



# Polygyny and strong genetic structuring within an isolated population of the wood ant *Formica rufa*

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## Abstract

Social structuring of populations within some *Formica* species exhibits considerable variation going from monodamous and monogynous populations to polydomous, polygynous populations. The wood ant species *Formica rufa* appears to be mainly monodamous and monogynous throughout most of its distribution area in central and northern Europe. Only occasionally it was mentioned that *F. rufa* can have both polygynous and monogynous colonies in the same geographical region. We studied an isolated polydomous *F. rufa* population in a deciduous mixed forest in the north-west of Belgium. The level of polydomy within the colonies varied from monodamous to 11 nests per colony. Our genetic analysis of eight variable microsatellites suggest an oligo- to polygynous structure for at least the major part of the sampled nests. Relatedness amongst nest mate workers varies considerable within the population and colonies but confirms in general a polygynous structure. Additionally high genetic diversity (e.g. up to 8 out of 11 alleles per nest for the most variable locus) and high within nest genetic variance (93%) indicate that multiple queens contribute to the gene pool of workers of the same nest. Moreover significant genetic structuring among colonies indicates that gene flow between colonies is restricted and that exchange of workers between colonies is very limited. Finally we explain how possible factors as budding and the absence of *Serviformica* can explain the differences in genetic structure within this polygynous *F. rufa* population.

## Keywords

*Formica rufa*, genetic differentiation, polygyny, budding, *Serviformica*, habitat fragmentation

## Introduction

Within the wood ant genus *Formica* s. str., monogynous colonies can only be founded when young and newly emerged queens disperse over long distances to find suitable conditions to start a new colony (Gösswald 1952, Rosengren and Pamilo 1983, Rosengren et al. 1993, Mabelis 1994). Moreover queens of the *Formica rufa* group are incapable of independently founding a colony. Hence, to establish a new colony, a queen must be accepted into a nest of a species belonging to the subgenus *Serviformica* (in Western Europe generally *F. fusca*), because she is unable to raise her own brood. High dispersal risks and low independent colony-founding success of individual wood ant queens are expected to promote a polygynous situation wherein daughter queens are adopted into the queens nest. This strategy is often accompanied by budding of the colony, whereby mated females of polygynous populations leave their natal nests with workers, and found a new nest in the vicinity of the ancestral nest. In particular when these queens have mated with males originating from outside the colony, such colony reproduction may strongly reduce the intranest relatedness within an ant population and even lead to multicolonial populations comprising several colonies each with several nest units that may or may not exchange workers.

Some western and northern European populations of mound building and other *Formica* species exhibit considerable intraspecific variation in colony founding and social structure within one region [for *Formica exsecta* (Seppä et al. 2004); *Formica truncorum* (Bargum et al. 2004); *Formica aquilonia* (Pamilo et al. 2005); *Formica lugubris* (Mäki-Petäys et al. 2005) and *Formica selysi* (Chapuisat et al. 2004)]. In some extreme cases, even “unicolonial” populations or supercolonies (Helanterä et al. 2009), where boundaries between populations are virtually absent, have been observed within the genus *Formica* (Chapuisat et al. 2005, Holzer et al. 2006, 2009, Bezděčková and Bezděčka 2011).

Shifts in the social structure of wood ant species are most commonly observed between populations living in nearby woodland patches that differ in management, vegetation characteristics or degree of isolation (Gyllenstrand and Seppä 2004). As these differences in social structure are frequently associated with a marked phenotypic divergence in morphological traits of the queen (i.e. the polygyny syndrome *sensu* Keller 1993), local adaptation to changing environmental conditions is frequently considered to be a main factor that allows intraspecific variation in social structure of wood ants (Seppä and Pamilo 1995, Mäki-Petäys et al. 2005, Holzer et al. 2006, Sorvari and Hakkarainen 2007a,b).

During the last decade, only a few studies reported extreme differences in social structure within a single ant population (Pirk et al. 2001, Chapuisat et al. 2004, Bargum et al. 2007, Kümmerli and Keller 2007, Saapunki et al. 2008). This persistence of sympatric social divergence within ant populations still remains poorly understood.

