Ann. Zool. Fennici 42: 179–187 ISSN 0003-455X
Helsinki 28 June 2005

Genetic patchwork of network-building wood ant populations

Pekka Pamilo[1,2]*, Danqing Zhu[1,3], Wilhelm Fortelius[4], Rainer Rosengren†, Perttu Seppä[1,5] & Liselotte Sundström[5]

[1)] *Department of Conservation Biology and Genetics, EBC, Uppsala University, Norbyvägen 18 D, SE-752 36 Uppsala, Sweden*
[2)] *Current address: Department of Biology, P.O. Box 3000, FI-90014 University of Oulu, Finland (*corresponding author's e-mail: pekka.pamilo@oulu.fi)*
[3)] *Current address: Neurobiology Laboratory, ANZAC Research Institute, NSW 2139, Australia*
[4)] *Sydväst Polytechnic, Aronia, Raseborgsvägen 9, FI-10600 Ekenäs, Finland*
[5)] *Current address: Ecology and Evolutionary Biology Unit, Department of Biological and Environmental Sciences, Biocenter 3, P.O. Box 65, FI-00014 University of Helsinki, Finland*

Received 7 Dec. 2004, revised version received 7 Mar. 2005, accepted 14 Mar. 2005

Pamilo, P., Zhu, D. Fortelius, W., Rosengren, R., Seppä, P. & Sundström, L. 2005: Genetic patchwork of network-building wood ant populations. — *Ann. Zool. Fennici* 42: 179–187.

Genetic organization of colonies and populations of the ant *Formica aquilonia* were studied at the edge of the urban area of the city of Helsinki within an area of about 400 km^2. Over six thousand old queens and workers were sampled from a total of 288 nest mounds from 14 populations (patches of forest) for an allozyme study, and workers from 13–15 nests in each of three populations were also characterized by microsatellite genotyping. Genetic relatedness among nest mates within populations was close to zero for both queens (estimates ranging from 0.02 to 0.13) and workers (from 0.01 to 0.22), with some of the estimates being significantly greater than zero. These results supported the view of a high level of polygyny within the nests. The populations showed significant genetic differences both at the allozyme loci (overall F_{ST} = 0.17) and at the microsatellites (F_{ST} = 0.24). The estimates of F_{ST} between pairs of populations varied from 0.01 to 0.61, the largest values being associated to reduced genetic variation and an apparent bottleneck within one population. The results showed that the local populations of this highly polygynous (multiple queens in a nest) and polydomous (multiple nests in a colony) ant can be differentiated genetically within potential dispersal distances, suggesting restricted dispersal and possible bottlenecks when colonizing new patches of forest.

Introduction

The evolutionary consequences of social interactions at the level of a colony depend on the genetic relationship of a colony and its neighbouring colonies. Theoretical models predict that the efficiency of kin selection in directing the evolution of social interactions within colonies depends on the geographical scales of population regulation and dispersal of individuals (*see* Kelly 1994, Chapuisat *et al.* 1997). The geographical scale is also essential for understanding popula-

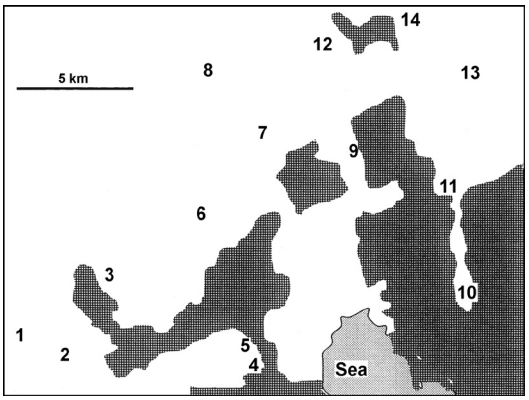

Fig. 1. Map of the study sites (1–14) on the north-western side of the city of Helsinki. Major urban areas are shown by shading.

tion dynamics, genetic differentiation, and speciation (Pamilo & Crozier 1997). These factors highlight the importance of studying the relationship between the social organisation of colonies and the spatial genetic structure of populations (Sundström *et al*. 2005).

