Inbreeding and kinship in the ant *Plagiolepis pygmaea*

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Abstract

In ants the presence of multiple reproductive queens (polygyny) decreases the relatedness among workers and the brood they rear, and subsequently dilutes their inclusive fitness benefits from helping. However, adoption of colony daughters, low male dispersal in conjunction with intranidal (within nest) mating and colony reproduction by budding may preserve local genetic differences, and slow down the erosion of relatedness. Reduced dispersal and intranidal mating may, however, also lead to detrimental effects owing to competition and inbreeding. We studied mating and dispersal patterns, and colony kinship in three populations of the polygynous ant *Plagiolepis pygmaea* using microsatellite markers. We found that the populations were genetically differentiated, but also a considerable degree of genetic structuring within populations. The genetic viscosity within populations can be attributed to few genetically homogeneous colony networks, which presumably have arisen through colony reproduction by budding. Hence, selection may act at different levels, the individuals, the colonies and colony networks. All populations were also significantly inbred (*F* = 0.265) suggesting high frequencies of intranidal mating and low male dispersal. Consequently the mean regression relatedness among workers was significantly higher (*r* = 0.529–0.546) than would be expected under the typically reported number (5–35) of queens in nests of the species. Furthermore, new queens were mainly recruited from their natal or a neighbouring related colony. Finally, the effective number of queens coincided with that found upon excavation, suggesting low reproductive skew.

Keywords: dispersal, Hymenoptera, inbreeding, kin structure, *Plagiolepis pygmaea*, polygyny

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Introduction

The formation of social groups has several consequences for the genetic structuring of natural populations (e.g. Sugg *et al.* 1996; Ross 2001; Hochberg *et al.* 2003). By definition, groups comprising related individuals arise when individuals forgo dispersal, and will, in conjunction with reproductive division of labour, lead to a reduction in the effective population size. Social insects are an extreme example of low population sizes relative to the census population size, because the vast majority of the group members permanently forgo reproduction. Furthermore, the effective number of reproductive individuals in a colony may be significantly lower than that observed, owing to unequal sharing of reproduction among reproductives. Small effective population size frequently leads to inbreeding and is considered detrimental to populations of most organisms. However, in social insects such as ants genetic viscosity and inbreeding may enhance genetic unity within the colony, a prerequisite for reproductive altruism (Hamilton 1964a, b, 1972; Bourke & Franks 1995).

The principle of inclusive fitness holds that individuals can pass copies of their genes to future generations without reproducing themselves, but by helping a relative to raise related but nondescendant young (Hamilton 1964a, b). Helpers gain inclusive fitness through the additional offspring that can be raised with the help given, devalued by the relatedness between the altruist and the recipient. Hence, the lower the relatedness the higher an increase in reproduction is required to overcome the loss in personal reproduction. Very low kinship is expected to result in selfish behaviour, nepotism, and social disruption (Hamilton 1972), and further, as individuals seldom interact with a

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relative, the heritability of worker traits declines such that selection can no longer act on them (Queller & Strassmann 1998).

The permanent presence of multiple reproductively active queens (polygyny) is common in ants, and the number of queens may vary both among and within species from only a few to several hundreds (Bourke & Franks 1995). Polygyny dilutes the relatedness between the workers and the brood they help to rear. Nevertheless, polygyny can emerge as a kin-selected trait such that the adoption of daughter queens is favoured by all parties, workers, resident queens and young queens alike, despite the reduction in inclusive fitness returns due to reduced relatedness within the colony (Rosengren & Pamilo 1983; Nonacs 1988; Pamilo 1991). Altogether the magnitude of impact on inclusive fitness returns attributable to polygyny is determined by the genetically effective number of breeders in the colony, in addition to colony survival and productivity. This quantity depends on the actual number of queens in the colony, their relatedness, the degree of reproductive skew (*sensu* Keller & Reeve 1994), and on the degree to which queens are related to their male mates, i.e. inbreeding (Queller 1993; Ross 1993).

The perhaps most important single factor contributing to the evolution of polygyny is increased risk of dispersal (Keller 1995); both high predation pressure during the nuptial flight as well as failures during colony founding may select for philopatry and the adoption of daughter queens to their natal colony (Wilson 1974; Nonacs 1988; Pamilo 1991; Perrin & Lehmann 2001). Reduced female dispersal is indeed strongly associated with polygyny, and colony queens often are related in many species (Hölldobler & Wilson 1977; Sundström 1993, 1995; Keller 1995; but see Fournier *et al*. 2003).

