Ann. Zool. Fennici 44: 462–474 ISSN 0003-455X (print), ISSN 1797-2450 (online)
Helsinki 19 December 2007

Phylogeography and population structure in the ant *Formica exsecta* (Hymenoptera, Formicidae) across Eurasia as reflected by mitochondrial DNA variation and microsatellites

Anna V. Goropashnaya[1,2], Vadim B. Fedorov[1,2,3], Bernhard Seifert[4] & Pekka Pamilo[5]

[1)] *Department of Conservation Biology and Genetics, EBC, Uppsala University, Norbyvägen 18D, SE-75236 Uppsala, Sweden*
[2)] *Institute of Arctic Biology, University of Alaska, Fairbanks, AK 99775–7000, USA*
[3)] *Institute of Plant and Animal Ecology, Ural Division of RAS, Yekaterinburg, Russia*
[4)] *Staatliches Museum für Naturkunde Görlitz, Postfach 30154, 02806 Görlitz, Germany*
[5)] *Department of Biology, P.O. Box 3000, FI-90014 University of Oulu, Finland (present address: Department of Biological and Environmental Sciences, P.O. Box 65, FI-00014 University of Helsinki, Finland; e-mail: pekka.pamilo@helsinki.fi)*

Received 5 May 2007, revised version received 10 Oct. 2007, accepted 11 October 2007

Goropashnaya, A. V., Fedorov, V. B., Seifert, B. & Pamilo, P. 2007: Phylogeography and population structure in the ant *Formica exsecta* (Hymenoptera, Formicidae) across Eurasia as reflected by mitochondrial DNA variation and microsatellites. — *Ann. Zool. Fennici* 44: 462–474.

Phylogeography and population structure of the ant *Formica exsecta* was studied across Eurasia by using mtDNA sequences and microsatellite genotypes. The phylogeny based on 1.5 kb mtDNA fragment including the cytochrome *b* and part of the ND6 gene showed significant division (1.63% of nucleotide divergence) between a haplotype from Tibet and all other haplotypes. Similar to findings in diverse array of species associated with forest in Eurasia, the mtDNA phylogeny revealed no evidence for vicariant events due to separation in different forest refugia over glacial periods. The haplotype network includes several small clades (with 2–4 haplotypes in each) with geographically limited distribution, but one geographical region may have received haplotypes from two or more of such clades. This pattern could indicate mixing of different gene pools during postglacial colonization of Europe from different forest refugia or from an ancestral source with some spatial genetic differentiation. The genealogy and the haplotype frequencies suggest postglacial colonization of Siberia from a single refugial source of limited size. Maternal and biparental DNA markers indicated a moderate but significant level of population differentiation (mtDNA Φ_{ST} = 0.42, microsatellite F_{ST} = 0.13) across Eurasia. However, no correlation between genetic differentiation estimated for mtDNA and microsatellites was found among the populations. Considerable reduction in microsatellite genetic diversity was found in the small population of *F. exsecta* in England, giving some basis to classify this population as near threatened.

Introduction

Quaternary environmental changes strongly influenced the geographical distribution, demographic history and pattern of genetic variation in extant species (Hewitt 1996, Bennett 1997). During cold and dry glacial periods, most of northern Eurasia was covered by treeless vegetation (West 2000) and the distribution ranges of forest species were restricted to refugial areas. Palaeoecological evidence suggests that the forest species could have survived during the glacial periods not only in southern peninsular refugia (Iberia, Italy and Balkans, Hewitt 1999) but also in other forest refugia located in central Europe (the Carpathians, Willis *et al*. 2000), the north east coast of the Azov and Black Seas, the southern Urals, south Siberian mountains and Mongolia (Tarasov *et al*. 2000). The past isolation in separate glacial refugia and the routes of post-glacial colonization are reflected in the geographic patterns of intraspecific genetic variation (Hewitt 1996). Several phylogeographic studies have addressed the refugial history of boreal forest species in Eurasia. While some species of small mammals ecologically associated with temperate deciduous forests demonstrated phylogeographic divisions in Europe (Deffontaine *et al*. 2005, Kotlik *et al*. 2006, Michaux *et al*. 2003), no phylogeographic structure was found across the Eurasian taiga zone, a continuous belt of coniferous trees from Scandinavia to the Pacific coast, in several avian species (Kvist *et al*. 2001, 2003, Zink *et al*. 2002a, 2002b), the red wood ant *Formica lugubris* (Goropashnaya *et al*. 2004b) and the flying squirrel *Pteromys volans* (Oshida *et al*. 2005), all of which are associated with the boreal forest. Another species of the red wood ants, *Formica pratensis*, demonstrated the phylogeographic split in central Europe but no substantial structure across a vast geographic area from Finland to Baikal Lake (Goropashnaya *et al*. 2004b). Taken together, the limited phylogeographic structure across the most of northern Eurasia and genetic signs of a demographic expansion imply contraction of the distribution range of each species to a single refugial area at different times during the late Pleistocene. This pattern of genetic differentiation gives no indication for vicariant separation in numerous forest refugia suggested by palaeoecological evidence. Co-distributed species can respond differently to the same historical environmental changes and it is necessary to study phylogeography of a number of species to elucidate general patterns in the biotic history of the Eurasian boreal forest.

The Palaearctic ant *Formica exsecta* Nylander, 1846 is a common species that lives in the continuous zone of mixed and deciduous forests all over Eurasia, often at the forest edges and forest openings.

The species shows substantial phenotypic polymorphism throughout its Palaearctic range which is reflected by publication of nine taxonomic names since 1846 (Dlussky 1967, Seifert 2000). Only one of these taxa — *F. mesasiatica* Dlussky, 1964 that occurs in the Central Asian mountains — has actually been recognized as a sister species because of differing, homogeneous morphology and assumed geographical isolation (Dlussky 1967). Seifert (2000) provisionally accepted Dlussky's position but noted incomplete discrimination and expressed the need for reassessment. Recent morphological investigation of extended material did not provide convincing clarification of this issue (B. Seifert unpubl. data).

We studied geographic patterns of mtDNA and microsatellite variation to address the following questions. First, we examined the phylogeography, association between genealogy and geographic distribution of mtDNA haplotypes, to reveal signs of possible vicariant separation in different glacial refugia and routes of post-glacial colonization, including both *F. exsecta* and *F. mesasiatica*. Second, we used population level analysis combining mtDNA haplotype frequencies with haplotype genealogies, and allele frequencies in microsatellite loci to assess population differentiation possibly reflecting refugial separation over the shallow historical time that was too short for generating phylogeographic structure.

