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The genetic population structure of the ant *Plagiolepis xene*-implications for genetic vulnerability of obligate social parasites

Kalevi Trontti[1,*], Serge Aron[2] & Liselotte Sundström[1]
[1]*Department of Biological and Environmental Sciences, University of Helsinki, P.O. Box 65 (Viikinkaari 1) FIN-00014, Finland;* [2]*Behavioural and Evolutionary Ecology, Université Libre de Bruxelles, Belgium, (*Corresponding author: Phone: +358(0)9-19157701; Fax: +358(0)9-19157694; E-mail: trontti@cc.helsinki.fi)*

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Abstract

Obligatory social parasites, such as ant species that need colonies of other ant species for reproduction, are rare and many of them are classified as vulnerable. This is especially the case with highly adapted permanent inquilines that are specialised on one or a few host species. Their rarity may be due to reduced dispersal abilities, as a result of reduced body size, altered wing morphology, and curtailed nuptial flight, eventually leading to inbreeding. Furthermore, the host populations may differ in their ability to resist the parasite, yet the conditions of successful parasite invasion are largely unknown. Here we investigated the population structure of the inquiline ant *Plagiolepis xene* and its host *P. pygmaea*, using microsatellite data. Genetic differentiation, inbreeding, the effective population size and nest kin structure were analysed. We found that populations of *P. xene* are established by a single or at most a few individuals, and that the populations were genetically highly differentiated. However, within individual host populations the parasite is able to maintain panmixia, although data on the host suggests that the local distribution of the parasite also follows patterns of substructuring in the host population. Altogether our results suggest that inquiline parasite populations are genetically highly vulnerable.

Introduction

Many parasites depend on a narrow range of host species as their resource. Consequently their distribution is determined by the abundance of the host, the level of fragmentation of the host populations, and the dispersal ability of the parasite in relation to the host individuals. In addition, the success of parasitism differs both spatially and temporally owing to variation in anti-parasite behaviours of the host (e.g. Payne 1997; Foitzik et al. 2001). Many ant species classified as vulnerable by The World Conservation Union (IUCN) are social parasites, which have evolved to exploit colonies of other ant species to raise their offspring. Perhaps the most committed mode of social parasitism is obligatory and permanent inquilinism, where the entire parasite life cycle takes place within the colonies of a single or few host species (Hölldobler and Wilson 1990). Inquiline queens infiltrate host colonies where they coexist with the host queen, and produce their sexual offspring, which is reared by the host workers simultaneously with their own brood (Wilson 1971; Hölldobler and Wilson 1990). Thus, permanent inquilinism is associated with the loss of the worker caste and the parasites completely rely on the host (Wilson 1971; but see Sumner et al. 2003).

Data on the genetically effective population sizes of inquiline ant species are scarce, yet such data are crucial for the assessment of the conservational status in these species. The effective population size is in turn determined by the spatial population structure, dispersal and mating patterns, and genetic drift (e.g. Hartl and Clark 1989). Given that inquiline ant species often occupy isolated populations with a restricted geographical distribution (Hölldobler and Wilson 1990), stochastic population effects are expected to play a crucial role in shaping the genetic structure of such populations. In addition, both inquiline queens and males are typically miniaturised and wings are rudimentary or absent in one or both sexes (Wilson 1971). Hence mating flights and dispersal are often significantly reduced in inquilines compared to most free living ants. Instead mating commonly takes place within the natal host colonies (Le Masne 1956; Passera 1964; Wilson 1971; Bekkevold and Boomsma 2000), which may lead to inbreeding depression and reduced population viability, unless harmful alleles are purged through regular inbreeding (e.g. Charlesworth and Charlesworth 1987; Pamilo and Crozier 1997). However, the reasons for concern regarding the vulnerability of inquiline parasites depend crucially on whether the inquiline populations are as fragmented as assumed. As pointed out by Hölldobler and Wilson (1990), ''the inquilines give the impression, *quite possibly false*, that they have no more than a toehold on their host populations and that they exist close to the edge of extinction''.

