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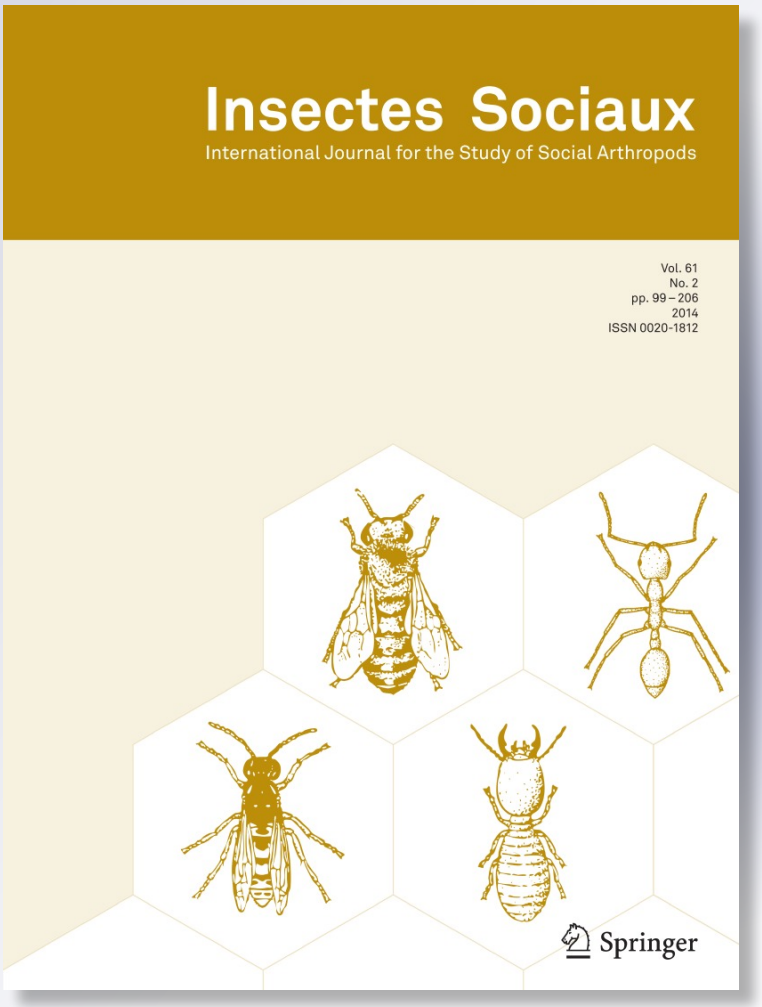

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RESEARCH ARTICLE

Dispersal in the inquiline social parasite ant *Plagiolepis xene*

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Abstract Inquiline ants are highly specialised social parasites. They usually do not produce their own worker caste but instead use the worker force of the host ant colony to ensure the rearing of their sexual progeny. Several barriers are expected to severely limit their migration, and the mechanism allowing them to disperse remains largely enigmatic. Here, we tested two hypotheses to account for the low level of infestation of inquiline parasites, in populations of the parasite ant *Plagiolepis xene* and its host *P. pygmaea*: (1) the establishment of a new *P. xene* colony is such a rare event that a single colonisation should be expected per population, and (2) once a *P. xene* colony is established in one location, it has very little chance to succeed in infecting a neighbour genetically unrelated colony. We sampled nests from both species along four separate transects, and genotyped host and parasite individuals at eight polymorphic microsatellite loci. Our genetic data contradict both hypotheses: multiple colonisation events were recorded in all four transects sampled and, in at least one case, *P. xene* has successfully migrated from one host colony of *P. pygmaea* to a spatially close unrelated nest. This shows that the dispersion capacity of the social parasite is sufficiently effective to ensure its long-term survival.