An important determinant of the social colonial structure is the number of reproductive females (queens) in the colony. Many ants have a single queen per colony and new nests are normally started independently by dispersing females. Many species, however, have polygynous colonies with several to many reproductive queens, and dispersal in their populations can be restricted for two reasons. First, females may stay in their maternal colonies without dispersing at all, and second, new nests are often established in the neighbourhood by budding of existing colonies. As a consequence, coexisting queens in polygynous colonies are expected to be related to each other (Crozier & Pamilo 1996). The males often disperse more freely, but their dispersal can be restricted if they inbreed, if mating is confined to special mating places (e.g. hill-topping), or if they are behaviourally and morphologically adapted to low dispersal (Sundström 1995). As a result, a social unit (a network of interconnected nests) may simultaneously function as a mating unit (a deme). Such a population structure gives good opportunities for genetic differentiation among local populations. Genetic studies in species with highly polygynous colonies have shown viscosity of populations with genetic differentiation increasing gradually with distance between nests in several cases (Pamilo 1983, Crozier *et al*. 1984, Pamilo & Rosengren 1984, Chapuisat *et al*. 1997).

Some ant species with polygynous colonies develop polydomy, they build colonial networks with interconnected nests (Rosengren & Pamilo 1983). Highly polydomous colonies are well known in the ant genus *Formica* as shown by the supercolonies of *F. paralugubris* in the Jura mountains (Cherix 1980, Pamilo *et al*. 1992), *F. truncorum* in Finland (Elias *et al*. 2005) and *F. yessensis* in Japan (Higashi 1983). The supercolony of *F. paralugubris* in the Swiss Jura mountains shows clear spatial differentiation that is due to viscosity and differentiation as a gradual function of distance within some hundreds of metres (Chapuisat *et al*. 1997). The pattern agrees with the interpretation that most matings take place within nests and most nests are produced by budding, although there must also be some long-distance dispersal resulting in nest-founding outside the boundaries of the supercolony (Chapuisat *et al*. 1997).

The mound-building red wood ant *F. aquilonia* has polydomous colonies that dominate boreal coniferous forests in northern Europe. The dynamics and dispersal of this species are thus of great importance to large ecosystems. We will here examine the genetic structure of colonies and genetic differentiation of populations in *F. aquilonia* within an area where the forest has been recently fragmented by human activities. The distances between the populations living in separate forest fragments are within the range of potential individual dispersal. The results show that ants with highly polygynous and polydomous colonies can show large genetic differences among closely located populations.

Material and methods

Samples

We sampled a total of 14 populations of *Formica aquilonia* from patches of coniferous forests within a 20 × 20 km^2 area around the city of Helsinki, southern Finland (Fig. 1). We refer to samples collected from a single forested area

as a population. Most of the material consisted of old reproductive queens (nine populations) that were sampled in spring 1991 (April to early May) from the mounds (Table 1). Only workers were sampled from five populations. Samples from all populations were analysed using enzyme electrophoresis. Based on these results, workers were later (1997) sampled from three populations (Solkulla, Martinlaakso, Veromiehenkylä) for a complementary microsatellite analysis (Table 1).

Enzyme electrophoresis

Enzyme electrophoresis was done as explained by Pamilo (1993). The enzymes used in this study were glucosephosphate isomerase (GPI), peptidase (PEP, glycyl-leucine as substrate), phosphoglucomutase (PGM), esterase (EST), phosphoglycerate kinase (PGK), and phosphogluconate dehydrogenase (PGD). The loci are denoted by italics, and the alleles are denoted according to the relative mobility of the respective allozymes using 100 for the predominant alleles. Earlier family studies showed that the loci are not linked to each other and that the alleles segregate in a Mendelian fashion (Pamilo 1993).

