

Unicoloniality, recognition and genetic differentiation in a native *Formica* ant

B. HOLZER,* M. CHAPUISAT,* N. KREMER,† C. FINET‡ & L. KELLER*

*Department of Ecology and Evolution, University of Lausanne, Biophore, Lausanne, Switzerland

†Laboratoire de Biométrie et Biologie Evolutive CNRS UMR 5558, Université Claude Bernard Lyon 1, Villeurbanne Cedex, France

‡Ecole Normale Supérieure de Lyon, Laboratoire de Reproduction et Développement des Plantes UMR CNRS 5667, Lyon Cedex, France

Keywords:

genetic structure;
recognition ability;
social insects;
unicoloniality.

Abstract

Some ants have an extraordinary form of social organization, called unicoloniality, whereby individuals mix freely among physically separated nests. This mode of social organization has been primarily studied in introduced and invasive ant species, so that the recognition ability and genetic structure of ants forming unicolonial populations in their native range remain poorly known. We investigated the pattern of aggression and the genetic structure of six unicolonial populations of the ant *Formica paralugubris* at four hierarchical levels: within nests, among nests within the same population, among nests of populations within the Alps or Jura Mountains and among nests of the two mountain ranges. Ants within populations showed no aggressive behaviour, but recognized nonnestmates as shown by longer antennation bouts. Overall, the level of aggression increased with geographic and genetic distance but was always considerably lower than between species. No distinct behavioural supercolony boundaries were found. Our study provides evidence that unicoloniality can be maintained in noninvasive ants despite significant genetic differentiation and the ability to discriminate between nestmates and nonnestmates.

Introduction

Kin selection is the key selective force allowing the evolution and maintenance of altruism in the animal kingdom. A necessary condition for kin selection to operate is the ability of individuals to discriminate kin from nonkin and preferentially direct altruism towards related individuals (Hamilton, 1964, 1987). Accordingly, in most ants and other social insects, individuals recognize nestmates from nonnestmates and aggressively reject the latter (Crozier & Pamilo, 1996). This allows the maintenance of the territorial colony boundaries, prevents the invasion and exploitation of the colony by unrelated conspecifics and ensures that altruistic acts are directed towards relatives (Crozier & Pamilo, 1996).

Some ants, however, have an extraordinary social organization, called unicoloniality, whereby individuals mix freely among physically separated nests (Hölldobler & Wilson, 1977; Pedersen *et al.*, in press). By reducing the costs associated with territoriality, unicoloniality allows high worker densities and effective habitat monopolization by the competitive exclusion of other ant species (Holway *et al.*, 1998; Holway, 1999; Holway & Suarez, 2004). Indeed, of the 17 land invertebrates species listed among the world's worst invaders (<http://www.issg.org>), five are ant species with documented or inferred unicolonial structures.

While being a key attribute responsible for the ecological dominance of some ants, unicoloniality is also an evolutionary paradox and a potential problem for kin selection theory because this mode of social organization leads to an extremely low relatedness between nestmates (Hamilton, 1964; Crozier, 1979; Bourke & Franks, 1995; Keller, 1995; Crozier & Pamilo, 1996; Queller & Strassmann, 1998; Queller, 2000). Understanding

Correspondence: Barbara Holzer, Department of Ecology and Evolution, University of Lausanne, Biophore, 1015 Lausanne, Switzerland.
Tel.: 41 21 692 4181; fax: 41 21 692 4165;
e-mail: barbara.holzer@unil.ch

how unicolonial populations have evolved and how they can be maintained by natural selection remains a major problem in evolutionary biology (Keller, 1995; Queller, 2000; Pedersen *et al.*, in press).

Until very recently it was thought that unicolonial species typically failed to exhibit any intraspecific aggression. However, this dogma has been challenged by recent findings showing that even introduced populations of the Argentine ant *Linepithema humile*, a species native to Argentina that has been introduced in various parts of the world (Suarez *et al.*, 2001), can exhibit aggression between supercolonies (Tsutsui *et al.*, 2000; Giraud *et al.*, 2002). The supercolonies are actually composed of a large number of nests forming a unicolonial population that can range over several thousand kilometres in the introduced range (Giraud *et al.*, 2002). Contrary to what was thought, native populations also form supercolonies, but their size is several orders of magnitude smaller than in the introduced range (Pedersen *et al.*, in press). Similar results have been obtained in the little fire ant *Wasmannia auropunctata*. No aggression was observed within a large introduced population in New Caledonia and within populations in the native range. However, aggression was high between populations in the native range (Le Breton *et al.*, 2004; Errard *et al.*, 2005).

The finding that unicolonial species may form supercolonies, which are aggressive to each other has important implications for our understanding of the origin and maintenance of unicoloniality. In particular, if supercolonies are relatively small it makes it a less daunting task to explain the evolution and stability of unicoloniality (Pedersen *et al.*, in press). To some extent those supercolonies can be considered as the end point of a continuum in social organization with strict monogyny at the other end and various levels of polygyny in between (Keller, 1995). This begs the question of the most relevant scale to be considered when measuring relatedness and investigating the role of kin selection. Theoretical studies have shown that relatedness should be measured at the level of the 'economic neighbourhood', that is the scale at which intraspecific competition generally takes place (Taylor, 1992; Kelly, 1994; Queller, 1994; Griffin & West, 2002). Hence, if competition occurs mostly within supercolonies, relatedness should be measured at the scale of the supercolony with the effect that individuals collected in the same nest are effectively completely unrelated. By contrast, if supercolonies compete with each other, for example for access to territory, then the genetic differentiation between supercolonies should be considered when measuring relatedness.