Keywords Social parasitism · Inquiline · Dispersal · Ants · Microsatellites

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Introduction

Dispersal is essential for the survival of all species (Comins et al., 1980), and the manner in which organisms disperse strongly influences the geographic distribution of their genetic variation (e.g., Clobert et al., 2012). The development of efficient dispersion strategies appears particularly challenging for species that specialise on specific resources, especially if that resource is scarce and/or if it has a highly fragmented geographic distribution. Species having developed a parasitic lifestyle often represent cases of strong resource specialisation, and are usually adapted to an extremely narrow range of hosts. Not only does the parasite needs to reach its host, possibly having to cross large distances in the process, but it also needs to identify and locate it, and to circumvent defensive mechanisms developed by the host in response to the selection pressure exerted by the parasite (Lively, 1999; Thomas, 2000; Thompson, 2005).

Intriguing cases of parasitism are observed in social Hymenoptera, with several species of ants, bees and wasps having independently developed a strategy of using the worker force from colonies of other species to rear their own reproductives (Wilson, 1971; Pedersen, 1996; Cervo and Dani, 1996; Buschinger, 2009). This so-called social parasitism is most spectacularly developed in inquiline ants. By contrast with other social insects, which are characterised by a reproductive division of labour between queens and workers, inquiline social parasites usually do not produce a worker caste; instead, queens invest most of their efforts and resources in producing new male and female sexuals that are reared by the host-colony worker-force (Bourke and Franks, 1995). Inquilinism has been documented in ca. 80 out of ca. 12,500 ant species described to date (Hölldobler and Wilson, 1990; Huang and Dornhaus, 2008). Most have a single host species and live permanently in their host colony. Most

inquiline social parasites are rare and hard to find under natural conditions, making them difficult organisms to study, and very little is known about their dispersal strategies [but see Bekkevold and Boomsma (2000) for a detailed study on dispersion strategy in the inquiline *Acromyrmex insinuator*]. Dispersion in parasitic ants is further complicated by the difficulties associated with invading a new host colony. In ants, recognition of nestmates from aliens relies on an efficient system of chemical cues. Workers defend their nest against intruders, which allows maintaining the genetic integrity of the colony and safeguarding of resources from competitors, robbers or parasites (e.g., Lenoir et al., 2001). As a consequence, the mechanisms allowing these highly specialised parasites to maintain their presence within their geographic range, whether more or less successful, remain largely enigmatic.

The typical inquiline ant *Plagiolepis xene* is a rare, obligate social parasite of its sister species *P. pygmaea* (Passera, 1964). In both the host and the parasite, colonies contain multiple queens (polygyny), mating takes place inside the nest among related individuals and populations are highly inbred (Passera, 1969; Trontti et al., 2005, 2006, 2007; Thurin and Aron, 2009). Dispersal in *P. xene* is likely to be restricted by the highly fragmented geographic distribution of its host (Passera, 1969), the small size of its female sexuals (Aron et al., 1999) while the males are wingless, and the absence of a mating flight. In laboratory experiments, all attempts to artificially introduce mated queens of *P. xene* in unparasitised colonies of *P. pygmaea* have failed (Passera, 1964) showing that chemical mimicry or invisibility is not easily achieved by the parasite. Altogether, these strong obstacles could result in a substantially reduced ability to disperse for this species, and may account for its apparent rarity, as it is extremely difficult to find in the wild: <1 % of host colonies sampled over more than 30 years were found parasitised, with 30–50 % of host colonies being parasitised in infected populations (Passera et al., 2001). In fact, a strongly reduced dispersal could even jeopardise its long-term survival (Trontti et al., 2006).

A first attempt to explore dispersal in *P. xene* confirmed the assumed difficulties for this species to disperse (Trontti et al., 2006): (1) all *P. xene* individuals sampled from a single location appeared to have a common origin, pointing towards a single colonisation event in the studied population, and (2) no nest of *P. pygmaea* that was spatially close to the parasitised nests, but genetically distant from them, was parasitised by *P. xene*, suggesting that it is difficult for *P. xene* to enter a new unrelated colony. However, this study was based on samples from a single location, and before general conclusions can be drawn on the dispersal of this typical social parasite, data from additional locations are needed.

