Contrasting population genetic structure for workers and queens in the putatively unicolonial ant *Formica exsecta*

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Abstract

The theory of inclusive fitness provides a powerful explanation for reproductive altruism in social insects, whereby workers gain inclusive fitness benefit by rearing the brood of related queens. Some ant species, however, have unicolonial population structures where multiple nests, each containing numerous queens, are interconnected and individuals move freely between nests. In such cases, nestmate relatedness values may often be indistinguishable from zero, which is problematic for inclusive fitness-based explanations of reproductive altruism. We conducted a detailed population genetic study in the polygynous ant *Formica exsecta*, which has been suggested to form unicolonial populations in its native habitat. Analyses based on adult workers indeed confirmed a genetic structuring consistent with a unicolonial population structure. However, at the population level the genetic structuring inferred from worker pupae was not consistent with a unicolonial population structure, but rather suggested a multicolonial population structure of extended family-based nests. These contrasting patterns suggest limited queen dispersal and free adult worker dispersal. That workers indeed disperse as adults was confirmed by mark–recapture measures showing consistent worker movement between nests. Together, these findings describe a new form of social organization, which possibly also characterizes other ant species forming unicolonial populations in their native habitats. Moreover, the genetic analyses also revealed that while worker nestmate relatedness was indistinguishable from zero at a small geographical scale, it was significantly positive at the population level. This highlights the need to consider the relevant geographical scale when investigating the role of inclusive fitness as a selective force maintaining reproductive altruism.

Keywords: dispersal, family groups, inclusive fitness, social insects, social organization, unicoloniality

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Introduction

The theory of inclusive fitness (Hamilton 1963, 1964) provides a powerful explanation for reproductive altruism in social insects (Bourke & Franks 1995; Crozier & Pamilo 1996; Foster *et al.* 2006). The theory posits that individuals can pass on their genes not only directly through their own offspring, but also indirectly by favouring the reproduction of relatives (kin selection). In nests of social insects, relatedness among nest members is generally high and workers, although foregoing their own reproduction, obtain high inclusive fitness benefits through rearing the brood of related queens (Bourke & Franks 1995).

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In many ant species, however, nests contain multiple reproductive queens (polygyny), which results in a decreased average relatedness among nest members (Keller 1993). Moreover, polygyny in ants is sometimes associated with high nest densities and the abolishment of nest boundaries, resulting in the mixing of individuals between nests such that multiple physically separated nests are interconnected (Hölldobler & Wilson 1977; Passera 1994; Holway *et al.* 1998, 2002; Chapman & Bourke 2001; Giraud *et al.* 2002; Debout *et al.* 2007). Such nest networks are called supercolonies if there are no behavioural boundaries (aggression) between nests within a supercolony and if direct cooperative interactions are impossible between individuals in distant nests (Pedersen *et al.* 2006). Within a population, one or several supercolonies may occur with such populations

being called unicolonial populations (Pedersen *et al.* 2006). Networks entailing up to several thousand interconnected nests have been observed in invasive ant species like the little fire ant, *Wasmannia auropunctata* (Le Breton *et al.* 2004; Errard *et al.* 2005), and the Argentine ant, *Linepithema humile* (Passera 1994; Holway *et al.* 1998; Tsutsui *et al.* 2000; Giraud *et al.* 2002; Jaquiery *et al.* 2005; Thomas *et al.* 2006), but also occur in ant species in their native range such as those in the genera *Lasius* (Yamauchi *et al.* 1981), *Myrmica* (Seppä & Pamilo 1995; Seppä & Walin 1996; Pedersen & Boomsma 1999a, b; van der Hammen *et al.* 2002), *Formica* (Higashi & Yamauchi 1979; Rosengren & Pamilo 1983; Chapuisat *et al.* 1997; Beye *et al.* 1998; Elias *et al.* 2005; Holzer *et al.* 2006), and *L. humile* (Pedersen *et al.* 2006).

Importantly, relatedness among members of the same supercolony (i.e. the cooperative unit; Pedersen *et al.* 2006) is often indistinguishable from zero (Bourke & Franks 1995; Keller 1995; Crozier & Pamilo 1996). This is potentially problematic to inclusive fitness-based explanations for reproductive altruism, and remains an unresolved issue in evolutionary biology. A first step towards resolving this problem is to obtain precise information on the genetic structuring among nests and on whether nests are grouped in supercolonies. To date, all studies that have investigated population genetic structures of potentially unicolonial ant species have used genotypes of adult workers for analysis. Unfortunately, such data do not allow one to disentangle the effects of sexual and adult worker dispersal. This is important because even if workers move freely between nests (which would suggest a unicolonial population structure), related queens might stay in the nest they founded or where they were born, which would lead to a multicolonial population structure with significant positive within-nest relatedness values among queens and among worker pupae.

