Population structure and evolution in the ant *Plagiolepis pygmaea* and its two social parasites *Plagiolepis xene* and *Plagiolepis grassei*

Kalevi Trontti

Department of Biological and Environmental Sciences
University of Helsinki
Finland

Academic dissertation

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Author's address:
Department of Biological and Environmental Sciences
P.O. Box 65 (Viikinkaari 1)
00014 University of Helsinki
Finland

E-mail: kalevi.trontti@helsinki.fi

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This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I   Trontti K., Tay W. T., and Sundström L.: Polymorphic microsatellite loci for the ant *Plagiolepis pygmaea*. Molecular Ecology Notes 3, 575-577.

II   Trontti K., Aron S., and Sundström L.: Inbreeding and kinship in the ant *Plagiolepis pygmaea*. Molecular Ecology 14, 2007-2015.

III   Trontti K., Thurin N., Sundström L., and Aron, S.: Mating for convenience or genetic diversity? Mating patterns of the polygynous ant *Plagiolepis pygmaea*. Manuscript.

IV   Trontti K., Aron S., and Sundström L.: The genetic population structure of the ant *Plagiolepis xene* - implications for genetic vulnerability of obligate social parasites. Conservation Genetics*,* in press.

V   Trontti K., Savolainen R., and Passera L.: Phylogenetic origins of the inquiline ants *Plagiolepis grassei* and *Plagiolepis xene*. Manuscript.

Contributions

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Idea	LS	KT	SA, KT	KT	LP, KT
Study design	TT	KT, LS	SA, KT	KT	KT, RS
Data collection	KT	KT, SA, LP	SA, KT, LP	KT, SA, LP	KT, LP, SA, RS, XE
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KT = Kalevi Trontti, LS = Liselotte Sundström, SA = Serge Aron, LP = Luc Passera,
RS = Riitta Savolainen, TT = Tek Tay, NT = Nicolas Thurin, XE = Xavier Espadaler

Supervisors
Prof. Liselotte Sundström, University of Helsinki, Finland
Prof. Serge Aron, Free University of Brussels, Belgium

Reviewers
Prof. Pekka Pamilo, University of Oulu, Finland
Dr. Jouni Aspi, University of Oulu, Finland

Examiner
Prof. Jacobus Boomsma, University of Copenhagen, Denmark

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Summary

Introduction

Social behaviour of animals affects dispersal and is therefore an important modifier of genetic population structures. In particular, the females of several species of mammals, birds and insects are philopatric and form breeding groups, whereas individuals of the male sex disperse. Such social organisation effectively maintains coancestry within the breeding groups, and subsequently promotes cooperative behaviours that are tied to kinship without exposing them to inbreeding (Sugg et al. 1996; Ross 2001).

Significant variation exists in social behaviour of animals across and within species, which is expected to result from variation in optimal fitness strategies given the ecological and social environments (Sherman et al. 1995; Keller and Perrin 1995; Cahan et al. 2002). In order to maximize personal reproduction individuals need to weigh the benefits of dispersal and solitary reproduction against delayed dispersal and reproductive opportunities in the natal group (Jennions and Macdonald 1994). Ecological constraints may select for group-living due to higher survivorship and foraging efficiency, and philopatry may entail benefits in future territory holding and reproductive success. Given that group members are also related, helping entails fitness returns even in the absence of any personal reproduction opportunities, by inclusive fitness. In addition, if there is a positive correlation between the number of helpers or group size and the reproductive success among the dominant individuals, they may also offer a share of the reproduction as an incentive to stay (Keller and Reeve 1994). The optimal strategies and the extent to which dispersal, helping, and reproductive sharing are put into action may change over time together with the changing ecological and social environments (Cahan et al. 2002). When the success of solitary breeding and direct reproduction are permanently outweighed by inclusive fitness benefits, these can lead to evolution of eusocial societies with reproductive altruism and permanent division between reproduction and labour (Hamilton 1964a, b).

The hymenopteran species (i.e. ants, bees and wasps) represent all social organisations at the continuum, starting from solitary life and loose associations of co-breeding females and ending to eusociality with complete and permanent reproductive altruism (Sherman et al. 1995; Keller and Perrin 1995). A further partitioning of reproduction may also occur above the level of eusociality when the group comprises more than one member of the reproductive caste (e.g. Keller and Reeve 1994; Sherman et al. 1995; Keller and Perrin 1995). Thus social hymenoptera provide unique schemes to investigate the conditions when genetic structure within the breeding groups promotes reproductive cooperation and altruism, and when it will lead to conflict. In addition, the social hymenoptera provide examples on how inclusive fitness strategies shape the structure of populations above the level of breeding groups.