Although many species have been described as forming unicolonial populations (Cherix, 1980; Kaufmann *et al.*, 1992; Passera, 1994; Ross *et al.*, 1996; Astruc *et al.*, 2001; Holway *et al.*, 2002; Van der Hammen *et al.*, 2002) there are only few cases where the level of aggression has been

studied at a large scale and no species other than the Argentine ant where behavioural tests have been combined with population genetic studies. Therefore, it is largely unknown whether native species described as unicolonial also form supercolonies aggressive to each other, and if so, what are the sizes of supercolonies. Such data are important to determine the scales at which cooperation and competition occur (Pedersen *et al.*, in press).

Several ant genera are of particular interest to study social evolution because they exhibit high variability in social organization (Bourke & Franks, 1995; Crozier & Pamilo, 1996). Among those, the genus *Formica* is of special interest because this genus is characterized by tremendous intraspecific and interspecific variation in social organization (Rosengren & Pamilo, 1983; Rosengren *et al.*, 1993; Bourke & Franks, 1995; Chapuisat *et al.*, 2004; Sundström *et al.*, 2005), with several species having been suggested to form supercolonies and unicolonial populations (Bourke & Franks, 1995; Elias *et al.*, 2005). One of the first supercolony was described in a population of *Formica lugubris* in the Swiss Jura mountains (Gris & Cherix, 1977; Cherix, 1980). Behavioural studies revealed no aggression within a 0.7 km² supercolony but some aggression with nests outside of the supercolony. However, later studies revealed the presence of two cryptic species (*F. paralugubris* and *F. lugubris* Pamilo *et al.*, 1992; Seifert, 1996) precluding to determine whether the aggression observed occurred within or between species. Later genetic studies showed that the ant forming the large supercolony is actually *F. paralugubris* and that it is characterized by a low intranest relatedness, significant isolation by distance (Chapuisat *et al.*, 1997) and low dispersal of both sexes (Chapuisat & Keller, 1999). Behavioural studies also showed that workers of this species usually are not aggressive towards workers from other nests of the same population (Chapuisat *et al.*, 2005) and that in the majority of the cases workers fail to discriminate between pupae of their own nests and pupae from other nests of the same population (Maeder *et al.*, 2005). An even larger supercolony with 45 000 nests has been found in *F. yessensis* on Hokkaido although no aggression test or any genetic studies are available (Ito, 1971; Higashi & Yamauchi, 1979). It is also unknown whether this supercolony exhibits aggression towards ants from other populations. Unicoloniality has also been investigated in populations of the ant *F. truncorum* (Rosengren *et al.*, 1985; Elias *et al.*, 2005). The genetic data showed that nestmate relatedness is very low or undistinguishable from zero and individuals move between nests. However, it is unknown whether this species form supercolonies, which are aggressive to each other. Interestingly, while conducting transfer experiments Rosengren *et al.* found a significant negative relationship between the acceptance index and the distance between nests at a scale of 100 km in *F. truncorum* (Rosengren & Pamilo, 1986).

Unfortunately, no genetic information is available on the genetic similarity between the nests tested.

The aim of this study was to conduct a large-scale study to determine whether *Formica* species can form supercolonies similar to those that have been described in the Argentine ant. In particular, we were interested in determining whether populations are organized in discrete supercolonies or whether there is a continuous gradation in the level of aggression that may correlate with genetic isolation by distance and/or spatial distance between nests. For this study we selected the ant *F. paralugubris* because previous work suggested the possible occurrence of both genetic isolation by distance at a small scale (Chapuisat *et al.*, 1997; Chapuisat & Keller, 1999) and well-defined supercolonies (Cherix, 1980, but see above discussion about the problem of cryptic species). To address these issues, we investigated the pattern of aggression and the genetic structure of six populations separated by up to 72 km. This allowed us to investigate the relationship between the level of aggression, geographic distance and genetic differentiation at four hierarchical levels: within and among nests within the same population, among nests of populations within the Alps or Jura Mountains and among nests of the two mountain ranges.

Materials and methods

Field collection

We collected *F. paralugubris* ants from four populations in the Swiss Jura Mountains and from two populations in the Swiss Alps between 7 July and 10 July 2003. All sites were situated between 950 and 1310 m above sea level (Fig. 1). For the aggression tests, we collected 300 workers from three nests at each site, except for the population of Lac de l'Hongrin where only two nests were found. The species was determined on the basis of morphological characters of workers or queens (Seifert, 1996). In order to measure the interspecific aggression, we also collected 10 nests of the sympatric species *F. lugubris* (Seifert, 1996) from a population close to Chalet à Roch. For the genetic analysis we collected workers from 20 nests at Bois de Peney, 20 nests at Chalet à Roch, 10 nests at Bois de Ban, seven nests at Champs Simon, nine nests at Château d'Oex and two nests from Lac de l'Hongrin. To estimate the geographic distance between nests, the GPS coordinates were noted (D-GPS; Garmin Ltd. Romsey, UK). Distances between nests ranged from 15 m to 72 km.