As a consequence of polygyny new modes of reproduction become available. In addition to colony foundation through single dispersing queens, a polygynous colony can also multiply through colony budding, where new daughter colonies are established in the vicinity of the maternal colony by workers and queens migrating on foot (Bourke & Franks 1995). This mode of reproduction leads to a population structure, where physically close colonies are genetically similar and distant colonies are genetically different (population viscosity *sensu* Hamilton 1964b). This in turn increases relatedness within groups of colonies, which has been proposed to favour altruism through selection at the level of colony networks (Hamilton 1964b). Indeed, polygynous ants frequently show structuring of populations (e.g. Ross & Keller 1995; Chapuisat *et al*. 1997; Liautard & Keller 2001; Gyllenstrand & Seppä 2003), and in species where both mono- and polygynous types of populations exist, local genetic structuring is often found only in the polygynous ones (e.g. Sundström 1993; Banschbach & Herbers 1996; Seppä *et al*. 2002; but see Sundström *et al*. 2003).

Inbreeding and genetic viscosity can thus counteract some of the reduction in relatedness caused by polygyny. However, any inclusive fitness benefits gained from inbreeding and philopatry are expected to be counteracted by costs associated with reduced dispersal. Most importantly, when colonies reproduce by budding such that neighbouring colonies are related, increased kin competition may mitigate the relatedness benefits (Pollock 1983; Taylor 1992a, b). This can be avoided only if local gene flow is weak enough to maintain genetic characteristics of colonies within the range of competition. In addition, inbreeding increases the level of homozygosity and, in Hymenoptera, subsequently increases the risk of producing sterile diploid male offspring instead of workers and queens. This follows from their complementary sex determination (e.g. Cook & Crozier 1995) where diploid individuals that are homozygous at the sex determination locus or loci develop into sterile males (Crozier 1971; Beye *et al*. 2003).

In this study we analyse population structure and kin structure in the polygynous ant *Plagiolepis pygmaea*. In particular we examine whether the mating system, breeding structure and mode of colony reproduction may alleviate the reduction in relatedness inevitably imposed by the presence of multiple reproductive queens. Indeed, Hamilton (1972) suggested that inbreeding may be a strategy to enhance social cohesion within insect colonies. Colonies of this species typically include up to 30 queens, or in some cases even more (Passera 1969), and reproduce both by independent colony founding and by budding. At present it is unclear whether daughter nests remain in contact with the mother nest, or whether these form independent units soon after budding. First, we analyse the degree of differentiation among populations at different locations and the spatial structuring within populations in terms of population viscosity. Second, we analyse the kin structure of the populations and colonies in terms of the relatedness, the effective number of queens and, the pattern of queen recruitment and inbreeding.
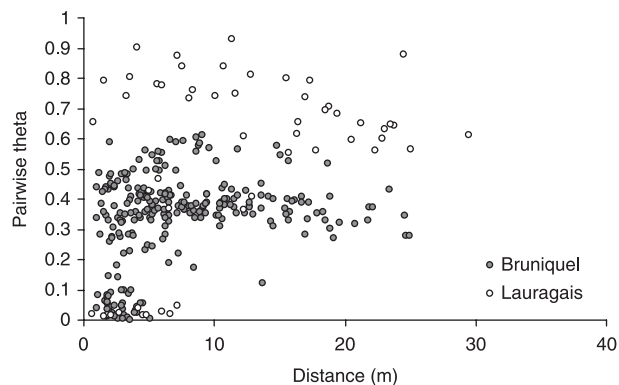
Materials and methods

Plagiolepis pygmaea occurs throughout the southern parts of Europe and is commonly found in arid areas with low vegetation. The subterranean colonies with 200–5000 workers (e.g. Passera 1969) are located under stones or in gravel. New worker brood is produced throughout the year, except in winter. The peak of worker production coincides with the production of sexuals that eclose in summer (June–July). Superimposed on colony reproduction by budding the nests also exhibit seasonal polydomy, i.e. they form summer nest units (usually within 0.5 m from the mother colony) that merge again for winter.