The social structure of *Formica rufa*, a wood ant species presumed to be monogynous throughout its distribution area, was studied in detail only once so far. Gyllenstrand et al. (2004) concluded that not all studied *F. rufa* colonies in Sweden are monogynous and

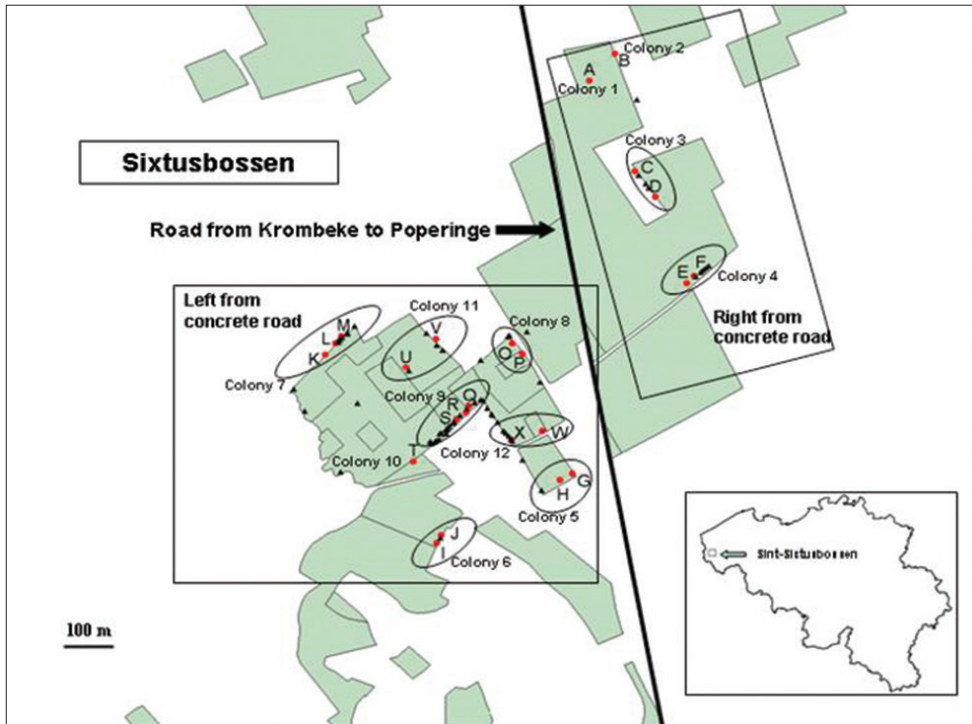
that their estimates of worker relatedness suggest a mix of polyandrous single queens nests and nests with a few coexisting queens. Indeed, other studies also mention that *F. rufa* might be very exceptionally polygynous (Seifert 1991, Seifert et al. 2010, Bezděčková and Bezděčka 2011) but genetic studies at colony level are lacking. In some regions of northern Belgium, both monodomous and oligodomous populations of the presumed monogynous species *Formica rufa* occur in highly fragmented, small forest habitats embedded within an agricultural matrix (Dekoninck et al. 2010). In this hostile matrix it is unlikely that dispersing young queens will encounter remote and isolated, suitable forest fragments. If habitat patches are too distant from each other to be bridged by dispersing queens, long distance dispersal will not be favoured (Rosengren et al. 1993, Mabelis 1994, Höfener et al. 1996). Consequently, even if dispersal is only successful within these suitable small patches, this may in the long term result in extinction of *Serviformica* ants due to the repeated parasitism by young queens and competition from neighbouring ant nests (Mabelis 1984, Czechowski and Vepsäläinen 1999, Czechowski and Markó 2006, Dekoninck et al. 2010). At one particular site in this wood ant hostile agricultural matrix, colonies of a *F. rufa* population vary extremely in degree of polydomy; multiple queens are often observed in a single nest and observations of nuptial flights and *Serviformica* are lacking. These preliminary observations suggest that this population might be another example of a shift in social organization of a wood ant, and in particular in a wood ant species that appears to be mainly monodomous and monogynous throughout most of its distribution area (Seifert et al. 2010).

Here in this study, our first aim was to infer by means of microsatellites if *F. rufa* shifted its social structure towards polygyny in this hostile fragmented forest complex. Second, we investigated the genetic structure of the population and explain its consequences. Furthermore, we analysed the variation in number of queens and number of interconnected nests in this population by relating intranest relatedness with the degree of polydomy and its persistence over multiple years. Finally we discussed the impact of budding and the lack of *Serviformica* in this context.

## Material and methods

### Study area

The *Formica rufa* population included in this study is situated in the forest of the Sixtusbossen at Poperinge-Vleteren (south of Western Flanders, Belgium) (Fig. 1). Former observations (Loones et al. 2008) showed that colonies within this populations vary in degree of polydomy and that multiple queens are often observed in a single nest, which suggests that multiple queens are present within most nests. New nests are primarily formed by budding from existing ones. Nuptial flights are short ranged and probably only performed by males. Copulations were only observed in the immediate vicinity of, and on the nest mounds. A relatively unique feature of the population is that *Serviformica* species, which are necessary for independent colony founding in *F. rufa*, have



**Figure 1.** All nests in the woodland patches of the Sixtusbossen in 2005–2006. Red ● are nests that have been sampled for genotyping, ▲ are the positions of the remaining nests present during the time of sampling.

never been found in the forest stands of the Sixtusbossen (Loones et al. 2008) and the closest known *Serviformica* population is located at least 15 km away from the Sixtusbossen (Dekoninck et al. 2003).