Aggression tests

Ants were kept at 22–24 °C in 26 cm × 41 cm × 15 cm compartments with flouon-coated walls (Whitford, Germany) and nest material on the ground. Workers were regularly fed with 8% sugar water and a mixture of agar,

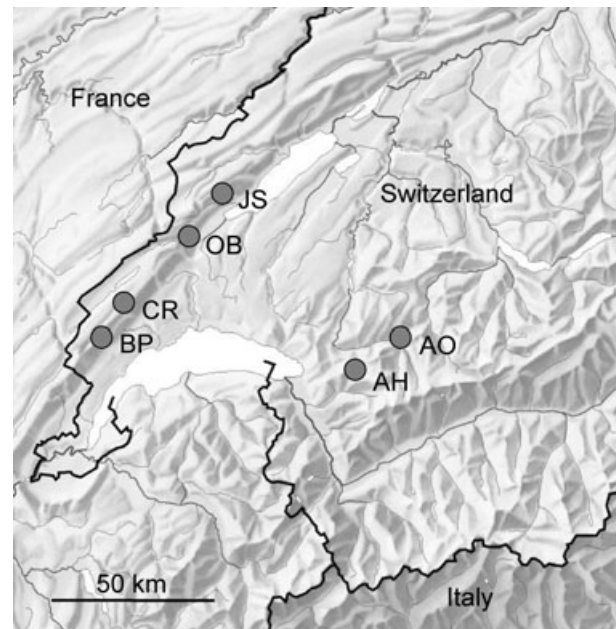


Fig. 1 Map of the study populations of the wood ant *Formica paralugubris* in the Swiss Jura Mountains: Bois de Peney (BP), Chalet à Roch (CR), Bois de Ban (OB), Champs Simon (JS) and in the Swiss Alps: Château d'Oex (AO), Lac de l'Hongrin (AH).

egg, honey and water. Aggression tests were performed (mean ± SD) 5 ± 4 days after field collection in a random order between each pair of nests. This resulted in an aggression matrix with 17 rows and columns with the following four categories: between nestmates, between nests of the same population, between nests of different populations from the same mountain range and between nests of different mountain ranges. We also conducted interspecific aggression tests between ten pairs of nests of *F. paralugubris* and *F. lugubris*.

In the aggression tests, one worker from each of two nests was selected and placed on opposite ends of a plastic cup (diameter: 6 cm) filled with nest material. Ants were observed for 2 min after the first encounter. To quantify the behaviour, each contact was scored as follows (Giraud *et al.*, 2002): 0 = ignorance (physical contact between individuals where neither ant showed any interest for the other ant), 1 = antennation (repeated tapping of the antennae somewhere on the other ant); 2 = avoidance (one or both ants retreated in opposite directions after contact); 3 = dorsal flexion (abdomen pointed out to the other ant in a threatening posture); 4 = aggression (biting of the other ant); 5 = fight (prolonged aggression, often involving locking the mandibles onto a body part of the other ant or carrying it). Six replicate tests were conducted for each nest pair, using the nest materials of each nest three times. After each test the cups were cleaned with ethanol to remove potential contamination with formic acid or alarm pheromones.

The observer of the aggression tests was blind with regard to the nest of origin of the workers. The level of aggression between pairs of nests was calculated as the mean of the highest level of aggression recorded during each of the six replicates.

In ants, antennation plays a crucial role in recognition behaviour and novel odours are generally investigated longer than familiar stimuli (Carlin & Hölldobler, 1987b). To determine whether ants may discriminate between different types of individuals we also recorded the proportion of long antennations, defined as the number of antennations that lasted for more than 3 s divided by the total number of antennations.

Genetic analysis

Eight workers from each nest were genotyped. DNA was extracted from individual workers by incubating three crushed legs in 250 μ L of 5% Chelex at 90 °C for 20 min. The suspension was stored at -20 °C and directly used for PCR amplification. The ants were analysed at four microsatellite loci developed for *F. paralugubris* (FL12, FL20, FL21, FL29, Chapuisat, 1996) and seven loci originally developed for *Formica exsecta* (FE7, FE8, FE11, FE19, FE37, FE38 and FE42, Gyllenstrand *et al.*, 2002). One marker (FE21, Gyllenstrand *et al.*, 2002) was discarded because of a null allele. Each locus had between two and 22 alleles with an average of six alleles and an expected heterozygosity ranging between 0.16 and 0.90 (mean heterozygosity: 0.59). Primers were labelled with fluorescent dyes and PCR products were visualized on an ABI 377XL sequencer.

Statistical analyses

The level of aggression and the proportion of long antennations between nestmates (control) and nonnestmates within the same population were compared with Wilcoxon signed-rank tests. The control nest was used only once and was paired with a randomly resampled value of the nonnestmates tests within each population. Each nonnestmates test within each population was used only once. Mantel tests (Sokal & Rohlf, 1995; Manly, 1997) were performed to analyse the effect of population on the level of aggression. We constructed a 'population matrix' containing '0' for pairs of nests from the same population and '1' for pairs of nests belonging to a different population. The aggression matrix was then correlated with the 'population matrix'. A similar matrix was used to test the effect of mountain range on the level of aggression. The 'mountain range matrix' contained '0' for pairs of nests from the same mountain range and '1' for pairs of nests from different mountain ranges. The same statistical procedure was also used to investigate the effect of population and mountain range on the proportion of long antennations.

Genetic variation was analysed at four hierarchical levels (individuals, nests, populations and mountain ranges) using a four-level hierarchical *F*-analysis of variance with the statistical software GENETIC DATA ANALYSIS (GDA, Lewis & Zaykin, 1999). The sigma values obtained with GDA were used to calculate the *F*-values according to the formulas described in ARLEQUIN (Schneider *et al.*, 2000). The coefficient at the first level measures the correlation of genes within individuals as compared to random genes within the nest ($F_{\text{ind-nest}}$). The coefficients at the upper levels estimate the genetic differentiation among nests within populations ($F_{\text{nest-pop}}$), among populations within mountain ranges ($F_{\text{pop-mountain}}$) and between the mountain ranges within the entire sample of populations ($F_{\text{mountain-total}}$). Confidence intervals were obtained with SPLUS (Insightful Corp., 2003) by bootstrapping 2000 times over loci.