We collected *P. pygmaea* from three locations in southern France: Cerbère in the vicinity of the border of Spain (April

1999), St Felix de Lauragais, 40 km south from the city of Toulouse (hereafter Lauragais; June 1999) and Bruniquel, 70 km northeast from Toulouse (March 2002). In Cerbère we collected workers from 49 colonies, most of which also included worker pupae and male brood. Virgin (winged) queens were found in only three colonies. In Lauragais we collected workers and old queens from 11 colonies. From Bruniquel we collected workers from 33 colonies. The spatial location of the nests was mapped in Lauragais and Bruniquel using 30 m transects across the populations. As we collected all colonies we could find, this yields an average nest density of $0.4/m^2$ in Lauragais, $1.2/m^2$ in Bruniquel. In Cerbère we collected all nests within an area of approximately 8000 m^2, which yields an estimate of about 0.006 nests per m^2. Two nests were never collected closer than 1 m from each other in Cerbère. In Bruniquel this occurred, however, here we analysed only one nest in each of these pairs to avoid sampling the same colony twice. In Lauragais the habitat was less rocky and we were able to collect complete colonies 40–50 cm deep until the layer of hard clay. Consequently, even if two pairs of the colonies were only 0.6 and 0.7 m apart (Fig. 1), we could ascertain that they were not connected underground. All samples were preserved in 94% ethanol for genetic analysis.

We scored genotypes of 8–20 workers from each of a total of 58 colonies (Lauragais $n = 11$, Cerbère $n = 23$, Bruniquel $n = 24$), 70 queens from eight colonies (Lauragais) and offspring from 12 colonies (20 worker pupae from four and 20 males from eight colonies; Cerbère). Individuals were genotyped at seven microsatellite loci (*P01*, *P06*, *P07*, *P11*, *P20*, *P23* and *P25*; Trontti *et al*. 2003) following the protocols described therein: for all samples from Lauragais and part of those from Cerbère the genotyping was carried out with autoradiography, and for all samples from Bruniquel and the remainder of those from Cerbère the genotyping was carried out applying fluorescent analysis. Allele sizes obtained with the two methods were standardized by running a set of the samples analysed by autoradiography in the automatic sequencer used for fluorescent analysis.

The data on workers comprises several hierarchical levels: individuals within colonies, colonies within populations, and populations within the entire sample. The intermediate level representing colonies within populations is called for because individuals in ant colonies are related, and therefore do not represent independent samples. Furthermore, when the sample comprises groups of related individuals the coefficient of identity by descent at the lowest level, in this case the colony, can only take zero or negative values (e.g. Sundström *et al*. 2003). To analyse the data we used two approaches: First, we used a three-level hierarchical *F*-analysis of variance (Weir & Cockerham 1984) to obtain coefficients of identity by descent (state) at each level of interest. Here the levels of interest are individuals within populations and populations within the entire sample. The former gives the inbreeding coefficient due to nonrandom mating devoid of the effects of kinship (F). The latter gives the allele frequency differences between populations ($\theta - P$). Second, we created 100 resampled data sets taking for each set one individual per colony. On these 100 data sets we run a two-level analysis to obtain estimates of inbreeding (f_R) and population differentiation (θ_R). The former estimate corresponds to the inbreeding coefficient F and the latter to the value $\theta - P$ of the three-level analysis. To obtain population-specific inbreeding coefficients F, we also analysed each population separately in a two-level analysis with colonies entered as subpopulations. In addition to worker data we had queen data from one population, which calls for a two-level analysis.

The three-level hierarchical analysis was performed with GENETIC DATA ANALYSIS version 1.1 (Lewis & Zaykin 2001), and the two-level analyses with FSTAT version 2.9.3 (Goudet 1995, 2001). The 95% confidence intervals were obtained by bootstrapping 15 000 times over loci. The significance for each of the 100 resampled data sets was tested by exact tests not assuming Hardy–Weinberg equilibrium (HWE). Finally, we analysed each population separately by PCA-GEN version 1.2 (Goudet 1999), which uses principal component analysis across loci to detect genetic subdivision within populations. We then run a two-level analysis with FSTAT using the above 100 resampled data sets separately for each population, but this time assembling colonies into groups as indicated by PCA-GEN This allowed us to test for the possibility that inbreeding is due to population subdivision rather than mating with relatives.