### Sampling design

Workers were sampled at different hierarchical levels to investigate the social structure and degree of genetic differentiation (Fig. 1). The isolated set of forest fragments that cover the Sixtusbossen was considered as the entire population. Within this population, between 20 and 37 workers were sampled from 23 separate nest mounds during spring 2005. The distance between two nests ranged from 5.7 m (nest O to nest P) to 1200 m (nest B to nest I).

Spatial separation and detailed observations during the last 10 years (Loones et al. 2008) allowed us to group nests into at least 12 colonies (C1–C12). Nests are considered as belonging to the same colony if historical observations revealed that they originated from budding from a former single nest. Colonies were further defined as nest aggregations based on spatial separation caused by the different forest patches

and forest edges and by following interconnecting trails. When a colony consisted of multiple nests, two or three nests were sampled.

Monitoring and detailed mapping of the nests during the past ten years (Loones et al. 2008) revealed that in one of the larger fragments, the number of nests increased from 29 in 1996 to 52 in 2006.

We also investigated temporal variation in genetic structure and relatedness by resampling two monodomous nests in the summer of 2009 (nest J and S) shortly after they budded and produced one and three daughter-nests respectively. For comparison, we also included nest I, which remained monodomous during these five consecutive years.

### Molecular analysis

All sampled workers were stored in 97% ethanol until DNA extraction. DNA was extracted from the legs of adult workers in 200 µl 6% Chelex (Biorad, Instagene Matrix™) and 10 µl proteinase K (Qiagen), incubated for two hours at 55 °C and subsequently for 15 min at 97 °C. Extracted DNA was kept frozen at -20 °C.

Specimens were genotyped with 8 microsatellite loci originally designed for *F. exsecta*: FE13, FE19, FE37, FE38 (Gyllenstrand et al. 2002) and for *F. paralugubris*: FL12, FL20, FL21 and FL29 (Chapuisat 1996).

Polymerase chain reactions (PCR) were carried out in 10 µl volumes. The PCR-mix for both FE13, FE19 and FE37 contained: 0.5 µl DNA, 1× PCR buffer (Qiagen), 1× Q-solution (Qiagen), 0.5 mM MgCl<sub>2</sub> (Qiagen), 100 µM dNTP (Fermentas), 0.4 µM forward and reverse primer and 0.5 U *Taq* polymerase (Qiagen). The other primers (FE38, FL12, FL20, FL21 and FL29) were used in a multiplex with 0.5 µM F&R FL20-primer, 0.2 µM F&R for the 4 other loci and 1× MP Master Mix (Qiagen). We repeated the samples that did not amplify the first time by adding 0.16 mg/ml BSA (100×) to the PCR mix and using only 0.25 µl DNA.

PCR amplification was performed under the following cycling conditions: initial denaturing at 94 °C for 3 min followed by 35 cycles of denaturing at 94 °C for 45 s, annealing at 50 °C (FE13, FE19, and FE37) or at 55 °C (FE38, FL12, FL20, FL21 and FL29) for 45 s and extension for 1 min at 72 °C followed by a last extension step of 10 min at 72 °C. Products were resolved and visualized by capillary electrophoresis on a SCE 9610 genetic analyzer (Spectrumedix) and using the Genospectrum 3.0.0 Software.

### Data analysis

The GENEPOP software package (Raymond and Rousset 1995) was used to calculate observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), and test for Hardy-Weinberg equilibrium and population differentiation (Goudet et al. 1996). FSTAT was also used to calculate fixation index ( $F_{ST}$ ) between pairs of colonies.

For each nest, we extracted information about (i) the degree of polygyny, (ii) the degree of genetic differentiation at the different hierarchical levels, (iii) relatedness amongst worker nestmates and (iv) population viscosity. Besides relatedness amongst nest mate workers, we used an alternative method to infer the number of queens that contributed in worker reproduction. This was done by identifying the absolute minimum number of queens (hereafter called AMQ) necessary to result in the observed worker genotypes per colony. This was performed by first assuming that only one single queen founded the colony, without restrictions on the degree of polyandry. If the given genotype data per colony did not fit with this assumption, there was evidence that the workers originated from at least a second queen and so forth. Although we realized that the number of queens obtained by this method clearly underestimates the effective number of reproducing queens as different queens can have identical alleles, it does not falsely reject the null hypothesis of monogyny.