DNA extraction and microsatellite amplification

DNA was isolated from frozen samples with 5% Chelex (BIO-Rad) according to Thorén *et al.* (1995). Four microsatellite loci (FL12, FL20, FL21 and FL29), designed originally for *Formica paralugubris* (Chapuisat 1996), were used. PCR was carried out in a volume of 10 μl containing 1 μl DNA template, 400 nM of each primer 75 μM each dGCT, dCTP and dTTP, 6 mM dATP, 1.5 mM $MgCl_2$, 20 μg ml^{-1} BSA, 1 X PCR buffer (following Finnzyme's recommendation) and 0.4 units of DynaZyme thermostable DNA polymerase (Finnzyme). The reaction was incorporated with α-^{33}P-ATP. PCR was performed under the following conditions: 94 °C for 3 minutes, then 35 cycles of 94 °C for 30 seconds, 52 °C for 30 seconds, 72 °C for 50 seconds, and a final extension of 72 °C for 5 minutes. When the amplification was unsuccessful, the reaction was repeated under the same conditions, except that 1/10 of one of the two PCR primers was endlabeled by γ-^{33}P-ATP (Sambrook *et al.* 1989) instead of α-^{33}P-ATP incorporation. This method gave products for some samples which failed to amplify when the incorporation method was used. The PCR products were run on 6% denaturing polyacrylamide gels. The allele sizes were determined by running samples against a size marker (pSK+/universal sequencing reaction).

Statistical analyses

Genetic variability was analysed with GENEPOP version 3.0 (*see* Raymond & Rousset 1995). Deviations from the Hardy-Weinberg genotype ratios were tested using the Markov-chain exact test of Guo and Thompson (1992) implemented in GENEPOP. GENEPOP was also used to test linkage equilibrium. We should note that these statistical tests may elevate type I error and be oversensitive as the tests were based on the number of individuals examined, and no correction was made to compensate for the relatedness of nest mates.

Table 1. The population samples. *n* = the number of nests sampled, *N* = the number of individuals genotyped.

Population	Queens		Workers/ allozymes		Workers/ microsatellites	
	n	*N*	*n*	*N*	*n*	*N*
1. Espoonkartano	12	188	–		–	
2. Pelto	17	374	–		–	
3. Solkulla	40	658	33	752	15	67
4. Mankkaa	–		6	90	–	
5. Klovis	17	362	–		–	
6. Träskända	22	512	47	908	–	
7. Askisto	–		11	216	–	
8. Kalajärvi	–		11	190	–	
9. Martinlaakso	16	260	–		15	58
10. Keskuspuisto	–		34	660	–	
11. Paloheinä	17	288	–		–	
12. Keimola	19	356	–		–	
13. Veromiehenkylä	–		14	272	13	47
14. Riipilä	21	384	–		–	
Total	181	3382	156	3088	43	172

The social structure of *F. aquilonia* was examined by estimating genetic relatedness (Pamilo & Crozier 1982) using the algorithm developed by Pamilo (1984) and Queller and Goodnight (1989). The standard errors were estimated by jackknifing over nests as suggested by Crozier *et al*. (1984) and Pamilo (1984). The relatedness estimate (r) was partitioned into components reflecting the number of matrilines and patrilines (r*) and the effects of putative inbreeding (Pamilo 1984, 1989).

Genetic differentiation among populations was estimated with F-statistics, using the algorithms of Weir and Cockerham (1984) as implemented in FSTAT (Goudet 1995). The estimates of F_{ST} (θ of Weir & Cockerham 1984) was calculated both by weighting each population with the sample size and by sampling a subset of nests from the populations in order to avoid the biasing effect of large samples. The standard errors were calculated by jackknifing over the loci (Pamilo 1984, Weir & Cockerham 1984). The program FSTAT (Goudet 1995) was also used to calculate the pairwise F_{ST} estimates between populations and to test deviations from zero by the use of permutations. Correlation between the genetic distances (pairwise F_{ST}) and geographical distances was tested with Mantel's test using the program of Manly (1985).

Results

Allozyme diversity

A total of 3382 queens from 181 nests and 3088 workers from 156 nests (Table 1) were examined for allozyme variation. Two allozyme loci had four alleles, three loci had three alleles and the remaining locus was biallelic (Table 2). Some of the alleles were very rare and only two loci were polymorphic in all populations. Pooled information from the allozyme loci allowed estimating parameters within populations (inbreeding, relatedness of nest mates) and differentiation among them. The frequencies of the most common alleles varied greatly among the populations, the population 13 (Veromiehenkylä) having the most deviating frequency at each locus except the largely monomorphic *Pgd*. Even when excluding this population, the frequencies ranged over wide spectra, from 0.23 to 0.89 at *Gpi*, from 0.41 to 0.94 at *Pep*, from 0.44 to 0.88 at *Pgm*, from 0.37 to 0.82 at *Est*, and from 0.46 to 1.00 at *Pgk* (Table 2). The mean expected heterozygosity over loci was 0.13 in Veromiehenkylä and ranged from 0.26 to 0.35 in the other populations (counting only the eleven populations in which all six loci were scored).