The aim of this study was to investigate the pattern of dispersal of both queens and workers. We used polymorphic microsatellite markers to infer the population genetic structure of worker pupae, which reflects the population genetic structure of their reproductive parents (provided that brood is not moved between nests) as well as adult workers, which also takes into account the dispersal pattern of the nonreproductive workers. We studied a polygynous population of the ant *Formica exsecta*, which is a particularly interesting species for such a study because it shows several characteristics usually associated with unicoloniality. For example, nests contain many queens (Brown & Keller 2000) and occur at high densities (Liautard *et al.* 2003). However, there is evidence that queens and workers differ in their dispersal behaviour: new queens are often recruited back to the nest where they were born (Brown & Keller 2002; Kümmerli & Keller unpublished data), whereas adult workers but not brood have been observed to move between nests (Cherix *et al.* 1980; Katzerke *et al.* 2006; personal observations).

Table 1 Classifications of different population structures based on estimates of *F*-statistic indices (F_{ST} and F_{IT}), within-nest relatedness (*r*), relatedness corrected for inbreeding (*r**) and the genetic effective queen number (N_e) following Pedersen *et al.* (2006)

Population structure	F_{ST}	F_{IT}	r	$r*$	N_e
Multicolonial	> 0	0	> 0	> 0	limited
One supercolony	0	0	0	0	∞
Several supercolonies	> 0	> 0	> 0	0	∞

We used highly polymorphic microsatellite loci to estimate nestmate relatedness (*r*), genetic differentiation between nests (F_{ST}), the overall inbreeding coefficient (F_{IT}) and the genetically effective number of queens (N_e) at two different spatial scales (entire population and a smaller scale within the population). These estimates can be used to determine whether a population consists of family-based nests (multicolonial) or of one or several supercolonies (unicolonial; see Pedersen *et al.* 2006 and Table 1 for classifications based on *r*, F_{ST}, F_{IT} and N_e). For these analyses, we used F_{IT} as a measure of inbreeding because we were interested in the reduction of individual heterozygosity relative to the total population rather than to the nest (e.g. F_{IS}). Populations consisting of family-based nests are typically characterized by a limited number of reproductive queens per nest ($1 < N_e < ∞$) that mated randomly in the population ($F_{IT} = 0$) with the effect that there is a significant genetic differentiation ($F_{ST} > 0$) between nests and significant positive nestmate relatedness (*r* > 0) (Bourke & Franks 1995). By contrast, if a population consists of nests belonging to a single supercolony, *r*, F_{ST} and F_{IT} are expected to be zero and N_e to be infinite. Finally, if a population consists of multiple supercolonies, the genetic differentiation ($F_{ST} > 0$, *r* > 0) between nests from different supercolonies should not result from nests containing a limited number of reproductive queens but from supercolonies being closed breeding units. Thus, limited dispersal of queens and males among supercolonies results in $F_{IT} > 0$ with the consequence that relatedness values must be corrected for such inbreeding (*r**) to obtain the true effective number of breeding queens per nest (see Pamilo 1985; Ross 2001). By this correction, *r** should become indistinguishable from zero, resulting in $N_e = ∞$. As the latter genetic pattern is also consistent with genetic isolation by distance between nests, we tested whether pairwise genetic differentiation between nests was correlated with pairwise geographical distance.

Finally, we used the obtained genetic values to estimate the proportion of migrating sexuals and workers and compared these estimates with direct measures of worker dispersal obtained in a mark–recapture experiment in the study population.
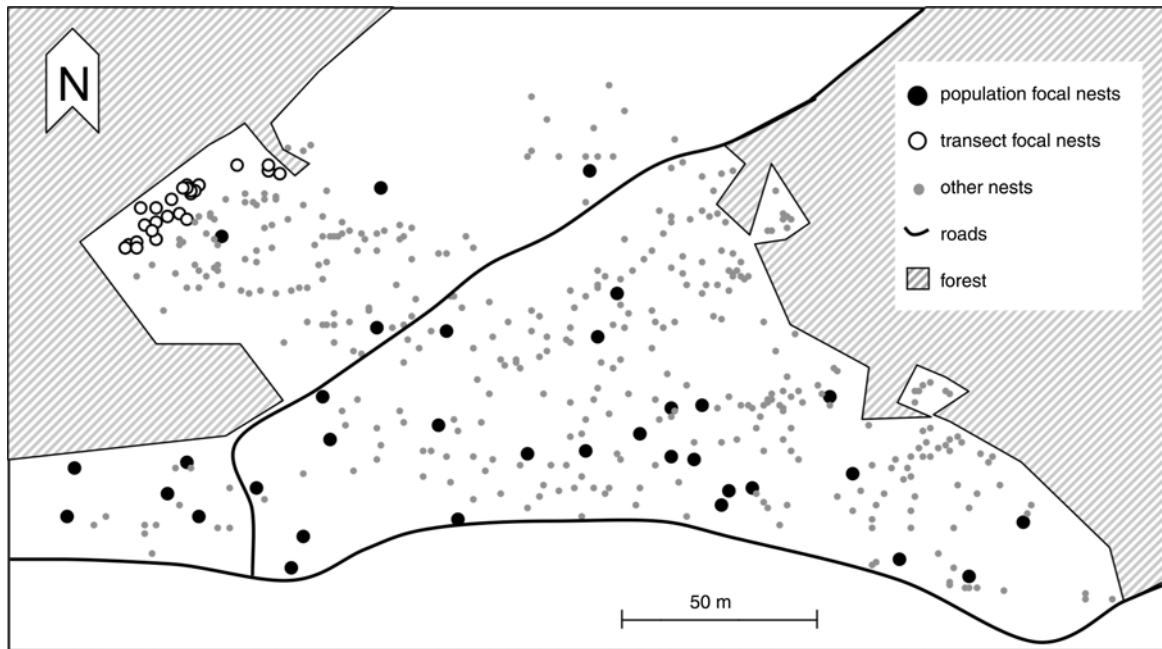
Fig. 1 Map of the studied *Formica exsecta* population on a cattle pasture at 'les Chenevières' in the Swiss Jura Mountains. The habitat with more than 400 nests is limited by a road in the south and by forest in the north, east and west.

