Highly variable social organisation of colonies in the ant *Formica cinerea*

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Social organisation of colonies was examined in the ant *Formica cinerea* by estimating the coefficient of genetic relatedness among worker nest mates. The estimates based on microsatellite genotypes at three loci ranged from values close to zero to 0.61 across the populations studied in Finland. These results showed that a fundamental feature of colonies, the number of reproductive queens, varied greatly among the populations. Colonies in some populations had a single queen, whereas the nests could have a high number number of queens in other populations. There was a weak but non-significant correlation between the genetic and metric distance of nests within two populations with intermediate level of relatedness. Differentiation among nearby populations (within the dispersal distance of individuals) in one locality indicated limited dispersal or founder effects. This could occur when females are philopatric and stay in the natal polygynous colony which expands by building a network of nest galleries within a single habitat patch.

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Many ants have colonies with a single reproductive queen (monogyny), and HÖLLDOBLER and WILSON (1977) hypothesised two categories where a shift to multiple-queen colonies (polygyny) have taken place. These are habitat specialists and tramp species with easily fragmenting colonies. Ant species with diverse social types, ranging from monogyny to high level of polygyny, provide interesting cases to test the proposed hypotheses. Variation of the colonial type is connected both to the social evolution by kin selection and to the ecology of the species in relation to the environment. Habitat saturation and nest site limitation have been suggested to lead to increased levels of polygyny in ants of the genera *Formica* (PAMILO 1981), *Leptothorax* (HERBERS 1986; HEINZE 1993) and *Myrmica* (PEDERSEN and BOOMSMA 1999). SEPPÄ et al. (1995) observed increased polygyny associated to the age of the habitat after human-caused disturbance and recolonisation. The number of queens per nest has a continuous distribution in these cases, whereas some species show extreme dichotomy in their social organisation as the polygynous colonies burst into networks of interconnected nests with very high numbers of queens in the genus *Formica* (PAMILO and ROSENGREN 1984; SUNDSTRÖM 1993) and in the introduced fire ant *Solenopsis invicta* (ROSS and KELLER 1995). Such shifts have not been clearly associated to any evident environmental factor.

Polygyny and monogyny are also connected to colony foundation. New nests can be established independently by dispersing females, or dependently with the help of workers (HÖLLDOBLER and WILSON 1977). In the latter case, dispersal of females is restricted. Dispersal can be yet more restricted if females are philopatric and remain in their natal nests, which can be the case in multiple-queen (polygynous) colonies (ROSS 2001). Dispersal and the mode of nest founding affect spatial genetic differentiation, and there is a link between the social organisation of colonies and the genetic structure of populations (PAMILO and CROZIER 1997). Genetic studies of ants indicate that species that build large polygynous colonial networks have commonly restricted dispersal and genetically well differentiated populations (SUNDSTRÖM 1993; SEPPÄ and PAMILO 1995; CHAPUISAT et al. 1997).

A preliminary enzyme electrophoretic study indicated that the ant *Formica cinerea* may show social polymorphism in its colonial structure and restricted gene flow between nearby populations (LINDSTRÖM et al. 1996). However, the low variability of allozymes prevented estimation of relatedness in most study populations. DNA microsatellites provide powerful tools for studying the organisation of social insect colonies (PAMILO et al. 1997; ROSS 2001). Here we apply microsatellites to study the genetic structure of colonies and populations of *F. cinerea*. Our aims are

Table 1. *Sample sizes and the observed variation (number of alleles and expected heterozygosity) in the F. cinerea populations for each locus.*

		locus FL12		locus FL20		locus FL29	
	nests	$N_{alleles}$	H_{exp}	$N_{alleles}$	H_{exp}	$N_{alleles}$	H_{exp}
Hanko A	4	3	0.17	3	0.30	2	0.20
Hanko B	11	4	0.61	5	0.52	2	0.31
Hanko C	6	4	0.67	5	0.64	2	0.40
Kalajoki	17	5	0.62	4	0.71	2	0.50
Siikajoki	9	4	0.56	5	0.71	2	0.30
Kontiolahti A	5	4	0.47	5	0.75	2	0.38
Kontiolahti B	5	3	0.43	5	0.72	3	0.46
Kontiolahti C	5	5	0.58	5	0.66	2	0.44
Sotkamo	21	5	0.56	5	0.74	2	0.46

to examine both variation in social structures of colonies and genetic differentiation within populations.