Here, we explore further the dispersion strategy in the highly specialised inquiline parasite ant *P. xene*. We extend the available data by sampling parasitised and non-parasitised nests from the host, collected along transects from 4 separate locations, and by genotyping sampled individuals of both the parasite and its host at several microsatellite loci. More specifically, we ask the following questions: (1) is the establishment of a new *P. xene* colony such a rare event that a single colonisation should be expected per population (i.e., are all *P. xene* individuals found in one location always genetically closely related because they share a common recent origin)?, (2) Once a *P. xene* colony is established in one location, can it only expand in the population by following the expansion of its host through budding (i.e., the establishment of a new nest a small distance away from the original nest, by a few newly mated females and workers), or are *P. xene* individuals sometimes capable of infecting a neighbour genetically unrelated colony of its host *P. pygmaea*?

Methods

Sampling

An extensive search for *Plagiolepis pygmaea* nests was conducted during the summers 2007 and 2008 along a transect of ca. 100 m in three sites (separated by 20–40 km from each other) in which the parasite *P. xene* had been found, in southern France: Bordeneuve (geographical coordinates: 43°28′17.75″N, 1°28′10.26″E), Tarabel (43°30′49.22″N, 1°39′26.33″E), and Lavalette (43°38′22.03″N, 1°34′57.31″E). The position and presence/absence of the parasite were carefully recorded for each nest. An additional site, more than 200 km to the southeast of the three others, was searched in April 2012: Valmy (42°31′43.38″N, 3°01′47.06″E). In this case, only the parasitised nests were recorded. For all 4 sites, 5–16 workers of *P. pygmaea* and 2–21 queens and/or males of *P. xene* were sampled for each parasitised nests, and 5–16 workers of *P. pygmaea* were sampled in 19 non-parasitised nests, for genotyping purposes. In total, 43 nests were sampled for the genetic analyses.

Molecular data

Genomic DNA was extracted from sampled individuals using a standard Chelex-extraction protocol. Individual ants were ground in 40 µl of a 5 % Chelex solution, then incubated at 85 °C for 90 min. For each extracted individual, eight microsatellite loci from Trontti et al. (2003; P01, P06, P07, P11, P20, P22, P23, P25) were amplified by multiplex PCR with the Multiplex PCR Kit (QIAGEN), following the protocol described in the manufacturer's manual and annealing temperatures of 55, 57 or 62 °C, depending on the

primer pair used. Amplified products were then separated by electrophoresis on an Applied Biosystems 3730 automated sequencer. Allele call was conducted with the software Peak Scanner version 1.0 (Applied Biosystems) and genotypes were manually entered in an Excel sheet as input to the program microsatellite analyser (MSA; Dieringer and Schlötterer, 2003).

Data analyses

For each transect, Nei's chord genetic distance (Nei et al., 1983) was computed between each pair of nests, both for the *P. pygmaea* and the *P. xene* samples, with MSA. A neighbour-joining tree was then generated with PAUP* (Swofford, 2003) for each transect and each species. This tree was used to visualise the genetic distances among all nests of one species at a site. It was therefore not taken as giving information on evolutionary relationships among nests, but rather as a dendrogram showing how similar nests are among each other. This genetic similarity was used as a proxy to identify nests that are likely to be related to each other, i.e., those that have a recent common origin because they are connected through recent budding events. This first assessment based on a visual inspection of the genetic dendrogram was further evaluated by determining whether genotypes from two different nests in a transect are more similar than expected by chance, given the genetic diversity within that transect. This was accomplished (1) by computing a weighted F_{ST} statistic over all loci for each pair of nests within a transect (based on the number of different alleles between haplotypes, Michalakis and Excoffier, 1996), using the program Arlequin 3.5.2.1 (Excoffier and Lischer, 2010), and (2) by generating 1,000 random permutations of the original data sets (permuting haplotypes between nests) and re-computing the same parameter on each of those, to assess the statistical significance of nests' differentiation (p value determined as the proportion of permuted data sets for which the re-computed pairwise F_{ST} was larger than or equal to the original value; statistical significance level set to 0.05). Nests that were not identified as statistically different were therefore considered related, possibly through recent budding events. Finally, to test whether all parasitised nests in close proximity are related to each other, for both species, we have also adopted a second approach based on a close examination of the genotype frequency data: two nests which did not share any allele at one or more loci, or that shared <10 % of their alleles with any other analysed nest for at least two loci, were considered unrelated (i.e., not sharing a recent common origin). The rationale behind this criterion is that two nests connected through recent budding events are expected to share at least a fraction of their alleles at each locus. In this second more conservative approach, only cases of strong genetic differences among nests were taken as evidence against this hypothesis of relatedness, providing a higher level of confidence for its potential rejection.

