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Genetic differentiation in sympatric wood ants, *Formica rufa* and *F. polyctena*

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Summary. Direct observations have suggested that the closely related wood ants *Formica polyctena* and *F. rufa* represent different social organizations, with high queen number in *F. polyctena* and a high frequency of monogynous nests in *F. rufa*. We examined social organization and genetic population structure in a setup where populations of the two species are sympatric and gene flow between the species is possible. Our aim was to compare social organization in the species, and study evolutionary relationships between them. The observed relatedness among colony workers suggested that the difference in the level of polygyny is quantitative rather than qualitative, with a higher queen number in *F. polyctena*. The observed difference in polygyny was not accompanied by a difference in spatial genetic differentiation which was weak in both species. The genetic distance between the species is consistent with limited interspecific gene flow. Identification of a few possible *F. rufa* migrants in *F. polyctena* populations suggests potential interspecific gene flow. Thus, reproductive isolation of the species may not be complete when they are sympatric.

Key words: Ants, DNA microsatellites, genetic differentiation; relatedness, social organization.

Introduction

Wood ants of the genus *Formica* form a group of ants with a great diversity of social organization. The level of polygyny of their colonies can vary not only between species but also within species, and in the latter case one social form often dominates the whole population. Intraspecific social polymorphism has been documented in several *Formica* species, including *F. cinerea* (Lindström et al., 1996), *F. exsecta* (Pamilo and Rosengren, 1984), *F. lugubris* (Pamilo et al., 1992) and *F. truncorum* (Sundström, 1993). Interspecific differences in social organization are common between sibling species of ants, where one species has monogynous and the other has polygynous colonies (Wilson, 1971). *Formica polyctena* and *F. rufa* are one example on such a species pair.

The specific status of the sibling species *Formica rufa* Linnaeus 1761 and *Formica polyctena* Förster 1850 was widely accepted only lately (Yarrow, 1955; Betrem, 1960). Though similar in morphology and ecology, the species differ in life history traits such as dispersal, colony founding and social organization. *F. rufa* is considered mainly monogynous, while *F. polyctena* is classified as obligately polygynous (Seifert, 1991). Based on morphology (number of erect hairs in different body parts), they could be clearly separated at least in their Swedish and Polish populations (Douwes, 1981a; Czechowski and Douwes, 1996). Further, there was no correlation between morphology and geographic location (Douwes, 1981a). Intermediate morphs interpreted as hybrids between the two species have been reported in Central Europe (Seifert, 1991; Czechowski, 1993; Czechowski and Douwes, 1996). It has also been suggested that the social structure of a colony can affect the morphology of workers in these species (Otto, 1960). For this reason, Czechowski and Douwes (1996) concluded: 'There still remains the main question: are these forms different species?'. A recent study of mtDNA haplotypes showed incomplete lineage sorting in these species and the haplotypes found in *F. polyctena* could be derived from one *F. rufa* haplotype by one or two nucleotide changes (Goropashnaya et al, in prep.).

As several *Formica* species are socially variable, we wanted to examine whether colonies morphologically assigned to *F. polyctena* or *F. rufa* do have different social organization. Social organization is examined by estimating genetic relatedness of colony workers. Based on previous literature, we expect the level of polygyny to be higher in *F. polyctena*. We

also wanted to study evolutionary relationships of the two morphologically, defined species, whether they represent polymorphic forms of a single species or two separate genetic units, answering the question posed by Czechowski and Douwes (1996). Furthermore, by sampling the two putative species from five nearby locations where they live in sympatry, we aim to test the prediction that high level of polygyny is associated to restricted dispersal and gene flow. Previous empirical tests of the association between polygyny and spatial differentiation have mainly compared different social forms of a single species (e.g. Sundström, 1993) or two related species (Seppä and Pamilo, 1995) in situations where the two social types live in geographical proximity but not in sympatry. We used microsatellite markers to estimate relatedness and genetic structure of sympatric populations of both morphologically defined species and to determine the genetic divergence between them.