Ants are eusocial and most species display a fixed morphological division of females into castes of reproductive queens and largely non-reproductive workers. However, variation exists in the social organisation of colonies, most notably owing to colony queen number, which can vary from only one to several hundreds (Bourke and Franks 1995). Ant species are traditionally separated into those that tend to have only one (monogyny) and those that tend to have several queens (polygyny) in their mature colonies (Hölldobler and Wilson 1977), however the queen number appears to be relatively flexible trait also within a species (Pamilo 1991b; Sundström 1993, 1995; Seppä et al. 2004; Sundström et al. 2005). The most extreme example of this social polymorphism is provided by the imported fire ant in North America where sympatric colonies are either strictly monogynous or polygynous with hundreds of queens (e.g. Ross 2001).

The only exception to eusociality among ants comes from the permanent and obligatory ant parasites, the inquilines. Most inquilines are secondarily worker-less, i.e. they have evolved to produce only sexual offspring, and raise their offspring in colonies of other ant species (Hölldobler and Wilson 1990). Therefore they display an intriguing reversal of eusocial life history, yet the parasite queens are now completely dependent on the eusocial colony organisation of their host. The inquiline life history is much comparable to avian brood parasitism, but with the notable difference that also the adult parasite is taken as kin and not just her offspring (Lenoir et al. 2001; Foitzik et al. 2001).

Genetic colony structure and inclusive fitness

Relatedness between the colony workers and the non-descendant brood they raise is the prerequisite for permanent reproductive altruism by kin selection (Hamilton, 1964a, 1964b, 1972). According to theory, individuals gain inclusive fitness by passing copies of their genes to future generations also by helping to raise related non-descendant young, instead of reproducing themselves (Hamilton, 1964a, b). The higher the relatedness between workers and the brood, the higher is the benefit from altruism, given that colony production is equal (Hamilton, 1964a, b). Therefore, a decrease in kinship may entail a reduction in the inclusive fitness of workers unless this is counteracted by a respective increase in colony reproduction, and can promote selfish behaviour such as nepotism (Keller 1995; Queller and Strassmann 1998).

The presence of multiple queens and also multiple male mates of queens dilutes colony relatedness. However, additional queens of polygynous colonies are typically recruited from their own offspring and therefore colony relatedness usually remains significantly higher than zero (Keller 1995; Bourke and Franks 1995). Adopting colony daughters is beneficial for resident queens and colony workers alike when dispersal is risky and the opportunities for independent colony founding for the new queens are small (Nonacs 1988). In addition, low dispersal of queens can favour altruism through selection at the level of colony networks, as then physically close colonies also remain genetically similar and individuals are more likely to interact with a relative (Hamilton 1964b, 1972; Queller 1992, 1993, 1994). In the extreme, queens of polygynous species do not disperse on wing but on foot, establishing

daughter colonies in the vicinity of the maternal colony with the assistance of workers (colony budding; Wilson 1971). Dispersal of queens is indeed reduced among polygynous ants compared to queens of monogynous species (e.g. Hölldobler and Wilson 1977; Keller and Passera 1989; Passera and Keller 1990; Bourke and Franks 1995; Sundström 1995; Sundström et al. 2005).

The daughter colonies may become functionally independent or remain connected after budding. In the latter case, the intermixing of queens and workers between colonies may abolish all population substructuring, and lead to unicolonial polydomy with colony networks covering entire populations (Hölldobler and Wilson 1977, 1990; Bourke and Franks 1995; but see Chapuisat and Keller 1999). Unicolonial polydomy is considered a challenge to kin selection theory as the relatedness within colonies is proportional to genetic differences between the colonies, and may be indistinguishable from zero among the sympatric colonies (Keller 1995; Bourke and Franks 1995). However, determining relatedness depends on the scale of intraspecific competition which varies according to the species. Hence, if competition takes place between population wide supercolonies, also the relatedness should be estimated over several populations instead of sympatric colonies that operate together (e.g. Sundström et al. 2005).