To investigate whether the level of aggression was correlated with genetic differentiation or geographic distance we calculated the pair-wise F_{st} between each pair of nest with FSTAT (Goudet, 2001). The geographic distance between each pair of nest was computed from the GPS coordinates of each nest. We used Mantel tests to analyse if the level of aggression and proportion of long antennations were correlated with genetic differentiation and/or geographic distance (Manly, 1997). All Mantel tests were performed with 5000 permutations using the software R Package (Casgrain & Legendre, 2001).

Results

Aggression

Aggressive behaviour between *F. paralugubris* workers was low at all the scales investigated (Fig. 2). Ants were never aggressive against their nestmates and the level of aggression between nonnestmates within populations was very low (aggression occurred in only one of the 16 tests). Hence, there was no significant difference in the level of aggression between nestmates and nonnestmates within populations (Wilcoxon signed rank test, $Z = -1.37$, $n = 16$, $P = 0.17$). Workers from nests in different populations were significantly more aggressive towards each other than ants from nests in the same population (Mantel, $r = 0.21$, $n = 17$ nests from six populations, $P < 0.01$). A similar result was found when restricting the analysis to the Jura Mountain populations (Mantel, $r = 0.28$, $n = 12$ nests from four populations, $P < 0.01$; sample sizes were too small to conduct a similar analysis within the Alps). Importantly, however, the level of aggression among nests of different populations was still low, as indicated by the mean \pm SD level of aggression being only 1.66 ± 1.04 . Finally, the level of aggression between nests from different mountain ranges was significantly higher than between nests from the same mountain range (Mantel, $r = 0.33$, $n = 17$, $P < 0.01$) although the overall level of aggression remained

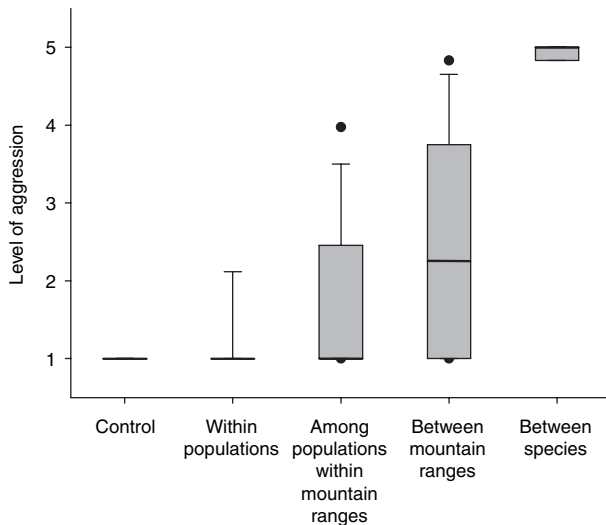


Fig. 2 Level of aggression among nestmates (control), among nests from the same population (within populations), among nests of different populations from the same mountain range (among populations within mountain ranges), among nests from different mountain ranges (between mountain ranges) and between the two species *Formica lugubris* and *Formica paralugubris* (between species). Aggression ranged from 1 (no aggression, antennations) to 5 (aggression, fight). The box plot indicates the median and the 5th, 25th, 75th and 95th percentiles.

moderate (mean \pm SD: 2.42 ± 1.43). These results contrast greatly with the data obtained in the interspecific tests, where aggression was very high. The level of aggression between *F. paralugubris* and its sibling species *F. lugubris* was (mean score \pm SD) 4.93 ± 0.09 with ants always fighting vigorously, often to death (Fig. 2).

Antennations

Ants were able to distinguish nestmates from nonnestmates, as shown by the significantly longer antennation bouts towards the latter (Wilcoxon signed rank test, $Z = -2.77$, $n = 16$, $P < 0.01$, Fig. 3). The length of antennations was also higher between ants originating from nests of different populations than between ants from nests of the same population (Mantel, $r = 0.19$, $n = 17$, $P < 0.01$). Finally, mountain range had an effect with longer antennations between ants from nests of the Jura and the Alps than between ants from nests of the same mountain range (Mantel: $r = 0.37$, $n = 17$, $P < 0.001$, Fig. 3).

Genetic structure

The hierarchical analysis revealed significant genetic differentiation among nests within populations, among populations within mountain ranges and between the two mountain ranges (Table 1). The highest genetic

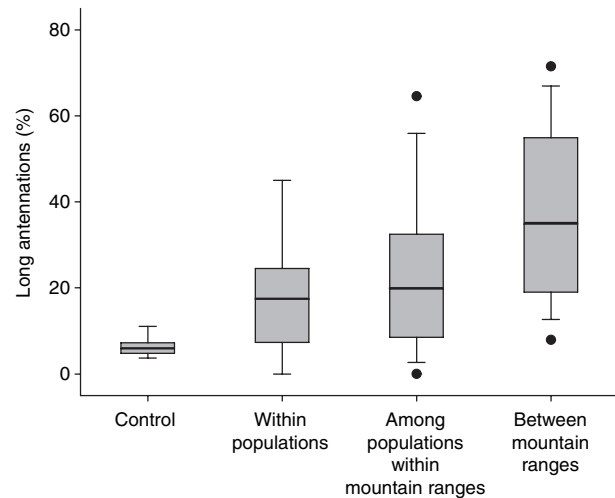


Fig. 3 The percentage of long antennations among nestmates (control), among nests from the same population (within populations), among nests of different populations from the same mountain range (among populations within mountain ranges) and among nests from different mountain ranges (between mountain ranges). The box plot indicates the median and the 5th, 25th, 75th and 95th percentiles.

