

Small-scale spatial genetic structure in an ant species with sex-biased dispersal

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In a population of the monogynous, polyandrous ant *Cataglyphis cursor*, we analysed the spatial genetic structure of queens, colony fathers and workers at a microgeographical scale to infer the extent of sex-biased dispersal and to assess the impact of limited dispersal on the patterns of relatedness within the colony. To this end, four microsatellite markers were scored for the queen and an average of 26 workers from each of 35 mapped colonies. We used pair-wise kinship coefficients between all pairs of genotypes, including the reconstructed colony father genotypes (1) to test and quantify isolation by distance patterns within each sex or caste through the analysis of kinship–distance curves, and (2) to compute the average relatedness between categories of colony members. The kinship–distance curve was much steeper for colony queens than colony fathers, indicating male-biased dispersal. However, colony fathers also displayed a non-random spatial genetic structure, so that even males show some dispersal limitation at the scale of the population, which extends over less than 250 m. The degree of relatedness between the different sexes and castes of colonies was well predicted from the number of mates per queen and the inbreeding of queens, and the impact of limited dispersal was very weak at this scale of observation. We discuss the interest of kinship–distance curves to assess sex-biased dispersal on a local scale and we compare our results with large-scale analyses of genetic structure in *Cataglyphis cursor* and other monogynous ant species. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **93**, 465–473.

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INTRODUCTION

Sex-biased dispersal, whereby one sex is more prone to disperse than the other, affects genetic variation within and among populations through asymmetrical gene flow (Slatkin, 1985; Prugnolle & de Meeus, 2002). In haplodiploid species, conventional *F*-statistics overestimate the number of migrants if dispersers are mainly of the haploid sex (i.e. males), and if migrants belong to the commoner sex (Berg, Lascoux & Pamilo, 1998). Thus, a method that allows estimating dispersal of both sexes separately is of high interest. Under sex-biased dispersal, uniparentally and bi-parentally inherited markers are expected to show different spatial patterns of genetic

variation because they differ in terms of both dispersal and drift processes. This phenomenon has led researchers to combine different classes of markers with alternate modes of inheritance, such as mitochondrial and nuclear DNA markers, to discern the maternal and paternal contributions to gene flow and population structure (e.g. Queller *et al.*, 1993; Petit, Balloux & Goudet, 2001; Ross, 2001a, b; Vargo, 2003; Clémencet, Viginier & Doums, 2005). Although this approach is theoretically powerful to investigate sex-biased dispersal, its inferential power depends on the genetic polymorphism of the uni-parentally transmitted genome, which is often low at a small geographical scale (e.g. Clémencet *et al.*, 2005).

Taken alone, the genetic structure at bi-parentally inherited markers might seem useless to infer sex-biased dispersal because genes transmitted by one

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sex are redistributed between sexes of the next generation. However, after the dispersal phase, the genetic structure assessed from individuals of the more dispersing sex should be slightly less developed than the one of the less dispersing sex because the next reproduction event has not yet mixed male and female alleles. This feature has been used to infer the extent of sex-biased dispersal among localities (e.g. Blundell *et al.*, 2002; Sundström, Keller & Chapuisat, 2003). Goudet, Perrin & Waser (2002) have compared the power of different estimation methods and concluded that comparing *Fst* of males and female individuals with highly polymorphic markers was the most efficient approach. Other sex-specific *Fst*-based approaches using individuals sampled before and after dispersal were developed by Vitalis (2002) and Fontanillas, Petit & Perrin (2004).

So far, studies investigating the sex bias of dispersal from the genetic structure of bi-parentally inherited nuclear markers have mainly considered a subdivided population sampling scheme, contrasting for instance *Fst* estimates for males and females (but see Ishibashi *et al.*, 1997; Knight *et al.*, 1999). Yet, to characterize the spatial genetic structure of a continuously distributed population, it may be more convenient to regress pair-wise relatedness coefficients between individuals on the spatial distance for at least two reasons: first, this method does not require subdividing artificially a population; second, it exploits the available information to the smallest spatial scale (Vekemans & Hardy, 2004). Under sex-biased dispersal, the lesser dispersing sex shows a steeper kinship–distance relationship, as confirmed by simulations (O. J. Hardy, unpublished). Comparing the kinship–distance curves of males and females therefore is a powerful approach to investigate sex-biased dispersal at a very local scale (Hazlitt, Eldridge & Goldizen, 2004; Neville *et al.*, 2006).