To estimate the degree of isolation by distance (Wright 1943) within the populations of Lauragais and Bruniquel, we calculated coefficients of identity (θ) between all pairs of colonies and combined this information with the metric distance between the colonies. Mantel tests were applied to obtain a correlation between the genetic and metric distance matrices. These analyses were performed using FSTAT 2.9.3 (Goudet 2001), with 2000 randomizations of the data to test for significance.

Fig. 1 Relationship between genetic differences (θ) and spatial distance (m) between pairs of colonies in Bruniquel and Lauragais.

We applied assignment tests to identify queens that were likely adopted from another colony by searching for divergent genotypes among colony queens. The tests were made with GENECLASS 1.02.1 (Cornuet *et al*. 1999), where we inferred the genetic origin of each queen as the 'probability-of-belonging' values (*P*), and tested whether each queen found in a colony was genetically similar to her nest mate queens (with $P \geq 0.05$) or whether she could be excluded from this group owing to lack of genetic similarity ($P < 0.05$). The alleles of the individual under investigation were removed from the data set during assignment in order to avoid bias due to the small number of queens per colony. A Bayesian probability function was used in the simulations (10 000 replications) and the allele frequencies were estimated directly from the data. The genotypes of individuals rejected by the analysis were checked to see if their genotypes were truly distinct from the other colony queens.

We estimated the regression relatedness among workers (Cerbère, Lauragais, Bruniquel), queens (Lauragais), and males (Cerbère) with RELATEDNESS 4.2 (Queller & Goodnight 1989), always using the respective population as the reference group. The 95% confidence intervals were obtained by jackknifing over colonies. We also calculated the inbreeding-corrected relatedness estimates (*r**) after Pamilo (1985). This allows an estimation of the effective number of queens (i.e. the number of unrelated diploid genomes contributed by colony queens) reproducing in the colonies (N_E), devoid of any effects due to inbreeding. This was calculated as an average over colonies for the Lauragais population from:

$$N_E = (4r_{fs} - r_q - 2r_m)/(4r_w - r_q - 2r_m) \qquad \text{(eqn 1)}$$

where r_{fs} is the relatedness among workers sharing the same mother, r_w and r_q are the observed inbreeding-corrected relatedness coefficients among colony workers and queens, and r_m is the relatedness among the males that have inseminated the queens (Ross 1993; Seppä 1994). We calculated N_E assuming both single and double mating of the queens hence r_{fs} equals 0.75 or 0.375 (assuming equal paternity share among males). Mother–offspring analyses have shown that the effective mating frequency is close

to two in this species (Trontti *et al*. in prep). Relatedness among fathers was unknown so we calculated N_E for a range of values from all male mates of the queens being unrelated ($r_m = 0$) to all male mates being full brothers ($r_m = 0.5$). This estimate of N_E does not include effects of reproductive skew among the queens, so if N_E is smaller than the harmonic mean number of queens (N_H), this means that queens share reproduction unequally. The confidence interval for N_E was calculated by first obtaining the 95% confidence intervals for the inbreeding-corrected relatedness estimates of Bruniquel by jackknifing across loci (calculated in FSTAT 2.9.1 following Queller & Goodnight 1989 and Pamilo 1985), and then applying the upper and lower values in equation 1. Finally, we tested for the presence of multiple queens based on worker genotype data with the program MATESOFT version 1.0, which performs parentage analysis for haplo-diploids (Moilanen *et al*. 2004).

Results

Altogether on average 7.4 alleles were detected at each of the seven loci across all three populations, with expected heterozygosities between 0.406 and 0.834 (Trontti *et al*. 2003). Within the populations the locuswise allelic richness varied from a minimum of 3 alleles to 10 (Cerbère), 6 (Lauragais), and 9 (Bruniquel). The three populations differed slightly but significantly in their allele frequencies ($\theta_R = 0.049$, SD = 0.010, exact *P* for 100 randomizations all < 0.017; $\theta - P = 0.042$, 95% CI = 0.021–0.062). The fixation indices *F* and f_R were very similar in magnitude ($F = 0.291$, 95% CI = 0.222–0.357; $f_R = 0.265$, SD = 0.030, exact *P* for 100 randomizations all < 0.001). Furthermore, the populationwise *F*-indices from the two-level *F*-analysis indicated strong heterozygote deficit among workers within all individual populations (Table 1; the pattern was similar across all loci). A similar level of inbreeding was also found for the queens in the Lauragais population (Table 1). We found no evidence for null alleles that could bias the estimate of *F*. First, strong heterozygote deficit was indicated by each marker independently. Second, the locuswise non-amplification rates (on average 4.3 individuals per locus; SD = 3.4), quantified from the hemizygous males, did not

