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RESTRICTED EFFECTIVE QUEEN DISPERSAL AT A MICROGEOGRAPHIC SCALE IN POLYGYNOUS POPULATIONS OF THE ANT *FORMICA EXSECTA*

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Abstract.—Ecological constraints on effective dispersal have been suggested to be a key factor influencing social evolution in animal societies as well as the shift from single queen colonies (monogyny) to multiple queen colonies (polygyny) in ants. However, little is known about the effective dispersal patterns of ant queens. Here we investigate the microgeographic genetic structure of mitochondrial haplotypes in polygynous populations of the ant *Formica exsecta*, both between pastures and among nests within pastures. An analysis of molecular variance revealed a very high genetic differentiation ($\Phi_{ST} = 0.72$) between pastures, indicating that queens rarely disperse successfully between pastures, despite the fact that pastures were sometimes as close as 1 km. Most of the pastures contained only a single haplotype, and haplotypes were frequently distinct between nearby pastures and even between groups of nests within the same pasture. In the three pastures that contained several haplotypes, haplotypes were not randomly distributed, the genetic differentiation between nests being $\Phi_{ST} = 0.17$, 0.52, and 0.69. This indicates that most queens are recruited within their parental colonies. However, a large proportion of nests contained more than one haplotype, demonstrating that colonies will sometimes accept foreign queens. The relatedness of mitochondrial genes among nestmates varied between 0.62 and 0.75 when relatedness was measured within each pasture and ranged between 0.72 and 1.0 when relatedness was assessed with all pastures as a reference population. Neighboring nests were more genetically similar than distant ones, and there was significant isolation by distance. This pattern may be due to new nests being formed by budding or by limited effective queen dispersal, probably on foot between neighboring nests. These results show that effective queen dispersal is extremely restricted even at a small geographical scale, a pattern consistent with the idea that ecological constraints are an important selective force leading to the evolution and maintenance of polygyny.

Key words.—Ant, ecological constraints, effective dispersal, *Formica exsecta*, genetic structure, mitochondrial DNA, sociality.

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The evolution of animal societies in which some individuals forego, to a greater or lesser extent, their own reproductive opportunities to help others to reproduce poses an evolutionary paradox that can be traced to Darwin (1859). Such societies have been described in approximately 220 bird species (Brown 1987; Emlen 1991), 120 mammals (Riedman 1982), and more than 10,000 insects (Wilson 1971). An important challenge for evolutionary biologists is to understand how such societies have emerged and subsequently evolved into elaborated social systems (Bourke and Franks 1995; Ross et al. 1996; Bourke 1997, 1999).

Ants provide an ideal system for such studies because they exhibit enormous variation in all of the components of social organization (i.e., number, relatedness, and reproductive roles of group members). One of the prominent differences between ant societies is the number of reproductive queens per colony. Queen number is a very labile trait and the evolution of multiple-queen colonies (polygyny) presents many of the same theoretical challenges as does the origin and maintenance of sociality (Ross 1989; Keller and Vargo 1993). Increased queen number is generally associated with a decrease in individual reproductive output of queens (Keller and Vargo 1993), raising the questions of why young queens join established colonies and why resident queens and workers accept them. For this reason, Rosengren and Pamilo (1983) referred to the evolution of polygynous societies from monogynous ancestors as ''sociality evolving a second time.''

Evolutionary explanations of polygyny, like those concerning the early stages of social evolution, must resolve the paradox of why individuals should risk some loss of personal reproduction by associating in groups. In the case of polygyny, queens should be unwilling to share reproductive privileges with other individuals, preferring instead to secure all of the colonies resources for their own direct reproduction. In general, the selective forces that have been invoked to explain the evolution of polygyny are the same as those implicated in the evolution of eusociality. It has been proposed that low rate of effective dispersal and independent colony founding by young queens is an important force selecting for polygyny (Crozier 1979; Pamilo and Rosengren 1984; Heinze 1993; Nonacs 1993; Rosengren et al. 1993; Keller 1995; Ross and Keller 1995; Ross et al. 1996). The lower the probability that young queens can successfully initiate a new colony on their own, the more frequently they should seek readoption into an established colony. This argument is akin to ecological constraints on independent breeding favoring the evolution of cooperative breeding in insects and vertebrates (Pamilo and Rosengren 1984; Emlen 1991, 1994; Reeve 1991; Reeve and Ratnieks 1993; Keller and Reeve 1994). Furthermore, members of an established colony should also be more likely to reaccept some of the young queens they produce when these queens have a very low prospect of successful independent colony founding (Seger 1993; Keller 1995).