The most extreme example of unicolonial polydomy is provided by the imported Argentine ant *Linepithema humile*, where the supercolonies lack genetic differences and kin discrimination even when the geographical scale is extended to cover continuous populations reaching from the coastline of Spain to Italy (Giraud et al. 2002; for American populations see Tsutsui and Case 2001; Buczkowski et al. 2004). Also other examples of high unicolonial polydomy are from species outside their natural range, such as *Lasius neglectus* (Boomsma et al. 1990; Espadaler and Rey 2001; see also review by Tsutsui and Suarez 2003). These populations may display suboptimal behaviour due to the relatively recent population bottleneck and expansion, and their extreme unicoloniality may be reduced after developing genetic differences over time. More modest levels of unicolonial polygyny seem however to be evolutionarily stable, e.g. in some species of the genus *Formica* in their natal range and in the native populations of the Argentine ants (*F. truncorum*, Elias et al. 2005, *F. paralugubris*, e.g. Chapuisat et al. 1997; Chapuisat and Keller 1999; Pedersen et al. 2006).

Effective populations and genetic variability

Many cooperative behaviours and reproductive altruism in particular is tied to relatedness. Subsequently the maintenance of eusocial organisation promotes a loss of genetic variation within the breeding groups compared to the total population. Genetic variability can however be important for social insects at the level of individuals and colonies. It may, for example, improve resistance to pathogens, tolerance to variation in environmental conditions, or increase the level of phenotypic plasticity of individuals, given a genetic component in worker caste polymorphism (Crozier and Page 1985; Hamilton 1987; Sherman et. al. 1988; see also Hughes et al. 2003; Cahan and Keller 2003; Cahan et al. 2004). Genetic variability is important for ants also owing to

their haplodiploid sex determination, because homozygosity at the sex determining locus leads to production of sterile diploid males that entail a reduction in colony reproduction and growth (Crozier, 1971; Page, 1980; Pamilo et al. 1994; Cook and Crozier 1995; for exception in sterility in a wasp see Cowan and Stahlhut 2004). These hypotheses are similar to the genetic factors that have been proposed to account for the evolution of polygyny (Bourke and Franks 1995).

The amount of genetic variation within breeding groups depends on the number of reproductive individuals. In addition, the size of breeding group determines the likelihood of inbreeding, and the rate at which genetic variation is lost owing to genetic drift. In ants, the female population is determined by the number of reproductively active queens. The size of the male population is in turn determined by the number of matings by the queens, as the queens can store sperm from several males before becoming egg layers (polyandry), and two or three matings for a queen occur across ant taxa (Boomsma and Ratnieks 1996; Strassmann 2001). However, the general view that the effective number of individuals is smaller than the census population is especially true for ants, as the reduction takes place at several levels. First, owing to the division of labour, the number of reproductive individuals is only a fraction of the population that can be sustained by the local resources (Pamilo and Crozier 1997). Second, owing to male haploid sex determination, the effective population size of males is only half the respective female population size. Third, reproduction in the colonies is often shared unequally, both among the queens and males (Keller and Reeve 1994).

Permanent social parasitism

Socially parasitic ants such as the inquilines use the colonies of other ant species to reproduce. In obligatory and permanent inquilinism the entire parasite life cycle takes place within the host colonies (Hölldobler and Wilson 1990). Inquiline queens infiltrate host colonies after dispersal, where they coexist with the host queens and produce their sexual offspring, which is reared by the host workers simultaneously with their own brood.

Most inquilines parasitize only one host species, which they resemble morphologically, suggesting that the species are closely related (Emery 1909; Hölldobler and Wilson 1990). A correlation between the host and parasite phylogenies may arise from two evolutionary schemes. First, adapting to the socially parasitic life history could be more pronounced between related than unrelated free-living species, e.g. due to similarities in ecology or in kin recognition mechanisms that may facilitate the social deception (Wilson 1971; Carpenter 1993). Second, the parasites could originate directly from the species that will later serve them as hosts, without a free-living intermediate ancestor (Emery 1909; "Emery's rule", Le Masne 1956a). The host species of inquilines have polygynous colonies that continuously recruit new queens, and hence inquilines are proposed to have speciated through intraspecific parasitism in which some queen types specialize in producing only sexual offspring, thus taking the advantage of other queens to invest in colony worker force (Buschinger 1986; Hölldobler and Wilson 1990; see also Savolainen and Vepsäläinen 2003).