Table 1 Genetic differentiation estimated in a four level F -analysis of variance at 11 microsatellite loci.

	$F_{\text{ind-nest}}$	$F_{\text{nest-pop}}$	$F_{\text{pop-mountain}}$	$F_{\text{mountain-total}}$
All	-0.039	0.062	0.049	0.086
Lower 95% CI	-0.043	0.050	0.034	0.063
Upper 95% CI	-0.012	0.067	0.056	0.174

$F_{\text{ind-nest}}$ is the correlation of genes within individuals compared to random genes within the nest. $F_{\text{nest-pop}}$ estimates the genetic differentiation among nests within populations, $F_{\text{pop-mountain}}$ the genetic differentiation among populations within mountain ranges and $F_{\text{mountain-total}}$ the genetic differentiation between mountain ranges within the entire sample of populations.

differentiation was found between mountain ranges. The significantly negative $F_{\text{ind-nest}}$ reflects an excess of heterozygotes within nests, as expected when nestmate workers are related. The F_{st} between pairs of nests ranged from -0.04 to 0.31 (average 0.12) and was positively correlated with geographic distance (Mantel, $r = 0.66$, $n = 68$, $P < 0.001$).

The level of aggression was correlated with the genetic differentiation (Mantel, $r = 0.39$, $n = 17$, $P < 0.001$) and geographic distance (Mantel, $r = 0.35$, $n = 17$, $P < 0.001$) between nests. The correlation between aggression and genetic differentiation remained significant when controlling for the effect of geographic distance (partial Mantel correlation, $r = 0.23$, $n = 17$, $P < 0.05$). However, the correlation between the level of aggression and geographic distance was no longer

significant when controlling for the effect of genetic distance (partial Mantel correlation, $r = 0.14$, $n = 17$, $P = 0.08$), suggesting that genetic differentiation explains more of the observed pattern of aggression than geographic distance.

Discussion

The first important finding of this study is that *F. paralogubris* workers showed almost no aggression towards conspecific individuals at all the scales investigated. There was a slight but significant increase in the aggressive scores with increasing distance, but even between the two mountain ranges the average aggressive score was lower than the value describing dorsal flexion, which is the first sign of truly antagonistic behaviour. The lack of aggression over large geographical scales contrasts strongly with most other ant species, which react aggressively towards individuals from other nests (Crozier & Pamilo, 1996). The finding of no aggression between individuals of different nests within populations, together with the earlier observations of extensive movement of workers and brood between nests in the Jura Mountains and the acceptance of brood originating from different geographical regions (Cherix, 1980; Maeder *et al.*, 2005), confirm that *F. paralogubris* is indeed unicolonial in Switzerland. The earlier reports of some nests being aggressive to each other (Gris & Cherix, 1977) most likely stemmed from the fact that both *F. lugubris* and *F. paralogubris* nests were used in the aggression experiments. Indeed, in aggression tests between the two closely related species, workers fought vigorously with the outcome being frequently the death of one of the two protagonists.

The second important finding of this study is the confirmation that unicoloniality is not associated with a complete breakdown of recognition ability (Chapuisat *et al.*, 2005). There were significant differences in behaviour at the spatial scales investigated. Aggression scores were significantly higher among nests from different populations than among nests of the same population, and also significantly higher among nests of the two mountain ranges than within. Furthermore, the data on antennation behaviour showed a significant increase in bouts length between nestmates and nonnestmates, between nests of different populations and between nests of the two mountain ranges. The peaceful investigation by antennation between ants from different nests might be caused by small differences in colony odour. Like trophallaxis or allogrooming, long antennations might be used to update the recognition template or to scramble recognition cues (Boulay *et al.*, 2000; Lenoir *et al.*, 2001) and thereby help to prevent conflicts that negatively affect colony performance (Keller & Chapuisat, 1999).

Interestingly, there was a significant correlation between the scores on the aggressive scale and both the

geographic distance and the genetic differentiation among nests. When the geographic distance and genetic differentiation were considered simultaneously in the analysis, the aggressive score remained significantly correlated with only the genetic distance, suggesting a slightly stronger effect of genetic differentiation. The outcome of similar analyses in other *Formica* species were variable, with a stronger impact of genetic differentiation in some cases (Beye *et al.*, 1997, 1998), and of geographic distances in others (Pirk *et al.*, 2001). These results are consistent with the current view that nestmate recognition in social insects can be based on environmentally – and/or genetically-derived cues (Gamboa *et al.*, 1986; Breed & Bennett, 1987; Adams, 1991; Liang & Silverman, 2000) with the respective importance of both type of cues being context-dependent (Carlin & Hölldobler, 1986, 1987a).

Overall, our findings contrast sharply with those obtained in the other unicolonial ant where precise information is available on the pattern of aggression and population genetic structure. In the European introduced population of Argentine ant similar tests to those performed in the present study showed that workers completely failed to discriminate between nestmates and nonnestmates workers of the same supercolony, even when nests were separated by several thousand km (Giraud *et al.*, 2002). Accordingly, the association between the level of aggression, spatial distance and genetic distance was not significant in the Argentine ant. Another major difference is that there are distinct supercolonies with aggression between but not within supercolonies in the invasive and native range of the Argentine ant (Pedersen *et al.*, in press), while we did not detect such a pattern in *F. paralogubris*. Importantly, a complete lack of aggression over a large geographic scale has never been reported in native populations of ants. Thus, even in the little fire ant *W. auropunctata*, studies in the native range reported the presence of aggressive supercolonies (Le Breton *et al.*, 2004; Errard *et al.*, 2005). It is currently impossible to determine whether other species of the genus *Formica* may also exhibit a social organization similar to that uncovered in *F. paralogubris*. The only species that has been studied over a larger geographic range is *F. truncorum* (Rosengren & Pamilo, 1986). In this species there was a negative relationship between the acceptance index and the distance between nests at a scale of 100 km, but the level of aggression between workers is not known.