Materials and methods

Study population and sampling

The study was carried out at 'les Chenevières', a cattle pasture at 1220 m above sea level in the Swiss Jura Mountains. In 2002, we individually marked more than 400 nests of the ant *Formica exsecta* distributed across approximately 3 ha (Fig. 1; maximum distance between two nests = 272 m), representing the entire population. In this population, nest sex ratio is bimodally distributed (Brown & Keller 2000) with most nests (approximately 90%) producing exclusively males and workers (hereafter referred to as male-producing nests) and some nests producing new queens (gynes), males and workers (hereafter referred to as female-producing nests).

To estimate the genetic structuring between nests at the population level, we collected worker pupae in July 2002 prior to the mating flight in 29 focal nests (Fig 1; 14 female- and 15 male-producing nests randomly selected with the distance between neighbouring focal nests being 16.8 m ± 10.9, mean ± SD). To determine the sex specialization of nests prior to brood emergence, we collected 50 pupae and assigned the sex and caste of late-stage pupae by moistening them in ethanol and observing their size, eye shape and genitalia (Brown & Keller 2000). We genotyped 8.2 ± 1.5 (mean ± SD) worker pupae per nest. We also genotyped 10.0 ± 1.8 (mean ± SD) adult workers collected from the dome of 21 nests (including 13 nests from which pupae were collected) in July 2002 and June 2003 (distance between

neighbouring focal nests = 14.8 m ± 6.5 m, mean ± SD). Adult workers were collected prior to the emergence of new workers and were therefore at least 1 year old; thus, they potentially could have migrated between nests.

To estimate the genetic relationship between nests at a smaller spatial scale, we genotyped worker pupae (11.6 ± 1.2, mean ± SD) and worker adults (11.8 ± 0.6, mean ± SD) collected from 25 nests along a 45 m long and 10 m wide transect (Fig. 1; distance between neighbouring nests = 2.1 m ± 1.5 m, mean ± SD) in June 2003. Worker pupae could only be collected from 21 nests because four nests had no brood.

Genetic analyses

We determined the genotypes of worker pupae and adults at six microsatellite loci FL21 (Chapuisat 1996) and FE17, FE19, FE21, FE37 and FE51 (Gyllenstrand *et al.* 2002). DNA was extracted from workers' heads or entire pupae in 250 μL or 500 μL of 5% Chelex, respectively. Samples were incubated for 10 min at 90 °C, vortexed, and incubated for another 10 min at 90 °C. Two multiplex polymerase chain reactions (PCRs) were used: multiplex-PCR1 combined FE19, FE21 and FE51 and multiplex-PCR2 combined FE17 and FE37. 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 described elsewhere (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 five to 28 with a mean of 11.8.

Genetic data analyses

We quantified genetic diversity and structuring within and between nests using the program FSTAT 2.9.4 (Goudet 1995). First, we estimated the expected and observed heterozygosities following Nei (1978). We then tested each of our four data sets (worker pupae and adults at the population and transect levels) for departures from the Hardy–Weinberg equilibrium by randomizing alleles among individuals within nests using 10 000 iterations. Linkage disequilibrium between pairs of loci in each sample was tested separately for the four different data sets using G statistics (Goudet *et al*. 1996) implemented in FSTAT 2.9.4. We calculated the F-statistic indices F_{ST} and F_{IT} following Weir & Cockerham (1984), which provide measures of the reduction in heterozygosity between subpopulations (F_{ST}) and of individuals relative to the total population (F_{IT}). For these analyses, we regarded each nest as a subpopulation consisting of the individuals genotyped and the entire sample of nests as the total population. Standard errors of F statistics were obtained by jackknifing over loci. Significant deviations from zero were assessed using randomization tests (Manly 1997) by applying 10 000 iterations. For F_{ST} values, genotypes were permuted whereas for F_{IT} values alleles were permuted among nests.