When polygynous nests recruit their own daughters as new reproductives and relatedness between nestmate queens equals that among workers ( $r$ ), the effective mean number of queens per colony or nest (hereafter called  $Q_n$ ) is a function of relatedness:  $Q_n = (1 + 2/m - r) / 3r$  where  $m$  is the effective paternity (Pamilo 1993, Seppä 1994, Gyllenstrand et al. 2004). For a typical *Formica rufa* nest, 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; a relatedness of 0.1 for oligogyne ( $Q_n = 7$ ) brood and 0.0079 for very polygynous brood ( $Q_n = 100$ ).

The genetic relatedness among individuals within a nest was estimated by means of the relatedness estimator developed by Queller and Goodnight (1989) with the program GenAlEx6 (Peakall and Smouse 2006). Relatedness records the degree of shared genetic material between individuals of the same nest with respect to randomly taken individuals from the total population.

Testing the correlation between relatedness and level of polydomy was performed by means of an exact Spearman rank order correlation (StatXact v.5). To avoid pseudoreplication of nests within a colony, only the average intranest relatedness per colony was used ( $n = 12$ ).

Patterns of genetic differentiation between nests (N) and colonies (C) were first investigated by means of visual inspection of a principal component analysis (PCA). This PCA was performed on average allele frequencies per nest with the programme GenAlEx6 (Peakall and Smouse 2006). Genetic structure of the population was further investigated using Wright's  $F$ -statistics and average  $F_{IN}$  (among individuals within nests),  $F_{NC}$  (among nests within colonies) and  $F_{CT}$  (among colonies within the population) were calculated. The significance of these  $F$ -statistics was tested by comparing the observed values against the null distribution as obtained by random permutation. The total genetic variation was partitioned according to these different hierarchical levels by means of Analysis of Molecular variance (AMOVA) with the program Arlequin version 3.0 (Excoffier et al. 2005).

Limited dispersal of individuals from their birth place results in genetic viscosity (Hamilton 1964). This induces genetic differentiation between geographically distant groups and consequently increases relatedness among neighbours. Genetic differentia-



tion in relation to geographic distance was investigated with a Mantel test (Liedloff 1999). This relationship was compared between nests from the same colony as well as between nests from different colonies. Comparing both relationships reveals whether the expected lower genetic differentiation between nests from the same colony are merely due to their limited geographic distance or due to the direct effect of an increased genetic relatedness of the budded nest.

## Results

### Hardy-Weinberg equilibrium and genetic diversity

We did not find significant differences between observed and expected genotype frequencies and hence no deviation from the Hardy-Weinberg equilibrium. Genetic diversity, calculated as expected heterozygosity ( $H_E$ ) at the nest level, ranged from 0.296 in the monodomous colony A up to 0.557 in a nest from a polydomous colony. The number of alleles per locus ranged from 1 (several loci) to 8 (FL20) at the nest level and from 3 (FE19) to 11 (FL20) for the total population. When focussing on the most diverse locus FL20, most nests contained more than 50% of the total number of alleles observed in the total population (11).

### Polygyny or monogyny estimation based on the AMQ, $Q_n$ and genetic relatedness

For the majority of the investigated colonies, the observed worker genotypes did not match with reproduction by a single queen based on AMQ (Table 1). For only five out of 23 nests, no clear evidence for polygyny could be observed. Worker genotypes from 14 nests originated at least from two different queens and for 4 nests even from three different queens. When assuming that this is the true number of queens that gave rise to these worker genotypes, the obtained levels of polyandry were unrealistically high (for example nest A, 1 queen would have mated with at least 4 different males) and hence indicates that these numbers clearly underestimate the effective number of reproductive queens within a single nest.

Relatedness estimates differed substantially among nests within the population and even within colonies and ranged from 0.49 to -0.218. Negative relatedness estimates indicates that individuals are more different than average individuals in the population. These values represent most likely random variation of estimates which are close to 0. With this estimation of relatedness we calculated  $Q_n$ . In general, this parameter confirmed the polygyn levels seen with AMQ, as for most of the nests the  $Q_n$  indicate a polygynous structure. Furthermore, three nests (nest A, B and P) of which the AMQ suggested they might be monogynous nests, had a  $Q_n$  between 1 and 2. Unfortunately, we could not calculate  $Q_n$  for each nest and this for two reasons. (i) For nest C, the relatedness estimate approaches zero and consequently the estimate of  $Q_n$  approaches

**Table 1.** The number of workers analysed for each nest and the number of the colony, the maximum number of alleles per locus, the level of polydomy within the colony, the absolute minimum number of queens (AMQ), relatedness ( $r$ ) and the estimated queen number according to Boomsma and Sudstrom (1998) for all genotyped nests.