Population	n	Caste	F	r	r*
Lauragais	11				
		Workers	0.290 (0.159–0.426)	0.529 (0.455–0.603)	0.140
		Queens	0.272 (0.148–0.408)	0.475 (0.336–0.614)	0.085
Cerbère	23				
		Workers	0.206 (0.144–0.253)	0.546 (0.478–0.615)	0.217
		Male offspring		0.360 (0.282–0.437)	
Bruniquel	24				
		Workers	0.293 (0.199–0.420)	0.542 (0.454–0.646)	0.162

Table 1 Populationwise inbreeding coefficients (*F*), relatednesses (*r*) and relatedness estimates corrected for inbreeding (*r**). Confidence intervals of 95% for the estimates are indicated in brackets. *N* indicates the number of analysed colonies

correlate positively with the locuswise inbreeding coefficients as would be expected in the presence of null alleles ($r = -0.36$; $P = 0.43$). Despite the high inbreeding coefficients, we detected no diploid individuals among the 160 male offspring that were genotyped in Cerbère. The probability of not identifying a diploid male due to homozygosity across all loci used was less than 1%, based on population allele frequencies. This estimate, however, does not take inbreeding into account, so we used the proportion of diploid workers that were homozygous at all loci for comparison. None of genotyped 232 diploid workers in this population was homozygous at all loci, which indicates that the probability of not detecting a diploid male is very low indeed.

The genetic distance between pairs of colonies (pairwise θ) increased with their spatial distances in the two populations where spatial data were available (Bruniquel: $r = 0.311$, $P < 0.001$, $n = 21$, $\beta = 0.009$; Lauragais $r = 0.438$, $P = 0.003$, $n = 11$, $\beta = 0.016$). This correlation was due to a fraction of colonies that formed clusters, in which colonies were both related and each other's nearest neighbours. In Lauragais we detected two such clusters containing two and three colonies, and in Bruniquel four clusters containing 11 and three times two colonies. Nevertheless, a considerable fraction of colonies located at equally short distances from each other were also genetically very different (Fig. 1). In contrast, colonies at greater distance from each other were always genetically differentiated. Thus the isolation by distance did not entail a gradual increase of genetic differences over spatial distance, but was rather due to the formation of a few clusters of genetically similar colonies with discrete boundaries within the populations.

The principal component analysis indicated further population subdivision in Bruniquel for a component that accounted for 32% of the total theta in the population ($P = 0.04$). By contrast, in Lauragais and Cerbère none of the principal component axes was significant (the probabilities for the most influential first component axes being $P = 0.09$ and $P = 0.46$, respectively). We re-analysed the data from Bruniquel with the observed boundaries for population subdivision to re-estimate f_R from the same resampled data sets as previously noted. The results indicated only a slightly lower average inbreeding coefficient, than without taking population subdivision into account ($f_R = 0.205$, SD 0.048; exact P for 99% of the randomizations < 0.05; $\theta_R = 0.181$, SD = 0.018, exact P for 100 randomizations all < 0.001) (cf. Table 1). Hence, the observed heterozygote deficit is only to a limited extent attributable to genetic viscosity, and was instead mainly due to nonrandom mating in favour of relatives.

The assignment tests in Lauragais excluded 19 (27%) queens from the colony in which they were found (probability-of-belonging value $P < 0.05$), the colony-specific percentage of exclusion varying from 9% to 44%. Of these excluded queens 15 were instead assigned to one or several of the genetically similar neighbouring colonies. It thus seems possible that colonies also recruit queens other than their own offspring, but that this would occur only between related colonies. The remaining four (6%) queens were left completely unassigned, i.e. did not get a value of belonging greater than $P = 0.05$ for any of the sampled colonies. However, inspection of their individual genotypes revealed that three of these carried alleles in common with other queens in the same colony. Only one of these queens carried alleles absent from all other queens in the colony. The possibility nevertheless remains that at least some of these four queens may have originated elsewhere.