Several lines of evidence suggest that polygyny may indeed be favored by ecological constraints on effective dispersal and independent breeding. Herbers (1986) showed that the degree of polygyny and the frequency of empty nest sites are inversely correlated across populations of *Leptothorax longispinosus*. Moreover, an experimental increase of nest-sites resulted in a decrease in the mean number of queens per nest.

In a comparative study of Leptothoracine ants, Bourke and Heinze (1994) also found that polygyny was associated with nest-site limitation, cold climate, and habitat patchiness, all of which increase effective dispersal cost. Similarly, in the fire ant *Solenopsis invicta,* the level of polygyny seems to be correlated with habitat saturation (Ross et al. 1996). Finally, the number of queens per colony in *Myrmica sulcinodis* was also found to be higher in habitats where all suitable nest-sites were occupied than in nonsaturated habitats (Pedersen and Boomsma 1999).

Recent studies have revealed that intra- and interspecific differences in queen number are frequently associated with a suite of changes in mating behavior and dispersal habits. Sexuals of polygynous species often do not take part in a mating flight, with mating sometimes occurring within the nest. New colonies are then formed by colony budding, a process whereby workers and queens depart on foot from their nest to initiate a new colony (Hölldobler and Wilson 1990; Keller 1991, 1993a,b; Bourke and Heinze 1994; Bourke and Franks 1995). In contrast, sexuals of monogynous species typically depart on large mating flights, and once mated the queens start a new colony without the assistance from workers. These different reproductive strategies are usually associated with phenotypic differences. Monogynous queens tend to have larger body sizes (Keller and Passera 1989b; Stille 1996), higher nutrient reserves (Passera and Keller 1990; Sundström 1995), longer life spans (Keller and Genoud 1997), higher fecundity (Michener 1964; Mercier et al. 1985; Keller 1988), and a later first age of sexual production (Keller and Passera 1990) than do their polygynous counterparts.

The lower effective dispersal rate of queens from polygynous species, together with the frequent occurrence of within-nest recruitment, intranidal mating, and reproduction via budding are expected to translate in a higher genetic structure of polygynous than monogynous populations (Rosengren and Pamilo 1983; Keller 1995; Chapuisat et al. 1997). Genetic studies with nuclear markers have indeed revealed that population genetic differentiation is generally higher in polygynous populations (e.g., Sundström 1993; Seppä and Pamilo 1995). However, studies based only on nuclear markers cannot determine whether the significant genetic structure of polygynous populations is predominantly due to a shift in male or female effective dispersal rate. Evidence of restricted effective dispersal by females comes from three studies that used maternally inherited mitochondrial DNA (mtDNA) markers. A high degree of differentiation has been found between nearby populations of a Ponerine ant, *Rhytidoponera* sp. 12 (Tay et al. 1997). However, this species has lost the queen caste, and reproduction is carried by mated but morphologically undifferentiated workers. Therefore, it is difficult to generalize these results to other ants. The second species, the fire ant *S. invicta,* displays monogynous and polygynous populations in its native habitat (Argentina and Brazil) as well as in its introduced (United States) range (Ross and Keller 1995). Genetic differentiation was high between populations or subpopulations, for both native and introduced polygynous populations (Ross and Shoemaker 1997; Ross et al. 1997; Goodisman and Ross 1998). Finally, Stille and Stille (1993) found that nests of *Leptothorax acervorum* close to each other often share the same mtDNA haplotype, suggesting that budding is common, but the degree of differentiation between nests was not quantified.

Although these data suggest that polygyny is indeed associated with restricted effective female dispersal, it is not known whether long-distance effective dispersal by females sometimes occurs or whether it has been completely replaced by local effective dispersal. The aim of this study was to determine genetic differentiation of maternally inherited markers within and between polygynous populations of the ant *Formica exsecta*. We selected this species for several reasons. First, *F. exsecta* is interesting for studying social evolution because it has a variable social structure. Some populations are monogynous (Pamilo 1983, 1984), whereas others are polygynous (Pisarski 1982; Brown and Keller 2000). In polygynous populations of the Swiss Jura Mountains the genetic relatedness between members of the colony is relatively low but significantly greater than zero, with estimates ranging from 0.02 to 0.30 (Brown and Keller 2000). The second interesting feature of this species is that it leaves in patchy habitats, which is typical of many polygynous species (Hölldobler and Wilson 1977). In the Jura Mountains, where we conducted our study, *F. exsecta* lives in cow pastures cut by humans in a region dominated by coniferous forest. This habitat has remained stable over time and pasture location has lasted at least 100 years. It is difficult to determine when the pastures had been colonized, but it is likely that the established populations are also at least 100 years old (C. Liautard, unpubl. data). Finally, *F. exsecta* provides an interesting system because females may have several reproductive options (Kutter 1969). They can disperse and found a new nest independently by parasitising other *Formica* nests (subgenus *Serviformica*), disperse and seek adoption in an established nest of their own species, or mate and stay in their natal nest and eventually disperse by foot to neighboring nests. We developed and used mtDNA markers to quantify the genetic structure and gene flow within and between pastures.