Previous studies have described *F. exsecta* as socially polymorphic, i.e. some populations consist of colonies with a single queen (monogyny) and some of colonies with many queens (polygyny) (Pamilo & Rosengren 1984, Seppä *et al*. 2004). Such shift in the social type may reflect behavioural plasticity depending on environmental factors, but it can also be to some extent

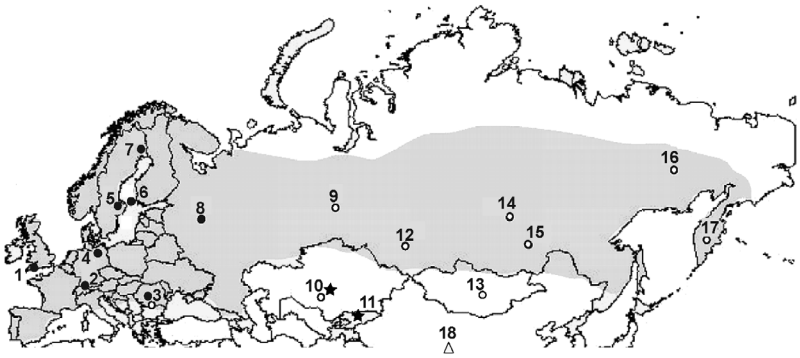

Fig. 1. The map showing the sampling localities of *Formica exsecta* and *F. mesasiatica*. The distribution area of *F. exsecta* is shown according to Dlussky (1967). The locality numbers are same as those in Table 1. The localities containing haplotypes from the geographic groups A–C (Fig. 2) are indicated with the following symbols: group A with a star, group B with a white circle, and group C with a black circle. The triangle designates a locality from Tibet.

genetically determined (Krieger & Ross 2002). We analysed the social organization of colonies in different geographical areas and considered its association with distinct phylogeographic or population groups to infer possible effects of population history on social organization.

Material and methods

Sampling and molecular techniques

For mtDNA analyses we examined samples (one specimen per nest) from 86 nests of *Formica exsecta* from 17 localities over most of the species distribution in Eurasia and from four nests of *F. mesasiatica* in Kyrgyzstan (Fig. 1 and Table 1). Exact geographic positions of sampling localities are available from the first author on request. All samples were stored in 70% ethanol until DNA extraction. Total genomic DNA was extracted from only the head and alitrank of single individuals with the DNeasy Tissue Kit (QIAGEN Inc.) or using Chelex-100 protocol (Thorén *et al*. 1995). A 1.5 kb mtDNA fragment including the cytochrome *b* gene (cyt *b*) and part of the NADH dehydrogenase subunit 6 (ND6) was amplified and sequenced with the following primers: Cytb-Fe-F (Liautard & Keller 2001), CB1, CB2, CB3, tR^S (Jermiin & Crozier 1994), and CB-11059, CB-11178, and CB-11449 (Goropashnaya *et al*. 2004b). Polymerase chain reaction (PCR) was carried out in 25 μl volumes containing 1 × PCR buffer, 2.0 mM $MgCl_2$, 0.4 μg/μl BSA, 0.2 mM dNTPs (Promega), 0.4 μM of each primer and 1.0 UTaq polymerase (Promega). A program for the amplification in a thermal cycler was used as follows: 3 min at 94 °C, 35 cycles of 30 sec at 92 °C, 30 sec at 45 °C, 1–2 min at 68 °C, and 10 min at 72 °C. Successful PCR products were cleaned with the QIAquick Gel Extraction Kit (QIAGEN Inc.) and then sequenced on an Applied Biosystems 3100 automated DNA sequencer using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). In total, 1481 base pairs were scored in 90 individuals.

Microsatellite variation in six populations of *F. exsecta* (localities 1–4, 6, 7; Fig. 1 and Table 2) was examined at six loci: FL20, FL21, FL29 (Chapuisat 1996), FE13, FE19, and FE49 (Gyllenstrand *et al*. 2002). The PCR was carried out for 4–5 workers from each nest (59 nests in total) according to Chapuisat (1996) and Gyllenstrand *et al*. (2002). The PCR products were analysed on 6% polyacrylamide gels and visualized using standard silver staining protocol (Bassam *et al*. 1991). Microsatellite data for Sweden (locality 5) were taken from the locality Bågskyttebana in Uppsala (Seppä *et al*. 2004).

Data analyses

Sequence variation and substitution patterns of the 1.5 kb mtDNA fragment were analysed

using the program MEGA ver. 2 (Kumar *et al.* 1993). Since the examined mitochondrial fragment combined two coding regions, we analyzed the segment both by treating each nucleotide site equally and according to the codon position, and included both transitions and transversions into the analyses. Genetic distances based on Jukes-Cantor substitution model (Nei & Kumar 2000) were calculated with the MEGA program. We chose Jukes-Cantor distances because they give similar values with lower variances than other distances when the estimates are smaller than 0.05 (Nei & Kumar 2000: p. 112). Estimates of divergence between haplotypes based on Kimura 2 parameter and Tamura Nei substitution models were similar to Jukes-Cantor distances.

Table 1. Haplotype frequencies, haplotype (h) and nucleotide (π) diversities with their standard deviations (SD) for the sampling localities of *Formica exsecta*. *N* is the number of individuals sequenced for a 1.5 kb mtDNA fragment including cyt *b* and part of ND6. The number of common haplotypes that are shared by at least two localities (H3, H4, H7, H17, H27) are shown in separate columns, unique haplotypes are listed for each locality (the number of individuals in parentheses if > 1). The haplotype codes are same as those in Fig. 2, and the locality numbers as those in Fig. 1.

Locality	*N*	Common haplotypes					Unique haplotypes	*h* (SD)	π (SD) %
		H3	H4	H7	H17	H27			
1. England	10	–	2	–	–	–	H6, H23(6), H24	0.64 (0.15)	0.29 (0.08)
2. Switzerland	11	3	–	–	1	–	H1, H18(4), H22(2)	0.82 (0.08)	0.37 (0.05)
3. Romania	10	–	–	–	8	1	H16	0.38 (0.18)	0.11 (0.07)
4. Germany	10	3	–	–	–	–	H21(7)	0.47 (0.13)	0.31 (0.09)
5. Sweden	11	2	1	–	2	–	H2, H5(2), H11(3)	0.89 (0.16)	0.28 (0.03)
6. Åland	1	–	1	–	–	–			
7. N.Sweden	3	–	–	–	–	–	H12, H30, H31	1.00 (0.27)	0.53 (0.18)
8. Moscow	1	–	–	–	–	–	H19		
9. Ural	7	–	–	–	–	2	H13(2), H20, H25(2)	0.86 (0.10)	0.24 (0.04)
10. Kazakhstan	5	–	–	1	–	–	H10, H15(2), H26	0.90 (0.16)	0.39 (0.08)
11. Kyrgyzstan*	4	–	–	1	–	–	H8, H9(2)	0.83 (0.22)	0.07 (0.02)
12. Novosibirsk	1	–	–	–	–	1			
13. Mongolia	1	–	–	–	–	1			
14. Baikal	1	–	–	–	–	–	H29		
15. Chita	1	–	–	–	–	1			
16. Kolyma	11	–	–	–	–	7	H14(2), H28(2)	0.58 (0.14)	0.23 (0.09)
17. Kamchatka	1	–	–	–	–	1			
18. Tibet	1	–	–	–	–	–	H32		

* *Formica mesasiatica* samples.