The genotype distributions were inconsistent with monogyny in 33% of the colonies according to the MATESOFT analysis. Of the remaining 67% worker genotypes were consistent with a single maternal and paternal genotype in only two nests in Cerbère and three nests in Bruniquel (9% of all colonies). In the remaining 58% of the colonies the genotype distributions could be explained by monogyny with multiple mating between 2 and 13 times. This would correspond to a harmonic mean mating frequency of 4.23, which is much higher than the observed harmonic mean mating frequency of two (Trontti *et al.* in prep.). Hence, the majority of the colonies are likely headed by several reproductive queens.

The average relatedness among colony workers was high, and did not differ between populations (Table 1), although colonywise relatedness estimates varied considerably (Cerbère $r = 0.174–0.792$; Lauragais $r = 0.386–0.755$; Bruniquel $r = 0.402–0.903$; all individual values were significantly higher than zero). As expected, relatedness in the possibly monogynous colonies (with single mating) was among the highest (Fig. 2); however, the population averages analysed without these colonies were not significantly lower than the estimates including them (Table 1): $r = 0.462$ (95% CI = 0.372–0.568; Bruniquel; $n = 21$) and $r = 0.502$ (95%
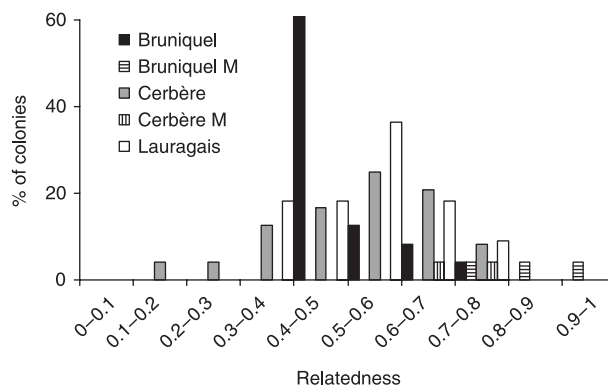

Fig. 2 Distribution of relatedness among workers of all colonies in the three study populations. Possibly monogynous colonies with a singly mated queen (M) of Bruniquel and Cerbère are presented separately.

CI = 0.451–0.546; Cerbère; n = 21). The distribution of colony-specific estimates of worker relatedness was unimodal in all populations, indicating that populations did not consist of a mixture of either monogynous or polygynous colonies (Fig. 2). However, the range of estimates was considerably narrower and their distribution strongly skewed in Bruniquel, with more than half the estimates coinciding with the minimum values of 0.4–0.5. The relatedness among colony queens in the Lauragais population was of the same magnitude as that among colony workers (Table 1), which provides further support for the conclusion that predominantly daughters are recruited as new queens. Moreover, male offspring collected from Cerbère was closely related, yet their relatedness was significantly lower than that for full brothers ($r = 0.5$) (Table 1), indicating the presence of multiple queens. The inbreeding-corrected relatedness estimates were significantly lower than the uncorrected estimates (Table 1), and given the observed queen number (below) and queen–queen relatedness, the expected relatedness among workers under outbreeding is significantly lower than the observed, $r = 0.11$ {calculated following Bourke & Franks 1995: $r = [0.75 + 0.25r_q(n_q - 1)]/n_q$, where n_q = number of queens and r_q = relatedness of queens; inbreeding corrected}.

The observed harmonic mean number of queens in the Lauragais population was $N_H = 8.4$ (range 5–15 per colony). Under single mating of the queens and assuming that all colony fathers were unrelated the effective number of queens per colony $N_E = 6.1$ (95% CI = 3.9–14.7). With even a moderate degree of relatedness among colony fathers ($r_m = 0.073$) the estimate of N_E converges to that of the harmonic queen number and as the male relatedness increases further, the estimate of N_E increases rapidly (e.g. when $r_m > 0.2$, $N_E > 30$). When assuming double mating of the queens and unrelated colony fathers the minimum estimate of N_E is 3.0 (95% CI = 1.9–7.0). Again, this estimate increases together with the relatedness among fathers, and equals 8.4 when assuming that colony fathers are related by $r_m = 0.174$. Given that male offspring within colonies are related by 0.36 and that extensive intranidal mating occurs, we conclude that the magnitude of N_E roughly equals or even exceeds the harmonic mean number of queens observed here. An effective number of queens in excess of the harmonic mean number queens would indicate queen turnover, i.e. that new queens take over reproduction in consecutive years.