Material and methods

Species

The two morphologically defined species, *Formica polyctena* and *F. rufa* belong to mound-building wood ants, so called *Formica rufa* group (Collingwood, 1979). The *F. rufa* group also includes at least four other species in Europe: *F. aquilonia*, *F. lugubris*, *F. paralugubris* and *F. pratensis*. Based on allozyme studies, *F. polyctena* and *F. rufa* form a genetically close pair (Pamilo et al., 1979; Tegelström et al., 1983), which supposedly also crossbreed (Seifert, 1991; Czechowski, 1993). Their distribution in Scandinavia is restricted to the southern parts of the area (Collingwood, 1979) where both species are well represented in the insect fauna. The two species differ in a number of biological characters (Seifert, 1991). *F. polyctena* mates after short-range flight and young queens are often recruited back to their natal nest or adopted in conspecific nests. Budding is common as a mode of dispersal in this species. *F. rufa*, on the other hand, is mainly monogynous and sexuals take part in long range dispersal during nuptial flights. Initiations of new nests are dependent through social parasitism of *Serviformica* nests (Seifert, 1991).

Species identification

Ten ants from each nest were examined for the number of erect hairs on back of head, pronotum, mesonotum and propodeum for species identification (Douwes 1981a, b). For separating *F. polyctena* and *F. aquilonia*, one additional character, hairs on the hind femur, was examined. Only nests where all ten workers could be classified as either *F. polyctena* or *F. rufa* were included in the genetic analysis. Less than ten percent of nests could not be identified with certainty and were left out from the analysis.

Samples

The study populations were located in boreal forests in the surroundings of Uppsala, eastern Sweden. Ants were collected in 1996–1997 from five localities (Fig. 1), where both species were found sympatrically. The distances between the localities were 4.8–28 km. Colonies of both species were present also in the areas between these sampling localities, although large parts were unsuitable for the species, including urban and cultivated areas and water bodies. In total, worker ants from 59 nests of *F. polyctena* and 53 nests of *F. rufa*, respectively were sampled.

Genetic analyses

DNA for PCR reactions was extracted from head and thorax of adult individuals using chelex protocol (Walsh et al., 1991). For genetic analysis, 8 microsatellite loci, FL12, FL20, FL21, FL29 (Chapuisat, 1996), FE13, FE19, FE37 and FE42 (Gyllenstrand et al., 2002) were used. From each nest, 3 individuals were genotyped. Amplification reactions were carried out in 10 µl volumes containing approximately 10 ng ant DNA, 1 × PCR-buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.08% Nonidet P40), 1.5 mM $MgCl_2$, 75 µM dNTP, 400 nM of each primer and 0.4 U of *Taq* polymerase (MBI Fermentas). Samples were PCR amplified under cycling conditions: initial denaturing at 94 °C for 3 min followed by 30 cycles of denaturing at 94 °C for 30 s , annealing at appropriate T_a for 30 s, extension at 72 °C for 30 s followed by a final extension step at 72 °C for 5 min. PCR products were analysed on 6% polyacrylamide gels and visualized using standard silver staining protocol (Bassam et al., 1991) or autoradiography.

Statistical analysis

Genetic relatedness, defined as the proportion of alleles shared by common descent (Pamilo and Crozier, 1982), was calculated using the algorithm of Queller and Goodnight (1989) as implemented in the software RELATEDNESS 4.2 (Goodnight, 1994). In the analysis, nests were weighted equally, and standard errors were obtained by jackknifing over nests.

Inbreeding within subpopulations inflates relatedness of workers nestmates, because relatedness depends both on the number of reproductive individuals (i.e. the level of polygyny and polyandry) and relatedness among them. Therefore the effective number of reproducers within a nest is better reflected by a relatedness estimate (r*) where the component caused by positive inbreeding coefficient (F) has been removed (Pamilo, 1984, 1985):

$$r^* = \frac{r - 2F/1 + F}{1 - 2F/1 + F}$$

High relatedness estimates suggest a small number of matrilines and patrilines per colony and non-independence of the nest mate genotypes. Such dependence of genotypes leads to pseudoreplication when testing Hardy-Weinberg equilibrium, allele frequencies and genetic differentiation. We used a resampling procedure to correct for such biases. From datasets of each species, genotypes of one individual per nest were drawn at random. One hundred such resampled datasets were produced per species.