Materials and Methods

Sampling

To quantify the genetic differentiation and level of effective dispersal between pastures, we sampled a total of 401 workers on the top of 83 nests from eight pastures along a 6-km transect (Fig. 1), in the vicinity of Bassin in the Swiss Jura Mountains in 1997. Within this sample, 45 workers from 10 nests were collected by Brown and Keller (2000) in the pasture at Les Chenevières. In all but one case, pastures contained a single cluster of nests. The exception was a pasture containing two distinct clusters of nests 500 m apart. These two groups were highly differentiated (see Results) and are considered as two pastures to simplify nomenclature.

To assess the level of effective dispersal within pastures, we selected the three pastures that contained several haplotypes in 1997. A total of 408 workers were sampled in 1998 from 45 nests in these three pastures. The Differential Geographic Position System (D-GPS; Garmin, Ltd., Romsey, U.K.) position was taken for each nest sampled in 1998 to determine the distance between each pair of nests. Because some nest are separated by less than 5 m, we also measured

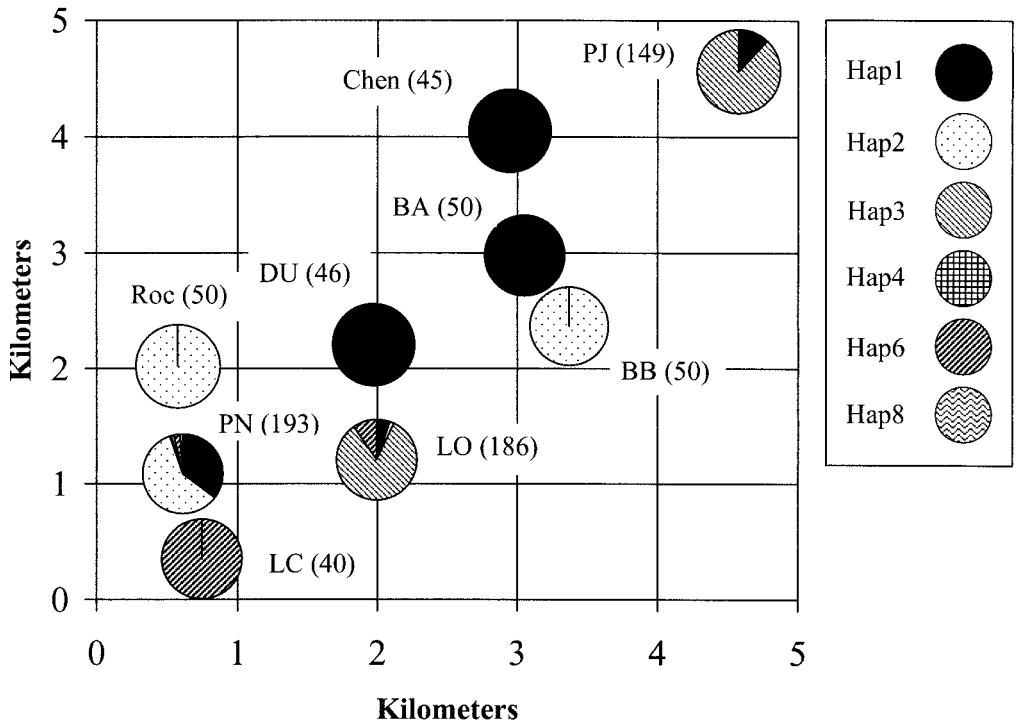

FIG. 1. Haplotype frequency in nine pastures in the Swiss Jura Mountains. Each circle represents a pasture, with the abbreviation of its name next to it (BA, Bugnonet subgroup A; BB, Bugnonet subgroup B; Chen, Les Chenevières; DU, La Dunanche; LC, La Conriéry; LO, Les Orgères; PJ, Pré de Joux; PN, Pré Nouveau; ROC, Combe au Roc) and the number of workers sampled. Pasture coordinates were determine according to the Swiss National Maps Service.

the distance between nine nests within Les Orgères with a tape-measure to assess the accuracy of the GPS measures. The relative position of all nests was the same for the GPS and tape-measure maps. When the distance between nests obtained for the two measures are compared, a maximum difference of 2.2 m between nests was observed between the two kinds of measures. This indicates that D-GPS measures provided reliable estimates of the distances between nests.