Table 2. Allele frequencies (%) at the allozyme loci within the populations, and the mean expected heterozygosity per locus. The heterozygosity values in parentheses are not directly comparable with the others as they are based on only five loci. – = the allele was not found; 0 = the frequency of the allele was below 0.5%; n.a. = not available, the locus could not be screened reliably.

	Gpi			*Pep*		*Pgm*				*Est*				*Pgk*			*Pgd*			Het
Pop	100	70	30	100	80	120	100	85	70	120	100	70	30	120	100	70	140	100	70	
1	84	16	–	92	8	32	64	–	4	37	62	1	–	–	57	43	–	100	–	0.31
2	83	17	–	94	6	21	63	0	16	34	64	2	–	–	46	54	–	100	–	0.32
3	78	22	–	85	15	17	75	–	8	35	63	2	–	0	55	45	–	99	1	0.33
4	70	30	–	55	45	–	44	–	56	16	81	3	–	–	100	–	n.a.	na	n.a.	(0.35)
5	71	29	–	75	25	–	59	–	41	13	82	5	–	–	100	–	–	100	–	0.26
6	76	24	–	82	18	4	80	1	15	15	68	17	0	–	65	35	–	98	2	0.33
7	77	23	–	79	21	–	81	–	19	24	60	17	–	–	98	2	–	74	26	0.33
8	23	77	–	41	59	n.a.	n.a.	n.a.	n.a.	31	69	–	–	–	82	18	1	95	4	(0.33)
9	89	11	–	58	42	9	88	–	3	27	70	3	–	–	88	12	–	100	0	0.26
10	47	53	–	70	30	–	69	–	31	35	55	10	–	–	76	24	n.a.	na	n.a.	(0.46)
11	57	43	–	83	17	–	74	1	25	37	55	8	–	–	77	23	–	97	3	0.35
12	31	69	0	80	20	–	86	3	11	19	72	9	–	–	96	4	–	96	4	0.26
13	12	88	–	100	–	–	100	–	–	83	17	–	–	–	16	84	–	100	–	0.13
14	73	27	0	72	28	1	60	1	38	20	72	8	0	–	88	12	–	100	–	0.32

Microsatellite diversity

Three to five workers from 13–15 nests in three populations (total 172 individuals, Table 1) were examined for microsatellite variation. In FL21 and FL29, 4% of the individuals failed to produce detectable microsatellite PCR products with either α-^{33}P-ATP incorporation or γ-^{33}P-ATP endlabeling method, which could be due to null alleles (e.g. Callen *et al.* 1993). However, only one locus × population combination (FL21 in Martinlaakso) showed a significant heterozygote deficit ($F_{IS} = 0.21$, $P < 0.001$).

The total number of alleles when pooling the four microsatellite loci was 26, with clear differences among the populations. The samples from Martinlaakso had a total of 24 alleles, whereas Veromiehenkylä had only eight (Table 3). The locus FL20 had the largest overall number of alleles but was monomorphic in Veromiehenkylä. Only two loci, FL21 and FL29 were polymorphic in all three populations. Significant linkage disequilibrium was observed between FL21 to FL29 in Martinlaakso and FL12 to FL29 in Veromiehenkylä ($P < 0.001$ for both). As the same patterns were not consistent in all populations, we kept all the loci in the later analyses.

Genetic structure of nests and populations

The genotypic data were used to estimate genetic relatedness of nest mates within each population. Based on the allozymes, relatedness among both queen and worker nestmates was low, ranging from 0.02 to 0.22 (Table 4), with the mean of all populations being 0.13 for workers and 0.07 for

Table 3. The number of alleles at the microsatellite loci, the total number of alleles observed, and the mean expected heterozygosity (H_e) per locus in the three populations studied.

	FL12	FL20	FL21	FL29	Total	H_e
3. Solkulla	1	5	5	4	15	0.44
9. Martinlaakso	5	9	4	6	24	0.54
13. Veromiehenkylä	2	1	3	2	8	0.23

Table 4. Average genetic relatedness ($r \pm$ S.E.), inbreeding ($F \pm$ S.E.), and relatedness adjusted for inbreeding ($r^* \pm$ S.E.) of nest mates. The values denoted with * depart significantly from zero ($P < 0.05$).