Genotypic disequilibrium was tested using FSTAT 2.9.3 (Goudet, 2001) employing sequential Bonferroni correction. Populations were tested for Hardy-Weinberg equilibrium using exact tests as in GENEPOP 3.1d (Raymond and Rousset, 1995). Genetic structure was studied by calculating F_{ST}, standardized allele frequency variance among populations, following Weir and Cockerham (1984) as implemented in FSTAT 2.9.3 (Goudet, 2001). Pairwise estimates of F_{ST} were calculated from the resampled dataset. We report the means of pairwise estimates and standard errors calculated over loci. Genetic distance within and between species were also obtained using resampled pairwise Nei's genetic distances (Nei, 1978) as implemented in GENETIX 4.01. The occurrence of possible hybrids and migrants between species was investigated using the computer software STRUCTURE (Pritchard et al., 2000) which assumes that loci are at linkage equilibrium and at Hardy-Weinberg equilibrium within populations, and uses a Bayesian clustering method to infer the number of populations (K). To make sure that we obtained consistent estimates for the number of populations, independent runs were performed for each estimated P(X|K) where X stands for the observed genotypes of the sampled individuals. First we used uniform prior allele frequency distributions to explore the data and second we used the prior information on species status to identify possible migrants.

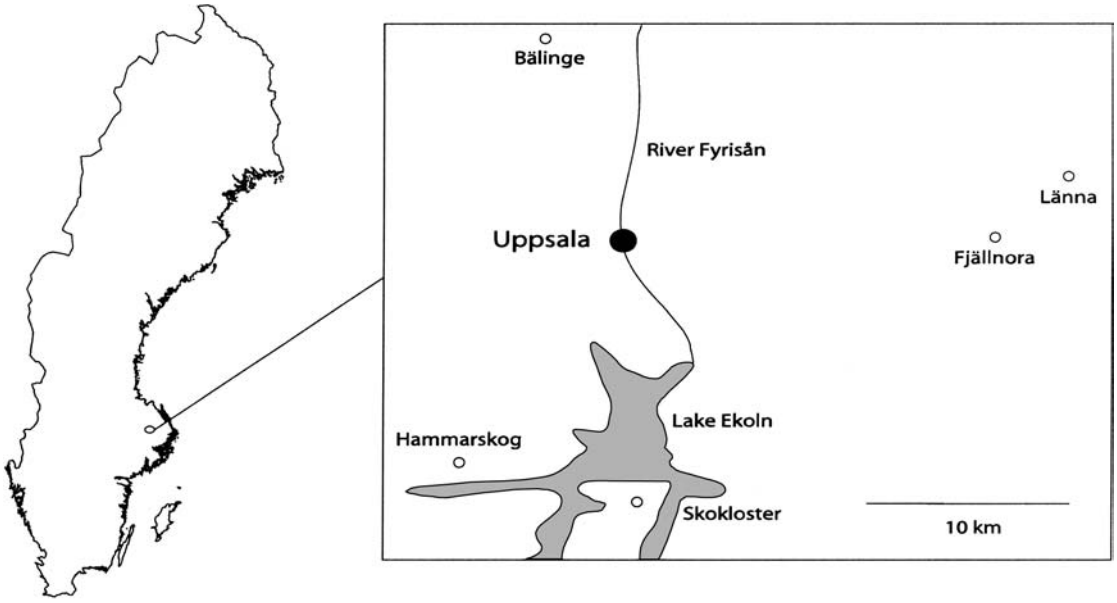

Figure 1. Map of the study area showing approximate distances between populations.