Results and discussion

Table 1 gives basic information on microsatellite data. Figure 1 shows for each transect (1) the spatial distribution of the *P. pygmaea* hosts' nests along the transect (with the exception of transect 4, for which the location of only the parasitised nests was recorded), (2) those nests that are parasitised by *P. xene*, (3) those nests for which we have genetic data, and (4) two dendrograms summarising the genetic similarity among nests, one for the host *P. pygmaea* and one for the inquiline social parasite *P. xene*. The molecular data sets, one for each transect and each species, are available as Supplementary Information S1, and pairwise F_{ST} values, calculated for each pair of nests within a transect, are shown as Supplementary Information S2.

The pattern observed in transect 1 is reminiscent of the one described in Trontti et al. (2006): Eight out of the nine parasitised nests (nests 5, 7–13) of *P. pygmaea* are genetically closely related, so much so that they are probably connected by recent founding events (with the possible exception of nest no. 10), and are infected by *P. xene* sexuals that are also genetically very similar. This suggests that this group of *P. xene* individuals have a recent common origin, and have expanded by following the establishment of new nests of the host by budding. That such a dispersal strategy accounts for a large portion of the parasitised nests makes sense: by following sexual and worker *P. pygmaea* individuals that leave the mother colony by walking to found a new one, the parasite avoids the difficult task of overcoming the chemical recognition mechanism of the host that is

Table 1 Average sample size (i.e., number of gene copies) per nest (n); mean number of alleles (A_e) and mean expected heterozygosity (H_e), calculated per nest and per locus

	n	A_e	H_e
P. xene			
Transect 1	14.0 ± 4.1	2.0 ± 0.8	0.237 ± 0.204
Transect 2	11.0 ± 5.8	1.8 ± 0.7	0.202 ± 0.221
Transect 3	22.4 ± 18.0	3.8 ± 1.8	0.515 ± 0.230
Transect 4	15.2 ± 1.1	4.3 ± 1.5	0.590 ± 0.254
P. pygmaea			
Transect 1	14.4 ± 5.2	4.8 ± 1.6	0.564 ± 0.154
Transect 2	25.2 ± 8.0	7.4 ± 3.0	0.685 ± 0.117
Transect 3	12.9 ± 1.9	5.5 ± 1.7	0.674 ± 0.110
Transect 4	15.2 ± 1.1	6.3 ± 2.2	0.670 ± 0.126