The inquiline parasites share several adaptations to parasitic life history (the inquiline syndrome; Wilson 1971; Hölldobler and Wilson 1990). In particular, most inquilines do not produce any workers of their own, since the parasites coexist with the host queens that secure a continuous production of host workers. In addition, inquilines are miniaturized compared to free living species, and several of their body structures are rudimentary, including wings (Wilson 1971). Smaller investment on size gives the parasite the advantage of developing into sexuals with less nutrition than is required to raise host worker brood (Bourke and Franks 1991; Nonacs and Tobin 1992). Subsequently, however, also the mating flights and dispersal are reduced in inquilines compared to free living ants, which may be connected to the trend that most inquilines seem to have very restricted distribution and many are listed by the IUCN list of threatened species (Hölldobler and Wilson 1990; Hilton-Taylor 2000; Chapman and Bourke 2001). The true concern regarding the vulnerability of inquiline parasites however depends on whether the inquiline populations are as fragmented as assumed, or whether our judgement on their rarity biased by their concealed life history (Hölldobler and Wilson 1990).

Genus Plagiolepis

The genus *Plagiolepis* (subfamily Formicinea; tribe Plagiolepidini) is distributed throughout the old world, with fourteen species and several subspecies currently described from Europe (Bolton 1995). Four of these, *P. xene*, *P. grassei, P. ampeloni* and *P. regis*, are inquilines, and the last three of these are classified as vulnerable (Hilton-Taylor 2000). The inquilines *P. grassei* and *P. xene* share *Plagiolepis pygmaea* as a common host species. *P. pygmaea* occurs throughout the southern parts of Europe from Spain to Austria and is commonly found in arid areas with low vegetation. Polygynous, subterranean colonies of the species with 5-35 queens are located under stones or in gravel from where they continue vertically underground approximately half a meter. Colony reproduction takes frequently place by budding but queens of the species are also capable of independent colony founding (Passera 1969; Seifert 1996).

The parasites *P. grassei* and *P. xene* both display several characters of the inquiline syndrome (Wilson 1971). However, the two species differ in the degree of the inquiline characters and in how well they are adapted to parasitize the host (Le Masne 1956a, b; Passera 1969; Aron et al. 1999). In particular, *P. grassei* produces a handful of workers together with sexual offspring, whereas *P. xene* produces exclusively sexual brood. Second, the gynes of *P. grassei* are relatively large compared to the strongly miniaturized gynes of *P. xene*, whose sexual offspring are smaller than the host's workers. Third, the sexual offspring of *P. grassei* develop from over-wintering larvae together with the host sexuals, whereas the offspring of *P. xene* develop from eggs laid in the spring together with the host workers. As the inquilines can harness the host workers to raise their brood, an investment on workers by *P. grassei* is considered a maladaptation (Wilson 1971; Hölldobler and Wilson 1990; but see Sumner et al. 2003 for an exception in *Acromyrmex*). In addition, owing to miniaturization and time-shift in reproduction, *P. xene* can reproduce also in host colonies that do not

produce queens or males. Altogether, these and other "intermediary" traits displayed by *P. grassei* (Le Masne 1956a; Passera 1969) may be due to more recent common ancestor with the host *P. pygmaea*, compared to the more specialized parasite *P. xene*.

Aims of the thesis

The first aim of this thesis was to develop a set of microsatellite markers to investigate both the host *P. pygmaea* and its social parasite *P. xene* (I). The rate and patterns of evolution of the repetitive sequences vary according to the locus, which calls for using common markers when comparing populations of more than one species. The second aim was to resolve population structure and mating strategies of *P. pygmaea* and *P. xene* (II, III, and IV). This provides information on the reproductive units, dispersal, and colony kinship of the host and parasite species, and may also reveal interaction between the parasite and host populations. The third aim was to construct a phylogeny of *P. pygmaea* and its two parasites (V), in order to test if the host is the closest free living relative to the parasites, as predicted by the Emery's rule, and if *P. grassei* has more recent common ancestor with the host than *P. xene*, as suggested by evidence in morphology and life histories.

Material and methods

Samples for the studies of population structure and mating frequencies of *P. pygmaea* were collected from Southern France, near the city of Toulouse and the French border against Spain. Samples of *P. xene* and *P. grassei* originate from the same area, and the former of these were used for both the population study of parasites and the phylogenetic reconstruction. Also other species of *Plagiolepis* were collected by the authors and contributed by collaborating researchers across Europe for the phylogenetic reconstruction.

The genetic population structure of *P. pygmaea* and *P. xene* and the parentage of the offspring of *P. pygmaea* were investigated with six to eight microsatellite markers. The applied molecular laboratory methods followed standard cloning and polymerase chain reaction based procedures developed for DNA (Sambrook and Russell 2000). DNA-microsatellites are the most widely applied genetic markers in population genetic studies due to several advantages (Goldstein and Schlötterer 1999). They are single-locus and co-dominant markers, meaning that alleles of a diploid individual can be distinguished from each other and specified to a given locus. In addition, microsatellites usually display high polymorphism, and are selectively neutral and therefore patterns of marker distribution should reflect random population events such as migration, mating patterns, and the effective population size. Microsatellite data were analysed using statistical methods based on the spatial distribution of genetic variation, such as F-analysis of variance (Weir and Cockerham 1984) and regression relatedness (Queller and Goodnight 1989).