Table 2. Microsatellite variation and relatedness among worker nestmates ($r \pm$ SE) in the *Formica exsecta* populations. N_n is the number of nests, N_t is the total number of individuals from a population, n_a is the average number of alleles per locus and H_E is average expected heterozygosity. Minimum and maximum values for relatedness are in boldface.

Population (locality)	N_n	N_t	n_a	H_E	$r \pm$ SE
England (1)	10	50	3.3	0.45	**0.72 ± 0.07**
Switzerland (2)	11	55	8.7	0.67	0.10 ± 0.05
Romania (3)	10	50	5.7	0.59	0.22 ± 0.06
Germany (4)	10	50	7.5	0.64	0.02 ± 0.03
Sweden (5)*	38	190	7.3	0.65	**0.01 ± 0.01**
Ural (9)	7	35	6.3	0.71	0.54 ± 0.09
Kolyma (16)	11	53	6.0	0.65	**0.72 ± 0.05**

* Data from Seppä *et al.* (2004).

We compared the log likelihood scores of trees constructed with and without molecular clock assumption (Felsenstein 1988) to evaluate constancy in rate of the mtDNA sequence evolution among lineages. Overall mean divergence within group, average divergence, and net distances between phylogenetic groups were estimated. Maximum parsimony median-joining haplotype network, designed primarily for intraspecific data, was constructed with the Network 3.1.1.1 program weighing characters equally (Bandelt *et al*. 1999).

Haplotype (h) and nucleotide (π) diversities and their variances within populations were calculated using the program DnaSP ver. 3 (Rozas & Rozas 1999). As only one nest of *F. exsecta* was available in each of the localities 6, 8, 12–15, 17, 18 (Table 1), these localities were excluded from the population analysis. In order to reveal any geographic structure, pairwise Φ_{ST} distances that include nucleotide differences were calculated using the program Arlequin (Schneider *et al*. 2000) and a minimum spanning network was constructed with a matrix of these distances as input. In order to assess the distribution of mtDNA variation, we used the analysis of molecular variance (AMOVA, Excoffier *et al*. 1992) both for the haplotype frequencies only (F_{ST}) and also incorporating nucleotide divergence between the haplotypes (Φ_{ST}). The two measures of differentiation were compared with the program PERMUT 2 (Pons & Petit 1996). Values of Φ_{ST} significantly larger than F_{ST} indicate that haplotypes with a small genetic divergence are commonly found in the same population or geographic region. Significance of genetic differentiation was tested by permuting the haplotypes among the populations. A hierarchical three-level analysis was performed by partitioning the total sum of squares into components representing variation among groups of genetically close populations, among populations within groups and among individuals within populations. Significance of genetic differentiation was tested by permuting the haplotypes among the populations for 1000 times.

The data set for a population analysis of microsatellites (localities 1–5, 9, 16; Fig. 1) comprised all genotyped individuals from the polygynous nests (*see* below) and a single randomly chosen individual from each monogynous nest (total of 229). Only one worker individual from a monogynous colony could be taken for the population analysis because only one matriline is present in the colony and the worker genotypes are not independent. Linkage disequilibrium, genetic diversity, and population differentiation were estimated using GENEPOP ver. 3.1d (Raymond & Rousset 1995). The Markov chain exact test was used to estimate the significance of deviation from Hardy-Weinberg expectations (Guo & Thompson 1992). Genetic diversity in populations was estimated as an average number of alleles per locus and average expected heterozygosity (H_E). Heterozygosity under Hardy-Weinberg equilibrium was calculated by $(1 - \Sigma p_i^2)2n/(2n - 1)$, where p_i is the frequency of the ith allele and n is the number of individuals in the sample (Li 1976). We created another set of data with five individuals from each nest and calculated genetic differentiation (as F_{ST}; Weir & Cockerham 1984) by sampling randomly one individual from each nest and repeating such sampling a hundred times. This method removes redundant genotypes from the analysis and reduces the sample size. Mean F_{ST} values and their standard errors were obtained by jackknifing over loci.

Minimum spanning networks with a matrix of pairwise Φ_{ST} and F_{ST} values as an input were constructed with the MinSpNet available at http://www.cmpg.unibe.ch/services/software.htm.

The genetic relatedness among nestmate workers was estimated with the method of Queller and Goodnight (1989) by using the program Relatedness 5.0 (Goodnight 1994) and weighting the nests equally. Standard errors were obtained by jackknifing over nests and loci. The relatedness among single queen broods in *F. exsecta* has been estimated as 0.64 (Sundström *et al*. 1996, Seppä *et al*. 2004). Therefore, nests with relatedness greater than 0.64 were considered as monogynous and lower than 0.64 as polygynous.

Correlation between pairwise genetic distances estimated for mtDNA and microsatellites as well as the association between genetic and geographic distances among populations were tested with the Mantel test (Sokal & Rolf 1995).