We estimated the relatedness (r) among nest members with the program RELATEDNESS 5.0.8 (Queller & Goodnight 1989) and obtained standard errors by jackknifing over nests. Relatedness (r) is the probability by which an allele is more likely to be shared among nest members than would be expected from the allele's frequency in the population (Bourke & Franks 1995; Crozier & Pamilo 1996). To obtain estimates of the true genetically effective queen number (N_e) corresponding to the number of equally reproducing queens needed to explain a given nest kin structure, we used the relatedness values corrected for inbreeding effects (r^*) following the method suggested by Pamilo (1985). N_e is then given as

$$N_e = \frac{4r_s - r_q - 2r_{m1}}{4r^* - r_q - 2r_{m1}},\qquad\text{(eqn 1)}$$

where r_s is the relatedness among female offspring of the same matriline, r_q is the relatedness among nestmate queens and r_{m1} is the relatedness among the mates of nestmate queens (Seppä 1994). The relatedness among female offspring of the same matriline (r_s) is $1/(2m_e) + 1/4$, where m_e is the mean effective mating frequency of queens in the nest (Queller 1993; Ross 1993). In our study population, r_q, r_{m1} and m_e have previously been estimated to be 0.084 ± 0.006 (mean ± SE), 0.020 ± 0.003 (mean ± SE) and 1.2, respectively (Liautard, Pedersen, Ovaskainen and Keller unpublished data).

To determine whether population structuring originated from division into genetically differentiated clusters (nests or supercolonies), we used the program BAPS 4.12 (available at http://www.rni.helsinki.fi/~jic/bapspage.html). This software implements a Bayesian approach to cluster groups of individuals (in our case groups of nestmate workers), with the optimal number of clusters (K, in our case the optimal number of estimated true nests or supercolonies) given by the highest log (ml = marginal likelihood) of the data (Corander *et al*. 2003). We conducted repeated spatial analyses (10 runs per data set) using the coordinates of nest locations as information (Corander *et al*. in press) and defined the maximal K as the number of nests from which samples were collected.

To determine whether population structuring originated from isolation by distance, we tested whether there was an association between pairwise genetic and geographical distances between nests. Matrices of pairwise F_{ST} between nests (obtained with FSTAT 2.9.4) were transformed by $F_{ST}/(1 - F_{ST})$ and the matrices of geographical distances were log-transformed as proposed by Rousset (1997) for two-dimensional habitats. Geographical distances between nests at the population level were measured by determining the geographical coordinates of each nest using a GPS 12XL (Garmin). For distance measures within the transect, we used a measuring tape to assess the exact position of each nest on a predefined grid. We calculated the correlation between the matrices of pairwise genetic and geographical distance with a Mantel test (r-statistics) using the program R-PACKAGE 4.0 (available on http://www.bio.umontreal.ca/Casgrain/en/labo/R/).

Estimating dispersal of sexuals

We adopted the dispersal model proposed by Chapuisat & Keller (1999) which assumes that gene flow is mediated by queens and males, with a proportion of queens originating from within the nest that mate with nestmate males (α), and the remaining queens (1 − α) coming from the panmictic population after having mated once at random (Chapuisat & Keller 1999).

These assumptions are reasonable because behavioural experiments revealed that some proportion of queens (35%) and males (66%) depart on mating flights, whereas the remaining queens and males mate within or on the surface of their parental nest (Schneider 2002). Moreover, genetic analyses also support the view that a high proportion of queens stay within their parental nest after mating (Liautard & Keller 2001; Brown & Keller 2002) and mate with related males (Kümmerli & Keller in press). Meanwhile, our recent genetic results also suggest that nests can recruit queens from other nests with these queens having probably mated randomly (Kümmerli and Keller unpublished data).

If α and the effective queen number (N_e) remain constant across generations, $1 − α$ (dispersal rate) can be estimated from the relatedness among brood (r) by using the following equation

$$r = \frac{3}{4N_e - \alpha - 4\alpha^2(N_e - 1)} \qquad \text{(eqn 2)}$$

(see Chapuisat & Keller 1999 for detailed explanations of the derivation of this equation). We chose $N_e = 23.6$, which corresponds to the harmonic mean queen number per nest for our study population (Liautard, Pedersen, Ovaskainen and Keller, unpublished data). This value matched well our estimate of the genetically effective number of queens contributing to brood production ($N_e = 23.7$, mean across male- and female-producing nests, see Kümmerli & Keller 2007). Finally, the assumption that queens are singly mated is reasonable because the harmonic mean mating frequency was estimated to be 1.20 in our study population (Liautard, Pedersen, Ovaskainen and Keller, unpublished data). Although queen dispersal is known to occur at a local level (Liautard & Keller 2001), there is currently no information available on the spatial scale at which male dispersal occurs. For that reason, we calculated the dispersal rate using relatedness values obtained from analyses at population and transect levels. Estimates of standard errors for dispersal rates were obtained by jackknifing over loci.