Genetic Analysis

To develop specific primers for *F. exsecta*, we sequenced 4 kb around the cytochrome *b* region from the NADH1 gene to the NADH4 gene for one individual, using ND1 and ND4 primers (Simon et al. 1994). This sequence was aligned with that of the honey bee (Crozier and Crozier 1993) to confirm that the target region of the mtDNA was amplified. With the help of the software Primer (available at: http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi), this sequence was used to design three pairs of primers in the cytochrome *b* gene and NADH1 gene (Table 1).

DNA from each ant was extracted using a standard salt extraction protocol. Head and thorax were ground in incubation buffer (50 mM Tris HCl, 20 mM EDTA, 1% SDS, 200 mM NaCl, 0.2 mg/ml proteinase K) and incubated overnight at 55°C. Proteins were then precipitated using NaCl solution (1.5 M), and DNA was precipitated and cleaned with 70% alcohol. The three regions (cytochrome *b*, ND1a, and ND1b) were amplified by polymerase chain reaction (PCR) for each individual. All PCR reactions were performed in a 25-μl volume using standard PCR conditions: PCR buffer (1X concentration), dNTPs (0.2 mM), primers (0.5 mM of each) and 1 unit of Gibco BRL (Rockville, MD) *Taq* DNA polymerase. ND1a PCR reaction was carried out with 1.25 mM of $MgCl_2$ in a thermal cycler programmed as follows: 94°C for 1 min, 35 cycles of amplification (92°C for 30 sec, 55°C for 20 sec, 71°C for 30 sec). The PCR product was incubated for 2 h at 37°C with *Nde*I restriction enzyme. ND1b PCR reaction was carried out with 1 mM of $MgCl_2$ in a thermal cycler programmed as follows: 94°C for 2 min and 35 cycles of amplification (92°C for 30 sec, 53°C for 20 sec, and 72°C for 40 sec). The PCR product was digested with *Hinf*I restriction enzyme for 2 h at 37°C. Cytochrome *b* PCR reaction was carried out with 2 mM of $MgCl_2$ in a thermal cycler programmed as follows: 92°C for 4 min, 35 cycles of

TABLE 1. Mitochondrial DNA loci yielding polymorphic haplotypes in the cytochrome *b* region of *Formica exsecta.*

Locus	F-primer	R-primer	Size
ND1a-Fe	CTT-TTA-GAG-ATG-CTA-TTA-AAT-TGC-TTA	TTG-AAT-TAG-ATG-ATC-ATC-CTA-TAA-AAA	234 bp
ND1b-Fe	CAT-TTA-TTC-TCC-TAA-CTT-GAG	GAG-AGA-GAT-ATT-CAT-TCT-TAG	757 bp
Cytb-Fe	CAG-TTT-AAT-TTC-TAA-TGA-ACA-AAC	GGA-TCT-CTA-AAA-ATA-TAT-GGG	~1100 bp

amplification (92°C for 1 min, 54°C for 1 min, +0.2°C/sec until 70°C, 70°C for 2 min, +0.5°C/sec until 92°C) and 72°C for 10 min. The PCR product was digested with *Hinf*I, *Rsa*I, and *Sau*3AI restriction enzymes for 2 h at 37°C. All digestion products were electrophoresed on a 2% agarose gel.

Population Structure

Genetic differentiation between nests within pastures and between pastures was assessed with two measures (Arlequin 1.1, Excoffier et al. 1992). First, an analysis of molecular variance (AMOVA), which incorporated information on DNA haplotype divergence into an analysis of variance (Φ_{ST}-values). Second, a classical analysis of variance without considering information on DNA haplotype divergence was done (F_{ST}-values). Because identical results were found with both methods, we will present only the Φ_{ST}-value. The probability that Φ_{ST} was significantly different from zero was estimated by permutation test using 10,000 random haplotype distributions. All analyses were performed by taking into account structure at both the nest and pasture levels. We also measured the similarity of nestmate workers regarding their mtDNA by estimating the relatedness between them with their mtDNA haplotype with the program Relatedness 5.0 (Queller and Goodnight 1989). To estimate the correlation between genetic distance between pastures (or nests) and geographical distance between pastures (or nests), we performed a Mantel's test (Genepop 3.1b, Raymond and Rousset 1995). The probability that the correlation coefficient was significantly different from zero was estimated by a permutation test using 10,000 randomly permuted matrices.

TABLE 2. Digestion profile on each haplotype with the approximate size in base pairs of each observed fragment longer than 100 bp. Fragments that vary in size among haplotypes are given in bold.