Results

In *F. polyctena*, the total number of alleles per locus ranged from 2 to 26 (mean 10.6) and the observed heterozygosity from 0.31 to 0.81 (mean 0.61) while the corresponding values for *F. rufa* were 3–19 alleles (mean 8.5) and heterozygosity from 0.34 to 0.78 (mean 0.58) (Table 1). No systematic deviations from linkage equilibrium were detected in either species across the resampled datasets. The local populations did not conform to Hardy-Weinberg proportions (Table 2). There was a consistent deficiency of heterozygotes at all the loci, suggesting that the departure was caused by non-random mating rather than by non-amplifying null alleles.

Relatedness estimates did not reveal any qualitative difference in social organization between species but indicated a small quantitative difference in the level of polygyny. Mean genetic relatedness (r) among worker nestmates in *F. polyctena* ranged between 0.27 and 0.42, and the inbreeding adjusted relatedness (r*) from 0.03 to 0.17 (Table 2). The corresponding estimates in *F. rufa* ranged between 0.45 and 0.60 for the mean genetic relatedness and from 0.27 to 0.54 for the inbreeding adjusted relatedness (Table 2). Estimates from the two species are non-overlapping, 95% confidence interval of mean relatedness in *F. polyctena* is 0.30 to 0.45 and in *F. rufa* 0.45 to 0.60. Similarly, 95% confidence interval of inbreeding adjusted relatedness in *F. polyctena* is 0.05 to 0.19 and in *F. rufa* 0.24 to 0.53.

Both species showed weak structuring among populations. Means and standard errors over resampled pairwise F_{ST}-values were 0.020 ± 0.003 and 0.023 ± 0.009 for *F. polyctena* and *F. rufa*, respectively (Table 3). The Bälinge population of *F. rufa* seemed to deviate most from the others with comparatively high F_{ST} values, while no such differences were evident among the *F. polyctena* populations. Interspecific comparisons revealed stronger genetic structuring, the mean and standard error over resampled pairwise F_{ST} values being 0.150 ± 0.045. Interspecific pairwise F_{ST} values within each sampling locality were of the same magnitude as the interspecific estimates between localities (Table 3), indicating that the geographical scale had no importance in pos-

Table 1. Comparison of genetic variability in *F. polyctena* and *F. rufa*. N_A is number of alleles, H_E and H_O are expected and observed heterozygosities, respectively

	F. polyctena			*F. rufa*		
	N_A	H_E	H_O	N_A	H_E	H_O
FL12	14	0.77	0.71	9	0.64	0.66
FL20	26	0.89	0.81	19	0.77	0.78
FL21	8	0.78	0.57	8	0.75	0.66
FL29	7	0.62	0.57	7	0.52	0.47
FE13	7	0.76	0.66	5	0.58	0.45
FE19	9	0.71	0.64	7	0.71	0.63
FE37	12	0.79	0.59	10	0.72	0.69
FE42	2	0.46	0.31	3	0.49	0.34

Table 2. Genetic relatedness among nestmate workers ($r \pm$ SE), inbreeding ($F \pm$ SE) and inbreeding adjusted relatedness (r^*) values. n is the sample size (number of nests) in each population

	Location	*n*	$r \pm$ SE	$F \pm$ SE	r^*
F. polyctena	Fjällnora	11	0.27 ± 0.06	0.08 ± 0.05	0.14
	Hammarskog	7	0.41 ± 0.09	0.20 ± 0.09	0.11
	Skokloster	12	0.42 ± 0.08	0.26 ± 0.04	0.03
	Länna	16	0.38 ± 0.05	0.16 ± 0.04	0.15
	Bälinge	13	0.39 ± 0.04	0.16 ± 0.04	0.17
F. rufa	Fjällnora	12	0.56 ± 0.08	0.03 ± 0.07	0.54
	Hammarskog	10	0.48 ± 0.07	0.17 ± 0.05	0.28
	Skokloster	9	0.51 ± 0.06	0.20 ± 0.07	0.27
	Länna	13	0.60 ± 0.06	0.14 ± 0.05	0.47
	Bälinge	9	0.45 ± 0.08	0.06 ± 0.06	0.38

Table 3. Intra- and interspecific pairwise genetic distances (above diagonal) and F_{ST} values between populations of *F. polyctena* and *F. rufa* (below diagonal). Estimates are means from 100 resampled datasets