Estimating dispersal of workers

We used two different approaches to test whether workers migrate between nests. First, we used the relatedness among nestmate adult workers to calculate the proportion of matings ($\alpha_{\text{worker adults}}$) that would occur between nestmate queens and males to explain this relatedness value if only sexuals but no workers disperse. Assuming that the same number of queens contributes to two consecutive worker cohorts and that worker lifespan is limited by one hibernation, $\alpha_{\text{worker adults}}$ will be equal to the α obtained from worker brood relatedness ($\alpha_{\text{worker brood}}$). However, if workers disperse, $\alpha_{\text{worker adults}}$ becomes lower than $\alpha_{\text{worker brood}}$ with the dispersal rate of adult workers (m_w) being

$$m_{\text{w}} = 1 - \frac{\alpha_{\text{workeradult}}}{\alpha_{\text{workerbrood}}} \qquad \text{(eqn 3)}$$

As workers can only migrate on foot, dispersal will mostly occur at a local spatial scale. For that reason, we used the relatedness estimates obtained from analysis at the transect level.

Second, we estimated the migration of adult workers along the transect by colour-marking a total of 12 198 workers from four nests in May 2003. These four nests were ran-domly chosen with one nest at the beginning, two nests in the centre and one nest at the end of the transect. We used four colours (white, green, yellow, red) of a nontoxic paint (Colodur Coop) to mark the workers of the four nests with a different colour each. Workers were counted, sprayed and returned to the nest from which they were collected. We recaptured workers 2 days (4555 individuals), 7 days (5157 individuals) and 1 month (4530 individuals) after the initial marking from the domes of all 25 nests of the transect. Recaptured workers were individually examined for the presence of colour marks, which were found on 322 workers (2.3% recapture rate). From the recapture data, we calculated the drifting ratio defined as the number of same-colour-marked workers recaptured in foreign nests divided by the total amount of marked workers recaptured of the corresponding colour (Elias *et al*. 2005). This method could lead to an overestimation of the dispersing rate if workers more likely to disperse are closer to the surface and are more likely to be marked and recaptured.

Results

Hardy–Weinberg and linkage equilibrium

There was no significant linkage disequilibrium between any pair of loci analysed within all four data sets. No significant departure from the Hardy–Weinberg equilibrium was found for the loci FE19, FE37 and FE51 (Table 2), whereas a significant departure was found for the loci FE17 and FE21 (in one out of the four data sets) and FL21 (in three out of the four data sets). We therefore carried out all analyses with and without FE17, FE21 and FL21 and found that F_{ST}, F_{IT} and r-values yielded equivalent results in both analyses for the loci FE17 and FE21. When including FL21, however, F_{IT} values were higher and yielded different results in terms of significance for several comparisons. The analysis of individual genotypes strongly supported the view that the high discrepancy between the expected and observed heterozygosities at locus FL21 was due to some alleles that were not or irregularly amplified (null alleles) and not due to an unusual mode of reproduction (see Keller 2007). For that reason, we excluded FL21 from further analyses.

Genetic structuring at the population and the transect level

F_{ST} values were small (range: 0.003–0.048, Table 3) but significantly greater than zero for worker pupae at both the population and transect levels as well as for worker adults at the population level (permutation tests: all $P = 0.0001$). In contrast, F_{ST} value for worker adults was not significantly different from zero (permutation test: $P = 0.08$) at the transect level.

Table 2 Expected (H_E) and observed (H_O) rates of heterozygosity for the six loci and the four different data sets analysed

| Locus | No. of alleles | Population level | | | | Transect level | | | |
| | | Worker pupae | | Worker adults | | Worker pupae | | Worker adults | |
		H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O
FE17	16	**0.79**	**0.74**	0.84	0.80	0.83	0.83	0.84	0.82
FE19	6	0.46	0.45	0.43	0.43	0.39	0.39	0.38	0.39
FE21	5	0.54	0.57	0.60	0.61	0.56	0.56	**0.55**	**0.49**
FE37	8	0.64	0.68	0.66	0.62	0.67	0.66	0.70	0.73
FE51	8	0.51	0.55	0.50	0.54	0.52	0.50	0.56	0.57
FL21	28	**0.85**	**0.79**	**0.84**	**0.70**	**0.83**	**0.75**	0.85	0.83
All loci		0.63	0.63	0.64	0.62	0.63	0.61	0.65	0.64
P (all loci)			0.72		**0.03**		0.10		0.25

Values in bold indicate significant deviations from the Hardy–Weinberg equilibrium. P values were obtained by randomization tests based on 10 000 iterations.