Nest (subpop)	N workers	Max number of alleles <sup>1</sup>	Level of Polydomy	AMQ	$r$	Queen number
A(1)	20	6 (55%)	1	1	0.493±0.060	1.26
B(2)	20	4 (36%)	1	1	0.395±0.044	1.66
C(3)	30	8 (73%)	5	2	0.009±0.047	87.09
D(3)	22	5 (45%)	5	1	0.242±0.043	2.92
E(4)	34	7 (64%)	7	3	0.035±0.035	22.15
F(4)	20	6 (55%)	7	3	0.068±0.057	11.24
G(5)	20	8 (73%)	3	2	0.089±0.057	8.51
H(5)	20	8 (73%)	3	2	0.160±0.061	4.58
I(6)	37	7 (64%)	3	2	0.037±0.028	20.93
J(6)	20	6 (55%)	3	2	-0.068±0.080	NA
K(7)	20	7 (64%)	10	2	0.351±0.046	1.91
L(7)	20	6 (55%)	10	1	0.290±0.052	2.38
M(8)	24	6 (55%)	10	2	0.038±0.058	20.37
O(8)	20	8 (73%)	3	2	0.085±0.047	8.92
P(8)	20	4 (36%)	3	1	0.445±0.056	1.43
Q(9)	20	7 (64%)	11	2	-0.149±0.076	NA
R(9)	20	8 (73%)	11	2	-0.012±0.063	NA
S(9)	20	8 (73%)	11	3	-0.033±0.069	NA
T(10)	19	6 (55%)	1	2	0.164±0.051	4.46
U(11)	20	5 (45%)	6	2	-0.218±0.080	NA
V(11)	20	5 (45%)	6	2	0.062±0.053	12.36
W(12)	20	7 (64%)	6	2	-0.149±0.056	NA
X(12)	20	8 (73%)	6	3	0.124±0.061	6.01

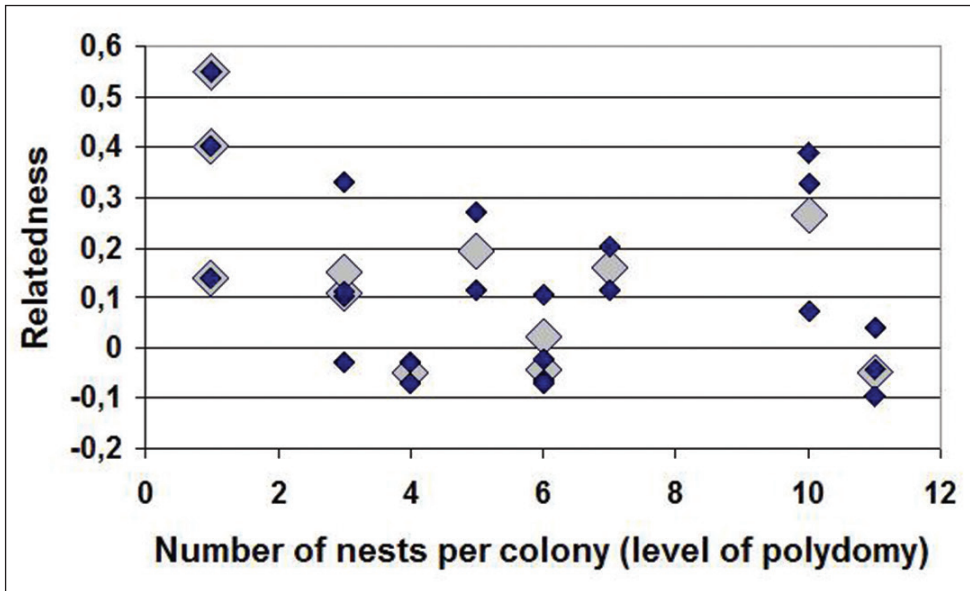
<sup>1</sup> = the % of total number alleles for this locus in the population

infinite. (ii) Due to negative relatedness estimates, Qn could not be retrieved for six other nests. Therefore, they are all marked with NA in Table 1. However, this still means that the estimate of Qn is very large (or approaches infinite).

### Relatedness versus level of polydomy

The correlation between the average intranest relatedness per colony ( $n = 12$ ) and the level of polydomy was significantly negative ( $r_s = -0.61$ ,  $p = 0.04$ , Fig. 2), indicating that monodomous colonies had a significantly higher relatedness. Although the average relatedness of highly polydomous colonies approached zero, relatedness of some nests within these highly polydomous colonies was much higher, suggesting lack of random mating.