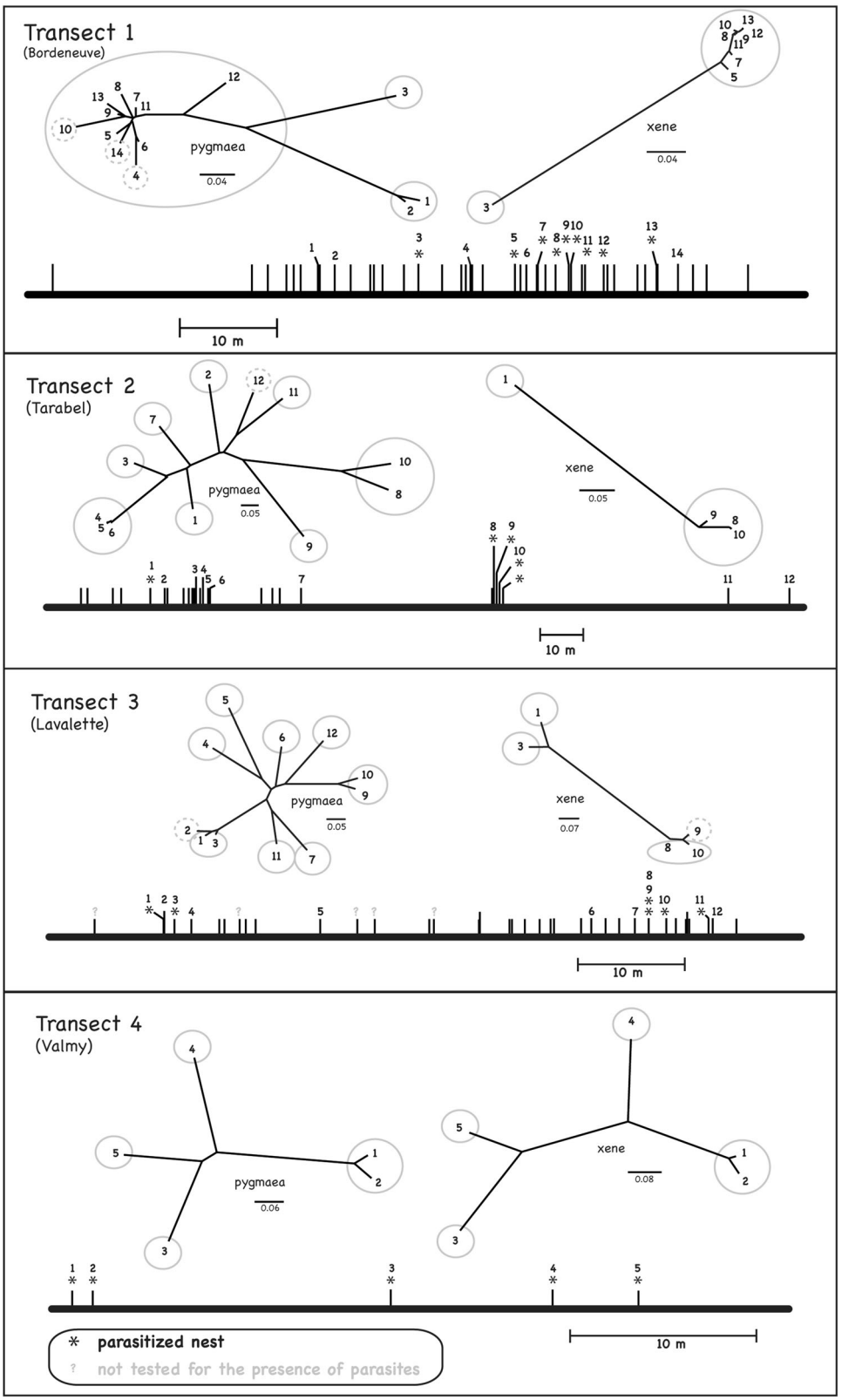

Fig. 1 Spatial distribution of the infected and non-infected nests of *P. pygmaea* recorded for this study along a transect conducted in each of four different sites. Each *vertical bar* indicates the location of a nest. Only the parasitised nests were recorded for transect 4. An *asterisk* indicates a parasitised nest, while a *question mark* specifies that we did not check for the presence of the parasite *P. xene* (only 5 nests in transect 3). A *number* identifies each nest sampled for the genetic analyses. For each transect, two *dendrograms* are shown, that summarise the genetic distances among pairs of nests, both for *P. pygmaea* and for *P. xene*. *Plain grey circles* on the *dendrograms* gather nests that are not statistically different from each other (see "Methods"), so that we may assume they share a recent common origin. *Dashed-line grey circles* identify nests for which relationships are ambiguous (e.g., the pygmaea nest 14 in transect 1 is significantly different from nests 11 and 13, but cannot be dissociated from all other non-ambiguous nests within the large circle depicted on the *dendrogram*; or the *pygmaea* nest 12 in transect 2 is statistically different from all other nests, except from nests 8 and 10)