MATERIAL AND METHODS

Samples

Formica cinerea inhabits open sandy areas where it digs nest galleries in the soil. In northern Europe, the species lives in largely isolated populations occupying sandy habitat patches, for example beaches by the sea or lakes. The species is known from only a few locations in Finland (LINDSTRÖM et al. 1996) and the nests are found within small patches of suitable habitat. We sampled populations in five localities in Finland, separated by 100–600 km from each other (Table 1, Fig. 1). Three of the localities (Hanko, Kalajoki, Siikajoki) are coastal areas along the Baltic Sea coast. The other two localities (Kontiolahti, Sotkamo) are inland by lake shores. In two localities (Hanko, Kontiolahti) we sampled three populations (designed A–C) each, the distances between populations being 2–5 km in Hanko and 10–20 km in Kontiolahti.

In each population, workers were collected from nest entrances that were separated by at least two metres, and in some cases there were other entrances in-between those that were used for sampling. The most distant nests within any population were maximally a few hundred metres apart.

DNA isolation and microsatellite analysis

Ants were stored at $-70°$ C or in absolute ethanol until analysis. DNA was extracted either by SDS/proteinase K digestion, phenol-chloroform purification and ethanol precipitation, or by a quick chelex method. Microsatellite variation was examined at the loci FL12, FL20 and FL29 using the primers designed originally for *Formica paralugubris* (CHAPUISAT 1996). The PCR conditions were the same as described for *F. paralugubris* (CHAPUISAT 1996). The amplification products were labelled internally by adding 0.02 µl of ^{33}P-dATP to each PCR, and were separated on 6 % denaturing polyacrylamide gels. In total, 477 worker individuals from 83 nests were genotyped (Table 1).

Statistical analyses

Genetic variability was measured as the number of alleles and heterozygosity expected under the Hardy-Weinberg assumptions. Measures of population allele frequency may be biased when samples consist of nest mates, because the genotypes sampled from a single nest are not independent of each other. To avoid this

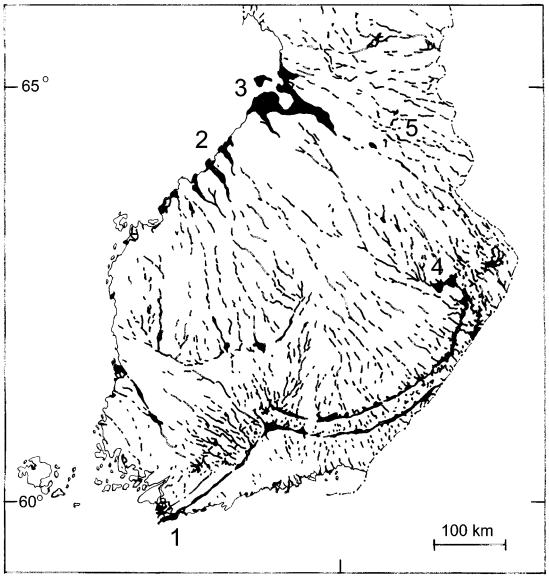

Fig. 1. Map of the sampling localities as follows: 1. Hanko, 2. Kalajoki, 3. Siikajoki, 4. Kontiolahti, and 5. Sotkamo. The black areas indicate the coastal dune fields and the glaciofluval eskers and end moraines that form suitable habitats for *Formica cinerea*. The map is from HELLEMAA (1998).

problem, we estimated the allele frequencies within populations by weighting each nest according to the formula (7) of PAMILO (1990) which takes into account the genetic redundancy of related nest mates. The results were very similar when we used this weighting scheme or weighted each nest equally.

Deviations from the Hardy-Weinberg genotype ratios were tested using the Markov-chain exact test of GUO and THOMPSON (1992) implemented in the program GENEPOP (RAYMOND and ROUSSET 1995, 1996). This program was also used to test linkage disequilibrium. These tests were based on the number of individuals examined, and no correction was made to compensate for the relatedness of nest mates. This increases the type II error of the tests.

The genetic relatedness of worker nest mates was estimated from the genotypic data with the program GENREL97 that uses the algorithm of PAMILO (1990), based on the methods of PAMILO (1984) and QUELLER and GOODNIGHT (1989). Standard errors were obtained by jackknifing over the nests.