The general mechanisms by which inquiline ants successfully invade host colonies or populations are largely unknown, as are the properties of the host that affect the success of parasite queens. Consequently, only a fraction of the host population may be available to the parasites. A coevolutionary arms race between host resistance and parasite specialisation is expected to take place, since the parasite can cause extensive damage to the host, e.g. by strongly inhibiting the production of host sexuals and/or workers (e.g. Wilson and Brown 1956; Passera 1964; Buschinger 1989; Hölldobler and Wilson 1990; Bekkevold and Boomsma 2000; Passera et al. 2001). One host character that may affect host susceptibility is colony kin structure, i.e. the number of reproductive queens in the colonies. This varies within and among species of ants from one to several hundred (Bourke and Franks 1995). The number of queens per colony may affect the chemical cues involved in nest mate recognition, and subsequent changes in cue composition may affect the chances of the parasite being identified by the workers. Defrauding the host's kin recognition system likely involves sophisticated chemical disguises, given that inquilines are generally specialised and restricted to a single host species (Hölldobler and Wilson 1990; Johnson et al. 2005). Indeed, most inquilines are characterised from hosts that have several queens per colony (polygyny), in which intra-specific aggression, and thus colony closure, tends to be less pronounced than in single-queen (monogyne) societies (Maeder et al. in press). Polygyny is also frequently associated with colony reproduction by budding so that colony networks comprising several nests are formed (polydomy) (Hölldobler and Wilson 1990). This may facilitate the spread of the parasite within colony networks, but may prevent spread across network boundaries.

The genus *Plagiolepis* (Formicidae; subfamily Formicinae) includes three species classified as vulnerable, all of which are inquilines characterised from Europe (*P. ampeloni*, *P. grassei*, and *P. regis*; http://redlist.org). *Plagiolepis ampeloni* is known to inhabit only three locations in Slovakia, South Tyrol, and Turkey, after the probable extinction of its type locality in Lower Austria (Faber 1969; Schlick-Steiner et al. 2003; Alfred Buschinger, pers. comm.). However, recent efforts to re-capture the species at South Tyrol failed (Alfred Buschinger, pers. comm.). *Plagiolepis grassei* has been detected during the past three decades only once in 1999 in Cerbère near the border of Spain, in the vicinity of its type habitat in Southern France (Luc Passera, pers. comm.). The present distribution of *P. regis*, originally described in the Soviet Union, is unknown. Other inquiline ants of the genus *Plagiolepis* are not considered vulnerable, but are nevertheless extremely rare.

Our study species, the obligate workerless social parasite ant *Plagiolepis xene* is highly specialised on its host species *P. pygmaea* (Aron et al. 1999; Passera et al. 2001). Like the vulnerable species, *P. xene* is rare and extremely difficult to find (Le Masne 1956; Passera 1964; Aron et al. 1999; Passera et al. 2001). Only approximately one out of a hundred host colonies is parasitized

(Passera, pers. comm.). In addition, the host colonies are located underground in soil of hard clay or rocks which makes extensive screening of populations practically impossible. Males of *P. xene* are wingless and mating takes place within the host nest where queens remain when fertilized (Passera 1964, 1969). Local dispersal of the parasite occurs in conjunction with host nest budding (i.e. the establishment of new neighbouring nests by the queens and workers from the mother colony) with *P. xene* queens following the host workers and queens when these establish a new nest (Passera et al. 2001). Colonization of new host populations may occur only through females. Here we use microsatellite markers to study the population genetics of *P. xene* and combine this information with genetic data on the host *P. pygmaea*. We use this genetic data to determine the breeding and dispersal patterns of the inquiline species and to assess whether colony kin structure of the host species is associated with the presence of the parasite.