One possible explanation is that peaceful intraspecific interactions have been selected for in *F. paralogubris* because of higher fitness of nonaggressive colonies compared to aggressive colonies. The costs and benefits of behaving aggressively towards nonnestmates are influenced by several features of the life-history of species. One of the key parameters is whether aggression may effectively prevent unrelated individuals from

entering the colony. When incoming reproductive individuals are less related to workers than are the resident reproductive individuals, workers should prevent their infiltration into the colony. In that respect, two characteristics of the biology of *F. paralugubris* suggest that the benefits of behaving aggressively towards nonnestmates might be lower than in ant species characterized by strong aggression between colonies. First, effective dispersal by male and female reproductives is limited. A genetic analysis in the Jura Mountains showed very strong isolation by distance indicating that few individuals successfully disperse in foreign colonies (Chapuisat *et al.*, 1997). In fact, the large majority of queens are recruited within their parental or from neighbouring nest (Chapuisat & Keller, 1999). Second, the relatedness within colonies is not significantly greater than the relatedness between individuals from closely located nests (Chapuisat *et al.*, 1997). Thus, workers of related neighbouring colonies may have evolved a high acceptance threshold because they encounter more often desirable related individuals than unrelated foreign individuals.

While the biology of *F. paralugubris* may decrease the benefits of rejecting foreigners and thus increase acceptance threshold, it is important to note that reduced aggression may also impinge on the population genetic structure of the species. Thus, acceptance of nonnestmates allows for individuals to move more frequently between neighbouring colonies, hence leading to reduced difference in relatedness between nestmates and individuals of closely located nests. Moreover, once colonies start to fail to discriminate against foreigners this may also set for alternative ways to maintain significant relatedness within colonies. For example, in the highly polygynous ant *F. exsecta*, colonies have a twofold mechanism to prevent invasion by unrelated queens. First, colonies reject all young queens attempting to enter when they do not themselves produce new queens (Brown *et al.*, 2003). Second, in the years where colonies produce new queens they produce many of them, hence diluting the effect of any foreign unrelated queens entering the colony (Brown & Keller, 2000; Brown *et al.*, 2002).

Overall, our findings have important implications for our understanding of the evolution of unicoloniality. First, this study shows that unicoloniality is not necessarily associated with a complete loss of recognition ability. Second, unicoloniality can exist in native populations and does therefore not require changes in the genetic composition or competitive environment associated with the introduction of species in new environments. Finally, unicoloniality can be associated with significant genetic differentiation and limited dispersal. It would be of great interest to conduct similar studies in other ants forming unicolonial populations in their native habitat to assess the generality of this pattern.

Acknowledgments

We thank Loic Degen for technical assistance, Anne Freitag and Arnaud Maeder for information on field sites, Bernhard Seifert for species determination, Jérôme Goudet for help with the statistical analyses and Rob Hammond and two anonymous reviewers for comments on the manuscript. This study was supported by several grants from the Swiss National Science Foundation to MC and LK.

References

- Adams, E.S. 1991. Nest-mate recognition based on heritable odors in the termite *Microcerotermes arboreus*. *Proc. Natl. Acad. Sci. USA* **88**: 2031–2034.
- Astruc, C., Malosse, C. & Errard, C. 2001. Lack of intraspecific aggression in the ant *Tetramorium bicarinatum*: A chemical hypothesis. *J. Chem. Ecol.* **27**: 1229–1248.
- Beye, M., Neumann, P. & Moritz, R.F.A. 1997. Nestmate recognition and the genetic gestalt in the mound-building ant *Formica polyctena*. *Insectes Soc.* **44**: 49–58.
- Beye, M., Neumann, P., Chapuisat, M., Pamilo, P. & Moritz, R.F.A. 1998. Nestmate recognition and the genetic relatedness of nests in the ant *Formica pratensis*. *Behav. Ecol. Sociobiol.* **43**: 67–72.
- Boulay, R., Hefetz, A., Soroker, V. & Lenoir, A. 2000. *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. *Anim. Behav.* **59**: 1127–1133.
- Bourke, A.F.G. & Franks, N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, NJ.
- Breed, M.D. & Bennett, B. 1987. Kin recognition in highly eusocial insects. In: *Kin Recognition in Animals* (D.J.C. Fletcher & C.D. Michener, eds.), pp. 243–285. Wiley, New York.
- Brown, W.D. & Keller, L. 2000. Colony sex ratios vary with queen number but not with relatedness asymmetry in the ant *Formica exsecta*. *Proc. R. Soc. B* **267**: 1751–1757.
- Brown, W.D., Keller, L. & Sundström, L. 2002. Sex allocation in mound-building ants: the roles of resources and queen replenishment. *Ecology* **83**: 1945–1952.
- Brown, W.D., Liautard, C. & Keller, L. 2003. Sex-ratio dependent execution of queens in polygynous colonies of ant *Formica exsecta*. *Oecologia* **134**: 12–17.
- Carlin, N.F. & Hölldobler, B. 1986. The kin recognition system of carpenter ants (*Camponotus* spp.). I. Hierarchical cues in small colonies. *Behav. Ecol. Sociobiol.* **19**: 123–134.
- Carlin, N.F. & Hölldobler, B. 1987a. The kin recognition system of carpenter ants (*Camponotus* spp.). II. Larger colonies. *Behav. Ecol. Sociobiol.* **20**: 209–217.
- Carlin, N.F. & Hölldobler, B. 1987b. The kin recognition system of carpenter ants (*Camponotus* spp.). III. Within-colony discrimination. *Behav. Ecol. Sociobiol.* **20**: 219–227.
- Casgrain, P. & Legendre, P. 2001. *The R package for multi-dimensional and spatial analysis*. version 4.0 [WWW document] <http://www.bio.umontreal.ca/Casgrain/R/index.html>.
- Chapuisat, M. 1996. Characterization of microsatellite loci in *Formica lugubris* B and their variability in other ant species. *Mol. Ecol.* **5**: 599–601.
- Chapuisat, M. & Keller, L. 1999. Extended family structure in the ant *Formica paralugubris*: the role of breeding system. *Behav. Ecol. Sociobiol.* **46**: 405–412.