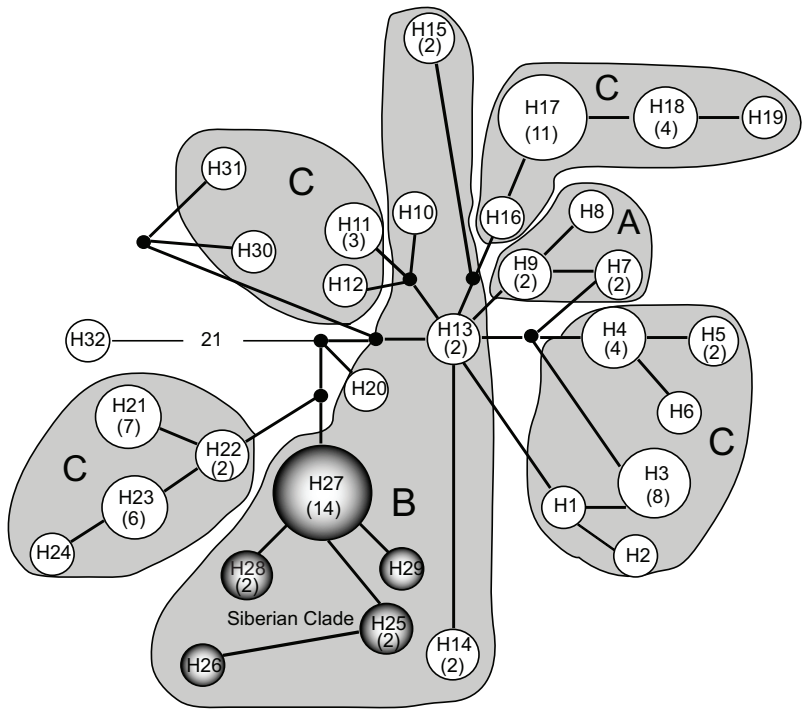

Fig. 2. Median-joining network showing phylogenetic relationships between the *Formica exsecta* and *F. mesasiatica* mtDNA haplotypes. Letters A–C indicate the geographical groups of haplotypes (Fig. 1), haplotypes H1–H32 same as in Table 1, and the number of substitutions between H32 and the closest hypothetical haplotype is 21. Circled areas are approximately proportional to the number of individuals bearing a particular haplotype. The numbers in the parentheses correspond to the haplotype frequencies. Branch lengths are approximately proportional to the number of substitutions involved between haplotypes. Shaded circles represent haplotypes of the Siberian clade.

Results

Geographical distribution of the haplotypes

We found 32 different haplotypes among 86 *Formica exsecta* and 4 *F. mesasiatica* ants (Table 1). Over the entire 1481-bp region excluding sites with alignment gaps and missing data, 61 nucleotide positions were variable with 25 parsimony informative sites. There were ten non-synonymous substitutions out of 43 in the 1122-bp cyt *b* gene and five out of 11 in the 289-bp part of ND6. One deletion was found in the haplotype H32 from Tibet in a 3´-flanking region of cyt *b*. The comparison of the likelihood scores of trees constructed with and without molecular clock assumption showed that the sequences have evolved at roughly constant rates ($P > 0.05$). Therefore, variation in mtDNA is suitable for approximate dating of historical events.

The haplotype H32 from Tibet (locality 18) was clearly distant to all the others, with a minimum of 22 nucleotide differences. The average distance between H32 and all the other haplotypes (H1–H31) was 1.63% ± 0.31%. Using H32 for rooting, it was possible to recognize several clades in the haplotype network (Fig. 2). The haplotype H13 had a central position in the network and several of the clades could be derived from it. Based on their geographical distribution the haplotypes can be tentatively grouped in three groups (A, B and C, Fig. 2): the group A contains haplotypes from Kyrgyzstan and Kazakhstan (locations 10, 11), the group B is distributed from the Urals across Siberia to Kamchatka (locations 9, 12–17) and the group C includes the haplotypes observed in Europe (locations 1–9).

Haplotypes H7–H9 (geographic group A) found in the individuals morphologically regarded as *F. mesasiatica* represented a non-reciprocally monophyletic group relative to the *F. exsecta* haplotypes. One individual of *F. exsecta* from Kazakhstan (locality 10) shared a haplotype (H7) with this clade. The average net distance between the *F. mesasiatica* (H7–H9) and all the other *F. exsecta* excluding the Tibetian H32, was 0.10% ± 0.07%, and this estimate was lower than the average divergence estimate of 0.48% ± 0.09% among all the *F. exsecta* haplotypes without H32.

The samples from the Urals, Siberia, and Mongolia had only haplotypes belonging to the

geographic group B (Fig. 1). Most of haplotypes found in Kazakhstan also belonged to the group B, and one haplotype (H27) was found in Romania. The clade of the mainly Siberian haplotypes H25–H29 resembled a star-like internal topology with the most common and widespread haplotype H27 (localities 3, 9, 12, 13, 15–17; Fig. 2) in the centre. Haplotypes in this Siberian clade represented 78% of all haplotypes observed in eastern Eurasia. The nucleotide diversity within the Siberian clade of haplotypes was 0.062% ± 0.02%. The only other haplotype found in Siberia was H14 which could be derived directly from the central (putatively ancestral) type H13 (Fig. 2).

The geographic group C included phylogenetically diverse set of haplotypes observed in Europe. Three haplotypes (H1–H3) from Switzerland, Germany and Sweden (localities 2, 4 and 5) as well as some haplotypes (H4–H6) from England, Sweden and the island of Åalnd in Finland (localities 1, 5 and 6), and four haplotypes (H21–H24) from Germany, England and Switzerland formed three separate clades, respectively. Notably, the insular population in England contained haplotypes from two different clades (H4 + H6 and H23 + H24). Northern Sweden had three haplotypes representing two clades. One of these clades (H30 + H31) was found only in northern Sweden, the other clade (H11 + H12) was present both in northern and southern Sweden.

Even though it was possible to distinguish several small clades (for example H30 – H31) with restricted geographical distributions (Fig. 2 and Table 1), all major geographical areas had haplotypes from at least two such clades. Thus, the clades did not represent distinct phylogeographic groups with allopatric distribution ranges.

Population structure

The populations of *Formica exsecta* showed mtDNA haplotype diversity ranging from 0.38 ± 0.18 in Romania (locality 3) to 1.00 ± 0.89 in northern Sweden (locality 7) (Table 1). Nucleotide diversity varied from 0.11% ± 0.07% in Romania (locality 3) to 0.53% ± 0.18% in northern Sweden (locality 7). Both haplotype and nucleotide diversities were highest in northern Sweden, Sweden, Switzerland and the Urals (localities 7, 5, 2, 9). The insular population in England (locality 1) also showed relatively high mtDNA diversity (Table 1).

The permutation test showed that all pairwise Φ_{ST} estimates based on mtDNA sequence divergence and haplotype frequencies were significant except for two pairs: Switzerland–Sweden and Urals–Kolyma (localities 2 & 5 and 9 & 16, Table 3). We clustered the populations into three regional groups according to the minimum spanning network of the populations: Urals–Kolyma, Sweden–Switzerland–Romania and Germany–England. Most of variation was found within populations (58.03%), smaller part of the variation was due to differentiation among the regions (30.39%), and 11.57% of variation was found among populations within the regions (Table 4). The analysis revealed moderate differentiation among all the populations of *F. exsecta* (Φ_{ST} = 0.420; $P = 0.001$ and $F_{ST} = 0.333$; $P < 0.001$). The estimate that included the number of nucleotide differences among haplotypes (Φ_{ST}) was slightly higher than the estimate based only on haplo-

Table 3. Pairwise mtDNA Φ_{ST} (below the diagonal) and microsatellite F_{ST} (above the diagonal) values among *Formica exsecta* populations. Values non-significantly different from zero are in boldface.