	Cytochrome *b*			ND1a	ND1b
	*Hinf*I	*Rsa*I	*Sau*3AI	*Nde*I	*Hinf*I
Haplotype 1	100 150 250 **290** 350	**100** **295**	**200** 220 510	**230**	**150** 600
Haplotype 2	100 150 **210** 250 350	**100** **295** 700	**130** 220 510	**100** **130**	**150** 600
Haplotype 3	100 150 250 **290** 350	**395** 700	**200** 220 510	**100** **130**	**150** 600
Haplotype 4	100 150 250 **290** 350	**100** **295** 700	**200** 220 510	**230**	**150** 600
Haplotype 6	100 150 **210** 250 350	**100** **295** 700	**200** 220 510	**100** **130**	**110** 600
Haplotype 8	100 150 250 **290** 350	**100** **295** 700	**200** 220 510	**100** **130**	**150** 600

RESULTS

Six haplotypes were found within the three PCR products (Table 2). Five of these were found in the 1997 samples, and the sixth was found in only a few individuals of the 1998 sample in the pasture Pré Nouveau.

Genetic Structure between Pastures

The number of haplotypes in polymorphic pastures varied between two and six (PJ, two haplotypes; LO, three; PN, six; Fig. 1). Genetic differentiation between pastures was extremely high ($\Phi_{ST} = 0.716$, $P < 0.00001$). Strong differentiation was also obvious when looking at haplotype distribution (Fig. 1). A single haplotype was found in six of the nine pastures and they were frequently distinct, even between closely located pastures (Fig. 1). The two populations BA and BB, which were in the same pasture (Fig. 1) and separated by only 500 m, were fixed for different haplotypes.

No significant correlation between genetic and geographical distance between pastures was observed (slope = 0.6, $P = 0.19$). However, the power of the analysis was low, due to the small sample size (nine pastures).

Within-Pasture Genetic Structure

Genetic differentiation among nests within each of the three pastures containing polymorphic haplotypes was considerable (LO: $\Phi_{ST} = 0.170$, $P < 0.001$; PJ: $\Phi_{ST} = 0.688$, $P < 0.0001$, PN: $\Phi_{ST} = 0.515$, $P < 0.0001$, Fig. 2a), indicating limited effective dispersal of queens between nests and some acceptance of daughter within established nests. A significant positive correlation between geographic distance and pairwised Φ_{ST} was found (LO: slope = 0.00088, $P = 0.0018$; PJ: slope = 0.00175, $P = 0.0018$, PN: slope = 0.0038, $P < 0.00001$; Fig. 2b). Greater genetic similarity between closer nests indicates that effective dispersal is generally local.

Strong differentiation between nests within the same pasture is also indicated by a high degree of similarity of workers regarding their mtDNA haplotype within nests—and thus their mtDNA relatedness. When ants from an individual pasture are taken as the reference population, the mtDNA relatedness varied between 0.62 and 0.75 (Table 3; note that mtDNA relatedness can be calculated only in the three pastures that contained more than one haplotype). When all pastures are taken as a reference population, the mtDNA relatedness was higher, ranging between 0.72 and 1.00 (Table 3). Obviously, the relatedness was 1.00 in all monomorphic pastures because all nestmate workers shared the same haplotype.

Nest Polymorphism

Several nests contained more than one haplotype. Among the random sample of nests collected in 1997, 95% ($n = 78$) contained a single haplotype and 5% ($n = 5$) two haplotypes. The proportion of nests containing several haplotypes was