		F. polyctena				*F. rufa*					
		Fjällnora	Hammar-skog	Skokloster	Länna	Bälinge	Fjällnora	Hammar-skog	Skokloster	Länna	Bälinge
F. polyctena	Fjällnora		0.048	0.079	0.035	0.061	0.579	0.502	0.516	0.516	0.590
	Hammarskog	0.014		0.082	0.075	0.073	0.609	0.486	0.504	0.537	0.478
	Skokloster	0.024	0.020		0.066	0.093	0.418	0.343	0.364	0.377	0.387
	Länna	0.011	0.023	0.020		0.042	0.494	0.420	0.441	0.463	0.516
	Bälinge	0.022	0.024	0.031	0.014		0.591	0.534	0.550	0.560	0.601
F. rufa	Fjällnora	0.177	0.179	0.135	0.158	0.185		0.016	0.020	0.013	0.103
	Hammarskog	0.144	0.135	0.101	0.126	0.156	0.008		0.035	0.021	0.123
	Skokloster	0.166	0.157	0.122	0.147	0.177	0.009	0.015		0.019	0.086
	Länna	0.158	0.157	0.119	0.145	0.171	0.006	0.008	0.008		0.073
	Bälinge	0.163	0.137	0.113	0.148	0.170	0.050	0.050	0.042	0.033	

sible gene flow across the species boundary and differentiation.

A high proportion of alleles were not shared between species which might underestimate measures of genetic differentiation from F-statistics (Hedrick, 1999) and genetic distance measures might better reflect genetic differences in such cases. The genetic distance between the species was high and ranged between 0.343 and 0.609 (mean = 0.495 ± 0.002) (Table 3). Within species the genetic distance between populations varied between 0.035 and 0.093 (mean = 0.065 ± 0.001) in *F. polyctena* and between 0.013 and 0.103 (mean = 0.051 ± 0.001) in *F. rufa*. The *F. rufa* Bälinge population revealed the highest values in line with the result from pairwise F_{ST} values.

Exploring the data using the STRUCTURE program without prior information revealed the highest probability for five clusters, which agreed with the morphological species identification. Two of the clusters contained 99% of all of the *F. rufa* individuals while the three other clusters contained 90% of the *F. polyctena* individuals. Including the prior information on species status and allowing for low migration rate (v = 0.05) resulted in five morphologically identified *F. polyctena* individuals assigned as *F. rufa*. *F. polyctena* individuals being assigned as *F. rufa* was robust to varying migration rate, because increasing the migration rate to 0.1 lead to seven *F. polyctena* individuals being assigned as *F. rufa* while decreasing the migration rate to 0.025 decreased that number to three. Assignments can be biased if the level of heterozygosity is very different in the target populations. As no big difference existed, it seems possible that some *F. polyctena* individuals had their ancestry in the *F. rufa* colonies.

Discussion

Social organization

Formica polyctena and *F. rufa* do not show any clear qualitative difference in social organization with two completely separate nesting strategies, but a small quantitative difference in the level of polygyny, contradicting our expectations. According to various sources, *F. rufa* is considered largely monogynous (Collingwood, 1979) with 75% of nests being monogynous in Germany (Seifert, 1996). *F. polyctena*, instead, is generally considered highly polygynous with up to

1000 or more (Collingwood, 1979) or even > 5000 queens per nest (Seifert, 1996), but a small fraction of nests, less than 5%, can be monogynous (Seifert, 1996).