	1	2	3	4	5	9	16
England (1)		0.230	0.290	0.242	0.250	0.236	0.233
Switzerland (2)	0.339		**0.014**	0.036	0.100	0.043	0.075
Romania (3)	0.625	0.185		0.062	0.109	0.048	**0.079**
Germany (4)	0.176	0.286	0.610		0.096	0.022	0.106
Sweden (5)	0.404	**0.066**	0.367	0.383		0.101	0.112
Ural (9)	0.319	0.259	0.557	0.309	0.332		**0.057**
Kolyma (16)	0.375	0.376	0.604	0.366	0.441	**0.041**	

type frequencies (F_{ST}), but the difference between these two estimates was insignificant ($P = 0.128$). This similarity implied that most of geographical differentiation was due to differences in the haplotype frequencies among populations.

All six microsatellite loci were polymorphic in each of the seven populations in which they were screened (Table 2). The Markov chain exact test revealed a significant deficiency of heterozygotes in five out of 42 locus–population combinations after Bonferroni correction. The test showed no evident association of the heterozygote deficiency with any particular locus or population, so we kept all the loci in the analyses. The exact test for linkage disequilibrium between pairs of loci within the populations gave significant results in only three out of 105 tests (15 locus pairs in 7 populations). Genetic independence of the loci was therefore assumed for the following analyses.

The number of alleles per microsatellite locus per population ranged from 2 to 15 (data on H_E estimates and allele frequencies at each locus are available from the authors on request). The average number of alleles per locus ranged from 3.3 in England ($n = 10$, locality 1) to 8.7 in Switzerland ($n = 11$, locality 2), and the average expected heterozygosity across all the loci from 0.45 in England (locality 1) to 0.71 in the Urals (locality 9) (Table 2).

Moderate population differentiation was found at the microsatellites. The overall F_{ST} estimate was 0.126 ± 0.022. The pairwise F_{ST} values (Table 3) varied from 0.014 between Switzerland (locality 2) and Romania (locality 3) to 0.290 between England (locality 1) and Romania (locality 3). The minimum spanning network of the populations based on the pairwise F_{ST} values did not distinctively group the populations, except that the population from England (locality 1) was clearly separated from all the others.

A matrix comparison between all pairs of populations revealed no association between the genetic distances estimated from mtDNA (pairwise Φ_{ST}) and microsatellites (pairwise F_{ST}: $r = –0.30$, $P = 0.120$). No correlation between genetic differentiation and geographic distances was found among the *F. exsecta* populations either for mtDNA or microsatellites ($r = 0.19$, $P = 0.180$; $r = –0.18$, $P = 0.210$, respectively).

Geographical distribution of the social types

Mean genetic relatedness among nestmate workers varied from 0.01 in Switzerland (locality 2) to 0.72 in both England (locality 1) and Kolyma (locality 16) (Table 2). The expected relatedness among single queen brood in *F. exsecta* is 0.64 (Sundström *et al*. 1996, Seppä *et al*. 2004). Therefore, colonies in the populations in England (locality 1) and Kolyma (locality 16) were considered monogynous (Table 2), whereas the populations from Switzerland, Romania, Germany, and Sweden (localities 2–5) with low relatedness estimates were regarded as polygynous. Relatedness in the Romanian population was somewhat elevated apparently due to one monogynous nest (inferred from the worker genotypes). Relatedness in the population from the Urals (locality 9) was somewhat lower than expected under strict monogyny, probably due to two polygynous nests among the seven nests sampled. This population was considered as socially polymorphic.

Discussion

Taxonomical implications

MtDNA variation of *F. exsecta* was studied

Table 4. Hierarchical analysis of mtDNA diversity in *Formica exsecta* populations. Groups were based on the minimum spanning network of the populations.

Variance component	% of total variance	Φ-statistics	*P*
Among groups	30.39	$\Phi_{CT} = 0.304$	0.008
Among populations within groups	11.57	$\Phi_{SC} = 0.167$	< 0.001
Within populations	58.03	$\Phi_{ST} = 0.420$	0.001

across most of the species distribution range. There are two divergent mitochondrial lineages one of which is represented by only one individual from Tibet. The divergence estimate of 1.63% ± 0.31% between the two lineages of *F. exsecta* is close to the mean interspecific divergence estimate of 0.98% ± 0.15% in the *Formica rufa* group ants (Goropashnaya *et al*. 2004a). This finding may indicate high level of intraspecific mtDNA divergence, existence of two cryptic species within morphologically defined *F. exsecta* or introgression of mtDNA from unknown species. However, with only one sample studied from Tibet, we cannot draw strong conclusions. This sample is not outside the range of phenotypic variation of Palaearctic *F. exsecta* in any of the 15 morphological characters tested but it shows some character expressions rarely seen (B. Seifert unpubl. data).

On the contrary, haplotypes of the morphologically distinct species *F. mesasiatica* formed a weakly differentiated monophyletic group among the *F. exsecta* haplotypes. This probably indicates that the time of geographic isolation of *F. mesasiatica* from *F. exsecta* in Kyrgyzstan has been too short to generate reciprocally monophyletic species. Since these two species are largely allopatric, extent of reproductive isolation is unknown. However, this study, even with small sample size from the interspecific contact zone in Kazakhstan (location 10), shows that one individual of *F. exsecta* had a *F. mesasiatica* haplotype (H7) and suggests either transfer of mtDNA between two morphological species through hybridization or incomplete lineage sorting since the separation of the populations.

Phylogeography and refugial history

This study revealed no strong phylogeographic structure in *F. exsecta*. This pattern is similar to species-wide phylogeography reported for sympatrically distributed species of the red wood ant *F. lugubris* but different from the phylogeographic structure in *F. pratensis* that clearly demonstrated phylogenetic division between haplotypes from western Europe and the rest of Eurasia (Goropashnaya *et al*. 2004b). As in the ants *F. exsecta* and *F. lugubris*, no strong phylogeographic structure across most of the Eurasian taiga zone was reported in other boreal forest taxa studied to date, including several avian species (Kvist *et al*. 2001, 2003, Zink *et al*. 2002a, 2002b) and the flying squirrel *Pteromys volans* (Oshida *et al*. 2005). This similarity across a diverse array of organisms implies a general pattern for many species ecologically associated with the boreal forest in Eurasia. The lack of pronounced phylogeographic structure gives no evidence for vicariant events due to separation in different forest refugia over glacial periods.

Rooting the obtained haplotype network with the sequence found in Tibet suggests that the basal haplotypes of the other *F. exsecta* include H13 which is central in the haplotype network (Fig. 2). H13 and some other closely related to it haplotypes (H10, H15, H20) were found in the Urals or in Kazakhstan south of the Urals. We calculated the time of coalescence among all haplotypes (H32 from Tibet excluded) using the ρ statistic (Saillard *et al*. 2000), the average distance to the basal node (H13). Under the conventional insect mtDNA divergence rate of 2% per Myr (DeSalle *et al*. 1987), the substitution rate for the whole sequence is one substitution every 67.5 kyr. The average distance to the root 4.1 ± 0.76 substitutions gives the coalescence time estimate of 276.6 ± 51.4 kyr. This time estimate is approximate but suggests that the time to common ancestry exceeds the time span of the last glaciation (10–115 kyr; Anderson & Borns 1997). It is tempting to suggest that the current populations of *F. exsecta* originateed from the south Urals (Fig. 1) prior to the last glaciation, became spatially separated with sorting of the derived haplotype clades and later colonized the continent in several waves from these genetically slightly differentiated sources.