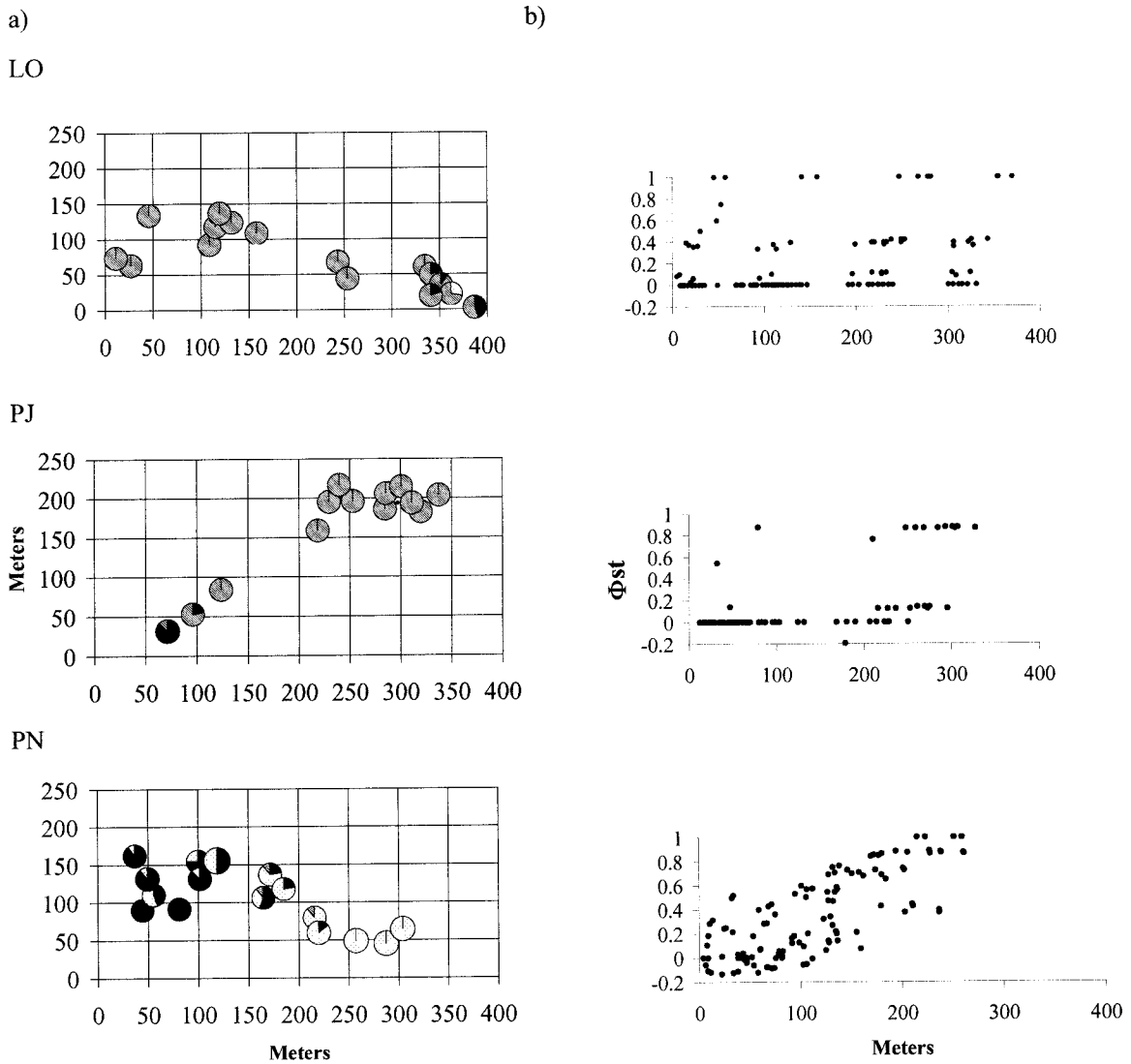

FIG. 2. (a) Haplotype frequency of ants from 16 nests for pastures LO and PN, and 13 nests for pasture PJ, each of which contains several haplotypes (see Fig. 1 for abbreviations). Circles indicate nest position and are filled according to haplotype frequency. Grid lines are spaced at 50 m. For pastures LO and PJ, black represents haplotype 1, white haplotype 2, and gray haplotype 3. For PN, black represents haplotype 1, white haplotype 2, and gray haplotype 3 or haplotype 8. (b) Correlation between genetic distance and geographic distance within three pastures with polymorphic haplotypes.

TABLE 3. Mean mitochondrial DNA relatedness between workers per pasture (see Fig. 1 for abbreviations). The entire sample (i.e., all eight populations) was taken as a reference population. When haplotypes were polymorphic within pastures, we also estimated relatedness using pasture as the reference population.

	Reference population	
	Pasture	Entire sample
BA	—	1.00 ± 0.00
BB	—	1.00 ± 0.00
Chen	—	1.00 ± 0.00
DU	—	1.00 ± 0.00
LC	—	1.00 ± 0.00
LO	0.75 ± 0.40	0.84 ± 0.26
PJ	0.85 ± 0.42	0.92 ± 0.20
PN	0.62 ± 0.43	0.72 ± 0.32
Roc	—	1.00 ± 0.00

higher in 1998, with 35% (n = 16) of nests containing two haplotypes and 4.4% (n = 2) of the nests containing three haplotypes. The higher proportion of nests with several haplotypes in 1998 is probably due to the sample being biased toward polymorphic pastures (Les Orgères, Pré de Joux, and Pré Nouveau). Indeed, when only the three polymorphic pastures were considered in 1997, the proportion of polymorphic nests (χ^2 = 2.08, df = 1, P = 0.15) was not significantly different between 1997 and 1998. The average number of haplotypes per nest was not different (t = 0.99, df = 86, P = 0.32) between 1997 and 1998 when only the three polymorphic pastures (Les Orgères, Pré de Joux, and Pré Nouveau) were considered. This suggests that the greater number of workers sampled in 1998 compared to 1997 (9.1 ± 1.3 versus 4.8 ± 0.5 workers per nest) had only a limited effect on the estimates of the number of haplotypes per colony.