In social animals, the mating system and the way individuals enter or leave a group are of special interest, because they affect the relatedness between members and, hence, evolution of altruistic behaviour through kin selection (Hamilton, 1964; Queller, 1992). Patterns of relatedness between individuals within and among groups are often used to infer mating system parameters, including the effective number of reproductive females and their mating frequency. Such inferences are particularly successful in social Hymenoptera (ants, bees, wasps), because the haploid nature of the males causes high relatedness values among sibs (Hamilton, 1964; Crozier & Pamilo, 1996; Ross, 2001b). For instance, models predicting the patterns of relatedness between social castes, for example relatedness values between queens, males and workers, usually assume that dispersal is not spatially restricted within a defined

population. Therefore, if males and/or young unmated reproductive queens (gynes) disperse out of their colony before reproduction, the relatedness between a queen and its mate(s), between queens in the case of polygyny and between the mates of a queen in the case of polyandry, is assumed to be zero (using the population as a reference gene pool). However, higher relatedness values can be expected whenever dispersal of at least one sex is limited. One way to investigate the combined effect of the mating system and of (potentially sex-biased) restricted dispersal is to characterize the patterns of relatedness between castes, both within and among colonies in a spatial perspective.

In ants, mating and dispersal habits are closely associated with queen number (Hölldobler & Wilson, 1990; Keller, 1991). The presence of a single queen per colony (monogyny) typically is associated with large nuptial flights where sexuals mate away from the nest, extensive queen dispersal and independent colony founding by queens (i.e. with no assistance from workers). In contrast, the presence of multiple queens per colony (polygyny) often coincides with a lack of nuptial flights, mating close or even within the natal nest, limited dispersal of mated queens and dependent colony founding (i.e. with the help of workers) by budding. Young mated queens leave the mother nest with adult workers to initiate new colonies nearby, resulting in a strong population structure (Bourke & Franks, 1995; Crozier & Pamilo, 1996). Therefore, the queen number in ants is a primary determinant of patterns of local gene flow and, hence, microgeographical genetic structure (Ross & Keller, 1995; Ross & Shoemaker, 1997).

In this study, we examined how sex-biased dispersal affects small-scale spatial genetic structure in the monogynous ant *Cataglyphis cursor*. An interesting feature of this species is that, while strictly monogynous, colonies reproduce by budding (Lenoir *et al.*, 1988). Queens are winged but do not fly (Lenoir *et al.*, 1988); they repeatedly leave the mother nest to mate with surrounding males and found new colonies by budding at a walking distance of their natal nest. By contrast, winged males fly and disperse their genes over longer distances. In *C. cursor*, queens mate multiply (Pearcy *et al.*, 2004a), which should lower relatedness among their daughters. Nevertheless, queens use sexual reproduction for workers and thelytokous parthenogenesis with central fusion for new queens' production (Pearcy *et al.*, 2004a, Pearcy, Hardy & Aron, 2006). Hence, relatedness between the queen and their sexual daughters is close to 1 (Pearcy & Aron, 2006). Such a mode of parthenogenesis increases the level of the queen's homozygosity over time, which in turn boosts relatedness between the queen and its worker daughters, and

among worker offspring (Pearcy *et al.*, 2006). Nevertheless, approximately 60% of new queens is also produced by workers through parthenogenesis when the colony queen is lost, a process permitting recovery of heterozygosity (Pearcy *et al.*, 2006). Males arise from unfertilized haploid eggs, as is usually the case in Hymenoptera. The original reproductive system of *C. cursor* is expected to enhance the difference in genetic structures between males and females sampled after the dispersal phase for two reasons. First, the parthenogenetic production of new queens results in that they are more related than sexually produced sisters. Given that reproductive females disperse on short distances, their high relatedness will substantially affect the average relatedness between nearby queens. Second, as long as new queens arise from queens, their alleles are not dispersed through males for several generations. Consequently, the difference in genetic structuring between sexes can build up on a few generations rather than just one generation.