Methods

Sampling and genetic analysis

Plagiolepis xene occurs throughout the Mediterranean and Central Europe and uses the subterranean ant *P. pygmaea* as its host. We collected the species in Southern France from three populations 50–100 km apart. First, we sampled altogether 31 host nests from a parasitized population in Bruniquel (Montauban) in March 2002. Samples were taken from all existing host nests from an area of approximately 90 m^2, each sample containing on average 109 host workers. The spatial location of each host nest was recorded. Eight of these nests were parasitized, and on average 20 (2–34) adult (dealate) *P. xene* queens per nest were found; the presence of parasites did not correlate with the host sample size ($r^2 = 0.053$; $P = 0.199$). Second, two parasitized nests were collected from a host population in Teyssonières (Toulouse) in July 1999. This sample included male and female offspring of the parasite, together with adult queens. Third, parasite queens were collected at Renery (near Toulouse), in January 1997. The latter two samples did not include host individuals or precise spatial data of the nests.

Three of the eight parasitized nests in Bruniquel were omitted from the genetic analyses because they were located within 0.5 meters from one of the remaining five colonies and based on the host worker genotypes were likely satellite nests of these (below; Figure 1a). In total, we genotyped 34 parasite queens from five nests: eight queens from the four largest samples, and all (2) queens from one nest. From the two nests of Teyssonières, we genotyped all adult parasite queens (3 and 8), eight female offspring from both nests, and four and five males, respectively. Four parasite queens were genotyped from each of the three parasitized nests of Renery. Finally, to infer the origin of all parasitized host nests, we genotyped eight *P. pygmaea* workers from sixteen unparasitized nests and from all eight parasitized nests of Bruniquel. All individuals were genotyped at 7–8 polymorphic microsatellite loci following Trontti et al. (2003) (P01, P06, P07, P11, P20, P22, P23 and P25 for *P. xene*; same set of loci for *P. pygmaea* except P22, which displays high levels of polymorphism only for the parasite species).

Statistical analyses

We used hierarchical *F*-analysis of variance (Weir and Cockerham 1984) to obtain coefficients of identity by descent at the levels of interest. In our data set, the lowest level comprised individuals

Table 1. Genetic variation in the three study populations. Number of nests and individuals analysed, maximum number of alleles per locus, average number of alleles over loci, and genetic diversity in each population sampled. Data on queens (Q) and female offspring (F) of Teyssonières are given separately

Population	n nests/ind	Allele maximum	Alleles av. (SD)	Gene diversity
Renery	3/12	3	1.8 (0.7)	0.203
Bruniquel	5/34	2	1.3 (0.5)	0.096
Teyssonières Q	2/11	4	2.8 (0.9)	0.400
Teyssonières F	2/16	5	2.9 (1.1)	0.323

within populations, the next level nests within populations, and the third level populations within the entire sample. Thus, the *F*-analyses give the inbreeding coefficient due to non-random mating among individuals within nests and among individuals within populations (f and F), and allele frequency differences between nests and between populations (θ and θ–P). *F*-analyses were performed only for adult (i.e. dealate) queens. In addition, we calculated pairwise θ and exact *P*-values for pairwise genetic differentiation between nests and between populations, to assess whether genetic differentiation observed at a given level of analysis is significant among all pairs of reproductive units. In this analysis all individuals within each population were pooled. Finally, we calculated pairwise θ and exact *P*-values for genetic differentiation between dealate queens and alate female offspring of Teyssonières to study temporal variation in allele frequencies. Analyses were performed with Genetic Data Analysis 1.1 (Lewis and Zaykin 2001; three-level hierarchical analysis) and FSTAT 2.9.3 (Goudet 1995, 2001; two-level hierarchical analyses, pairwise θ, and exact *P*-values not assuming Hardy-Weinberg equilibrium). The 95% confidence intervals for the *F*-analysis were obtained by bootstrapping over loci for 15,000 times, and the level of significance for the exact *P*-values with permutation tests resampling the data up to 1000 times.