Discussion

Our analyses reveal an extremely high microgeographic genetic differentiation ($\Phi_{ST} = 0.72$) between pastures. Such a high differentiation indicates that effective queen dispersal is extremely restricted among pastures, despite their close proximity. In fact, two-thirds of the pastures contained a single haplotype and some pastures were fixed for different haplotypes even though they were only a few hundred meters away and in one case shared the same clearing. These results clearly show that effective queen dispersal between pastures and between distinct clusters of nests within a pasture is very uncommon, suggesting that one or very few queens initially colonize these locations.

High mtDNA genetic differentiation among populations has also been reported in two other polygynous ants. In *Rhytidoponera* sp. 12 the differentiation was high ($F_{ST} = 0.5$) between populations distant by a few kilometers (Tay et al. 1997). In *S. invicta* the differentiation varied between $\Phi_{ST} = 0.192$ and $\Phi_{ST} = 0.422$ among groups of nests distant by 5 km in native populations (Ross et al. 1997); in introduced populations it equaled 0.136 at the scale of 2 km, and 0.503 along a 21-km transect (Ross and Shoemaker 1997; Goodisman and Ross 1998). The high differentiation in *Rhytidoponera* sp. 12 was suggested to stem from the flightlessness of the gamergates (reproductive workers), which may prevent their dispersal between populations. However, the genetic differentiation was nearly as high in *S. invicta* and higher in *F. exsecta* than in *Rhytidoponera* sp. 12, and females of both *S. invicta* (DeHeer et al. 1999) and *F. exsecta* (Fortelius 1987) have retained the ability to fly. This suggests that flightlessness is more likely to be a consequence than the cause of low effective dispersal.

Because *F. exsecta* forms small populations that are patchily distributed in pastures, our sampling design and the hierarchical analysis with pastures treated as one level of structure corresponds to a real geographic subdivision between breeding populations. In contrast, *S. invicta* and *Rhytidoponera* sp. 12 do not form discrete populations separated by forests or other geographic barriers that strongly prevent gene flow. Therefore, hierarchical analyses of population subdivision were based on arbitrary subdivision not directly associated with effective ecological constraints on dispersal. In fact, it is likely that the significant genetic differentiation of mtDNA found in *S. invicta* and *Rhytidoponera* sp. 12 reflects isolation by distance that is detectable on a scale of a few kilometers. Our data in *F. exsecta* demonstrate that in addition to isolation by distance (see below), relatively long-range effective dispersal between pastures, and even groups of nests within the same pasture, is very rare. This contrasts with the well-developed colonizing ability of queens from monogynous species that are typically more prone to fly (Sundström 1995) and disperse several kilometers away from their natal colony (Boomsma and Van der Have 1998). Interestingly, one of the few monogynous species in which significant genetic differentiation has been found is *F. exsecta*. Using one nuclear allozyme marker, Pamilo (1983, 1984) found a small but significant differentiation between islands in a monogynous population. However, the level of differentiation found ($F_{ST} = 0.09$) was much lower than the differentiation found between pastures in Switzerland. This may be due to specific female restricted effective dispersal (thus translating into a higher differentiation at mitochondrial than nuclear markers) and/or a lower effective dispersal associated with polygyny in Switzerland.

Mitochondrial DNA genetic differentiation similar to that found in *F. exsecta* is very unusual in animals other than polygynous ants. Generally, it only occurs at a continental scale, between populations separated for several millions years (e.g., Ellsworth et al. 1994; Baker et al. 1995; Arctander et al. 1996; Jansen van Vuuren and Robinson 1997). In rare cases, very high mtDNA genetic differentiation has been due to high fidelity to a feeding ground (humpback whales, Baker et al. 1998) or reproductive site (e.g., colonial sea birds, Friesen et al. 1996; green turtles, Fitzsimmons et al. 1997). Aside from these studies, high mtDNA genetic differentiation has been reported only in one other species, Betchstein's bats (Kerth et al. 2000). Interestingly, this species is also social: groups of females living together in the same roost. Levels of microgeographical differentiation at maternally inherited markers similar to that found in *F. exsecta* has also been documented in some plants. For example, high differentiation using chloroplast markers has been found in three *Silene* species (*S. alba*, McCauley 1994, 1997; *S. vulgaris*, McCauley 1998; *S. dioica*, Giles et al. 1998; Ingvarsson and Giles 1999). The distribution of a male sterility gene in a population of *Plantago lanceolata* also suggests a strong differentiation at a microgeographic scale (Van Damme 1986). In these species strong microgeographic differentiation is probably due to low seed dispersal ability, with seed dispersing by gravity.