The phylogenetic reconstruction was conducted by sequencing parts of two mitochondrial genes (cytochrome oxidase c subunit I, COI and cytochrome b, Cyt b) and one nuclear gene (internal transcribed spacer, ITS). Mitochondrial genes and introns are suitable for studies at the species taxon, because the substitution rates are higher in them compared to coding regions of nuclear genes (e.g. Ballard and Whitlock 2004). The inclusion of also nuclear sequences in the data set is important since the two genomes sometimes produce different phylogenetic patterns (Ballard and Whitlock 2004; Rubinoff and Holland 2005). The phylogenetic reconstruction was conducted according to maximum parsimony.

Main results and discussion

Genetic population structure of Plagiolepis pygmaea

Microsatellites have proved valuable in studies of social insects such as ants, where exact data on colony kin structure, queen mating frequencies or effective dispersal would be difficult to obtain with other means than genetic markers (Queller et al. 1993). Also the markers developed for this work displayed mostly high polymorphism and proved effective tools to detect genetic structures within and between colonies and populations (I). In addition, most of the markers designed from sequences of *P. pygmaea* amplified also in *P. xene*. Applicability of microsatellite markers across related species is shown also in other Formicine ants (Chapuisat 1996; Gyllenstrand et al. 2002).

The analysis of populations of *P. pygmaea* revealed strong substructuring in all studied populations (II), which is expected under polygyny owing to queen philopatry and colony reproduction by budding (Crozier and Pamilo 1996). Indeed, the assignment tests of our study suggest that most if not all queens were adopted by their natal colony (II). However, in contrast to other polygynous species, we also detected high levels of inbreeding in the populations of *P. pygmaea* (II, III), which further enhances the observed population structure. This indicates exceptionally low dispersal by males in the species, which usually is strong enough in ants to avoid significant inbreeding (Bourke and Franks 1995). According to the analysis of mating patterns the males were, in terms of genetic correlation, on average brothers to the queens (III). Given such high relatedness, most of the matings must have taken place within colonies, as outside the colonies such high proportion of the male mates would unlikely be related.

Reduced dispersal is common in polygynous ants, and as suggested by Hamilton (1972), it may maintain genetic cohesion of colonies across several generations. As a result of low dispersal of *P. pygmaea* queens, high kinship is maintained within the colonies regardless of the presence of multiple reproductives (II). In addition, inbreeding further enhances population substructuring and subsequently also the relatedness (II, III), which in many colonies did not differ statistically from the value expected under monogyny and single or double mating (II). The consistency between the effective and the observed number of queens further suggests that reproductive

skew within the colonies does not contribute significantly to within-colony relatedness (II).

Low dispersal is proposed to favour altruism through selection at the level of colony networks because it counteracts the reduction in relatedness caused by polygyny, by increasing relatedness within groups of colonies (Hamilton 1964b). However, the inclusive fitness benefit gained from philopatry and inbreeding is predicted to suffer from costs associated with reduced dispersal. In particular, 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 enhanced relatedness are mitigated by group costs. First, 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; Taylor 1992a, b). Second, inbreeding may entail costs due to detrimental effects on colony performance, such as inbreeding depression and increased risk of producing sterile diploid males in replacement of female offspring (diploid male load; e.g. Ross et al. 1993; Pamilo et al. 1994; Cook and Crozier 1995).

We, however, suggest that benefits of low dispersal for inclusive fitness override the costs of in *P. pygmaea* (II). First, the scales of competition and relatedness differ so that competition partly occurs between non-kin (cf. Queller 1992, 1994). Neighbouring colonies are not always related owing to strong substructuring within short distance, and the species is able to found colonies independently, which takes reproductive individuals outside the range of related colonies. Second, the negative impact of inbreeding on colony performance may be smaller for social insects than for other animals because the haploid males promote purifying selection of harmful recessive alleles, except for those genes that are expressed exclusively in females (Crozier 1970; Cook and Crozier 1995; see also Keller and Passera 1993; Keller and Fournier 2002). In addition, the diploid male load imposed by inbreeding may be insignificant at least for mature colonies as any diploid male offspring can be eliminated at low cost under offspring overproduction (Keller and Passera 1993; Keller and Fournier 2002). Indeed, we found no diploid individuals among the male brood of *P. pygmaea*, suggesting that either no diploid male eggs are laid or they are removed before maturation (II). Conversely, recent evidence from *Formica exsecta* and *Bombus terrestris* suggests that inbreeding causes a load to colony founding, size, longevity, and production (Liautard et al. pers. comm.; Gerloff and Schmid-Hempel 2005). However, inbreeding depression is considered to be weaker for species that inbreeds regularly such as *P. pygmaea* (Charlesworth and Charlesworth 1987).