Our results show that not all *F. rufa* colonies are monogynous. When nests recruit there own daughters as new reproductives and relatedness between nestmate queens equals that among workers (*r*), the effective mean number of queens per colony (n) is a function of relatedness: $n = (1 + 2/m - r)/ 3r$, where m is the effective paternity (Pamilo, 1993; Seppä, 1994). In *F. rufa*, the effective paternity has been estimated to be 1.47 (Boomsma and Sundström, 1998), giving the expected relatedness of 0.59 among the single-queen brood. The upper and lower 95% boundaries of the inbreeding adjusted relatedness within our study populations suggest that the effective queen number is between 1.2 and 2.9. Thus, our estimates of worker relatedness suggest that *F. rufa* populations are a mix of polyandrous single queen nests and nests with a few coexisting queens. The *F. polyctena* populations, on the other hand, showed slightly lower levels of relatedness and a higher number of coexisting queens, which agrees with the classification of the species as obligate polygynous (Rosengren et al., 1993). Inbreeding adjusted relatedness suggests that in *F. polyctena* the effective queen number is between 3.7 and 15.2, assuming the same level of multiple paternity as in two other species of the *F. rufa* group, *F. rufa* (see above) and *F. aquilonia* (Pamilo, 1993).

The apparent discrepancy between our relatedness estimates and the earlier reports on very high queen numbers in *F. polyctena* may have several explanations. First, geographical variation in polygyny is known in many species, including *Formica* (Collingwood, 1979; Pamilo et al., 1992). Such variation has not been demonstrated in *F. polyctena*, but it is possible that the colonies in our study area are less polygynous than elsewhere. Second, the high observed queen numbers probably include some unmated females that do not contribute to the worker production. The frequency of such unmated queens has been estimated to 14–45% (Rosengren et al., 1993; Yamauchi et al., 1994), and it is unlikely that unmatedness alone explains the apparent discrepancy. Third, genetically effective queen number inferred from worker nestmate relatedness may be considerably lower than the actual queen number and may explain the apparent discrepancy between our relatedness estimates and the high level of polygyny. The model used to interpret the estimates of worker relatedness in terms of the number of queens, assumed that each queen contributes equally and that the queen number is stable. If reproduction is skewed and a small number of queens dominate reproduction (Keller and Reeve, 1994; Reeve and Keller, 1995), the effective queen number can be much smaller than the actual number. Fluctuations of the queen number can also lead to a discrepancy between the actual number of queens and the estimates based on the above model. When the number fluctuates, a large number of new queens can be recruited from a small number of sibships, resulting in a high relatedness among them. This will further result in relatedness among workers that is higher than expected on the basis of the model with constant queen numbers.

The estimates of the fixation index were positive in our study, suggesting intranidal mating. In that respect our *F. polyctena* populations resemble those of *F. paralugubris*, where Chapuisat et al. (1997) estimated that 99% of matings can take place within the nests, resulting in high viscosity, positive inbreeding coefficients and elevated relatedness among workers even though the number of coexisting queens is high. However, the estimate of the effective number of queens in *F. polyctena* was only a few tens at most when the effects of inbreeding were taken into account. We estimated positive inbreeding coefficients also in *F. rufa*, but in that species the inbreeding adjusted relatedness estimates did not much alter the view on the level of polygyny. It remains to be seen which one of the explanations could best bridge the gap between the genetic and field observations.

As noted by Keller (1995) the degree of polygyny in ants spans a continuum. It has earlier been suggested that the level of polygyny in *Formica* ants could de regulated by the availability of nest sites (Pamilo, 1981) and habitat localization (Pamilo and Rosengren, 1984). Herbers (1986) showed that the degree of polygyny and number of empty nest sites are inversely correlated, an experimental increase of nest sites resulted in the reduction of number of coexisting queens in *Leptothorax longispinosus*. Since *F. polyctena* and *F. rufa* occur sympatrically in our study area, the difference in polygyny can not be readily explained by habitat constraints. The relatively higher level of polygyny in *F. polyctena* compared to *F. rufa* can be attributed to several morphological and physiological characteristics. The *F. rufa* queens are larger (Seifert, 1991) which implies higher contents of fat and glycogen that increases the probability of successful colony foundation.