Genetic differentiation between populations was estimated with the F-statistics, using the algorithm of WEIR and COCKERHAM (1984). As nest mate workers were closely related to each other in some cases (see below), the individuals could not be considered as independent genotypes. The estimation of population differences was therefore done by sampling at random one individual from each nest, and repeating the sampling 1,000 times (ROSS and SHOEMAKER 1993). These samples were used to calculate the mean values of the numerator and denominator of the Weir-Cockerham estimator of F_{ST}, and the final estimate was obtained as the ratio. The standard errors were calculated by jackknifing over loci and over populations. Geographic differentiation within populations was examined by correlating genetic and metric distances between pairs of nests. The correlation between the two distance matrices (genetic and metric) was tested with the Mantel test using the program of MANLY (1985).

RESULTS

Genetic diversity

All the loci were polymorphic in all the populations studied (Table 1). Altogether five alleles were detected at locus FL12, eight alleles at FL20 and three alleles at FL29. Ten out of the 16 alleles were detected in all five geographical locations, and six of the alleles (two at each locus) were detected also in each population. Unique alleles were present with low frequencies (less than 5 %) at the locus FL20 (one allele in Siikajoki and two alleles in Hanko-B) and locus FL29 (one allele in Kontiolahti-C).

The frequencies of some common alleles varied considerably among the populations. The most notable examples were given by two alleles at locus FL20 that had low frequencies in the pooled samples in Hanko (1 % and 4 %) while their frequencies in the other populations ranged from 9 to 42 % and from 14 to 41 %, respectively. The expected mean heterozygosity was lowest at each locus in the population Hanko-A, while all the other populations showed very similar levels of variation (Table 1).

Linkage disequilibrium between pairs of loci within populations was tested with the exact test by using all the individuals as independent observations. Four out of 27 tests gave a significant result, namely between the loci 12 and 20 in Kalajoki, Hanko-B and Kontiolahti-B, and between the loci 20 and 29 in Hanko-B. As the genetic data were partly redundant because of relatedness of nest mates (see below), we concluded that there was no clear evidence for population-wide linkage disequilibrium between the markers. Significancies also disappeared after Bonferroni correction.

Exact tests for the probability of deviation from the Hardy-Weinberg expectations revealed significant departures from equilibrium in Sotkamo, Hanko-A and –C (Table 2). In Hanko this deviation could be due to small samples consisting of few families. The departure in Sotkamo was moderate ($F = 0.09$), and was mostly due to the deficiency of heterozygotes at locus FL29 ($F = 0.29$, $P = 0.0037$).

Table 2. *The mean estimates of inbreeding (F) and genetic relatedness among nest mates (r). The three sites of Kontiolahti are pooled for this analysis, and the population of Sotkamo includes only the patch A with 16 nests.*

	locus FL12		locus FL20		locus FL29		all loci	
	F	*r*	*F*	*r*	*F*	*r*	$F \pm$ s.e.	$r \pm$ s.e.
Hanko A	−0.08	−0.15	0.37	−0.02	0.33	0.22	0.24 ± 0.07	0.03 ± 0.09
Hanko B	−0.09	0.71	0.11	0.58	−0.23	0.45	-0.05 ± 0.10	0.61 ± 0.08
Hanko C	−0.11	0.50	−0.36	0.36	−0.01	0.06	-0.18 ± 0.17	0.34 ± 0.11
Kalajoki	0.05	0.24	0.10	0.30	0.11	0.20	0.09 ± 0.04	0.25 ± 0.07
Siikajoki	−0.05	0.23	0.08	0.26	0.29	0.24	0.07 ± 0.09	0.25 ± 0.09
Kontiolahti	−0.12	0.21	0.17	0.11	0.02	−0.04	0.04 ± 0.04	0.10 ± 0.05
Sotkamo A	0.03	0.09	0.10	0.09	0.16	0.04	0.09 ± 0.03	0.08 ± 0.03

There was a slight deficiency of heterozygotes in most other populations, but the average inbreeding coefficients were below 0.1 and not significantly different from zero (Table 2).