	F_{ST}	F_{IT}	r	$r*$	N_e
Population level					
Worker pupae	**0.048 ± 0.007**	0.026 ± 0.037	**0.095 ± 0.019**	0.046	41.9
Worker adults	**0.022 ± 0.007**	0.036 ± 0.024	**0.051 ± 0.025**	−0.020	∞
Transect level					
Worker pupae	**0.022 ± 0.004**	**0.028 ± 0.012**	0.054 ± 0.036	−0.001	∞
Worker adults	0.003 ± 0.005	0.007 ± 0.030	0.006 ± 0.009	−0.008	∞

Table 3 Estimates of the F-statistic indices (F_{ST} and F_{IT}), relatedness (r), relatedness corrected for inbreeding ($r*$) and the genetic effective queen number (N_e)

F and r values in bold are significantly different from zero.

F_{IT} values ranged from 0.007 to 0.036 (Table 3) and were marginally greater than zero for worker adults at the population level and worker pupae at the transect level (permutation tests: both $P = 0.053$). F_{IT} values for worker pupae at the population level (permutation test: $P = 0.08$) and worker adults at the transect level (permutation test: $P = 0.33$) were not significantly different from zero.

Relatedness values (r) ranged from 0.006 to 0.095 (Table 3) and were significantly greater than zero for worker pupae and adults at the population level (one-tailed t-tests, worker pupae: $t_{28} = 4.95$, $P < 0.0001$, worker adults: $t_{20} = 2.02$, $P = 0.028$). At the transect level, however, relatedness among nest members was not significantly greater than zero for worker pupae ($t_{20} = 1.47$, $P = 0.08$) and worker adults ($t_{24} = 0.69$, $P = 0.25$).

Inbreeding-corrected relatedness ($r*$) was only significantly greater than zero for worker pupae at the population level (one-tailed t-test: $t_{28} = 2.42$, $P = 0.011$, Table 3). As a consequence, estimates of the genetically effective queen numbers based on $r*$ yielded a finite value for worker pupae at the population level, whereas estimates of the genetically effective queen numbers became infinite for adult workers at the population level and for worker pupae and worker adults at the transect level (Table 3).

Genetically isolated subpopulations vs. isolation by distance

For worker pupae at the population and transect level, clustering analyses using BAPS revealed significant structuring among nests with the most likely number of clusters being $K = 2$ and the probability (p) of the log (ml) being $0.71 ± 0.21$ (mean ± SD, population level) and $P = 1.0 ± 0.0$ (transect level). By contrast, for adult workers, the most likely number of clusters was $K = 1$ (p [log(ml)] $= 1.0 ± 0.0$) both at the population and transect levels.

Mantel correlations between pairwise genetic and geographical distances of nests revealed low correlation coefficients (ranging from –0.07–0.07, Table 4) for analyses of worker brood and adults at the population and transect levels. None of the correlation coefficients were significantly greater than zero, indicating that there was no significant genetic isolation by distance between nests.

Dispersal behaviour

When assuming that dispersing sexuals disperse randomly within the population (i.e. the entire population is the

Table 4 Test for association between pairwise genetic and geographical distance between nests (n) using Mantel-tests (r-statistics)

	Mantel r	N	P
Population level			
Worker pupae	0.025	29	0.38
Worker adults	−0.066	21	0.20
Transect level			
Worker pupae	0.073	21	0.27
Worker adults	−0.012	25	0.43

relevant geographical scale), the genetic data on worker pupae are best explained by a high proportion of queens ($\alpha = 82.7\% \pm 1.2\%$) mating with nestmate males and returning into their parental nest and a low proportion of queens ($1 - \alpha = 17.3\% \pm 1.2\%$) dispersing and mating randomly within the population. When assuming that dispersal mainly occurs at a smaller geographical scale (represented by the transect in our study), the genetic data on worker pupae are best explained by 64.6% ± 5.0% of the queens mating with nestmate males and returning to their parental nest and the remaining 35.4% ± 5.0% queens dispersing and mating randomly within the population.

Our mark–recapture experiment revealed that a high proportion of workers migrated between nests at the transect level. Two days after the worker marking, the proportion of marked workers found in other nests than the nest they originated from (drifting ratio) was 5.9% ± 4.1% (mean ± SE). The drifting ratio increased to 37.3% ± 8.9% after 7 days and reached 85.1% ± 7.7% after 1 month (31 days), which results in an average drifting ratio per day of 3.7% ± 0.8%. Marked workers were found on all nests within the transect and workers marked on an individual nest where recaptured on average on eight other nests with a maximal detected dispersing distance of 20.2 m (maximal detectable dispersal distance = 39.6 m).