We also estimated the regression relatedness (r) among nest-sharing parasite queens with the allele frequencies of the local population as reference (''colony relatedness''; Queller and Goodnight 1989). In addition, to estimate the number of genetically effective reproductive parasite queens for each population (see below), we pooled all queens within each population and then calculated the average relatedness within the three populations (''population relatedness''). To compare the kin structure between the infected and uninfected host nests we estimated the within-colony relatedness also for the host nests of Bruniquel. The relatedness calculations were performed in Relatedness 4.2 (Queller and Goodnight 1989) and confidence intervals were obtained by jack-knifing over loci.

The effective number of reproductive parasite queens (N_E) present in the three populations was calculated following Pamilo (1993) and Seppä (1994) from the regression relatedness of female offspring (i.e. in our case the relatedness among resident queens):

$$N_E = [4(0.25 + (0.5/(M_E)) - r]/3r \qquad (1)$$

where M_E is the effective mating frequency of the parasite queen and r is the relatedness among queens. Here we calculated N_E assuming that $M_E = 1$. This assumption yields the maximum estimated female founder population, as any value of M_E greater than one would result in a decrease in N_E. Furthermore, if queens mate with several related males, the estimate of M_E will approach one as the relatedness among her male mates increases. We constructed the 95% confidence intervals for the estimate of founders (N_E) by recalculating N_E with both the upper and the lower 95% confidence limit of the relatedness estimates entered in Equation 1.

To test whether parasites are distributed non-randomly within the host population in Bruniquel, we first inferred host colony genealogy and then tested whether parasites were aggregated according to host genealogy. Because the host species can reproduce by budding, neighbouring nests sharing a recent common origin are genetically more similar to each other than to the rest of the population (Trontti et al. 2005). We applied two methods to infer and visualize the budding history of the host population. First, we calculated Nei's pair-wise genetic distances (Nei 1988) between the host nests, and constructed an unrooted UPGMA dendogram from the data by using the program Genetic Data Analysis 1.1 (Lewis and Zaykin 2001). Second, we used Bayesian inference for detecting genetic structuring within the host population. We used the program Bayesian Analysis of Population Structure (BAPS) ver. 3.0, with default settings (Corander et al. 2003, 2004). The distribution of parasites was then matched against the kin structure of the host nest network, to assess whether parasites occur across all host colony aggregations or are confined to specific host colony lines. Finally, we used logistic regression to test whether parasitized nests were aggregated within the host by comparing the minimum distances of each parasitized and unparasitized host nest to the closest parasitized host nest. Hence, if the parasite is aggregated within the host population, we expect the parasitized nests to have a generally shorter distance to another parasitized nest than to unparasitized ones. All eight observed parasitized nests were included in this analysis.

Results

The three parasite populations in Bruniquel, Teyssonières and Renery were highly differentiated with an overall θ–P of 0.63 (95%, CI = 0.46–0.74), and pairwise θ ranging between 0.44 and 0.78 ($P < 0.001$ for all pairwise comparisons). On average, 3.5 alleles (range 2–6) per locus were found in the three populations, but the degree of polymorphism varied greatly (Table 1). In Bruniquel, six out of eight analysed microsatellite loci were completely fixed for one allele and the remaining two polymorphic loci had only two alleles each. The lack of genetic variability indicates that this population was either initiated by single or very few immigrant queens or has lost variation due to extensive drift. In contrast, both nests in Teyssonières were genetically polymorphic, none of the loci was fixed, and up to five alleles were detected in male and female offspring. In Renery, the level of polymorphism was intermediate between the other two. The estimated number of foundress queens indicated that individual populations may have been initiated by only one or few individuals (Table 2).