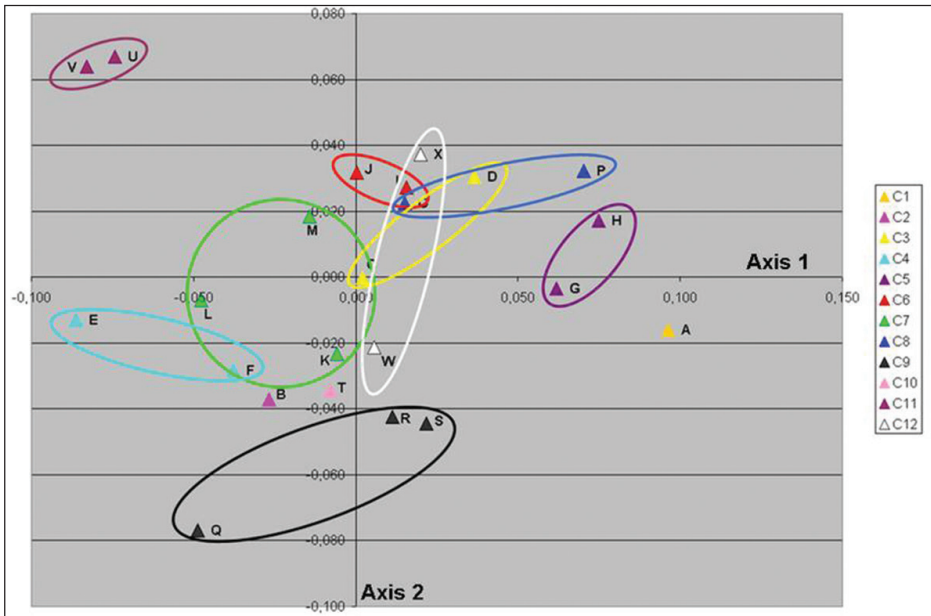
**Figure 2.** Correlation between the relatedness and the level of polydomy. Large grey symbols are the average intranest relatedness per colony, smaller dark symbols are estimated genetic intranest relatedness per nest.

### Genetic structuring

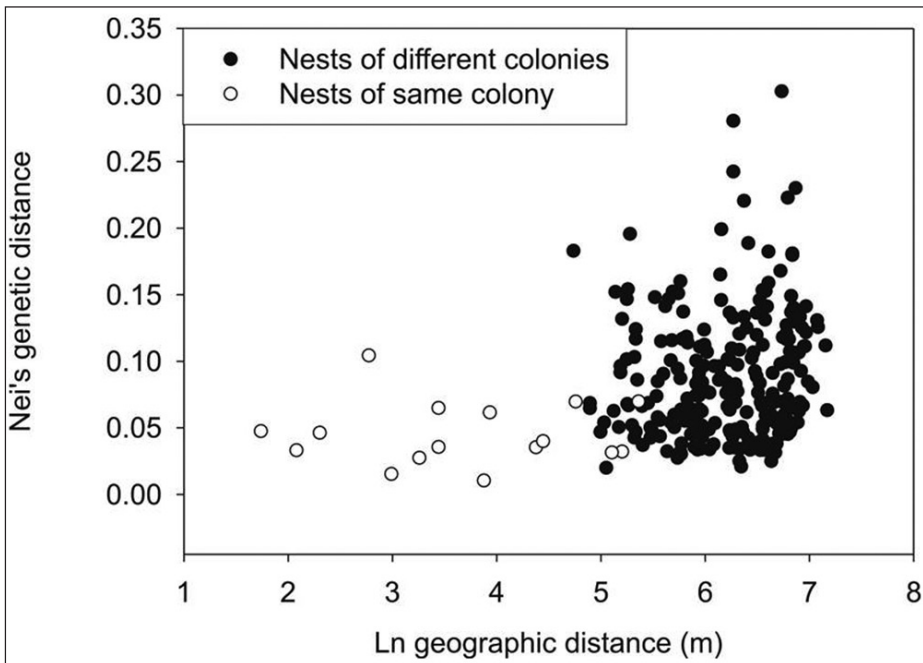
Visual inspection of the PCA revealed, in general, that nests from the same colony (e.g. C11) were often more similar in allele frequency compared to nests from different colonies (Fig. 3). However, substantial genetic variation in allele frequency still remained among nests within a colony, suggesting their independent genetic structure (e.g. nests from C4 and C7).

Hierarchical analyses of variance indicated that the major part of the total genetic variation (93.5%) was found within nests. Genetic variation among nests within colonies was low ( $F_{NC} = 0.027$ ) and explained 1.72% of the total genetic variation. This estimate was significantly higher ( $p < 0.0001$ ) than expected from random mating among nests members within a colony. At the highest hierarchical level, differentiation among colonies was higher ( $F_{CT} = 0.077$  and  $p < 0.0001$ ) and contributed to 4.88% of the total genetic variation. The within nest inbreeding coefficient  $F_{IN}$  was estimated as  $-0.004$  and not significantly different from zero ( $p = 0.5$ ).