When estimating worker dispersal based on our relatedness values (equation 3), it turned out that the relatedness among nestmate workers ($r = 0.006$) was smaller than the minimal possible relatedness ($r = 0.032$) given under the model's assumptions ($N_e = 23.6$). This discrepancy is most likely due to stochastic errors in relatedness calculations arising from limited number of pupae and loci genotyped because a mean effective queen number of $N_e = 124$ (the value required to obtain $r = 0.006$) seems very unlikely on the basis of previous genetic studies of the same population (Brown & Keller 2000, 2002; Kümmerli & Keller 2007). In any case, the data strongly indicate that workers mix freely between nests at the transect level.

Discussion

Our population genetic analyses revealed different patterns of genetic structuring between nests depending on whether we used genotypes of worker pupae or adult workers. Overall, genetic structuring among nests was higher when using worker pupae than when using adult workers. Because analyses based on worker pupae describe the dispersal pattern of reproductive queens and their mates, whereas analyses based on worker adults describe the dispersal pattern of adult workers following the dispersal of sexuals, our data strongly indicate that workers move between nests.

For worker pupae at the population level, we detected a significant genetic structuring among nests. Moreover, the relatedness among worker pupae and its corrected value for F_{IT} (inbreeding coefficient) were significantly greater than zero, which translated into finite estimates of the mean genetically effective queen number (Table 3). According to the classification of Pedersen *et al.* (2006) (Table 1), this pattern of genetic structuring is consistent with a multicolonial population structure with queens forming extended family-based groups, whereby relatedness among the many queens that contribute to brood production is relatively low but significantly greater than zero.

For adult workers at the population level, we detected a low but significant genetic structuring among nests and a marginally significant overall inbreeding coefficient. Although relatedness among workers was significantly greater than zero, the relatedness value corrected for inbreeding became indistinguishable from zero, which translated into an infinite estimate of the mean genetically effective queen number (Table 3). According to the classification of Pedersen *et al.* (2006) (Table 1), this pattern of genetic structuring corresponds to a unicolonial population structure possibly consisting of more than one super-colony. Importantly, the observed pattern of genetic structuring between nests does not seem to be explained by population viscosity (see Chapuisat *et al.* 1997; Pedersen *et al.* 2006), as there was no positive relationship between geographical and genetical nest distances (Table 4).

Our analyses of the genetic structuring among nests at the transect level confirmed that the genetic structuring among nests is different depending on whether worker pupae or adult workers were analysed. For worker pupae, we detected a low but significant genetic structuring among nests and a marginally significant positive inbreeding coefficient (Table 3). For adult workers, by contrast, we detected no genetic structuring among nests at all, which is consistent with a unicolonial transect structure.

Based on our dispersal model, our genetic data, irrespective of the geographical scale considered, are consistent

with a high proportion of queens mating with nestmate males (transect level, 83%; population level, 65%). This dispersal pattern largely contrasts with the one found for workers, where genetic and recapture data indicate free mixing of individuals at the transect level.

Our findings suggest a biological scenario characterized by the following features. First, while some new queens successfully disperse to foreign nests, the majority of them disperse very locally and are recruited back to either their parental or a nearby nest where they form extended family groups. This dispersal behaviour is consistent with the observed significant genetic structuring among nests (F_{ST} > 0) at the transect and population levels as well as with a significant positive relatedness value and finite estimate of the genetically effective queen number at the population level. These findings strengthen the conclusion from previous studies showing restricted queen dispersal (Liautard & Keller 2001) and queen recruitment (Brown & Keller 2002; Kümmerli and Keller unpublished data). Second, many queens mate with nestmate males, which is compatible with positive F_{IT} values. However, it seems also that some proportion of queens mate randomly with non-nestmate males from the population, which might explain the absence of significant genetic isolation by geographical distance when using nuclear markers (this study), but the observation of significant isolation by distance for maternally inherited genetic markers (mtDNA, Liautard & Keller 2001). Third, workers produced by queens in extended-family-structured nests freely mix between neighbouring nests forming a unicolonial population structure. However, workers do not mix completely between all nests at the population level, which might be due to two reasons. First, the population is structured into different areas (see Fig. 1) separated by small-scale geographical separations such as roads, forest and bush patches, with these separations possibly hindering free migration. Second, workers are short-lived (Keller & Genoud 1997), and because they emerge in family structured nests, their free dispersal might be insufficient to completely erode population structure at the population level. This worker dispersal scenario is consistent with weak but significant genetic structuring (F_{ST} > 0) between nests and a low but significant positive within-nest relatedness coefficient at the population level. Furthermore, free dispersal in certain areas but not in the entire population due to time or geographical constraints is consistent with a significant inbreeding coefficient (F_{IT} > 0) at the population level. Finally, this pattern of worker dispersal is compatible with the abolishment of any genetic structuring between nests at a smaller (transect level) spatial scale. The fact that *Formica exsecta* workers move freely between neighbouring nests conforms to previous findings where aggression among workers was found to be low irrespective of the distance between nests within the same population (Brown *et al.* 2003).