Evolutionary relationships between F. polyctena and F. rufa

The results show clearly that the morphologically defined samples of *F. polyctena* and *F. rufa* fall into two distinct genetic groups. The observed association between the morphology and genetics confirms the species status and answers the question raised e.g. by Czechowski and Douwes (1996). Previous microsatellite studies have revealed F_{ST} values between conspecific *Formica* populations with different social forms within a single locality as large as 0.19 in *F. cinerea* (Goropashanaya et al., 2001), 0.11 in *F. exsecta* (Seppä et al., submitted) and 0.14 in *F. truncorum* (Gyllenstrand et al., submitted). Thus, the difference between *F. polyctena* and *F. rufa* matches the commonly observed intraspecific level.

The existence of two widely different social forms, either as social polymorphism within a single species or as species specific nesting strategies in a pair of closely related species, has lead to speculation about the connection between colony-level social organization and speciation (Shoemaker and Ross, 1996). Gene flow, particularly by females, is generally much lower among polygynous than monogynous populations (e.g. Sundström, 1993) and the neighbouring conspecific populations can show drastic genetic differences as in

F. truncorum (Sundström, 1993), *F. exsecta* (Liautard and Keller, 2001), *Solenopsis invicta* (Shoemaker and Ross, 1996) and *Myrmica rubra* (Seppä and Pamilo, 1995). These differences are particularly strong in mtDNA, reflecting restricted female gene flow, and male gene flow can still keep the gene pools connected. The question is whether a shift in the social organization can lead to gradual genetic differentiation and ultimately speciation, resulting in pairs of sibling species with different social organization patterns. Our working hypothesis was that genetic differentiation is higher among *F. polyctena* than among *F. rufa* populations, and that the difference in social organization has initially contributed to the separation of these two species. However, as already discussed, the species did not differ greatly in the social organization and the patterns of spatial intraspecific differentiation were also very similar in them.

The observation of morphologically intermediate forms interpreted as hybrids (not included in our genetic analyses) indicates possible gene flow between the species, in agreement with earlier observations by Seifert (1991) and Czechowski (1993). The construction of linkage free populations identified a few possible migrants and detected a few *F. polyctena* individuals with possible ancestry in *F. rufa*. This implies unidirectional gene flow from less to more complex systems, which is in accordance with reports from intraspecific studies of socially polymorphic species (Ross and Shoemaker, 1997, Seppä et al., unpubl.). Possibly reproductive isolation between the species is not complete, but hybridization is too infrequent to result in similarity of the sympatric populations in the geographical scale of our sampling.

The observed degree of genetic differentiation between geographic populations was very similar in both species and we found no evidence that gene flow would have been restricted in the more polygynous *F. polyctena*. In fact, the level of genetic differentiation was comparable with that in strictly monogynous *Formica* species (Sundström, 1993). Somewhat higher inbreeding values in *F. polyctena* indicate that this species may experience more intranidal mating and local mate competition (LMC). When new queens are drawn from the same genotype pool as workers, the fraction of matings taking place among nestmates (a) is related to the inbreeding coefficient F as $a > 4F/(1 + 3F)$ (Pamilo, 1985). With the estimate of F of 0.17 in *F. polyctena*, the fraction of intranidal matings is over 45%, but it is unlikely that it reaches the level of 99% estimated for *F. paralugubris* (Chapuisat et al., 1997). The two species differ also in population wide sex ratios, being strongly female biased in *F. polyctena* (investment ratio 0.22), and close to 1:1 (investment ratio 0.46) in *F. rufa* (Pamilo and Rosengren, 1983; Pamilo 1990). This difference contradicts the expectation based on general effects of polygyny (Trivers and Hare, 1976), according to which the sex allocation ratio under worker control is expected to be more female biased when the level of polygyny is low. However, according to the dispersal model of sex allocation (Pamilo, 1990), the population sex ratio depends on the success of dispersing queens rather than on the level of polygyny. Female bias in *F. polyctena* could result from i) higher dispersal success (d) of females (predicted investment in females being $d/(1 + d)$ under queen control, Pamilo, 1990), ii) from worker control of investment (investment in females being $1.67d/(1 + 1.67d)$ assuming an effective paternity of 1.5), or iii) from LMC. Thus, possible explanations for the female biased sex ratio in *F. polyctena* include LMC and a significant role of workers in controlling the investment.

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