Here, we used the information of microsatellite loci (1) to compare the small-scale spatial genetic structure of queens, males and workers in a population, and (2) to assess the impact of limited dispersal and the mating system on the levels of relatedness within colonies. Based on the asymmetrical sex dispersal pattern of *C. cursor*, we expect the kinship–distance relationship of individuals sampled after the dispersal phase to be steeper for queens than for males, while the spatial genetic structure of workers should reach intermediate levels. The levels of relatedness within colonies are predicted from the number of colony fathers, the inbreeding of queens resulting from parthenogenesis and the relatedness induced by limited dispersal between the colony fathers and between the queen and the colony fathers. These predictions are compared with observed values and the relative impact of dispersal limitation is assessed. Our results are discussed with regard to previous studies of the genetic structure at mitochondrial and nuclear DNA markers (Clémencet *et al.*, 2005) at larger spatial scales.

MATERIAL AND METHODS

SAMPLING

Thirty-five nests of *C. cursor* were excavated in the end of April/early May 2001 at St-Hyppolite (Southern France), before the emergence of the first sexuals. Adults (queens and workers) as well as brood at various stages (eggs, larvae and both worker and sexual pupae) were collected and brought into the laboratory. A sample of workers from each nest was immediately stored at -80°C for subsequent genetic

analyses. Colonies were housed in artificial nests and maintained under laboratory conditions ($26 \pm 2^{\circ}\text{C}$ and 12 h : 12 h light : dark); they were fed on cockroaches, sugar water and grape.

DNA EXTRACTION AND MICROSATELLITE ANALYSIS

To assess the kin structure of the colonies, a sample of workers ($X \pm \text{SD} = 26.4 \pm 20.36$; range: 7–70; $n = 926$) and the queen ($n = 33$; two queens died before genetic analyses and their genotype was inferred from the workers' pedigree) from each nest were genotyped at four polymorphic microsatellite loci (Ccur-46, Ccur-11, Ccur-58 and Ccur-63b; see Pearcy *et al.*, 2004b for detailed protocol).

The genotype of the males that inseminated each queen, the colony fathers, was inferred at each locus on the basis of the queen and workers genotypes. This is straightforward because of the haploidy of males, as, for each locus, a male gives the same allele to all his offspring. The number of distinct male genotypes inferred per colony provides the minimal number of mates of each queen. Hereafter, the term 'males' will denote the mates of a colony queen (not the males produced by this colony).

DATA ANALYSIS

The relatedness between individuals is expressed by the mean kinship coefficient, F_{ij} , which is estimated from the genotypes by J. Nason's estimator (Loiselle *et al.*, 1995) using the software SPAGeDi (Hardy & Vekemans, 2002). The latter is also used to compute the inbreeding coefficient, F_i , of queens and of workers. F_{ij} indicates the degree of relatedness between individuals i and j relative to the mean relatedness of randomly sampled individuals from the study population, which is assumed to be zero (Rousset, 2002; Hardy, 2003). The symbol F_{ij} is used because it represents fundamentally the same metric as the F -statistics of Wright (1965) (e.g. the F_{ST} among populations is equivalent to the average F_{ij} between individuals of the same population). In ant genetics literature, relatedness between individuals is usually expressed by the 'relationship' coefficient, r_{ij} , which represents a standardized proportion of the genes from i that are identical by descent to genes from j (Queller & Goodnight, 1989). For the purposes of the present paper, F_{ij} is more convenient than r_{ij} because r_{ij} is asymmetrical when comparing individuals differing in ploidy or inbreeding levels (i.e. $r_{ij} \neq r_{ji}$), whereas F_{ij} is affected neither by the ploidy nor the inbreeding. In addition, F_{ij} allows an easier comparison of the spatial genetic structures of different sexes when differences reflect contrasted dispersal abilities. The connection between F_{ij} and

r_{ij} is straightforward: $r_{ij} = k_j F_{ij} / (1 + (k_j - 1)F_{ij})$, where k_j and F_{ij} are the ploidy and inbreeding levels of individual j , respectively. Thus, $r_{ij} = 2F_{ij}$ for two non-inbred diploids, whereas $r_{ij} = F_{ij}$ if j is an haploid or a completely inbred diploid. F_{ij} was computed for all possible pairs of individuals, including the (inferred) colony fathers. To characterize the mating system, the pattern of relatedness within each colony was investigated by averaging F_{ij} values over pairs of individuals belonging to the same nest, considering all possible comparisons between individuals (queens, males and workers). To help readers accustomed to r_{ij} coefficients, the latter are also provided using the conversion formula shown above.