Allele frequencies in the adult queens and the offspring queens did not differ statistically in the two nests of Teyssonières ($\theta = 0.022$ and 0.068; $P = 0.258$ and 0.292, respectively), which indicates that the same matrilines persist across years. Thus, we combined the data on old and young queens for the hierarchical F-analysis. Overall, all three parasite populations were at most very weakly structured. In Renery and Teyssonières, the nests were statistically differentiated, but only 5% of the total genetic variation was attributable to differentiation between nests (Table 2). Furthermore, none of the pairwise estimates of differentiation between nests differed significantly from zero in any of the populations (Teyssonières $\theta = 0.13$, $P = 0.14$; Renery $\theta = 0.005$–0.10, $P = 0.25$–0.59; Bruniquel $\theta = -0.082$–0.047, $P = 0.26$–1). These results indicate that individuals are able to disperse within populations and so homogenize allele frequencies between nests. Consequently, the colony relatedness among nestmate individuals was indistinguishable from zero in all locations (Table 2). Because the populations were not significantly substructured, we considered it justified to pool all individuals in a given population for the analysis of inbreeding. We detected no inbreeding among individuals within populations ($f = -0.021$; 95% CI = -0.096–0.046), indicating panmixia within populations. However, mating was nonrandom among individuals within the total set of populations ($F = 0.623$; 95% CI = 0.457–0.741), which is consistent with the observed strong population differentiation.

Analysis of the host population structure in conjunction with the parasite in Bruniquel reveals that parasitized nests of *P. pygmaea* were clustered within the population. The average distance from a parasitized host nest to the nearest other parasitized nest was much shorter than the distance of any unparasitized nest to the nearest parasitized nest (0.50 m, SD = 0.31; 4.84 m, SD = 3.90 m, respectively; logistic regression $b = -0.009$; df = 32; $P = 0.029$). Comparing the distribution of the parasites against the host population structure revealed that the parasite was only found in eight nests of one genetically homogeneous cluster of eleven host nests (Figure 1). Genetic differentiation within this cluster ($\theta = 0.026$; SE 0.039) was much lower than the average differences among nests across the host population ($\theta = 0.350$; SE = 0.035). Thus, these eleven nests probably share a common origin by colony budding. This cluster was also supported by the Bayesian inference of population substructuring, where the optimal partition of the population contained 11

Table 2. Nest differentiation within the populations (θ), colony and population level relatedness estimates (r), and the estimated number of founding females. Teyssonières queens and offspring are analysed together

Population	θ 95% CI	Colony r 95 % CI	Population r 95 % CI	Founders 95 % CI
Renery	0.05	0.08	0.76	1.0
	0.05–0.07	0.08–0.09	0.51–1.00	0.7–1.7
Bruniquel	−0.01	−0.05	0.92	0.8
	−0.04–0.00	−0.06 – −0.04	0.68–1.16	0.6–0.9
Teyssonières	0.05	0.10	0.36	2.5
	0.01–0.08	0.03–0.15	0.14–0.58	1.4–7.1

groups each comprising a single nest, one group comprising two nests, and one group comprising eleven nests (n = 24; marginal log-likelihood of optimal partition = −3234). Hence, the cluster infected by the social parasite encompassed many more nests than any other cluster in the population (Figure 1). Given that we sampled all nests within the area, this indicates that the parasitized host nests have gone through more frequent budding events than the rest of the population. Finally, the average relatedness was similar in the parasitized ($r = 0.504$; SD = 0.139; n = 8) and the unparasitized host nests ($r = 0.561$; SD = 0.156; n = 16), when the total population was used as the reference population. Likewise, the nestwise gene diversities (Nei 1988) averaged over loci, which may be