It is important to note that, unless the benefits attributable to inclusive fitness of workers benefit also the queens for example at the level of colonies by enhanced colony performance, the question whether workers can actually drive inbreeding depends completely on whether they are able to control the mating patterns of their new queens. This control could be achieved if the workers repel any queens attempting to enter the colony, whether their own daughters or alien queens. Interestingly, behavioural observations indicate that workers actively repel at least heterocolonial males when these arrive at an unrelated nest even if virgin queens are present (S. Aron pers. comm.), which reduces the opportunities for outbreeding.

The analysis of mating patterns revealed extensive polyandry in *P. pygmaea* (III). The observed average of nearly three matings for one queen is the highest mat-

ing frequency reported for a polygynous ant species. Additional patrilines may also have remained undetected owing to inbreeding, as it reduces the resolution of paternity assignment. Multiple mating by ant queens is generally considered to result from genetic or other benefits associated with it, because prolonged mating flight entails cost to the queen by increasing energy consumption and exposure to predation. However, polyandry is unlikely to be selectively favoured based on colony-level benefits attributable to enhanced genetic diversity in *P. pygmaea* (III). This is because polyandry appears to have little effect on the genetic diversity in the colonies owing to high levels of inbreeding, or on their effective population size which could potentially alleviate the loss of genetic variation by genetic drift (III). In turn, selection for polyandry due to genetic benefits can not be completely excluded as each additional mate increases the chance of obtaining a rare unrelated mate, and may therefore increase the variance in inbreeding among offspring. Indeed, female polyandry is in general considered unlikely to occur for purely non-genetic benefits as it always leads to increased genetic variation and to the possibility of also genetic benefits (Jennions and Petrie 2000).

However, non-genetic hypotheses are appealing to explain high polyandry in *P. pygmaea*. First, each queen is likely attended simultaneously by multiple males while in their natal nest due to highly biased population sex ratios (90 % of sexuals are males; S. Aron, in prep.), whereas the queens are not expected to actively avoid additional matings owing to low costs of polyandry by intranidal mating and natal philopatry (cf. "convenience polyandry"; Alcock et al. 1978; see also Pedersen and Boomsma 1999 for similar conditions in *Myrmica* ants). Second, the queens may also benefit from multiple mating owing to increased sperm supply and the subsequently prolonged personal reproductive life, especially because the extra copulations are likely not costly in the species (Cole 1983; see also Boomsma and Ratnieks 1996; Fjerdingstad and Boomsma 1998). Third, the males might distribute their sperm across several females to increase the chance of inseminating a female which will produce gynes, because paternal genes are transferred only to female offspring under haplodiploidy and less that 10 % of colonies produce gynes. This would promote polyandry if the queens do not obtain enough sperm from single copulations (Jennions and Petrie 2000; see also Pitnick and Markow 1994 for possible male bet-hedging in *Drosophila*).

Population structure and evolution of the inquilines

Populations of the inquiline parasite *Plagiolepis xene* were found to be genetically isolated, indicating that dispersal can seldom mediate gene flow between populations (IV). This becomes especially evident when the parasite populations are compared to the host, the populations of which are only marginally differentiated within the same geographical study range (II). Furthermore, the analysis of the effective population size indicated only one or few foundresses for each parasite populations (IV). Subsequently, although up to one hundred or more individuals can be found in a single parasitized host colony (Passera 1969), new populations of *P. xene* apparently face a genetic bottleneck that reduces the effective population size. Our results therefore

support the general assumption that populations of inquiline parasites are highly fragmented (Hölldobler and Wilson 1990). In contrast, substructuring within parasite populations was weak and indicated almost complete panmixia.