	$r \pm$ S.E.	$F \pm$ S.E.	$r^* \pm$ S.E.
Workers, allozymes			
3. Solkulla	0.04 ± 0.02*	0.01 ± 0.02	0.03 ± 0.05
4. Mankkaa	0.22 ± 0.12	0.17 ± 0.09	–0.11 ± 0.19
6. Träskända	0.01 ± 0.01	0.03 ± 0.02	–0.05 ± 0.04
7. Askisto	0.19 ± 0.08*	0.05 ± 0.04	0.10 ± 0.09
8. Kalajärvi	0.13 ± 0.08	0.01 ± 0.05	0.11 ± 0.08
10. Keskuspuisto	0.15 ± 0.03*	0.09 ± 0.03*	–0.03 ± 0.06
13. Veromiehenkylä	0.15 ± 0.19	–0.01 ± 0.07	0.15 ± 0.19
Workers, microsatellites			
3. Solkulla	0.05 ± 0.06	0.04 ± 0.05	–0.03 ± 0.10
9. Martinlaakso	0.18 ± 0.06*	0.03 ± 0.08	0.13 ± 0.10
13. Veromiehenkylä	0.20 ± 0.16	0.06 ± 0.13	0.10 ± 0.35
Queens, allozymes			
1. Espoonkartano	0.04 ± 0.03	–0.01 ± 0.05	0.04 ± 0.03
2. Pelto	0.04 ± 0.02	0.01 ± 0.03	0.01 ± 0.07
3. Solkulla	0.04 ± 0.02*	–0.02 ± 0.03	0.04 ± 0.02
5. Klovis	0.02 ± 0.02	0.01 ± 0.04	0.01 ± 0.09
6. Träskända	0.05 ± 0.03	0.02 ± 0.03	0.00 ± 0.06
9. Martinlaakso	0.05 ± 0.08	0.05 ± 0.04	–0.05 ± 0.13
11. Paloheinä	0.13 ± 0.07	0.02 ± 0.05	0.09 ± 0.09
12. Keimola	0.10 ± 0.04*	0.01 ± 0.03	0.08 ± 0.06
14. Riipilä	0.13 ± 0.05*	0.00 ± 0.03	0.12 ± 0.05*

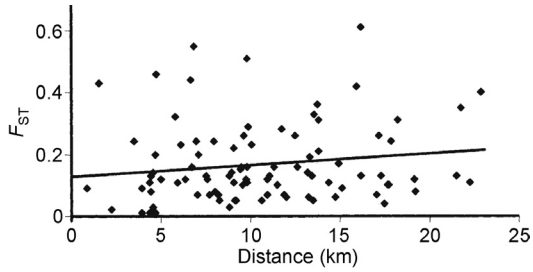

Fig. 2. Genetic differentiation (F_{ST}) as a function of the geographical distance between the populations (rank correlation $r_s = 0.23$, $P = 0.04$).

queens. Six out of the 16 estimates were significantly greater than zero (*t*-test: Solkulla queens $t_{39} = 2.07$ and workers $t_{32} = 2.29$, Askisto $t_{10} = 2.34$, Keskuspuisto $t_{33} = 5.37$, Keimola $t_{18} = 2.23$, Riipilä $t_{20} = 2.77$). The four largest relatedness estimates were from workers, but the overall worker and queen relatedness did not differ significantly from each other (Mann-Whitney's test: $U_{7,9} = 15$, $P > 0.05$). The inbreeding coefficient was fairly small in most populations, but the estimate was significant in Keskuspuisto. Removal of the effect of inbreeding on relatedness (Pamilo 1984) reduced all except one (Riipilä) of the relatedness estimates indistinguishable from zero (Table 4). Microsatellite estimates of worker relatedness generally agreed with the allozyme estimates in the sense that two of the estimates were moderately high (0.18 and 0.20), and the estimates adjusted for inbreeding were low (Table 4).