To determine the spatial genetic structure of each sex and castes (workers or queens), F_{ij} values between individuals from different colonies were (1) averaged over a set of distance intervals (maximal distance of each interval in metres: 20, 40, 60, 80, 100, 130, 160 and 300), and (2) regressed on the natural logarithm of the distance between i and j , $\ln(d_{ij})$. This regression is justified because F_{ij} is expected to decrease approximately linearly with $\ln(d_{ij})$ when a genetic structure develops under limited gene dispersal (Vekemans & Hardy, 2004). The magnitude of spatial genetic structuring among castes and sex was compared using the S_p statistic defined as $S_p \equiv -b/(1 - F_{(N)})$, where b is the regression slope of F_{ij} on $\ln(d_{ij})$, and $F_{(N)}$ is the mean F_{ij} between individuals from neighbouring colonies (Vekemans & Hardy, 2004). S_p is adequate to compare the spatial structures of individuals differing by their levels of ploidy (males vs. queens and workers) or inbreeding (queens vs. workers), because F_{ij} values are not affected by these parameters (contrary to r_{ij}). Approximate standard errors (SE) were obtained by a jackknife procedure over loci and approximate 95% confidence intervals (CI) were constructed using the estimate ± 2 SE. To test whether there is a non-random spatial genetic structure within each sex, colonies positions were randomized 9999 times to get the distribution of b under the null hypothesis that there is no spatial structure. The P -value of the test is given by the proportion of the b distribution inferior to the observed b .

To assess the impact of limited dispersal on the pattern of relatedness within colonies, the values observed are compared with their predictions from pedigree relationships. Figure 1 shows the different kinship coefficients between categories of individuals within a monogynous (single queen), polyandrous colony, using the indices q , m and w to denote comparison involving a queen, a male (i.e. a queen's mate) and a worker, respectively. As a convention, we use f to denote kinship or inbreeding coefficients as model parameters and F for the corresponding estimators obtained from genotypic data. Thus, f_{q-m} is the

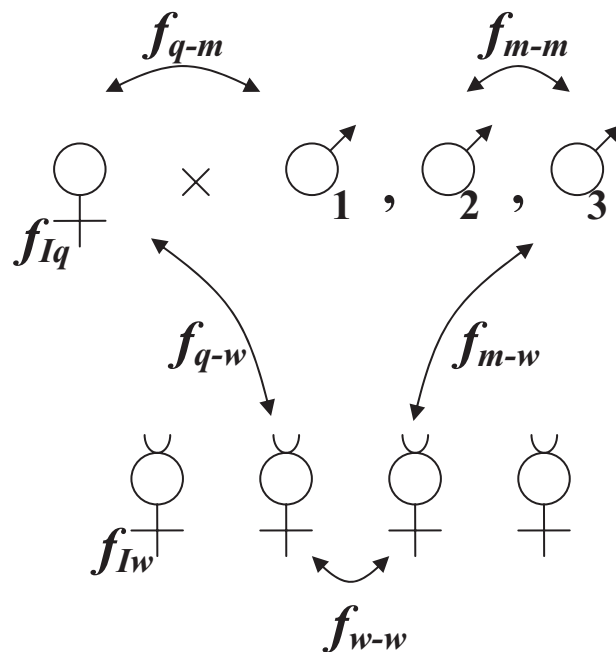


Figure 1. Representation of the different inbreeding (f) and kinship (f) coefficients characterizing the genetic structure within a colony of *Cataglyphis cursor*. The subscripts q , m and w represent queen, queen's mate and worker, respectively.