Discussion

The degree of genetic differentiation among populations of the ant *Plagiolepis pygmaea* was only slight at geographical distances over 50 km, yet the species exhibits within-population structuring at distances less than 30 m. Several lines of evidence suggest that this population substructure is further enhanced by the breeding system of the species. First, the high levels of inbreeding in both workers and queens indicate extensive mating among relatives, and philopatry in both sexes. Second, the assignment tests suggest that most queens are adopted by their natal colony. As a result high kinship is maintained both among workers and queens despite the presence of multiple queens. The consistency between the effective and the observed number of queens further suggests that reproductive skew within the colonies does not contribute significantly to the within-colony relatedness. Interestingly, we found no diploid males among the 160 individuals that were analysed. This suggests that either no diploid male eggs are laid or they are removed before maturation.

Genetic structuring at a local scale, combined with less pronounced structuring at a global scale is most likely due to differences in the degree of genetic drift within and across populations. The effective population size within each of our study populations is smaller than the total population and so will increase the effects of drift (Hartl & Clark 1989). The observed genetic differentiation within populations thus arises from three sources. First, colony reproduction by budding leads to aggregations of related colonies, which are initiated by a limited number of colony foundresses. Second, the breeding system involving inbreeding will further enhance the effect of drift (Hartl & Clark 1989). Third, in one population, significant substructuring was detected, as indicated by the principal component analysis. This suggests that populations may be established by queens from different source populations.

Relatedness in *P. pygmaea* colonies was high both among workers and queens, and in many colonies the estimates did not differ statistically from those expected under monogyny with single mating. The species is, however, not functionally monogynous. First, the worker genotypes were generally inconsistent with a single once-mated reproductive queen, and the average mating frequency required to explain the observed genotype distributions among workers was twice as high as that observed in the species (Trontti *et al.* in prep.). It is also likely that the sample of eight workers per colony did not include all genotypes present in the colony. This, together with inbreeding, underestimates the level of polygyny detected by parentage analysis. Second, the relatedness among males was significantly lower than that expected for full brothers, which indicates that more than one queen contributes to male brood (unless some males are produced by workers). Third, the estimated effective number of reproductive queens is clearly higher than one, and coincides with or even exceeds the observed harmonic mean number of queens. This rules out high reproductive skew among the queens as a cause of the high relatedness among nest mate workers and queens.

The exceptionally high inbreeding found here suggests very low effective gene flow also within populations, and

philopatry by both sexes. Strong philopatry among queens is also supported by the similarity of nest mate queen genotypes. Furthermore, if queen recruitment from other colonies occurs, these queens arrive from neighbouring related colonies, as indicated by the assignment tests. The resolution of assignment tests, however, depends on the genetic differences among the groups under comparison and the markers used. In our case the differences among neighbouring nests were commonly small. The risk of incorrect assignment is higher when groups of related individuals are sampled and when the populations violate Hardy–Weinberg equilibrium (e.g. Waits *et al.* 2001), so the exclusions indicated by the assignment tests should be interpreted with caution.

Low effective male dispersal in *P. pygmaea* is peculiar since the males are capable of flying, and do swarm in late June (personal observation). Possibly males first mate in their natal colony and later disperse, with very few being successful. The low male dispersal observed here stands in contrast with many previously investigated polygynous ants, where gene flow seems to homogenize gene frequency differences among populations with respect to nuclear markers (e.g. Krieger & Keller 2000; Gyllenstrand & Seppä 2003; Rüppell *et al.* 2003) A similar case of low effective dispersal by both sexes has, however, been described in the wood ant *Formica paralugubris* (Chapuisat & Keller 1999), in which more than 99% of the matings were estimated to take place among nest mates.