While phylogeography based on relatively slow evolving insect mtDNA has a limited resolving power over the shallow time span of the last glaciation, an analysis combining the haplotype genealogies with haplotype frequencies provides an insight to the recent refugial history. Most of the haplotypes (78%) found to the east of the Urals belonged to the Siberian clade resembling a star-like internal phylogeny (Fig. 2) with the most common (72%) and geographically widespread haplotype H27 in the centre

surrounded by rare haplotypes differing by a small number of substitutions (not more than 3). The star-like phylogeny and low mtDNA divergence in the sample containing most haplotypes from the Siberian part of species distribution range indicate a reduction in its historical effective size followed by a population expansion (Slatkin & Hudson 1991). The time to common ancestry in recently expanded population can be estimated from nucleotide diversity divided by the divergence rate for mtDNA (Rogers & Jorde 1995). Using the divergence rate of 2% per Myr, the expansion time could be estimated as 31 kyr (95% CI = 11–50 kyr). While this time estimate is highly approximate, its 95% confidence interval includes the beginning of the glacial retreat (13–14 kyr) and the shift towards the Holocene environment in Eurasia (Andersen & Borns 1997). It is reasonable, therefore, to assume that the ancestors of the Siberian haplotypes went through reduction of effective size surviving the last glaciation in a single forest refugium that later served as the main source for postglacial colonization of a vast area to the east of the Urals. This finding is consistent with genetic signs of range contraction followed by demographic expansion at different times during the Pleistocene reported for a taxonomically diverse set of forest-associated species, such as the ants of the *Formica rufa* group (Goropashnaya *et al*. 2004a, 2004b) birds (Kvist *et al*. 2001, 2003, Zink *et al*. 2002a, 2002b), and the Siberian newt (Berman *et al*. 2005).

Another approach to infer the recent refugial history is to analyse genetic differentiation at the population level by taking into account both haplotype genealogies and haplotype frequencies. The estimates of population differentiation among all populations of *F. exsecta* were significant and close to those (Φ_{ST} = 0.56, F_{ST} = 0.43) reported for *F. lugubris*, another ant species without significant phylogeographic structure across Eurasia (Goropashnaya *et al*. 2004a). A considerable part of total mtDNA variation was allocated among the three groups of populations revealed by the minimum-spanning network (not shown) based on pairwise Φ_{ST} distances.

The two populations studied in north Asia, the Urals and Kolyma, were not significantly differentiated from each other and formed a tight group separated from the European populations. It is notable that among all the pairwise comparisons, the populations from the Urals and Kolyma did not differ significantly from each other by either mtDNA or microsatellite distances. Close genetic affinities between the two populations separated by a distance over 4500 km imply recent historical connections and further support postglacial colonization of most of Siberia from a single refugial source. According to the palaeoecological evidence, forest refugia were located during the last glaciation in the southern Urals, south Siberian mountains and northern Mongolia (Grichuk 1984, Efimik 1996, Tarasov *et al*. 2000).

The analysis of population differentiation in Europe revealed two groups of populations without obvious correspondence to geographic positions: Sweden–Switzerland–Romania, Germany–England. This pattern could indicate mixing of different gene pools during postglacial colonization from different forest refugia. Recently, a glacial forest refugium in the Carpathians was revealed by palaeoecological and phylogeograpic studies (cf. Goropashnaya *et al*. 2004b). As compared with species strictly associated with forest environment, *F. exsecta* could potentially survive glacial periods in larger refugial areas in Europe. While *F. exsecta* is confined to the forest zone and mountain intrazonal forest, this species prefers forest openings and builds nest mounds mainly of grass stems (Dlussky 1967). According to palaeoecological reconstructions, during the last glaciation grassy parkland with patches of forest covered much of continental Europe south of the Alps (Andersen & Borns 1997) and could represent additional refugial areas for *F. exsecta*. A population level study with a more detailed sampling design is required to reveal the detailed refugial history of this species in Europe.

Population structure and social types

Unlike mtDNA data, analysis of population differentiation by the use of microsatellite allele frequencies did not reveal any clear association between geographic positions and genetic affinities among populations. The estimates obtained

in our study for the microsatellite F_{ST} (0.13) and mtDNA Φ_{ST} (0.42) are close to those observed for *F. exsecta* on a small local scale in Sweden (F_{ST} = 0.11, Φ_{ST} = 0.31; Seppä *et al.* 2004). A number of studies in other ant species showed contrasting difference between microsatellite and mtDNA estimates of genetic differentiation on a local scale, with microsatellite F_{ST} being much lower than mtDNA Φ_{ST}. Examples include *Solenopsis invicta* and *Leptothorax rugatulus*, two ant species with both monogynous and polygynous colony types (F_{ST} = 0.03, Φ_{ST} = 0.21, Ross *et al.* 1997; F_{ST} = 0.00, Φ_{ST} = 0.25, Rüppell *et al.* 2003; respectively), *Formica lugubris* (F_{ST} = 0.03, Φ_{ST} = 0.72; Gyllenstrand & Seppä 2003), *Diacamma cyaneiventre* (F_{ST} = 0.26, Φ_{ST} = 0.97; Doums *et al.* 2002), *Nothomyrmecia macrops* (F_{ST} = 0.06, Φ_{ST} = 0.98; Sanetra & Crozier 2003). The substantial and consistent differences between values of these estimates are largely due to restricted female gene flow induced by dependent colony foundation. It is notable that strong mtDNA structure was observed in *F. exsecta* on a local scale in Switzerland (Liautard & Keller 2001) with Φ_{ST} estimate of 0.72 considerably exceeding the estimate of 0.42 reported for differentiation on continental scale in our study. Strong differentiation on a local scale reflects local colonization dynamics, where suitable habitat patches can be colonized by few queens, resulting in a strong effect of stochastic events on the haplotype diversity. On the global scale, at least some of our geographic samples were from areas larger than a single habitat patch. This implies larger effective population size, weaker effect of genetic drift and, therefore, smaller estimates of genetic differentiation as compared with differentiation on a small spatial scale.

The relatedness estimates showed that all four populations studied from continental Europe were of the polygynous social type. The populations from the opposite limits of species distribution range (England and Kolyma) demonstrated the monogynous type of social organization. This is consistent with the previous finding of variation in social structure between geographic populations of *F. exsecta* (Pamilo & Rosengren 1984). Both types of social organization were found in the population from the Urals. Previously, social polymorphism was described in population from Sweden sampled over the area comparable to the size of the sampling site in the Urals (Seppä *et al.* 2004). No clear association between the type of social organization and geographic location of populations can be seen from our data.