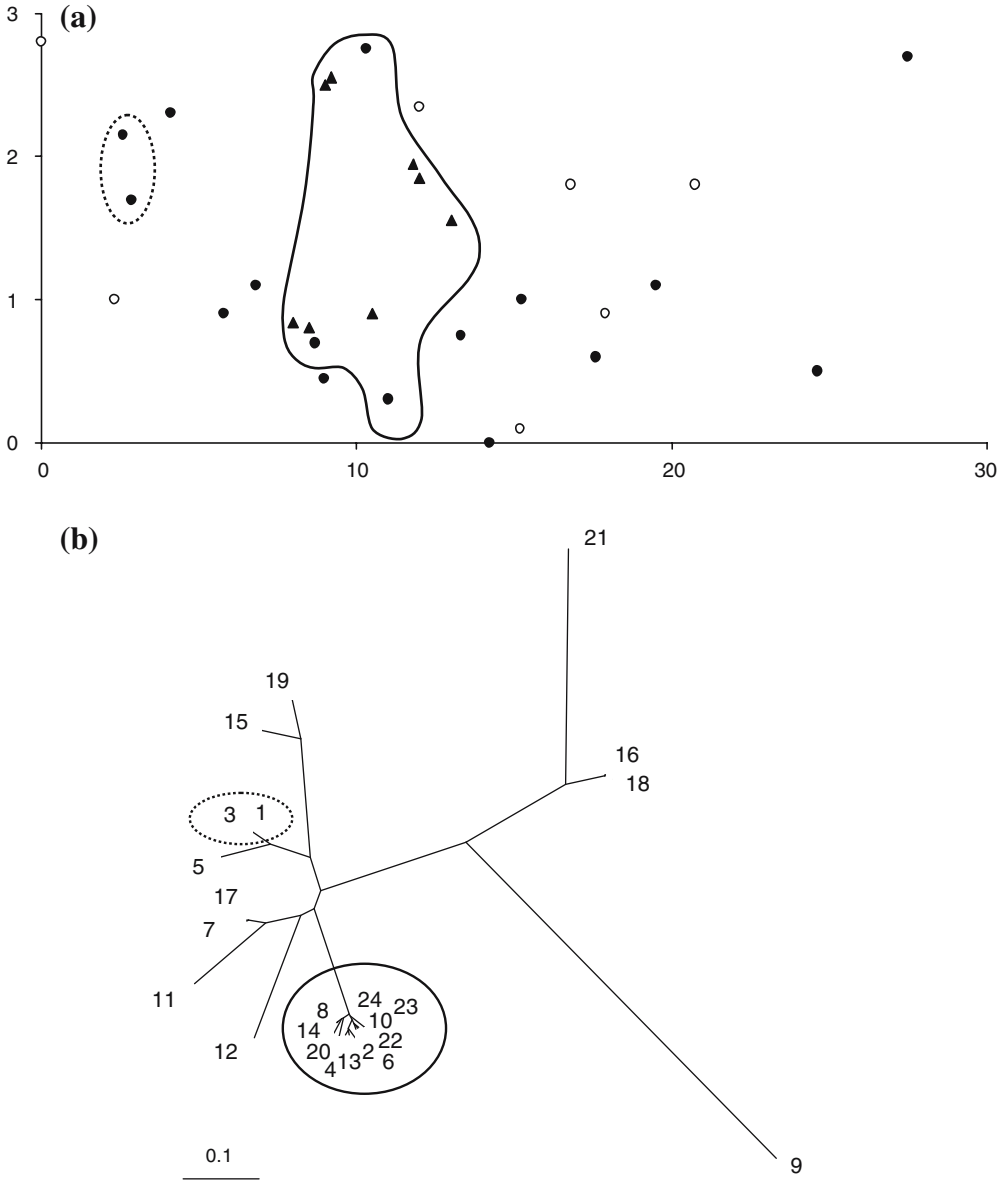

Figure 1. Map (a, scale in meters) and unrooted distance dendogram (b) of host colonies of Bruniquel. The two significant clusters of related colonies consisting of two (dash line) and 11 (solid line) nests are outlined, the latter included all the parasitized nests. (a) Triangles mark parasitized and circles unparasitized nests, of which the solid ones were genotyped. Note the different scales of x and y-axes. (b) Bar corresponds to genetic distance of D = 0.1 (Nei 1988).

affected by the effective number of reproductives, were also similar for the two groups (parasitized $H = 0.377$; SD = 0.065; n = 8, unparasitized $H = 0.445$; SD = 0.091; n = 16).