average kinship between a queen and one of its mate, f_{m-m} between the different mates of a queen, f_{q-w} between a queen and its worker daughters, f_{m-w} between a queen's mate and a worker (which can be its daughter or not), and f_{w-w} between two workers (which can be half sisters or full sisters). In *C. cursor*, queens are produced through a mode of parthenogenesis causing inbreeding (Percy *et al.*, 2006). The individual inbreeding coefficients of queens and workers are represented by f_{iq} and f_{iw} , respectively. In a large population without dispersal limitation f_{q-m} and f_{m-m} are expected to be zero. However, under dispersal limitation of males and females, a spatial genetic structure (genetic viscosity) builds up so that f_{q-m} and f_{m-m} can be positive. Given that workers result from sexual reproduction between a diploid queen and a number, M_p , of haploid males, the kinship coefficients involving workers can be predicted by decomposing pedigree relationships (assuming that the sperm of the different males contribute equally to the pool of workers):

$$f_{q-w} = \frac{1}{2} \left(\frac{1}{2} + \frac{1}{2} f_{iq} \right) + \frac{1}{2} f_{q-m} \quad (1)$$

where the first term corresponds to the direct genetic contribution of the queen to its daughter, including the impact of the queen's inbreeding coefficient as a

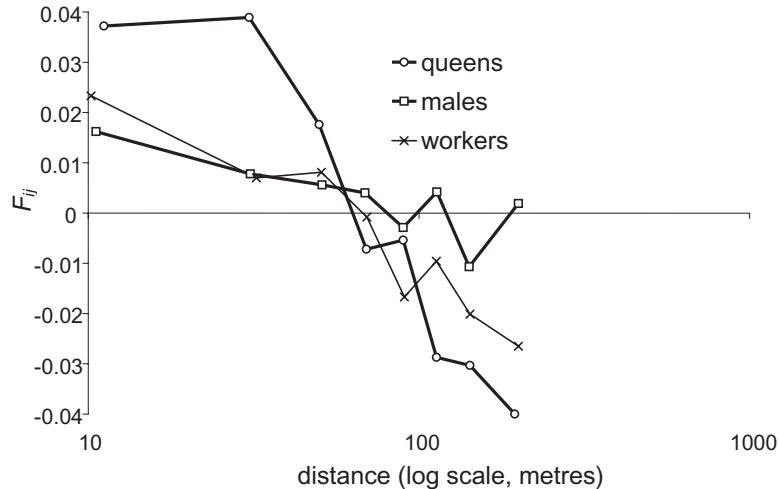


Figure 2. Comparison of the spatial genetic structures of queens, males (the queen's mates) and workers in a population of *Cataglyphis cursor*, described by the average kinship coefficients between individuals according to the spatial distance between colonies.

result of parthenogenesis, and the second term corresponds to the additional queen–worker kinship when the worker's father is related to the queen.

$$f_{m-w} = \left(\frac{1}{2} + \frac{1}{2}f_{q-m}\right)\frac{1}{M_p} + \left(\frac{1}{2}f_{m-m} + \frac{1}{2}f_{q-m}\right)\left(1 - \frac{1}{M_p}\right) \quad (2)$$

where the first term expresses the kinship between a worker and its father multiplied by the probability that a male is the father of a given worker, and the second term expresses the kinship between a given worker and one of the queen's mate that is not his father, multiplied by the probability that a male is not the father of a given worker. Both terms also include the additional male–worker kinship when the worker's mother (i.e. the queen) is related to the male.

$$f_{w-w} = \frac{1}{4}\left(\frac{1}{2} + \frac{1}{2}f_{iq}\right) + \frac{1}{2}f_{q-m} + \frac{1}{4}\left[\frac{1}{M_p} + \left(1 - \frac{1}{M_p}\right)f_{m-m}\right] \quad (3)$$

where the first term corresponds to the kinship through the queen's genetic contributions (including the impact of the queen's inbreeding), the second term corresponds to the additional kinship between workers when their father(s) is related to the queen and the third term corresponds to the kinship through the worker's father(s), which is direct when the workers have the same father (with probability $1/M_p$) or indirect when they have distinct but related fathers. Note that the expected inbreeding coefficient of workers is the kinship coefficient between the queen and its mates: $f_{lw} = f_{q-m}$. The (harmonic) mean number of fathers detected per colony in the study population was $M_p = 5.6$ (Pearcy *et al.*, 2004a).