In one population we were able to study the parasite colony network together with the underlying host colonies. Here the parasite was found only in a cluster of related host colonies that shared a common origin through budding (IV). This coincides with observations that *P. xene* is transmitted within populations mainly through host colony budding (Passera et al. 2001). In addition, the parasite dispersal within the host population appeared in this case to be limited to host nests within the same colony network, suggesting that not all host colony networks are suitable for the parasite. However, this study was not able to specify why the parasite was restricted to these particular host colonies, and this would need further studies with several populations. An intriguing finding was also that the parasitized host colony had possibly gone through more colony budding events than other colonies in the population. This coincides with observations that host queens seek into the parts of colony where the *P. xene* is not present (Passera et al. 2001). This inquiline analogue to avian nest desertion may explain why colonies can produce high number of offspring even if *P. xene* inhibits the host reproduction by oophagy under laboratory conditions (Passera et al. 2001).

As a consequence of low number of founding queens in each population, the effective population size of *P. xene* is only a fraction of the census population size (IV). Hence this study, the first one to describe population structure of any inquiline species with genetic markers, highlights the potential genetic vulnerability of the fairly common *P. xene.* The observed pattern is likely to hold also for other inquilines, especially the related and much rarer *P. grassei*. However, how much of a risk inbreeding contributes to the populations, or whether ecological factors act before the influence of inbreeding becomes significant remains debatable among social insects as well as in other animals (Pamilo and Crozier 1997; Keller and Waller 2002).

One explanation for the rarity of the parasites and the differences in their relative abundance could result from cryptic host subtypes that would differ in their suitability for the parasites. However this is not supported by this study as the colonies that were parasitized by *P. xene* did not differ from the sympatric unparasitized colonies except in being related (III), and the same appeared to be the case with respect to the one sample of *P. grassei*. Moreover, the host population that included *P. xene* was not genetically differentiated from the unparasitized ones or from the one that contained *P. grassei*, and all populations had the same overall genetic population structure with respect to inbreeding coefficients and relatedness values (II, III).

It has been suggested that the inquilines arose from intraspecific parasites through sympatric speciation because they depend completely on the eusocial colony organisation of the host (Bourke and Franks 1991), and the finding that the host is the closest free-living relative to the parasite is considered to provide support for this (Buschinger 1986; Hölldobler and Wilson 1990). Indeed, *P. pygmaea* was found to be the closest free-living species to both its inquiline parasites (V). However, the evidence for sympatric speciation is conditional on the likelihood that no free-living species between the parasite and its host has existed, as the parasites could alternatively have speciated in allopatry from free-living intermediate species that were not in-

cluded in this study. The possibility of sympatric speciation has inspired also other phylogenetic studies on the relationships of inquilines and their hosts (Parker and Rissing 2002; Savolainen and Vepsäläinen 2003; Sumner et al. 2004), of which two support the Emery's rule.

The phylogenetic reconstruction also indicates that parasitism has evolved twice, when mitochondrial and nuclear sequences were analysed together, and such that *P. grassei* has more recent common ancestor with the host than *P. xene* (V). However, the nuclear data conflicts with the mitochondrial genes suggesting a common origin for the two parasites instead, and we are not able to resolve with the current data whether the parasites are sister species or not. Conflicts between nuclear and mitochondrial gene trees are not uncommon (Ballard and Whitlock 2004; Rubinoff and Holland 2005), and in this case may indicate introgression of either nuclear genes from *P. xene* to *P. grassei* or mitochondrial genome from *P. pygmaea* to the common ancestor of the parasites, depending whether the true tree of the parasites is mono or polyphyletic (V).

Whichever is the evolutionary origin of the parasites, the shortcomings in parasitic adaptations of *P. grassei*, e.g. in miniaturization and worker production (Le Masne 1956a; Passera 1969), are likely to entail a load for its reproduction in host colonies compared to *P. xene*. Subsequently the differences in traits related to parasitism may partly explain the rarity of *P. grassei* compared to relatively more abundant *P. xene* even if the two parasitize the same common host species.

Conclusions

Altogether this study reveals intriguing patterns on population structuring and mating strategies of the polygynous ant *P. pygmaea*, and provides information on the populations of its inquiline parasite *P. xene*. In particular, the approaches based on genetic markers provide detailed data on the size of the reproductive units, effective dispersal, and kinship of the breeding groups, and also suggest possible interactions between the populations of the host and the parasite.