We also found high mtDNA genetic differentiation among nests within the same pasture. This indicates that effective dispersal is not only restricted between pastures, but also between nests of the same pasture. The average relatedness of nestmate workers, as assessed with mtDNA markers ranged between 0.62 and 1.00, indicating that queens are mainly recruited within their maternal colony. These values are much higher than the relatedness values obtained with nuclear markers. Using microsatellites and allozymes, Brown and Keller (2000) found that the relatedness of workers was $r = 0.065 \pm 0.027$ at Les Chenevières. Although the relatedness values obtained with nuclear and mtDNA markers are not comparable, the higher relatedness obtained with the mtDNA markers suggests that males disperse significantly more than females.

Within pastures, differentiation of the mtDNA markers among nests was associated with isolation by distance, close nests being genetically more similar than distant ones. Strong differentiation indicates that queens disperse at a very short distance within pastures. This pattern can arise from the formation of new nests mostly by budding or by queens entering neighboring nests after having mated in their maternal nest (Keller 1991). Several studies using nuclear markers showed that polygyny is often associated with isolation by distance at a small geographical scale (Sundström 1993; Seppä and Pamilo 1995; Chapuisat et al. 1997). Our results on a maternally inherited genetic marker support the view that this pattern is at least partly due to limited effective female dispersal.

Information on the mtDNA genetic differentiation between

closely located nests is available in three other ants. In *Leptothorax acervorum* some clustering of nests with identical mtDNA haplotypes has been found, suggesting limited effective queen dispersal within population (Stille and Stille 1993). However, the differentiation between nests was not quantified. In *Rhytidoponera* sp. 12 colonies were clumped with respect to mtDNA haplotypes in one of the seven sites studied, but the lack of significance in the six other sites are most likely due to the smaller number of colonies sampled at these sites (Tay et al. 1997). Finally, the average mtDNA relatedness at the level of nest within sites, that is, at a scale of less than 100 m, was not different from zero in introduced populations of *S. invicta* (Goodisman and Ross 1998). Moreover, within the six sites studied the relationship between geographic distance and their genetic similarity was only very weak, with a significant association in only one site. Hence, the microgeographic mtDNA genetic structure of introduced *S. invicta* populations apparently contrasts with those of *F. exsecta*, *L. acervorum*, and *Rhytidoponera* sp. 12. However, in native Argentinean populations, nestmate relatedness values are similar to the ones found for *F. exsecta*. A possible explanation for this difference is that the social organization of *S. invicta* has been modified by the new ecological conditions encountered in the introduced range (Keller 1995; Ross et al. 1997; Goodisman and Ross 1998).

Although *F. exsecta* nestmates have similar mtDNA haplotypes (high mtDNA relatedness), several haplotypes were present in some nests. This indicates that nests occasionally accept foreign, unrelated queens. It is likely that the presence of several haplotypes is due to the acceptance of unrelated queens over several generations especially because it seems that nests of *F. exsecta* are relatively long lived. Additionally, there are probably several unrelated queens because minority haplotypes are found in high frequency in some of the nests. The presence of several haplotypes in the same colony has also been reported in *L. acervorum* (Stille and Stille 1992), *Rhytidoponera* sp. 12 (Tay et al. 1997), *Iridomyrmex purpureus* (Carew et al. 1997), and *S. invicta* (Goodisman and Ross 1998). This raises the question of why colonies do accept unrelated queens. It is assumed that social insects workers use cuticular hydrocarbon cues to recognize related individuals (Carlin and Hölldobler 1983; Stuart et al. 1993; Dahbi and Lenoir 1998). However, it seems that in polygynous colonies, the discrimination capacity of workers decreases due to the increased diversity of odor cue profiles already existing within the colony (Keller and Passera 1989a; Starks et al. 1998; Goodisman and Ross 1999). In some highly polygynous colonies, nestmate recognition seems to be very low and it is possible that workers use indirect cues to prevent foreign queens from entering their nest. For example, in the highly polygynous ant *Formica paralugubris*, young queens are much more likely to be accepted in foreign colonies producing queens than in colonies producing no queens (Fortelius et al. 1993). Selective destruction of all young queens in colonies producing no queens may have evolved as a simple rule of thumb to decrease the probability of acceptance of foreign unrelated queens. It is interesting to note that three of the studied population of *F. exsecta* (Les Orgères, Pré Nouveau, and Les Chenevières) has indeed been found to exhibit poor nestmate recognition capacity (W. D. Brown, C. Liautard, and L. Keller, unpubl. data).

In conclusion, this study reveals very high genetic differentiation both between nests within pastures and between pastures, even though the pastures studied were close to each other. Moreover, the majority of the pastures contained a single haplotype, supporting the view that successful dispersal and independent breeding by young queens is rare and that ecological constraints may indeed be an important force selecting and maintaining polygyny.

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