RESULTS

SPATIAL GENETIC STRUCTURE

The level of relatedness between individuals from different colonies decreases with the spatial distance, whatever the sex and caste considered (Fig. 2) and, accordingly, the S_p statistics are significantly positive (Table 1). However, the kinship–distance curve is much steeper for queens than for males: the magnitude of spatial structuring expressed by the S_p statistic is five times higher for queens than for males, with no overlap in their confidence intervals [S_{p_q} (95% CI) = 0.031 (0.015, 0.047), S_{p_m} (95% CI) = 0.006 (0.000, 0.012); Table 1]. Although males clearly disperse further than queens, the randomization test still reveals some spatial genetic structuring among males, thus some male dispersal limitation at the scale of the population (Table 1). As for workers, their degree of spatial genetic structure appears intermediate between the ones of queens and males (Fig. 1; Table 1). The relatedness between individuals belonging to different sex or castes also decreases with the spatial distance between colonies, at a rate higher for pairs involving queens and lower for pairs involving males (Table 1).

PATTERN OF RELATEDNESS WITHIN COLONIES

Mating queen–males pairs are unrelated [mean F_{q-m} (95% CI) = 0.000 (−0.032, 0.032), or $r_{q-m} = 0.000$, for queens relative to males; Table 1]. As expected, the inbreeding coefficient of their worker progeny, F_{lw} , is not significantly different from zero. By contrast, queens can be inbred because of their production by thelytokous parthenogenesis, which increases the level of homozygosity; accordingly, their mean

Table 1. Pattern of relatedness within and between colonies of a population of *Cataglyphis cursor* assessed by microsatellite markers

Comparison	Mean kinship coefficient		Spatial genetic structure	
	Within colony $F_{(C)}$	Between nearby colonies $F_{(N)}$	Sp	P -value
$q-q$		0.037 ± 0.010	0.031 ± 0.008	< 0.001
$m-m$	0.026 ± 0.017	0.016 ± 0.007	0.006 ± 0.003	0.007
$w-w$	0.177 ± 0.017	0.023 ± 0.005	0.017 ± 0.004	< 0.001
$q-m$	0.000 ± 0.016	0.022 ± 0.009	0.014 ± 0.005	
$q-w$	0.277 ± 0.022	0.031 ± 0.010	0.023 ± 0.005	
$m-w$	0.082 ± 0.016	0.017 ± 0.003	0.011 ± 0.003	

m , males (queen's mates); q , queens; w , workers.

For every possible comparison between castes and sex, the average (\pm SE) kinship coefficient between individuals sampled within colonies [$F_{(C)}$] and between nearby colonies < 20 m apart [$F_{(N)}$] are given, as well as the Sp statistic (\pm SE) describing the magnitude of spatial genetic structuring and the associated P -value of the randomization tests. The latter tests can only be applied for comparisons within categories of individuals.

inbreeding coefficient over the four studied loci averages $F_{Iq} = 0.276$ (Pearcy *et al.*, 2006). Males that mated with the same queen are somewhat related ($F_{m-m} = r_{m-m} = 0.026$; randomization test, $P = 0.029$; Table 1). The mean kinship coefficient between queens and their daughters [F_{q-w} (95% CI) = 0.277 (0.233, 0.321), or $r_{q-w} = 0.554$; Table 1] is lower but not significantly different from the value predicted by Eqn 1: $f_{q-w} = (1 + 0.276)/4 + 0/2 = 0.319$. The mean kinship between workers and the queen's mates [F_{m-w} (95% CI) = 0.082 (0.050, 0.114), or $r_{m-w} = 0.082$ for workers relative to males; Table 1] is close to the prediction of Eqn 2: $f_{m-w} = (1/5.6)(1/2 + 0) + (1 - 1/5.6)(0.026/2 + 0) = 0.100$. Finally, the mean kinship between workers [F_{w-w} (95% CI) = 0.177 (0.143, 0.211), or $r_{w-w} = 0.354$] is lower but not significantly different from the value predicted by Eqn 3: $f_{w-w} = (1/4)((1 + 0.276)/2) + (1/4)[(1/5.6) + (1 - 1/5.6)0.026] = 0.209$. It is worth noting that these values are mostly affected by the inbreeding of queens and the number of queen's mates. By contrast, the impact of limited dispersal is very small, as can be assessed from the predicted pattern of relatedness assuming no dispersal limitation (by applying Eqn 1–3 with $f_{q-m} = f_{m-m} = 0$): $f_{q-w} = 0.319$, $f_{m-w} = 0.089$ and $f_{w-w} = 0.204$; these values are very similar to those predicted above using the observed estimates of f_{q-m} and f_{m-m} (respectively, 0.319, 0.100 and 0.209).