associated with entering a new colony (Passera et al., 2001; Trontti et al., 2006). However, contrary to what was observed in the study of Trontti et al. (2006), another parasitised nest (nest number 3) genetically radically different from the eight others, both for the parasite and its host, was found in the same site, only metres away (<10 m)

from the others. Our analyses show that both *P. pygmaea* and *P. xene* individuals from nest 3 are statistically different from those of the other parasitised nests, suggesting that this nest is not related to the other parasitised nests through recent budding events. Moreover, all the *P. xene* alleles found in nest 3 for locus 6 are absent in the other nests, which reinforces that conclusion with the meeting of our second stricter criterion (see "Methods"). Similar patterns suggesting the presence of more than one genetic unit of *P. xene* (each identified by a grey circle in Fig. 1, and hereafter referred to as a "genetic lineage") are also observed in the three other transects: two lineages in transect 2, three in transect 3, and four in transect 4. If we use the stricter definition of a genetic lineage identified within a transect, based on the sharing of alleles among nests (see "Methods"), the number of *P. xene* genetic lineages in transect 3 is reduced to 2, as we cannot anymore exclude the possibility that nests 1 and 3 are in fact related. On the other hand, all other genetic lineages of *P. xene* identified in Fig. 1 are confirmed by our stricter criterion. Indeed, in transects 2 and 3, the two genetic lineages of *P. xene* are clearly differentiated because they share no allele at three and four loci, respectively. In transect 4, four genetic lineages can be identified, either because they share no allele for at least one locus, or because they share <10 % of their alleles at two or more loci. Consequently, we conclude that all *P. xene* individuals found in one location do not share a recent common origin.

Two alternative hypotheses can explain this pattern: (1) though reduced, the dispersion capabilities of *P. xene* are sufficient to generate at least two colonisation events per site (and up to 4 such colonisations in Valmy), or (2) the groups of *P. xene* that appear genetically unrelated do have an ancient common origin (i.e., they are all connected to a single ancestral colonisation of this site) but have diverged through mutation and/or genetic drift for a sufficient amount of time that they are today no longer genetically similar. To differentiate these two hypotheses, we compared the genetic variation across transects to that found within each transect. According to hypothesis 2, we expect the genetic lineages (grey circles) to be genetically less distant within transects than between transects, because the divergence time separating the lineages within a transect should be smaller than that among transects. As shown in Fig. 2, which is a dendrogram constructed just like those in Fig. 1 but including the *P. xene* nests of all four transects, two genetic lineages from the same transect are, on average, not less distant than a pair of genetic lineages coming from different transects. In fact, no significant difference was found between the mean genetic distance (Nei's Chord distance) calculated between pairs of genetic lineages within transects (0.558 ± 0.176) and the mean genetic distance calculated among transects (0.540 ± 0.129) (t test, $p = 0.72$). These observations give

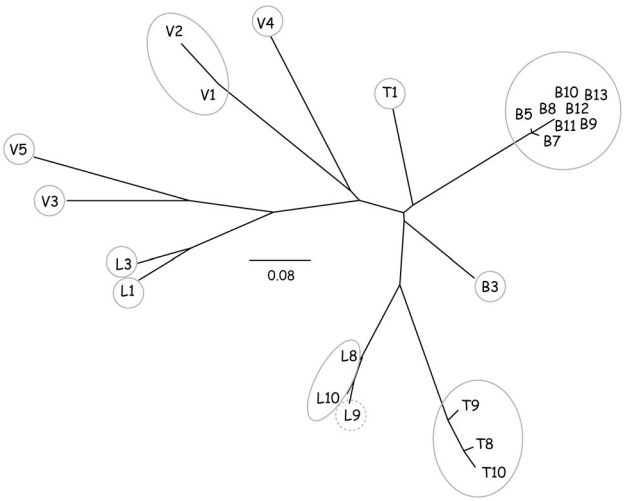

Fig. 2 *Dendrogram* summarising the genetic distances separating the *P. xene* individuals from pairs of nests sampled across all four transects. *Grey circles* are transferred from Fig. 1. Each nest is identified by a *letter* that refers to the site it was sampled from (*B* Bordeneuve, *T* Tarabel, *L* Lavalette, *V* Valmy) and by a *number* corresponding to those shown in Fig. 1