The presence of mtDNA haplotypes from two different phylogenetic clades and relatively high mtDNA diversity estimates in the population in England gave no indication for reduction of historical effective size due to founder events during postglacial colonization. Only a few populations of *F. exsecta* are currently known in England (Collingwood 1979) and they have probably undergone reduction in the effective population size due to habitat fragmentation. Moreover, our study clearly shows that the English population consists of monogynous colonies and this, together with a small number of nests, implies a small current effective population size. Even though the level of mtDNA diversity was still high in England, microsatellite polymorphism was greatly reduced compared to the continental populations. The estimates of the average number of alleles per locus and average heterozygosity were the lowest in the English population (Table 2). It is somewhat unexpected that the reduction of nuclear diversity in England was not paralleled with a similar reduction in mtDNA diversity, even though the smaller effective population size should make mtDNA variation sensitive to a reduction of population size (Birky *et al.* 1989). Nevertheless, sparsity of populations in England and the observed reduction in nuclear genetic diversity give some basis to classify *F. exsecta* as near threatened similar to several other *Formica* ants, e.g. *F. lugubris, F. rufa,* and *F. aquilonia* (Hilton-Taylor 2000).

Acknowledgements

We thank N. Dokuchaev, A. Gilev, N. Gyllenstrand, A. Lazutkin, K. Liautard, M. Lund, B. Marko, P. Neumann, V. Semerikov, P. Seppä, D. Stradling, and A. Zakharov for providing material, J. Wallén for the laboratory assistance, K.G. McCracken for providing laboratory space in the Institute of Arctic Biology, Fairbanks, Alaska for a part of the work. The study has been supported by grants from the Natural Science Research Councils of Sweden and Finland (77311),

Sven and Lilly Lawski's Foundation and from the European Commission. V. Fedorov was supported by NSF (grant EPS-0346770).

References

Anderson, B. G. & Borns, H. W. Jr. 1997: *The Ice Age world. An introduction to Quaternary history and research with emphasis on North America and Europe during the last 2.5 million years*. — Scandinavian University Press, Oslo, Norway.

Bandelt, H.-J., Forster, P. & Röhl, A. 1999: Median-joining networks for inferring intraspecific phylogenies. — *Molecular Biology and Evolution* 16: 37–48.

Bassam, B. J., Caetano-Anolles, G. & Gresshoff, P. M. 1991: Fast and sensitive silver staining of DNA in polyacrylamide gels. — *Analytical Biochemistry* 196: 80.

Bennett, K. D. 1997: *Evolution and ecology: the pace of life*. — Cambridge University Press, Cambridge.

Berman, D. I., Derenko, M. V., Malyarchuk, B. A., Grzybowski, T., Kryukov A. P. & Miscicka-Sliwka, D. 2005: Intraspecific genetic differentiation of Siberian newt (*Salamandrella keyserlingii*, Amphibia, Caudata) and cryptic species *S. schrenckii* from the Russian South-East. — *Zoologichesky Zhurnal* 84: 1374–1388. [In Russian with English summary].

Birky, C. W. Jr., Fuerst, P. & Maruyama, T. 1989: Organelle gene diversity under migration, mutation and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparisons to nuclear genes. — *Genetics* 121: 613–627.

Chapuisat, M. 1996: Characterization of microsatellite loci in *Formica lugubris B* and their variability in other ant species. — *Molecular Ecology* 5: 599–601.

Collingwood, C. A. 1979: The Formicidae (Hymenoptera) of Fennoscandia and Denmark. — *Fauna Entomologica Scandinavica* 8: 1–174.

Deffontaine, V., Libois, R., Kotlík P., Sommer, R., Nieberding, C., Paradis, E., Searle, J. B. & Michaux, J. R. 2005: Beyond the Mediterranean peninsulas: evidence of central European glacial refugia for a temperate forest mammal species, the bank vole (*Clethrionomys glareolus*). — *Molecular Ecology* 14: 1727–1739.

DeSalle, R., Freedman, T., Prager, E. M. & Wilson, A. C. 1987: Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. — *Journal of Molecular Evolution* 26: 157–164.

Dlussky, G. M. [Длусский, Г. М.] 1967: [*The ants of genus Formica*]. — Nauka, Moscow. [In Russian].

Doums, C., Cabrera, H. & Peeters, C. 2002: Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*. — *Molecular Ecology* 11: 2251–2264.

Efimik, V. E. 1996: Pliocene and Pleistocene relict species in spider fauna of the South Urals. — *Zoologichesky Zhurnal* 75: 1138–1148. [In Russian with English summary].

Excoffier, L., Smouse, P. E. & Quattro, J. M. 1992: Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. — *Genetics* 131: 479–491.

Felsenstein, J. 1988: Phylogenies from molecular sequences: inference and reliability. — *Annual Review of Genetics* 22: 521–565.

Goodnight, K. F. 1994: *Relatedness 4.2b*. — Goodnight Software, Houston, TX.

Goropashnaya, A. V., Fedorov, V. B. & Pamilo, P. 2004a: Recent speciation in the *Formica rufa* group ants (Hymenoptera, Formicidae): inference from mitochondrial DNA phylogeny. — *Molecular Phylogeny and Evolution* 32: 198–206.

Goropashnaya, A. V., Fedorov, V. B., Seifert, B. & Pamilo, P. 2004b: Limited phylogeographic structure across Eurasia in two red wood ant species *Formica pratensis* and *F. lugubris* (Hymenoptera, Formicidae). — *Molecular Ecology* 13: 1849–1858.

Grichuk, V. P. 1984: Late Pleistocene vegetation history. — In: Velichko, A. A., Wright, H. E. Jr. & Barnosky, C. W. (eds), *Late Quaternary environments of the Soviet Union*: 155–179. University of Minnesota Press, Minneapolis.

Guo, S. W. & Thompson, E. A. 1992: Performing the exact test of Hardy-Weinberg proportions for multiple alleles. — *Biometrics* 48: 361–372.

Gyllenstrand, N., Gertsch, P. J. & Pamilo, P. 2002: Polymorphic microsatellite DNA markers in the ant *Formica exsecta*. — *Molecular Ecology Notes* 2: 67–69.

Gyllenstrand, N. & Seppa, P. 2003: Conservation genetics of the wood ant, *Formica lugubris,* in a fragmented landscape. — *Molecular Ecology* 10: 2931–2940.

Hewitt, G. M. 1996: Some genetic consequences of ice ages, and their role in divergence and speciation. — *Biological Journal of the Linnean Society* 58: 247–276.