- Chapuisat, M., Bernasconi, C., Hoehn, S. & Reuter, M. 2005. Nestmate recognition in the unicolonial ant *Formica paralugubris*. *Behav. Ecol.* **16**: 15–19.
- Chapuisat, M., Bocherens, S. & Rosset, H. 2004. Variable queen number in ant colonies: no impact on queen turnover, inbreeding, and population genetic differentiation in the ant *Formica selysi*. *Evolution* **58**: 1064–1072.
- Chapuisat, M., Goudet, J. & Keller, L. 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. *Evolution* **51**: 475–482.
- Cherix, D. 1980. Note préliminaire sur la structure, la phénologie et le régime alimentaire d'une super-colonie de *Formica lugubris* Zett. *Insectes Soc.* **27**: 226–236.
- Crozier, R.H. 1979. Genetics of sociality. In: *Social Insects* (H. R. Hermann, ed.), pp. 223–286. Academic Press, New York.
- Crozier, R.H. & Pamilo, P. 1996. *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*. Oxford University Press, Oxford.
- Elias, M., Rosengren, R. & Sundström, L. 2005. Seasonal polydomy and unicoloniality in a polygynous population of the red wood ant *Formica truncorum*. *Behav. Ecol. Sociobiol.* **57**: 339–349.
- Errard, C., Delabie, J., Jourdan, H. & Hefetz, A. 2005. Intercontinental chemical variation in the invasive ant *Wasmannia auropunctata* (Roger) (Hymenoptera Formicidae): a key to the invasive success of a tramp species. *Naturwissenschaften* **92**: 319–323.
- Gamboa, G.J., Reeve, H.K., Ferguson, I.D. & Wacker, T.L. 1986. Nestmate recognition in social wasps: the origin and acquisition of recognition odours. *Anim. Behav.* **34**: 685–695.
- Giraud, T., Pedersen, J.S. & Keller, L. 2002. Evolution of supercolonies: the Argentine ant of southern Europe. *Proc. Natl. Acad. Sci. USA* **99**: 6075–6079.
- Goudet, J. 2001. *FSTAT, a program to estimate and test gene diversities and fixation indices*. version 2.9.3. [WWW document] <http://www2.unil.ch/popgen/softwares/fstat.htm>.
- Griffin, A.S. & West, S.A. 2002. Kin selection: fact and fiction. *Trends Ecol. Evol.* **17**: 15–21.
- Gris, G. & Cherix, D. 1977. Les grandes colonies de fourmis des bois du Jura (groupe *Formica rufa*). *Mitt. Schweiz. Entomol. Ges.* **50**: 249–250.
- Gyllenstrand, N., Gertsch, P.J. & Pamilo, P. 2002. Polymorphic microsatellite DNA markers in the ant *Formica exsecta*. *Mol. Ecol. Notes* **2**: 67–69.
- Hamilton, W.D. 1964. The genetical evolution of social behavior. *J. Theor. Biol.* **7**: 1–52.
- Hamilton, W.D. 1987. Discriminating nepotism: expectable, common, overlooked. In: *Kin Recognition in Animals* (D. J. C. Fletcher & C. D. Michener, eds.), pp. 417–437. Wiley, New York.
- Higashi, S. & Yamauchi, K. 1979. Influence of a supercolonial ant *Formica (Formica) yessensis* Forel on the distribution of other ants in Ishikari coast. *Jap. J. Ecol.* **29**: 257–254.
- Hölldobler, B. & Wilson, E.O. 1977. The number of queens: an important trait in ant evolution. *Naturwissenschaften* **64**: 8–15.
- Holway, D.A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. *Ecology* **80**: 238–251.
- Holway, D.A., Lach, L., Suarez, A.V., Tsutsui, N.D. & Case, T.J. 2002. The causes and consequences of ant invasions. *Annu. Rev. Ecol. Syst.* **33**: 181–233.
- Holway, D.A. & Suarez, A.V. 2004. Colony-structure variation and interspecific competitive ability in the invasive Argentine ant. *Oecologia* **138**: 216–222.
- Holway, D.A., Suarez, A.V. & Case, T.J. 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science* **282**: 949–952.
- Insightful Corporation. 2003. *s-PLUS*. version 6.2, Seattle, Washington.
- Ito, M. 1971. Nest distribution of *Formica yessensis* Forel in Ishikari shore in reference to plant zonation. *J. Fac. Sci. Hokkaido Univ. Ser.* **18**: 144–154.
- Kaufmann, B., Boomsma, J.J., Passera, L. & Petersen, K.N. 1992. Relatedness and inbreeding in a French population of the unicolonial ant *Iridomyrmex humilis* (Mayr). *Insectes Soc.* **39**: 195–200.
- Keller, L. 1995. Social life: the paradox of multiple-queen colonies. *Trends Ecol. Evol.* **10**: 355–360.
- Keller, L. & Chapuisat, M. 1999. Cooperation among selfish individuals in insect societies. *Bioscience* **49**: 899–909.
- Kelly, J.K. 1994. The effect of scale dependent processes on kin selection: mating and density regulation. *Theor. Popul. Biol.* **46**: 32–57.
- Le Breton, J., Delabie, J.H.C., Chazeau, J., Dejean, A. & Jourdan, H. 2004. Experimental Evidence of Large-Scale Uniclonality in the Tramp Ant *Wasmannia auropunctata* (Roger). *J. Insect Behav.* **17**: 263–271.
- Lenoir, A., Hefetz, A., Simon, T. & Soroker, V. 2001. Comparative dynamics of gestalt odour formation in two ant species *Camponotus fellah* and *Aphaenogaster senilis* (Hymenoptera: Formicidae). *Physiol. Entomol.* **26**: 275–283.
- Lewis, P.O. & Zaykin, D. 1999. *Genetic data analysis: computer program for the analysis of allelic data*. version 1.1. [WWW document] <http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php>.
- Liang, D. & Silverman, J. 2000. You are what you eat: diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften* **87**: 412–416.
- Maeder, A., Freitag, A. & Cherix, D. 2005. Species- and nestmate brood discrimination in the sibling wood ant species *Formica paralugubris* and *Formica lugubris*. *Ann. Zool. Fennici* **42**: 201–212.
- Manly, B.F.J. 1997. *Randomization, Bootstrap and Monte Carlo Methods in Biology*, 2nd edn. Chapman and Hall, London.
- Pamilo, P., Chautems, D. & Cherix, D. 1992. Genetic differentiation of disjunct populations of the ants *Formica aquilonia* and *Formica lugubris* in Europe. *Insectes Soc.* **39**: 15–29.
- Passera, L. 1994. Characteristics of tramp species. In: *Exotic Ants. Biology, Impact, and Control of Introduced Species* (D.F. Williams, ed.), pp. 23–43. Westview Press, Boulder, CO.
- Pedersen, J.S., Krieger, M.J.B., Vogel, V., Giraud, T. & Keller, L. in press. Native supercolonies of unrelated individuals in the invasive Argentine ant. *Evolution*, in press.
- Pirk, C.W.W., Neumann, P., Moritz, R.F.A. & Pamilo, P. 2001. Intranest relatedness and nestmate recognition in the meadow ant *Formica pratensis* (R.). *Behav. Ecol. Sociobiol.* **49**: 366–374.
- Queller, D.C. 1994. Genetic relatedness in viscous populations. *Evol. Ecol.* **8**: 70–73.
- Queller, D.C. 2000. Pax argentinica. *Nature* **405**: 519–520.
- Queller, D.C. & Strassmann, J.E. 1998. Kin selection and social insects. *Bioscience* **48**: 165–175.
- Rosengren, R. & Pamilo, P. 1983. The evolution of polygyny and polydomy in mound-building *Formica* ants. *Acta Entomologica Fennica* **42**: 65–77.
- Rosengren, R.C.D. & Pamilo, P. 1986. Insular ecology of the red wood ant *Formica truncorum*: II. Distribution, reproductive