Discussion

Our results show that populations of the inquiline parasite *Plagiolepis xene* are isolated within the study range. All populations are characterized by distinct alleles as only a minority of the alleles are shared by all populations and many loci are locally fixed. Subsequently the genetic similarity among parasite populations was low compared to the host species at the same geographical scale (θ–$P = 0.042$; Trontti et al. 2005), which is most likely due to a lower number of foundresses and/or a higher rate of genetic drift. In contrast, weak within-population substructuring and regression relatedness, and low inbreeding coefficients indicated almost complete panmixia within populations. A weak but significant genetic differentiation between nests was observed in Renery and Teyssonières. However, our data indicate that only 5% of the total variation was attributable to differentiation between nests. This suggests that the parasites actively shuffle between invaded host nests or, if the parasitized host nests are functionally connected (i.e. are part of the same colony network), the parasite offspring may be transported from nest to another by the host workers. Altogether the structuring of the parasite populations differs significantly from the host *P. pygmaea*, the populations of which are strongly subdivided but show very little differentiation (Trontti et al. 2005).

Our results also reveal a strong founder effect in all parasite populations, as the effective number of parasite queens was low. These estimates are consistent with the observed level of genetic variation. The maxima of three and two alleles respectively in Renery and Bruniquel can be explained by population establishment by one singly mated parasite queen, where two alleles derive from the queen and one from the haploid male. In Teyssonières the observed maximum of five alleles suggests that the population may have been initiated by more than one queen. The alternative possibility, that this population was originally initiated by one, multiple-mated queen, seems unlikely. It would assume that the founding queen had mated with at least three males all of which were carrying a different allele. Considering that intranidal mating and therefore mating between relatives is prevalent in inquilines, all male mates of individual queens are unlikely to carry different alleles. Altogether it appears that each parasite population commonly descends from one or a few founding queens and that gene flow seldom takes place between populations. Therefore, it may be biologically more meaningful to measure relatedness at the level of metapopulations, i.e. as the genetic similarity of individuals within populations compared to the total set of populations. Analyzed this way, the relatedness reaches $r = 0.77$ (95% CI = 0.64–0.85), which equals the expectation of hymenopteran full sisters ($r = 0.75$).

The analysis of the underlying host colony network in Bruniquel revealed that the parasite was found only from a cluster of related host colonies that shared a common origin through budding, although host nests of a different origin were present in the vicinity. It is not clear whether some of these host nests are functionally independent and only share a common origin, or if they are interconnected parts of the same colony. Whichever the case, the parasite's dispersal within the host population seems to be restricted by the host colony kin borders. This suggests that parasites are transmitted vertically in the population, i.e. within the colony network to new host colony buds (Passera et al. 2001). In addition, host nest budding appears to be more frequent among infected colonies than among the uninfected ones in this population, as only one other significant clustering of only two nests could be inferred from the analysed nests. Interestingly, this pattern coincides with the results and prediction by Passera et al. (2001), that the host queens of *P. pygmaea* accumulate into those parts of the colony where parasite is absent to avoid competition with parasites (''queen escape''). The increased budding may thus represent a host response to parasitism, although budding in this cluster may also have occurred before parasite invasion. Similarly, increased parasite pressure by the ant *Protomognathus americanus* has been proposed to alter the population structure of its host *Leptothorax longispinosus*, by promoting budding and thus reducing the average size and age of host nests (Foitzik and Herbers 2001).