Population differentiation

As noted above, the allele frequencies showed considerable differences among the populations both for allozymes and microsatellites. All the loci and alleles gave a rather uniform pattern of differentiation. The allozyme loci were not completely independent of each other but some correlation between loci and alleles existed (Table 2). This can be at least partly explained by genetic similarity at several loci in closely located populations. In addition to the wide range of the frequencies of the common alleles, it is particularly worth pointing out the negative correlation between the frequencies of the alleles Pgm^{70} and Pgm^{120}, even when the frequency of the most common allele Pgm^{100} remained almost unchanged (Table 2). A high frequency of the allele Pgm^{120} (populations 1–3) was associated with a high frequency of the allele Pgk^{70}, whereas the populations 4 and 5 with a high frequency of Pgm^{70} lacked variation at *Pgk*. It was, however, not possible to identify clear haplotype groups as should be the case if the observed differentiation would be caused by an admixture of two genetically diverged source populations.

The overall F_{ST} from the allozymes was calculated by using both queen and worker data and resampling nests to equalize the sample sizes. Using an upper limit of 15 nests per population, resulted in $F_{ST} = 0.17 \pm 0.04$ (jackknifed over the loci). The result was the same with sample sizes ranging from 6 to 20 nests per population. The pairwise estimates between the populations showed a wide range of values from 0.03 to 0.60 (Fig. 2). The populations showing the largest genetic differences to any other population were Veromiehenkylä (mean of the pairwise estimates 0.42, the range being from 0.29 to 0.61) and Kalajärvi (mean 0.25, range 0.11 to 0.51). The most differentiated populations were at the northern edge of the study area (13. Veromiehenkylä, 8. Kalajärvi, 12. Keimola). The rank correlation between the genetic distance (F_{ST}) and the geographical distance between the populations was significant (Mantel's test: $r_s = 0.23$, $P = 0.04$). Some closely located populations formed genetically very homogeneous clusters (populations 1–3 and 10–11).

The microsatellites screened in three populations showed large differences in the allele frequencies. For example, only one allele was shared by all three populations at the locus FL20, although a total of ten alleles were found altogether. The dominant allele in both Solkulla and Veromiehenkylä (71% and 100%, respectively), was not the dominant allele in Martinlaakso (12%). The overall estimate of F_{ST} from microsatellites was significant ($F_{ST} = 0.24$, $P < 0.001$ by permutation test) but smaller than for the same populations from allozymes ($F_{ST} = 0.33$). Two of the three pairwise F_{ST} estimates based on microsatellites were higher than those based on allozymes (Table 5).

Discussion

Formica aquilonia is a common mound-building wood ant in northern Europe and it has been described as highly polygynous (Rosengren *et al.* 1993). The existence of a high number of queens means that the nestmates are expected to be only distantly related. This was also demonstrated in the present study. Particularly, relatedness of coexisting queens was close to zero. Polygyny is often associated with female philopatry, and as a result males also tend to mate with nest mate females (Chapuisat *et al.* 1997). Limited dispersal of polygynous species leads to population genetic viscosity and therefore increases relatedness among neighbours, maintaining the genetic cohesion of the colonies (Sundström *et al.* 2005).

The relatedness among both queens and workers was generally low, suggesting a high level of functional polygyny and exchange of queens between the nests within each population. Several estimates from workers were, however, greater than 0.15 while the relatedness of coexisting queens was consistently close to zero. There can be several explanations for this, although it is important to point out that the difference between queen and worker relatedness was not significant. First, reproductive skew among the queens could lead to uneven contributions and to somewhat elevated relatedness among the offspring (Reeve & Keller 2001). It is not obvious why the queens should contribute unequally to worker production, unless it is correlated with a corresponding unequal contribution to sexual production, or if subordinate queens produce mainly workers. The frequency of unmated queens has been observed to approach 20% (Rosengren & Pamilo 1986) which means that the effective level of polygyny is less than that indicated by the number of queens. Possible reproductive skew has not been estimated. Second, the average inbreeding coefficient among the workers (for allozymes) was slightly higher than among the queens, and the adjusted relatedness estimate r^* in workers was close to that of queens. Inbreeding among workers would agree with a similar observation in *F. paralugubris* (Chapuisat *et al.* 1997). The lack of significant inbreeding among the queens could then indicate stronger selection against inbred individuals in that caste. Third, the inbreeding estimates for workers from the microsatellites could have been affected by null alleles. Null alleles have been found in quite a number of studies (e.g. Callen *et al.* 1993, Pemberton *et al.* 1995). We could not amplify the loci FL21 and FL29 from all the individuals in Martinlaakso and Veromiehenkylä, and the Martinlaakso population showed deviations from the Hardy-Weinberg equilibrium at FL21 This suggests that null alleles may be present. However, the estimates of inbreeding and relatedness from the microsatellites did not markedly differ from those based on allozymes, so it is reasonable to conclude that null alleles did not play a major role in our results.