DISCUSSION

IMPACT OF SEX-BIASED DISPERSAL ON THE SPATIAL GENETIC STRUCTURE

From a methodological point of view, our results show that sex-biased dispersal can be detected efficiently

within a continuous population by comparing the kinship–distance curves between males and females sampled after dispersal. This approach is similar in essence to the one of Goudet *et al.* (2002) based on F_{ST} , except that it considers a continuous space in a spatially explicit way, so that it is more appropriate to investigate what happens within continuous populations. By contrast with the methods based on the comparison between uni-parentally and bi-parentally inherited markers, the kinship–distance relationship does not depend on the polymorphism of uni-parentally transmitted genomes, which is often low or absent at a local scale. However, because bi-parentally inherited alleles are redistributed between sexes after each sexual reproduction event, this approach requires males and females to be sampled after their dispersal phase. Ideally, the spatial range of the sampling scheme (the minimal and maximal distances between individuals) should at least encompass the spatial range of dispersal distances between males and females. The ability of the method to detect sex-biased dispersal also depends on the degree of dispersal asymmetry, and dispersal of the less dispersing sex must be limited enough to generate a spatial genetic structure. Other studies have used the same principle to assess sex-biased dispersal by regressing pair-wise relationship coefficients on spatial distance separately for each sex (Sundström *et al.*, 2003; Hazlitt *et al.*, 2004), or by comparing the spatial autocorrelation of male genotypes and females genotypes (Neville *et al.*, 2006), two approaches totally equivalent (Hardy & Vekemans, 1999). It is noteworthy that, when males and females differ in their ploidy level, care must be taken in the choice of the relatedness measure because the relationship coefficient (r_{ij}) is affected by the ploidy

and inbreeding of the individuals, contrary to the kinship coefficient (F_{ij}). For example, in the absence of sex-biased dispersal in an ant species, r_{ij} between females is expected to decay approximately two times faster with the spatial distance than r_{ij} between males, a pattern that could erroneously be interpreted as evidence for male-biased dispersal. Hence, for haplodiploid organisms, it is advisable to use pair-wise kinship coefficients for comparing the spatial genetic structures of sexes.

From a biological point of view, our results are consistent with sex-biased gene flow with reduced female dispersal at very small scales in *C. cursor*. Reduced queen dispersal during mating combined with colony founding by budding generates significant microgeographical genetic structure above the nest level. This pattern is expected, because dispersal in this species is associated with restricted local gene flow mainly as a result of limited vagility of queens. In contrast, males of *C. cursor* can fly and disperse much further than females. Accordingly, spatial genetic structuring is much more pronounced for queens than for males. Although males are the dispersing sex in *C. cursor*, our data show that male–male relatedness decreases with distance, indicating that male dispersal is somewhat limited at the scale of the study population (which extends over 250 m). Workers show a level of spatial genetic structuring intermediate between the ones of queens and males, a natural consequence of the fact that workers combine alleles of the queens and their mates. Our results on sex-biased dispersal in *C. cursor* are consistent with those of Clémencet *et al.* (2005) who compared population differentiation at mitochondrial and nuclear DNA at two spatial scales (populations separated by 300–4500 m, and by 2500–120 000 m), sampling 1–2 workers per colony. These authors found much more differentiation at mitochondrial than nuclear DNA, indicating male-biased dispersal. Moreover, their analyses showed isolation by distance for both markers at both scales, implying that male dispersal is also limited for the 300–4500 m spatial scale. Our sex-by-sex study allows investigating dispersal on a smaller scale (1–300 m), where mitochondrial DNA usually lacks polymorphism (which indeed occurs in Clémencet *et al.*, 2005). Even at this scale, isolation by distance was detected in males, implying that they probably disperse over no more than a few tens of metres, while reproductive females probably disperse over a few metres at most.