strong support to the hypothesis that the different genetic lineages found at one location (i.e., along a transect of ca. 100 m) stem from two or more separate colonisation events for *P. xene*. Furthermore, the same pattern is found in all four sampled transects, suggesting that it is widespread across the species range.

Our data also indicate that *P. xene* individuals are capable of switching to an unrelated neighbour nest: as shown in Fig. 1, in transect 2, all *P. xene* found in nests 8, 9 and 10 are clearly genetically similar, yet the *P. pygmaea* individuals from nest 9 are very distant from those of nests 8 and 10, so much so that they share no allele at three different loci (P06, P23, and P25). *P. pygmaea* workers from nest 9 are genetically too distant from those of nests 8 and 10 for sharing a recent common origin. Yet, nest 9 is only 55 and 75 cm away from the two others. This spatial proximity, combined with the genetic similarity of the *P. xene* individuals from nests 8, 9 and 10, suggests that *P. xene* individuals from one colony have successfully infected a new unrelated neighbour colony of *P. pygmaea*.

Overall, these results offer at least two important insights into the dispersal strategy of the inquiline social parasite *P. xene*. They first confirm the results of Trontti et al. (2006), showing that the easiest way for the parasite to spread in one location is to follow the colony budding of its host (evidence mostly from transect 1). Passera et al. (2001) field observations and later Trontti et al.'s (2006) data even suggested that the presence of the parasite prompted an increase in nest budding by the host, because only infected colonies were spatially widespread in the studied area. Because we only genotyped a small portion of the non-infected nests, our data

do not test this hypothesis further. Although it is not known whether the infected nests sharing a recent common origin (e.g., nests 5, 7–9, 11–13, in transect 1) are each functionally independent, or if they are still interconnected in a single colony, the spread of the parasite within the boundaries of this genetic entity appears clearly much easier than between unrelated nests. This is presumably a consequence of an effective chemical recognition mechanism within the host colony that prevents foreign individuals to enter.

Remarkably, however, in contrast to the results of Trontti et al. (2006), our data also show that *P. xene* is sometimes able to circumvent the barriers to infecting a new unrelated colony. First, they offer strong evidence that *P. xene* is capable to migrate from one host colony of *P. pygmaea* to a spatially close unrelated nest, and succeed to infect it. Second, in all four transects, each surveying nests over a distance of <100 m, multiple colonisation events (2 in transects 1–3, 4 in transect 4) have been identified. While the detailed mechanism by which the species is capable of entering a new unrelated nest/colony still needs to be investigated, the dispersion capacity of the parasite appears sufficiently effective to maintain its presence within its geographic range in the future, but also to maintain a sufficient level of intra-population genetic variability to ensure its long-term success.

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References

Aron S., Passera L. and Keller L. 1999. Evolution of social parasitism in ants: size of sexuals, sex ratio and mechanisms of caste determination. *Proc. R. Soc. Lond. B* **266**: 173–177

Bekkevold D. and Boomsma J.J. 2000. Evolutionary transition to a semelparous life history in the socially parasitic ant *Acromyrmex insinuator*. *J. Evol. Biol.* **13**: 615–623

Bourke A.F.G. and Franks N.R.F. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey

Buschinger A. 2009. Social parasitism among ants: a review (Hymenoptera: Formicidae). *Myrmecol. News* **12**: 219–235

Cervo R. and Dani F.R. 1996. Social parasitism and its evolution in *Polistes*. In: *Natural History and Evolution of Paper-Wasps* (Turillazzi S. and West-Eberhard M.J., Eds). Oxford University Press, Oxford, UK, pp 98–112