Hewitt, G. M. 1999: Post-glacial re-colonization of European biota. — *Biological Journal of the Linnean Society* 68: 87–112.

Hilton-Taylor, C. (compiler) 2000: *2000 IUCN Red List of Threatened Species*. — IUCN, Gland, Switzerland and Cambridge, UK.

Jermiin, L. S. & Crozier, R. H. 1994: The cytochrome *b* region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: sequence divergence in Hymenoptera may be associated with nucleotide content. — *Journal of Molecular Evolution* 38: 282–294.

Kotlik P., Deffontaine V., Mascheretti S., Zima, J., Michaux, J. R. & Searle, J. B. 2006: A northern glacial refugium for bank voles (*Clethrionomys glareolus*). — *Proceedings of the National Academy of Sciences of The United States of America* 103: 14860–14864.

Krieger, M. J. & Ross, K. G. 2002: Identification of a major gene regulating complex social behaviour. — *Science* 295: 328–332.

Kumar, S., Tamura, K. & Nei, M. 1993: *MEGA: molecular evolutionary genetics analysis, v. 2*. — Pennsylvania State University.

Kvist, L., Martens, J., Ahola, A. & Orell, M. 2001: Phylogeography of a Palaearctic sedentary passerine, the willow tit (*Parus montanus*). — *Journal of Evolutionary Biology* 14: 930–941.

Kvist, L., Martens, J., Higuchi, H., Nazarenko, A. A., Valchuk, O. P. & Orell, M. 2003: Evolution and genetic structure of the great tit (*Parus major*) complex. — *Proceedings of the Royal Society of London Series B* 270: 1447–1454.

Li, C. C. 1976: *First course in population genetics*. — Boxwood, Pacific Grove, California.

Liautard, C. & Keller, L. 2001: Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica exsecta*. — *Evolution* 55: 2484–2492.

Michaux, J. R., Magnanou, E., Paradis, E., Nieberding, C. & Libois, R. 2003: Mitochondrial phylogeography of the Woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region. — *Molecular Ecology* 12: 685–697.

Nei, M. & Kumar, S. 2000: *Molecular evolution and phylogenetics*. — Oxford Univ. Press, Oxford, New York.

Oshida, T., Abramov, A., Yanagawa, H. & Masuda, R. 2005: Phylogeography of the Russian flying squirrel (*Pteromys volans*): implication of refugia theory in arboreal small mammal of Eurasia. — *Molecular Ecology* 14: 1191–1196.

Pamilo, P. & Rosengren, R. 1984: Evolution of nesting strategies of ants: genetic evidence from different population types of *Formica* ants. — *Biological Journal of the Linnean Society* 21: 331–348.

Pons, O. & Petit, R. J. 1996: Measuring and testing genetic differentiation with ordered versus unordered alleles. — *Genetics* 144: 1237–1245.

Queller, D. C. & Goodnight, K. F. 1989: Estimating relatedness using genetic markers. — *Evolution* 43: 258–275.

Raymond, M. & Rousset, F. 1995: GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. — *Journal of Heredity* 86: 248–249.

Rogers, A. R. & Jorde, L. B. 1995: Genetic evidence on modern human origins. — *Human Biology* 67: 1–36.

Ross, K. G., Krieger, M. J. B., Shoemaker, D. D., Vargo, E. L. & Keller, L. 1997: Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. — *Genetics* 147: 643–655.

Rozas, J. & Rozas, R. 1999: DnaSP version 3. — *Bioinformatics* 15: 174–175.

Rüppell, O., Strätz, M., Baier, B. & Heinze, J. 2003: Mitochondrial markers in the ant *Leptothorax rugatulus* reveal the population genetic consequences of female philopatry at different hierarchical levels. — *Molecular Ecology* 12: 795–801.

Saillard, J., Forster, P., Lynnerup, N., Bandelt, H. J. & Norby, S. 2000: MtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. — *American Journal of Human Genetetics* 67: 718–726.

Sanetra, M. & Crozier, R. H. 2003: Patterns of population subdivision and gene flow in the ant *Nothomyrmecia macrops* reflected in microsatellite and mitochondrial DNA markers. — *Molecular Ecology* 12: 2281–2295.

Schneider, S., Roessli, D. & Excoffier, L. 2000: *Arlequin v. 2000. A software for population genetic data analysis*. — Genetics and Biometry Laboratory, Department of Ecology and Anthropology, University of Geneva, Switzerland.

Seifert, B. 2000: A taxonomic revision of the ant subgenus *Coptoformica* Mueller, 1923. — *Zoosystema* 22: 517–568.

Seppä, P., Gyllenstrand, M., Corander, J. & Pamilo, P. 2004: Coexistence of the social types: genetic population structure in the ant *Formica exsecta*. — *Evolution* 58: 2462–2471.

Slatkin, M. & Hudson, R. R. 1991: Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. — *Genetics* 129: 555–562.

Sokal, R. R. & Rolf, F. J. 1995: *Biometry: the principles and practice of statistics in biological research*. — W. H. Freeman and Company, New York.

Sundström, L., Chapuisat, M. & Keller, L. 1996: Conditional manipulation of sex ratios by ant workers, a test of kin selection theory. — *Science* 274: 993–995.

Tarasov, P. E., Volkova, V. S., Webb, T., Guiot, J., Andreev, A. A., Bezusko, L. G., Bezusko, T. V., Bykova, G. V., Dorofeyuk, N. I., Kvavadze, E. V., Osipova, I. M., Panova, N. K. & Sevastyanov, D. V. 2000: Last glacial maximum biomes reconstructed from pollen and plant macrofossil data from northern Eurasia. — *Journal of Biogeography* 27: 609–620.

Thoren, P. A., Paxton, R. J. & Estoup, A. 1995: Unusually high frequency of $(CT)_n$ and $(GT)_n$ microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera: Vespidae). — *Insect Molecular Biology* 4: 141–148.

Weir, B. S. & Cockerham, C. C. 1984: Estimating *F*-statistics for the analysis of population structure. — *Evolution* 38: 1358–1370.

West, K. J. 2000: *Plant life of the Quaternary cold stages*. — Cambridge University Press, Cambridge.

Willis, K. J., Rudner, E. & Siimegi, P. 2000: The full-glacial forests of central and southwestern Europe. — *Quaternary Research* 53: 203–213.

Zink, R. M., Drovetski, S. V. & Rohwer, S. 2002a: Phylogeographic patterns in the great spotted woodpecker *Dendrocopos major* across Eurasia. — *Journal of Avian Biology* 33: 175–178.

Zink, R. M., Rohwer, S., Drovetski, S. V., Blackwell-Rago, R. C. & Farrell, S. L. 2002b: Holarctic phylogeography and species limits of three-toed woodpeckers. — *Condor* 104: 167–170.