Social structures

The mean relatedness of worker nest mates varied widely among and within the populations (Table 2). The lowest and highest estimates came from different populations in Hanko, 0.03 in Hanko-A and 0.61 in Hanko-B. The estimate from Hanko-C was intermediate (0.34). It should be noted that the estimates from Hanko-A and –C were based on small numbers of nests. The high estimate in Hanko-B was not associated to any sign of inbreeding.

Two other coastal populations, Kalajoki and Siikajoki, showed intermediate relatednesses, $r = 0.25$. Both populations also showed an excess of homozygotes. If the positive values of F were due to inbreeding, the relatedness estimates can be partitioned into components reflecting the number of matrilines and patrilines within colonies on the one hand, and the effects of inbreeding on the other (PAMILO 1984). The estimates of the component reflecting the number of matrilines and patrilines, given as $[r - 2F/(1 + F)]/[1 - 2F/(1 + F)]$, were 0.13 and 0.11 for these two populations, respectively.

All three populations in Kontiolahti had uniformly low relatedness, as did the estimate after pooling these populations (Table 2). The relatedness in the Sotkamo population appeared moderately high ($r = 0.21$) and the population also showed an excess of homozygotes ($F = 0.10$). Both values were affected by a subdivision in the population as the sample consisted of 16 nests in one continuous patch (patch A) along the beach and five nests located on some 250 m away (patch B). Using only the nests from patch A removed most of the homozygote excess and the relatedness estimate dropped to $r = 0.08$ (Table 2). The distribution of relatedness, when calculated separately for each nest, had a larger standard deviation in the samples with intermediate relatedness (Kalajoki $r = 0.25$, s.d. $= 0.29$) than in samples with low relatedness (Sotkamo $r = 0.08$, s.d. $= 0.12$).

Dispersal and differentiation

Three nearby populations were sampled at two Finnish study localities, Hanko and Kontiolahti. The three populations in Kontiolahti had very similar allele frequencies, and the estimates of F_{ST} among them ranged for the three loci from -0.013 to 0.023. The mean value was $F_{ST} = 0.003$ with the standard error of 0.010 over loci and 0.003 over populations.

In contrast to the genetic homogeneity of the Kontiolahti populations, those in Hanko showed clear genetic differentiation. The single-locus estimates of F_{ST} ranged from 0.005 to 0.085, with a mean of 0.042. The standard error over loci was 0.030, but that over populations was 0.070, indicating that the differentiation was largely due to departing allele frequencies in the population Hanko-A.

Differentiation within populations was examined as a function of distance. Restricting the analysis to the contiguous populations, the correlation between the genetic distance (measured as pairwise F_{ST}) and the metric distance between pairs of nests was slightly positive although not significant in Sotkamo-A (correlation 0.12, $P = 0.08$ with Mantel's test) and in Kalajoki (correlation 0.16, $P = 0.10$).

We pooled the nearby populations to obtain average allele frequencies for each of the major geographical localities. Such mean allele frequencies gave pairwise F_{ST} values between the Finnish localities within the range 0.014–0.144, with the mean of 0.077 (Table 3). This mean had a standard error of 0.012 over loci and 0.043 over localities. The pairwise differences showed that the pooled Hanko population differed most from all the others, with the pairwise F_{ST} values > 0.1. Estimates between all the other populations were below 0.07, and the closest pair was formed by the northern populations of Sotkamo and Kalajoki ($F_{ST} = 0.014$). The differentiation of the Hanko populations was largely due to the sample from Hanko-A. If we take the Hanko populations separately, the distances to the other populations ranged from 0.18 to 0.38 for Hanko-A, from 0.09 to 0.19 for Hanko-B and from 0.027 to 0.10 for Hanko-C.

DISCUSSION

The relatedness among worker nest mates varied widely, both within and among populations. The average relatedness ranged from values close to zero to 0.61 across populations (Table 2). The relatedness estimated with microsatellites in Hanko-B and Kontiolahti (0.61 ± 0.08 and 0.10 ± 0.05, respectively) agreed with the preliminary estimates based on one biallelic enzyme locus (0.81 ± 0.11 and 0.01 ± 0.05, respectively, LINDSTRÖM et al. 1996). Relatedness

Table 3. *Pairwise F_{ST} values between the major localities.*

Sotkamo	Kontiolahti	Siikajoki	Hanko	
Kalajoki	0.014	0.045	0.069	0.111
Sotkamo	–	0.037	0.034	0.105
Kontiolahti		–	0.063	0.144
Siikajoki			–	0.131

could not be estimated by allozymes in the other populations due to lack of polymorphism. Microsatellites thus provided more detailed insight into the variation in social structure.