Many studies have associated polygyny with genetic viscosity both in nuclear (e.g. Pamilo 1983; Pamilo & Rosengren 1984; Seppä & Pamilo 1995; Chapuisat *et al.* 1997; Pirk *et al.* 2001; Gyllenstrand & Seppä 2003) and mitochondrial markers (e.g. Stille & Stille 1993; Liautard & Keller 2001; Gyllenstrand & Seppä 2003; Rüppell *et al.* 2003). Population viscosity and inbreeding are described also in at least one monogynous species, *Formica exsecta* (Sundström *et al.* 2003). In fact, genetically completely homogenous local populations are found only in a minority of polygynous species, such as *Linepithema humile* (e.g. Krieger & Keller 2000; Tsutsui & Case 2001; Giraud *et al.* 2002); *Lasius neglectus* (Boomsma *et al.* 1990), a few others in their non-native, introduced range (review by Tsutsui & Suarez 2003), and also most recently in *Formica truncorum* (Elias *et al.* 2005). Altogether reduced dispersal is common in polygyne ants, and as suggested by Hamilton (1972), inbreeding may be a viable strategy to maintain genetic cohesion of colonies across several generations.

However, although inbreeding may increase inclusive fitness returns for workers, it is also likely to entail costs due to detrimental effects on colony performance by increasing the risk of producing sterile diploid males instead of female offspring (e.g. Ross *et al.* 1993; Cook & Crozier 1995). Despite the high degree of inbreeding we found no diploid individuals among the male brood of *P. pygmaea*, but we cannot rule out the possibility that diploid male offspring had been eliminated at earlier developmental stages, as we only had mature brood. Alternatively the colonies may have retained sufficient diversity at the sex determining loci by assortative mating, as may be the case in *Linepithema humile* (Keller & Passera 1993; but see Keller & Fournier 2002). However, we consider this unlikely as the high inbreeding coefficients argue against any preference for unrelated individuals. In addition, low dispersal and genetic viscosity increases competition among kin, which may cancel out inclusive fitness benefits derived from philopatry (e.g. Pollock 1983; Kelly 1992; Queller 1992, 1994; Taylor 1992a, b). Thus, selection at the level of individuals may be counteracted by selection at the level of groups or higher entities, such that the individual benefits from high relatedness are mitigated by group costs of inbreeding or competition. Nevertheless, when the scales of competition and relatedness differ so that competition partly occurs between non-kin, the benefits of genetic viscosity may override the costs of competition (e.g. Queller 1992, 1994).

Not surprisingly many organisms have some means of reducing the risk of inbreeding, such as sex-biased dispersal (e.g. Sugg *et al.* 1996), and also show inbreeding depression (Charlesworth & Charlesworth 1987; Crnokrak & Roff 1999; Keller & Waller 2002). In contrast, studies on the negative consequences of inbreeding on individual performance and mate choice in ants suggest that inbreeding depression may be less common in them (Keller & Passera 1993; Keller & Fournier 2002). Indeed, the impact of inbreeding on colony performance has been hypothesized to be smaller in social insects compared to other animals, possibly because the haploid males allow purifying selection of lethal recessive alleles between each generation (e.g. Crozier 1970), or a lower cost of decreased egg and larval viability due to offspring overproduction (Keller & Passera 1993). Hence, ant colonies may be in a position to take advantage from the inclusive fitness benefits mediated by inbreeding. More studies on the consequences of inbreeding on colony performance are desirable, as well as a more precise understanding of the function of the complementary sex determination in ants, in order to fully weigh the costs against benefits involved in inbreeding in ants.

Our data on *P. pygmaea* shows that reproductive strategies involving low dispersal and sib mating may maintain high kinship within groups even if reproduction is shared by many individuals. Thus their breeding system based on philopatry may alleviate the loss in inclusive fitness returns caused by dilution of relatedness due to polygyny. However, the selective pressures that shape breeding strategies likely differ among workers, queens, and males. Hence the question whether inclusive fitness can act as the driving force for inbreeding depends on whether workers are able to control these patterns. Interestingly, preliminary behavioural observations indicate that workers actively repel

heterocolonial males when these arrive at an unrelated nest, even if virgin queens are present (S. Aron, in prep.). Given that female philopatry has evolved for other reasons this may be a way for workers to reduce the erosion of relatedness by preventing outbreeding. Research on both behaviour and population genetics of ants and other social animals could shed light on this still unexplored question in social evolution.

Acknowledgements

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