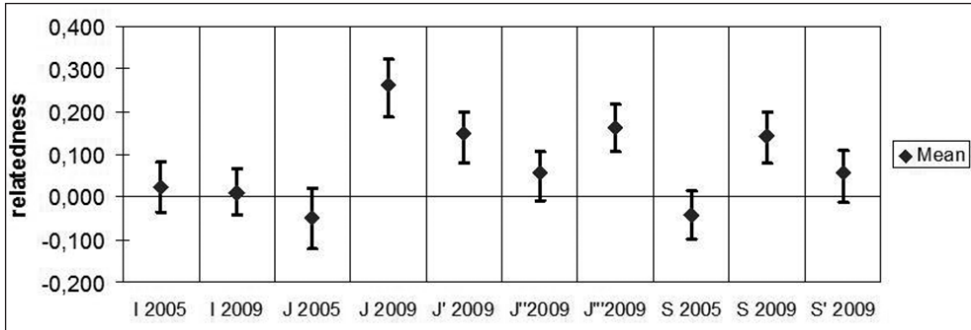
To investigate whether the lower differentiation among nests within colonies is merely an effect of larger distances between nests of different colonies, we compared the relationship between Nei's genetic distance and geographic distance of pairs of intracolony nests and pairs of intercolony nests (Fig. 4). For intracolony nest comparisons, the Mantel test showed no obvious relationship between genetic distance and geographic distance (all except one genetic distance below 0.1). This pattern contrasted



**Figure 3.** Genetic structure of the *Formica rufa* population at Sixtusbossen as revealed by Principal Component Analysis of the allele frequencies per nest. Nests with the same colour originate from the same colony. First and second PCA axes explained 36.5% and 20% of the total among nest genetic variation, respectively.



**Figure 4.** Mantel test showing the relationship between the Nei genetic distance and the geographical distance  $\ln(m)$ .



**Figure 5.** Mean relatedness values of the nests sampled in 2005 and 2009 and their daughter-nests in 2009.

with the intercolonial nest comparisons, wherein nests located at a comparable geographic distance (about 200 m) as intercolonial nests, often showed a genetic distance higher than 0.10.

### The impact of budding on relatedness

The relatedness of the mother nests that had budded between 2005 and 2009 (nest J and S) has increased significantly ( $p < 0.001$ ) in 2009 (respectively from -0.050 to 0.262 and from -0.042 to 0.142; Fig. 5). Moreover the relatedness of the daughter nests J', J'', J''' and S' also show a positive relatedness that is higher than the relatedness of the mother nest in 2005. The relatedness of nest I where no budding was observed in 2009 has decreased.

### Discussion

In this forest complex where opportunities for independent colony founding are virtually absent, our results demonstrated that the presumed monogynous species *Formica rufa* has shifted its social structure towards polygyny for at least a part of the population. This polygynous social structure results in a strong reduction of the intra-nest relatedness and the majority of the colonies showed relatedness values that were only marginally higher than zero. The average intra-nest relatedness of worker nestmates in the population was  $0.129 \pm 0.014$ , suggesting weak polygyny (Rosengren et al. 1993). This loss of genetic identity within colonies was confirmed by the distribution of the genetic variation, wherein almost all of the genetic variation within the population (93.5%) was observed within colonies. Remarkably, some colonies still attained high relatedness values that are primarily observed among workers of monodomous colonies. The number of different alleles in these colonies is still considerably higher than expected under a single queen – single male mating scenario. This suggests that the

high relatedness observed in these monodomous colonies is most likely due to adoption of daughter queens from inside the colony.

The effective number of queens per colony can be inferred from estimates of the relatedness of workers within a colony (Pamilo 1985, Queller 1993, Seppä 1994, Keller 1995) and here it ranges from 1 to infinite. This estimator however relies on strong assumptions about the degree of polyandry and population structuring, which could not be validated in this study. A high relatedness within a nest or colony for example does not necessarily imply a low absolute number of queens. If all reproducing queens have similar genotypes this results in high relatedness and hence estimates of a low number of queens. Therefore we used a second estimator AMQ. Although we did not intend to estimate the effective number of breeding queens per nest (according to Rosengren et al. (1993) this can usually not be determined because of the large nest volume) based upon AMQ, it can be expected that most, if not all nests of this population, are polygynous. Our approach only revealed the absolute minimum number of queens required to reconstruct the observed worker genotypes per nest. Assuming that this equals the true number of reproducing queens that gave rise to the observed worker genotypes would however imply a strong degree of polyandry. In nests categorised as monogynous based on AMQ, up to six different alleles could be observed for the most polymorphous locus. In this case, a single queen should have mated with at least four different males. However the effective paternity has been estimated to be 1.47 for a typical *F. rufa* (Boomsma and Sundström 1998). Although polyandry has been observed and assumed for this species (Gyllenstrand et al. 2004), here assumed levels of polyandry would be unrealistically high. It is therefore much more likely that for nests where we could not provide unequivocal evidence for a polygynous colony structure, multiple reproducing queens generated the genotyped workers sampled at each nest.