IMPACT OF LIMITED DISPERSAL ON PATTERNS OF RELATEDNESS WITHIN COLONIES

Males mating with a queen are slightly but significantly related. Likely, this stems from limited male

dispersal, which results in a reduction of the number of male sources for each colony producing gynes at the time of mating. The effect of limited dispersal of males is reinforced by the fact that only a small proportion of colonies (30–40%, Pearcy & Aron, 2006) produce sexuals in *C. cursor*. From those, most produce males taking part to nuptial flight, whereas very few nests produce female sexuals and the number of females reared per nest is usually low. This increases the probability that the mates of a queen be somewhat related. A surprising result of this study is that, despite the limited dispersal of both sexes, mating queen–males pairs are on average unrelated. Mating avoidance between relatives might be involved. However, the power to detect a weak level of relatedness between queens and their mates might be insufficient. For instance, if males moved less than 20 m before mating, their expected kinship with a reproductive female would approach 0.022 (Table 1), a value lying within the approximate 95% confidence interval of mating queen–male kinship estimate (–0.032 to 0.032, using twice the standard error in Table 1). Interestingly, our data also show that, at the microgeographical scale studied, patterns of caste relatedness within colonies are only slightly affected by limited male and female dispersal. Indeed, the predicted kinship coefficients are very similar when dispersal limitation is assumed or not. Rather, the inbreeding of queens and the number of queen's mates appear much more prominent on patterns of relatedness within colonies. Especially, male–worker and worker–worker relatedness values are well predicted from queen-mating frequency.

Genetic studies with nuclear and/or mitochondrial markers have revealed significant population genetic differentiation in a diversity of ant species belonging to different genus (e.g. *Formica*, Sundström *et al.*, 2003; *Plagiolepis*, Trontti, Aron & Sundström, 2005; *Myrmica*, Seppä & Pamilo, 1995; *Pheidole*, Fournier, Aron & Milinkovitch, 2002; *Solenopsis*, Ross & Shoemaker, 1997). These studies have provided evidence that variation in social organization (monogynous vs. polygynous) results in differences in gene flow regime and patterns of relatedness within colonies. Strong microgeographical structure was found in all polygynous species or form, which are characterized by queens mating in or near the parental nest, readoption of newly mated queens and budding as the mode of colony founding. Polygynous social systems also show moderate or even zero relatedness among colony members, because nest-mate queens may be unrelated and queen number may be very high. By contrast, significant microgeographical structure is rare in monogynous ant species, where queens and males disperse widely; this social form usually shows high nest-mate relatedness. Nevertheless, viscous local

population structures have been reported in a few monogynous species, the queenless ant *Diacamma cyaneiventre* (Doums, Cabrera & Peeters, 2002), the monogynous social form of *Formica exsecta* (Sundström *et al.*, 2003) and *C. cursor* (Clémencet *et al.*, 2005). In *D. cyaneiventre* and *C. cursor*, female reproductives have extremely restricted dispersal compared with males, which disperse genes over longer distance. In the wood ant *Formica exsecta*, both sexes participate in mating flights and mated queens found new colonies independently; yet, a significant spatial genetic structure occurs for queens but not for colony fathers.

In conclusion, although constraining in terms of sampling effort because the queen must be captured, our approach allows a detailed investigation of sex-biased dispersal through its impact on the spatial genetic structure of males and females sampled after dispersal. Its main advantages are that it does not suffer the low polymorphism of mitochondrial DNA at a local scale and that it can predict the impact of limited dispersal on the genetic structure within colonies.

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