queen number across nests, the harmonic queen number was six times smaller than the arithmetic mean queen number. The estimated queen number was greater than previously estimated (Brown and Keller, 2000). Some of the discrepancy may stem from the fact that Brown and Keller (2000) assumed that nestmate queens were unrelated when estimating queen number.

Genetic analyses allowed us to determine the remaining key parameters of the population's breeding system. First, we determined the relatedness among queens within nest and found that they were significantly related. The relatedness among queens did not significantly correlate with nest queen number. This finding is in line with the known biology of this population because previous studies showed that only few nests produce new queens and these are the ones with low queen number (i.e., those that benefit most from the production and recruitment of new queens Brown and Keller, 2000, 2002; Brown et al., 2002). Thus, the queen number is expected to vary greatly over years within nests, and because new queens are produced only when queen number is low, the relatedness of queens should depend only on the queen number present in the year when new queens were produced and not the actual queen number observed in a nest.

Second, we determined the number of males with which queens had mated. Two-thirds of the mated queens had sperm from a single male. The average mating frequency of mated queens (1.34) was similar and not significantly different from the value observed in a monogynous population of *F. exsecta* in Finland (1.26; $\chi^2_3 = 2.56$, $P = 0.46$; Sundström et al., 1996). Because we did not analyse the female offspring of single mother queens, we cannot directly assess the effective number of

matings per queen in this population. However, in the Finnish population of *F. exsecta* it has been shown that males that mated with the same queen differ considerably in their relative contribution to female offspring, resulting in a lower effective mating frequency than the average number of males with which queens mated (Keller et al., 1997). Assuming that the reproductive skew among males is similar in the Finnish and Swiss populations, queens' effective number of matings can be estimated as 1.16. This suggests that multiple mating has only little effect on the observed brood relatedness.

Third, we determined the relatedness among males that mated with the same queen and males that mated with different queens from the same nest. Interestingly, the relatedness among the mates of a given queen was significantly higher than that of males mated with different queens. A similar pattern has been found in *Myrmica sulcinodis* (Pedersen and Boomsma, 1998). In *F. exsecta*, the relatedness among mates was not significantly different from the average relatedness of male pupae in queen producing nests, suggesting that queens mate mostly within their natal nest. There are several possible explanations for the difference in relatedness among males that mated with the same queen and males that mated with different queens of the same nest. First, nests may contain queens from different generations that have mated with males that were produced during different years. Second, related males may be reproductively active at the same time in a queen-producing nest, making it more likely for a queen to mate with related males. Third, it is possible that some of the queens changed nests after having mated in their natal nest. This would explain why the relatedness among reproductive queens is slightly lower than the relatedness among queen pupae.

Fourth, we estimated the relatedness between the males and the queen they mated with, which represents the expected level of inbreeding of the brood (Liautard and Sundström, 2005). This analysis revealed that mates were not significantly related, which is surprising given that there was a significant deficit of heterozygotes in both worker pupae and adult queens ($F_{IS} = 0.128$ and 0.114 , respectively). This observed deficit of heterozygotes, although slightly higher, was not significantly different from previous estimates in the same population ($t_{29,33} = 0.89$, $P = 0.38$; Brown and Keller 2000). A study at a larger scale in the same area showed that the deficit of heterozygotes increases with the size of the reference population so that the F_{IS} value is also greater than 0.15 when considering a geographic scale similar to the present study (Kümmerli and Keller, 2007). We are unable to provide an explanation for why our study failed to detect a significant relatedness between queens and their mates.

The final and important component of the breeding system estimated in this study was the reproductive status of queens. Dissections showed that 11% of the queens were unmated and 10% lacked developed ovaries, suggesting a small degree of reproductive skew. This was confirmed by a significant difference between the

observed and expected brood relatedness under no reproductive skew for male pupae, and non significant difference for workers and queen pupae, when analysed at the nest level. The relatively low reproductive skew found in *F. exsecta* might be a common feature of highly polygynous social insects. Although not estimated precisely, the available data are also suggestive of low reproductive skew in the Argentine ant (Fournier and Keller, 2001) and *Formica paralugubris* (Chapuisat and Keller, 1999a), two species with highly polygynous nests and low relatedness among nestmates. Additionally, relatedness among nestmates of many highly polygynous social insects is close to zero (e.g. Chapuisat and Keller, 1999a; Giraud et al., 2002). Those results are in contradiction with the only model available for groups with many potential breeders (Reeve and Emlen, 2000), this model predicting that in such associations reproductive skew should be high, with the dominant breeder monopolizing the majority of the reproduction. The low reproductive skew observed was not correlated with either queen number or queen relatedness for sexual brood. The observed low skew for worker pupae was however negatively correlated with queen number. Additionally there was no significant association between the mating status of queens and whether they had developed ovaries. The proportion of queens that were mated and had developed ovaries was also not significantly correlated with the queen number per nest or their relatedness.

The characterisation of all key components of the breeding system of this population of *F. exsecta* allowed us to determine how kin structure was influenced by the various components of the breeding system. An interesting finding was that almost all classes of individuals were related to each other despite the fact that the average nest queen number was very high. The most important factor accounting to this apparent discrepancy was the considerable variation in queen number across nests, with the harmonic mean queen number being six times lower than the arithmetic mean. Brown and Keller (2000) found a lower estimate of effective queen number per nest with values ranging from two to seven. However, they had not any information about the nests' breeding structure, they therefore just estimated effective number of queens by dividing 0.75 by the observed relatedness of diploid pupae.

Theoretical studies have demonstrated that variation in queen number either across nest or temporally within a nest is an important factor contributing to a significant population average relatedness among nest members because this value depends on the harmonic mean queen number (Queller, 1993; Ross, 1993). This has then been confirmed empirically in several species of polygynous wasps (Gastreich et al., 1993; Queller et al., 1993; Henshaw et al., 2004), although the queen number in such colonies is much lower than in nests of our population of *F. exsecta*. In swarm founding wasps, queen number varies greatly during the season and new queens are preferentially produced when colonies contain few queens. As a result, queens are more related to

each other than workers (e.g. Queller et al., 1993; Henshaw et al., 2000). In our study we did not follow queen number over several years. However, several lines of evidence suggest that the number of reproductive queens varies among years with new queens being preferentially produced and recruited when their number falls below a given threshold (Brown and Keller, 2000; Brown et al., 2002; Kümmerli and Keller, 2007). Temporal variation in queen number has also been observed in *Myrmica* ants (Elmes, 1987).

The estimation of queen number suggested that marked queens were somewhat less likely to be captured than unmarked queens (see Supplementary online material). This result may be explained by workers and queens moving among nests, as it often occurs in polygynous species. Thus, if marked queens tend to escape the nest where they have been captured (disturbed), the probability to recapture them will be lower than for unmarked queens.

In conclusion, the repeated mark-release-recapture of queens used in this study proved a highly useful technique to obtain information on the queen number per nest. Indeed, the actual queen number per nest was found to be much higher than expected based on previous studies. Mark-release-recapture has been used in several other studies in ants, but primarily for the purpose of estimating worker or brood number, foraging range or queen dispersal distance (e.g. Glancey and Lofgren, 1988; Billick, 1999). To our knowledge this is the first application for estimating nest queen number. This method could be used to obtain information in other studies of highly polygynous ant species where nest size or structure precludes excavation and sorting of all nest members.

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Supplementary online material

A detailed description of the estimation of queen number by Bayesian methods can be found as supplementary material in the online version at www.birkhauser.ch/IS (DOI 10.1007/s00040-008-1010-9)

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