- strategy and competition. *Mitt. Schweiz. Entomol. Ges.* **59**: 63–93.
- Rosengren, R., Cherix, D. & Pamilo, P. 1985. Insular ecology of the red wood ant *Formica truncorum*: I. Polydomous nesting, population size and foraging. *Mitt. Schweiz. Entomol. Ges.* **58**: 147–176.
- Rosengren, R., Sundström, L. & Fortelius, W. 1993. Monogyny and polygyny in *Formica* ants: the result of alternative dispersal tactics. In: *Queen Number and Sociality in Insects* (L. Keller, ed.), pp. 308–333. Oxford University Press, Oxford.
- Ross, K.G., Vargo, E.L. & Keller, L. 1996. Social evolution in a new environment: the case of introduced fire ants. *Proc. Natl. Acad. Sci. USA* **93**: 3021–3025.
- Schneider, S., Roessli, D. & Excoffier, L. 2000. *Arlequin: A software for population genetics data analysis*. version 2.000 [WWW document] <http://lgb.unige.ch/arlequin>.
- Seifert, B. 1996. *Formica paralugubris* nov. spec. – a sympatric sibling species of *Formica lugubris* from the western Alps (Insecta: Hymenoptera: Formicoidea: Formicidae). *Reichenbachia* **31**: 193–201.
- Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*, 3rd edn. W.H. Freeman and Company, New York, USA.
- Suarez, A.V., Holway, D.A. & Case, T.J. 2001. Patterns of spread in biological invasions dominated by long-distance jump dispersal: Insights from Argentine ants. *Proc. Natl. Acad. Sci. USA* **98**: 1095–1100.
- Sundström, L., Seppä, P. & Pamilo, P. 2005. Genetic population structure and dispersal patterns in *Formica* ants – a review. *Ann. Zool. Fennici* **42**: 163–177.
- Taylor, P.D. 1992. Altruism in viscous populations – an inclusive fitness model. *Evol. Ecol.* **6**: 352–356.
- Tsutsui, N.D., Suarez, A.V., Holway, D.A. & Case, T.J. 2000. Reduced genetic variation and the success of an invasive species. *Proc. Natl. Acad. Sci. USA* **97**: 5948–5953.
- Van der Hammen, T., Pedersen, J.S. & Boomsma, D.I. 2002. Convergent development of low-relatedness supercolonies in *Myrmica* ants. *Heredity* **89**: 83–89.

Received 17 February 2006; revised 7 March 2006; accepted 13 March 2006