Mäki-Petäys *et al.* (2005) studied spatial genetic differentiation of *F. aquilonia* in a fragmented forest in Russia where the demography of the population had been followed for 30 years. The study showed that fragmentation had led to local population decline, colonisation of new forest fragments and differentiation associated to bottlenecks. Yet, the most notable genetic differences in that study were between subpopulations that had existed already before the recent disturbances. Those results showed that marked differences ($0.15 < F_{ST} < 0.20$) may develop under natural conditions between populations separated by less than a kilometre, and that recent human influence can initiate such differentiation by fragmenting the previously continuous habitat (Mäki-Petäys *et al.* 2005).

Our present study area was located in and around the urban area of the city of Helsinki. Forest fragmentation has increased within this area significantly within the last few decades, but the forests have been fragmented for considerably longer time because of various human

Table 5. Pairwise F_{ST} estimates between populations screened for microsatellites; above diagonal are the values based on microsatellites and below diagonal those based on allozymes.

	3	9	13
3	–	0.18	0.21
9	0.10	–	0.32
13	0.31	0.55	–

activities (e.g. agriculture). Thus, we do not know when the observed genetic differentiation has emerged. It is noteworthy to point out that the genetically most different population, based on both allozymes and microsatellites (Veromiehenkylä), had also least genetic variation. This indicates a bottleneck associated to colonisation and genetic differentiation, a pattern consistent with the populations with known demography (Mäki-Petäys *et al*. 2005).

Limited gene flow between established nests leads to genetic differentiation at a small geographic scale. Such differences have been reported in a number of polygynous species (e.g. Sundström 1993, Seppä & Pamilo 1995, Gyllenstrand & Seppä 2003, Seppä *et al*. 2004, *see* Sundström *et al*. 2005 for a review). The group of mound-building red wood ants (the *Formica rufa* group) which *F. aquilonia* belongs to, is known to be taxonomically problematic with hybridisation between several pairs of species (Seifert 1991, Czechowski 1996, Goropashnaya *et al*. 2004) and description of new species in spite of the large number of ecological studies (Seifert 1996). The mean estimate of F_{ST} among the populations agrees with, and the largest values even exceed, the interspecific estimates of F_{ST} between two other *Formica*, *F. polyctena* and *F. rufa*, living sympatrically within an area comparable to that of our present study (Gyllenstrand *et al*. 2004). However, the interlocus and interallele correlations did not show any evident pattern that would divide the populations studied here in separate groups that might represent a deeper taxonomical division. The association of differentiation with reduced variation suggests that bottlenecks, founder effects when colonising new forest patches and limited dispersal could explain the patterns of differentiation.

Highly variable microsatellites are known to reflect poorly genetic differentiation as measured by F_{ST} when the level of heterozygosity within populations is high, the upper limit of detectable F_{ST} equalling the homozygosity within populations (Hedrick 1999). The homozygosity at single loci and populations were all greater than 0.34, with mean values per population being greater than 0.46. We may thus expect that the estimates from allozymes and microsatellites could still be comparable.

We can conclude that the results agree with the findings from *F. aquilonia* at Peshki (Mäki-Petäys *et al*. 2005) in that nearby populations can show large genetic differences that have developed in the past history and that can be enhanced by human-caused habitat fragmentation. The results are also consistent with those detected in a related species *F. paralugubris*, which forms large supercolonies and shows spatial genetic differentiation within a continuous habitat due to restricted dispersal of sexuals and mating within nests (Chapuisat *et al*. 1997).

Acknowledgements

The study has been supported by grants from the Natural Sciences Research Council of Sweden, the Wenner-Gren Foundation and the Academy of Finland (grants 77311 to PP and 206505 to LS).

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