The observed multicolonial genetic structure in this study seems to be identical to that observed in other wood ants such as *Formica polyctena* (Beye et al. 1997), *F. lugubris* (Gyllenstrand and Seppä 2003) and *Formica pratensis* (Beye et al. 1998).

We found a significant negative correlation between the level of polydomy and the relatedness amongst nest mate workers per nest and per colony. Interestingly, almost all sampled monodomous colonies showed relatedness estimates that were higher than 0 while polydomous colonies were on average characterised by lower relatedness estimates that, in most cases, did not differ from zero. Nevertheless, some nests within these polydomous colonies exhibited relatedness estimates that are larger than 0. Our data from the sampling and analyses of 2005 indeed point in the direction that budding probably occurs when relatedness among workers drops due to immigration of extra-nest males or queens. In such cases, nests of polydomous colonies with high relatedness could be recently budded nests. A restricted data set of nests resampled in 2009 suggests this. Seppä (2008) mentions in this context that if the number of reproducing queens becomes extremely high or the queens originate from very different genetic pools, the relatedness amongst queens and nest workers becomes 0, and this induces budding. However to confirm this, a more profound and longer-term sampling and identical follow-up of the *Formica rufa* population in Westvleteren is necessary.

In ant species that have a unicolonial population structure, each nest contains numerous queens, are interconnected and individuals move freely between nests (Chapuisat et al. 2005, Holzer et al. 2009). Moreover the nestmate relatedness values are often indistinguishable from zero and unicoloniality is often associated with low overall genetic variability. Elsewhere in Europe, *Formica* species which follow a sessile life history in a stable habitat, indeed have the potential to develop unicoloniality as was confirmed by Chapuisat et al. (2005) and Elias et al. (2005). Our study suggests that *F. rufa* has the potential to do so when a population is hosted in a isolated forest complex without *Serviformica* in the near environment. In the small forest patches at Westvleteren long-term domination of long-lived resources (tending of Homoptera) leads to habitat saturation so that the colony might increase continuously. However, in such cases independent colony foundation becomes increasingly difficult because of a lack of territory and food. Furthermore, independent colony foundation is completely impossible because of lack of *Serviformica*. This situation promotes the reacceptance of queens by the maternal colony or maybe, in some cases, even nests of nearby colonies and the establishment of networks of nests originating from budding as is typical for unicolonial ant populations (Jackson 2007). However in the field aggression and wood ant wars were sometimes observed. These wars were as described for *F. polyctena* by Mabelis (1979) and can be very intense. These wars can even be between very nearby nests from the same and sometimes from different colonies. This latter aspect is lacking in real unicolonial ant populations (Jackson 2007) or almost lacking (Holzer et al. 2006). Unicoloniality suggests limited queen dispersal and free adult worker dispersal. In general free adult worker dispersal between different colonies is not observed in Westvleteren. Probably this can be confirmed in the near future with mark-recapture measures and a comparative study of workers and eggs of summer- and winter-nests of several colonies (cf. Elias et al. 2005). We conclude that in Westvleteren we have a multicolonial population structure of extended family-based nests.

## Conclusions and further research

Most colonies and nest of this population of *Formica rufa* appear to be polygynous. Moreover our genetic analyses suggest the presence of genetic structuring in the Westvleteren population. The allelic diversity was high compared to that found at the same loci in other monogynous wood ant populations elsewhere in Belgium (Flanders). Further research on a large geographic scale by extensive genetic sampling of mono- and polydomous *F. rufa* populations in Flanders (e.g. near Bruges Dekoninck et al. 2010) could explain whether the rarely reported polygynous structure of *F. rufa* as in Westvleteren can indeed be attributed to fragmentation and/or lack of *Serviformica* ants like *Formica fusca*. Our results indicate that more detailed temporal analyses of relatedness at the nest and colony levels for this and other wood ant species, will teach us more about the driving factors that might induce a shift in social structure (here from monogynous to oligogynous and polygynous) and variation within one population.

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