Clobert J., Baguette M., Benton T.G. and Bullock J.M. 2012. *Dispersal Ecology and Evolution*. Oxford University Press, Oxford, UK

Comins H.N., Hamilton W.D. and May R.M. 1980. Evolutionary stable dispersal strategies. *J. Theor. Biol*. **82**: 205–230

Dieringer D. and Schlötterer C. 2003. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes* **3**: 167–169

Excoffier L. and Lischer H.E. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Res*. **10**: 564–567

Hölldobler B. and Wilson E.O. 1990. *The Ants,* Springer-Verlag, Berlin

Huang M.H. and Dornhaus A. 2008. A meta-analysis of ant social parasitism: host characteristics of different parasitism types and a test of Emery's rule. *Ecol. Entomol*. **33**: 589–596

Lenoir A., D'Ettorre P., Errard C. and Hefetz A. 2001. Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol*. **46**: 573–599

Lively C.M. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. *Am. Nat*. **153**: S34–S47

Michalakis Y. and Excoffier L. 1996. A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. *Genetics* **142**: 1061–1064

Nei M., Tajima F. and Tateno Y. 1983. Accuracy of estimated phylogenetic trees from molecular data. *J. Mol. Evol*. **19**: 153–170

Passera L. 1964. Données biologiques sur la fourmi parasite *Plagiolepis xene* Stärcke. *Insect. Soc.* **11**: 59–70

Passera L. 1969. Biologie de la reproduction chez *Plagiolepis pygmaea* Latr. et ses deux parasites sociaux *Plagiolepis grassei* Le Mas. Pas. et *Plagiolepis xene* St. (Hymenoptera: Formicidae). *Ann. Sci. Nat. Zool. 12e sér.* **11**: 327–482

Passera L., Gilbert M. and Aron S. 2001. Social parasitism in ants: effects of the inquiline parasite *Plagiolepis xene* St. on queen distribution and worker production of its host *Plagiolepis pygmaea* Latr. *Insect. Soc*. **48**: 74–79

Pedersen B.V. 1996. A phylogenetic analysis of cuckoo bumblebees (*Psithyrus* Lepeletier) and bumblebees (*Bombus* Latreille) inferred from sequences of the mitochondrial gene cytochrome oxidase I. *Mol. Phylogenet. Evol*. **5**: 289–297

Swofford D.L. 2003. *PAUP*: phylogenetic analysis using parsimony (*and other methods).Version 4*. Sinauer Associates, Sunderland, Mass

Thomas C.D. 2000. Dispersal and extinction in fragmented landscapes. *Proc. R. Soc. Lond. B* **267**: 139–145

Thompson J.N. 2005. *The Geographic Mosaic of Coevolution—Interspecific Interactions*. University of Chicago Press, Chicago

Thurin N. and Aron S. 2009. Sib-mating in the ant *Plagiolepis pygmaea*: adaptive inbreeding? *J. Evol. Biol*. **22**: 2481–2487

Trontti K., Tay W.T. and Sundström L. 2003. Characterisation of polymorphic microsatellite markers for the ant *Plagiolepis pygmaea*. *Molec. Ecol. Notes* **3**: 575–577

Trontti K., Aron S. and Sundström L. 2005. Inbreeding and kinship in the ant *Plagiolepis pygmaea. Mol. Ecol*. **14**: 2007–2015

Trontti K., Aron S. and Sundström L. 2006. The genetic population structure of the ant *Plagiolepis xene*-implications for genetic vulnerability of obligate social parasites. *Cons. Genet*. **7**: 241–250

Trontti K., Thurin N., Sundström L. and Aron S. 2007. Mating for convenience or genetic diversity? Mating patterns in the polygynous ant *Plagiolepis pygmaea. Behav. Ecol*. **18**: 298–303

Wilson E.O. 1971. *The Insect Societies*. Harvard University Press, Cambridge, Mass