Coancestry promotes cooperative behaviours in breeding groups, and according to the kin selection theory, explains why some individuals can under natural selection afford to sacrifice personal reproduction for the good of others (Hamilton 1964a, b). In many species of cooperative breeders, the individuals may choose to delay dispersal and stay as helpers in the natal group when the success of dispersal and solitary breeding is low due to ecological constraints (Jennions and Macdonald 1994; Cahan et al. 2002). However, this is not a matter of choice for workers of eusocial species with morphological caste determination, and dispersal may also be very limited for queens of many polygynous ant species. Hence the importance of coancestry is amplified under eusociality, and the behaviours of individuals are expected to be refined to maximize inclusive fitness at a given social environment (Cahan et al. 2002). For example, workers seem to control the offspring sex ratios for their own genetic interests (Sundström 1995; Bourke and Franks 1995).

The populations of *P. pygmaea* are the first example to demonstrate that also low dispersal of males can substantially increase kinship within the breeding groups, in addition to the more traditional female philopatry (II). Due to intranidal mating it seems plausible that workers of *P. pygmaea* could control the mating patterns of colony queens for their own fitness purposes. Given also that inbreeding is regular across populations, the risk of inbreeding depression that could counteract these benefits may be low. However, many other polygynous species demonstrate that low male dispersal is not a common strategy to enhance colony kinship, and high relatedness is not a necessity for a stable social organisation as the returns from reproductive altruism are determined also by other factors (e.g. Bourke and Franks 1995).

The results on *P. pygmaea* also caution that the contribution of non-genetic factors should not be underestimated (III). The highly male biased sex ratios of *P. pygmaea* are congruent with local resource competition, in which reduced dispersal and colony reproduction by budding result in kin competition over local resources (Clark 1978; Pamilo 1991a). This has two consequences relevant to our studies. First, low number of females may promote female polyandry because each female will attract several males. However, most of the benefits proposed to explain polyandry in ants are attributable to genetic variability, and the role of these is difficult to exclude unambiguously even in *P. pygmaea* where additional matings contribute on average very little to genetic variation. Second, the male biased sex ratio of *P. pygmaea* may promote inbreeding because the males likely have difficulties in finding mating partners, and thus may have an incentive to mate already within the natal colonies if it produces also queens. This may be the driving force of inbreeding in the species.

The results of *P. xene* support the common view that the inquiline populations are highly fragmented and genetically vulnerable (IV). Dispersal abilities of many inquilines seem reduced based on their morphology and loss of mating flights (Hölldobler and Wilson 1990). However, obligatory parasites in many other taxa have managed to secure their dispersal by evolving even very complex strategies such as modification of host behaviour and intermediary hosts (Begon et al. 1996). Therefore making a generalisation that the rarity of inquilines follows from weak dispersal abilities seems controversial. Instead, the rarity of certain inquilines may partly result from their interactions with the host species, rather than difficulties in reaching the host colonies. Indeed, the rarity of *P. grassei* compared to *P. xene* could result from differences in how well the two are adapted to reproduce in the host colonies, given that any host effect is excluded due to the common garden (IV, V).

Finally, the phylogeny of *P. grassei* and *P. xene* follows the Emery's rule. This supports that the evolution of obligatory and permanent social parasitism is more pronounced between two related species, or even that these parasites evolve in sympatry from the host species by intraspecific parasitism (Buschinger 1986; Hölldobler and Wilson 1990; see also Savolainen and Vepsäläinen 2003). In addition, the phylogeny suggests that *P. grassei* may be a younger parasite than *P. xene*. In this case the weakly developed parasitic traits of *P. grassei* and its rarity may follow from its younger phylogenetic age compared to *P. xene* (V). Such link between the rarity of an inquiline species and its parasitic features, and also possibly between its evolutionary age has not been established before.

In conclusion, the organisations of social insect colonies are connected in many ways to behaviour and dispersal. Among the ants, the polygynous organisation seems to be particularly connected to many aspects of species abundance. First, most of the vulnerable ant species are social parasites (Hilton-Taylor 2000; Chapman and Bourke 2001), which calls for knowledge also on their polygynous hosts. Second, the second largest group of vulnerable ants comprises of the polygynous mound-building ants (Hilton-Taylor 2000). Queens of these species are less adapted for long-range dispersal and independent colony founding, compared to queens of monogynous species, which may accentuate the effect of habitat fragmentation on establishment of new populations (Sundström 1995; Gyllenstrand and Seppä 2003; Mäki-Petäys et al. 2004; Sundström et al. 2005). Third, ironically, high queen number is also the precondition to unicoloniality with reduced aggression towards unrelated colonies, which seems to explain the emergence of severe ant pests and to facilitate their spread outside the natural range (Suarez et al. 2001; Holway et al. 2002; Tsutsui and Suarez 2003). Altogether, knowledge on organisations of social insect colonies can contribute both for issues of conservation biology and population management.

Acknowledgements

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