Two parameters, the number of matings by queens and the number of queens in the nest principally affect the relatedness among nest mate workers. Therefore, variation in relatedness reflects important changes in these elements of the breeding system. Maximally polyandry (multiple mating by a queen) could decrease the within-brood relatedness from 0.75 to 0.25. However, most *Formica* species studied so far have been largely monandrous or have showed paternity skew, and in the most polyandrous species the within-brood relatedness has reduced to 0.6 (PAMILO 1982, 1993; SUNDSTRÖM 1993; CHAPUISAT 1998). In contrast, polygyny may result in any level of relatedness depending on the number of queens, the relatedness among them, and the amount of skew in the partitioning of reproduction (ROSS 2001).

The genetic data revealed that the social organisation of *F. cinerea* varies greatly. The relatedness among nest mates and the array of genotypes found within nests suggest that each nest includes predominantly a single mated queen in some populations (e.g. Hanko-B). In contrast, workers came predominantly from a large number of queens in some other populations (e.g. Kontiolahti and Sotkamo), whereas the Kalajoki population was intermediate in the sense that the nests had, on average, a few queens with some variation among the nests. Similar variation of relatedness was detected among the *F. cinerea* populations in Sweden (GOROPASHNAYA et al. 2001).

Intraspecific variation of polygyny is known in ants. There are also a few species that show extreme social polymorphism with some populations being characterised by monogynous and monodomous colonies (each colony has a single queen and occupies one nest, these are called type M), whereas other locations are inhabited by polygynous and polydomous colonial networks (a colony occupies many nests and there are many queens per nest, these are called type P) (ROSS et al. 1996). Interestingly, such variation is found in several *Formica* species (PAMILO and ROSENGREN 1984; ROSENGREN et al. 1985; SUNDSTRÖM 1993). The pattern found in *F. cinerea* was not equally clear-cut and relatedness varied over a wide range of values. The variation of the social organisation of *F. cinerea* seemed not to be connected to obvious differences in the age, type or size of the habitat, as all sampling sites consisted of relatively young habitat along coasts.

Genetic studies have connected variation in the social organisation to differences in dispersal and spatial differentiation. *Formica* species that build type P colonial networks tend to show some viscosity and the genetic differentiation increases with the distance within populations (PAMILO and ROSENGREN 1984; CHAPUISAT et al. 1997). This was also seen in *F. cinerea* in Kalajoki and Sotkamo, even though the correlations were not significant. Such a pattern could result from drifting of workers between nearby nests, or from limited dispersal of sexual individuals (particularly of queens), or both. At a somewhat larger scale, several species with polygynous and polydomous colonies have shown clear differentiation between habitat patches within potential dispersal distances of ant queens. Estimates of F_{ST} among populations with type P colonies were 0.19 in *F. truncorum* (SUNDSTRÖM 1993) and 0.18 in *Myrmica rubra* (SEPPÄ and PAMILO 1995) within study areas of a few square kilometres. The estimates within similar geographic scales among populations with predominantly type M colonies were 0.03 in *F. truncorum* and 0.03 in *M. ruginodis*, a close relative of *M. rubra*. Furthermore, gene flow between the M and P types within a species is restricted in the introduced fire ant *Solenopsis invicta* (ROSS et al. 1996).

The preliminary allozyme study showed a clear difference between two populations of *F. cinerea* in Hanko. One peptidase allele had a frequency of 0.43 at one site but was missing at the other site some five kilometres away (LINDSTRÖM et al. 1996). The present microsatellite results confirmed partly this initial observation, as there was a strong genetic differentiation among the Hanko populations ($F_{ST} = 0.061$). Departing allele frequencies in the population with least polygynous colonies, Hanko-A, largely caused this differentiation. The populations in Kontiolahti, where the relatedness was uniformly low and consequently the level of polygyny high, showed no spatial differentiation. The results did not in this respect follow the pattern detected in other species. It seems that genetic differentiation depends not only on the social type of colonies (M or P) but also on the fragmentation of the habitat and discontinuities of the populations.

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