Given the fact that there is no isolation by distance, the significant genetic structuring between nests observed for worker pupae and adult workers should result in a number (K > 1) of genetically distinct clusters when analysing the data with BAPS. According to the classification of Pedersen *et al.* (2006) (Table 1), K should equal the number of extended family groups for the genetic structuring observed for worker pupae at the population and transect level, whereas K should equal the number of supercolonies for the genetic structuring observed for worker adults at the population level. The results obtained from the BAPS analyses did not however, meet these expectations. Although BAPS detected some structuring between nests using worker pupae genotypes, the number of clusters (K = 2) was much lower than the number of nests studied (population: n = 29, transect: n = 21). When using adult worker genotypes, our data were best explained when all nests were grouped into a single cluster (K = 1). One possible explanation for these findings is that BAPS was unable to detect the correct population structure, which might be due to the overall low genetic differentiation between nests and the low number of loci analysed. Limited sensitivity is known to be a problem in another clustering programme using a similar mathematical approach (Evanno *et al.* 2005). Another potential explanation might be that a given population does not consist of a discrete number of nests or supercolonies and population genetic structure can therefore not simply be classified as being either multicolonial or unicolonial. Instead, there might be a continuum of different types of population structures with one end point being characterized by family-based nests where all queens mate randomly in the population and return to their parental nest afterwards, while the other end point is characterized by supercolonies in which individuals mate and mix randomly. From that perspective, the genetic population structure of *F. exsecta* lies somewhere between the two end points with the structuring between nests using worker pupae lying closer towards the multicolonial end and the structuring between nests using adult workers lying closer towards the unicolonial end of the continuum.

Our study is the first showing that genetic structuring between nests differs depending on whether worker pupae or adult workers were analysed with the data conforming to restricted queen but free worker dispersal. This dispersal behaviour is in sharp contrast to invasive unicolonial ant species like the argentine ant (*Linepithema humile*) and the little fire ant (*Wasmannia auropunctata*), where apart from workers also queens and brood freely mix between nests (Giraud *et al.* 2002; Errard *et al.* 2005; Jaquiery *et al.* 2005). This discrepancy might be a fundamental difference between native and introduced unicolonial species. However, because there are no other studies on native species differentiating between queen and worker dispersal, the latter conclusion remains speculative.

Although reports of ant species exhibiting unicolonial population structures are increasing, the question remains: what is the benefit of free dispersal? It has been suggested that by reducing the costs associated with territoriality, unicoloniality allows higher worker densities and more effective habitat monopolization (Holway *et al*. 1998; Holway & Suarez 2004; Debout *et al*. 2007). Although this might be true and probably also applies to *F. exsecta*, it does not explain the effect of free dispersal on the erosion of relatedness, which leads to an evolutionary paradox in that reproductive altruism can no longer be explained by kin selection. However, our analyses show that the erosion of relatedness is incomplete such that there was a significant genetic structuring between nests at the population level, whereas no structuring was observed between nests at the transect level. These findings raise the question of the most relevant scale to use when investigating the role of kin selection in maintaining reproductive altruism in unicolonial species. Theoretical studies have shown that relatedness relevant for kin selection should be measured at the level of the 'economic neighbourhood', the scale at which intraspecific competition generally takes place (Pollock 1983; Taylor 1992; Kelly 1994; Queller 1994; West *et al*. 2002). If the relevant spatial scale at which competition in *F. exsecta* occurs is small (i.e. represented by the transect level in our study), the nestmate relatedness estimate ($r = 0.006$) is undistinguishable from zero. Thus, the maintenance of reproductive altruism could not be explained by kin selection at this spatial scale of competition. By contrast, if the relevant spatial scale at which competition in *F. exsecta* occurs is larger (i.e. represented by the population level in our study), our nestmate relatedness estimate ($r = 0.051$) is significantly greater than zero, with kin selection being a plausible mechanism explaining the maintenance of reproductive altruism in *F. exsecta*. Although the exact scale at which intraspecific competition occurs in unknown, the studied population is quite small (Fig. 1, maximum distance between two nests = 272 m); thus, it seems possible for intraspecific competition to occur at the population scale.

In conclusion, this study reveals a new type of social organization among group-living animals, whereby queens of the ant *F. exsecta* form extended family-based nests (multicolonial population structure), whereas workers mix freely between nests (unicolonial population structure). Such a social organization might be a common characteristic of other ant species forming unicolonial populations in their native habitats. However, our analyses also showed that the population genetic structure cannot simply be classified as being either multicolonial or unicolonial, but rather lies on a continuum of social organizations ranging from strictly family-based nests where all individuals return to their parental nest, to supercolonies where all individuals mix freely between nests. While this study emphasizes the importance of considering the type of individuals used when interpreting genetic data, it also adds a new example to the incredible diversity of social organizations (Keller 2007) existing in social insects.

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