The greatest challenge for social parasites is to overcome the nestmate discrimination code of their hosts, allowing them to successfully achieve social integration in a host colony. Cuticular hydrocarbons are considered to be the main chemical cues involved in nestmate recognition in social Hymenoptera (Vander Meer and Morel 1998; Lenoir et al. 1999; Lenoir et al. 2001; Johnson et al. 2005). Indeed, in the slave-making ant *Polyergus rufescens*, the parasite rapidly acquires the host hydrocarbon profile after invasion (D'Ettorre et al. 2002; Johnson et al., 2002; 2005), suggesting that correct chemical signalling is crucial for social parasites. Consequently parasite transmission may be more pronounced in genetically uniform host populations where, given that the recognition cues are heritable, the chemical profiles of colonies overlap. However, authenticating this hypothesis requires data from several host and parasite populations.

The observations that *P. xene* had not dispersed into unrelated nests outside the parasitized colony cluster of *P. pygmaea* is consistent with the role of the chemical recognition mechanisms as a local dispersal barrier for the inquilines. Evidence for the importance of the source host colony in the interaction between *P. pygmaea* and*P. xene* has been previously provided by Passera (1964). Attempts to introduce *P. xene* queens into alien host colonies always resulted in the parasite being killed, even if the host colony was already parasitized. However, the parasites in this experiment may have originated from a different population, so the outcome may be a population effect, rather than a colony effect (cf. Foitzik and Herbers 2001). Altogether the mechanism by which *P. xene* infests new populations remains unsolved. Host workers may fail to recognize an invading parasite simply because newly emerged parasite queens lack odor cues, and acquire their chemical signature from the host only after invasion (Lenoir et al. 1999; Lenoir et al. 2001; Johnson et al. 2005).

Finally, we found no association between parasite infection and the number of queens in the host colony, as expressed by intracolony relatedness. A similar result has been reported for the inquiline parasite ant *Acromyrmex insinuator* and its host *A. echinatior*, where the parasitized host colonies do not have more queens (Bekkevold and Boomsma 2000). Variation in host susceptibility may thus result from other factors than kin structure. Indeed, a few key behaviours may significantly affect the success of parasites. Guarding behaviour and aggression levels seem to determine the interactions between the host *Leptothorax longispinosus* and its obligate slave maker *Protomognathus americanus*, such that host colonies from a population with high parasite pressure more effectively resist parasitism than conspecifics that seldom encounter the parasite (Foitzik et al. 2001). Furthermore, the parasites in this location were more effective in parasitizing, which suggests host-parasite co-evolution (Foitzik et al. 2001).

In conclusion, our main results on *P. xene* support the assumption that populations of inquiline parasites are genetically isolated. In addition, parasite dispersal also within populations appears to be limited to host kin groups within the same colony network. This suggests that only a fraction of the host colonies may be available to the parasite. Finally, although up to one hundred or more individuals can be found in a single parasitized host colony (Passera 1964; Passera et al. 2001), new populations of *P. xene* apparently face a severe genetic bottleneck as they all seem to descend from only one or a few foundresses. Consequently the ratio of effective versus census population size of *P. xene* is only 0.01. The reliability of our conclusions concerning within-population structure and the parasite-host interactions may suffer from the small number of samples available. However, as inquiline species are very rare, more comprehensive data sets are difficult to obtain.

Our results highlight the potential genetic vulnerability of the still fairly common species *P. xene*. The pattern observed for *P. xene* is likely to hold also for the other social parasites, especially the closely related, and much rarer, *P. grassei*, which shares its host with *P. xene*. The effective population sizes of this and the other inquiline *Plagiolepis* are probably even smaller than recognized today. Unique genetic variation is prone to be lost owing to high population differentiation in any one of these populations. Thus our study highlights the need for conservation measures of social parasites, as well as parasitic species in general. Data on the population genetic structuring of other inquilines, and in particular data on the factors involved in parasite population establishment would be beneficial for conservation planning of these species.

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