

# Mating for convenience or genetic diversity? Mating patterns in the polygynous ant *Plagiolepis pygmaea*

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Several genetic and nongenetic benefits have been proposed to explain multiple mating (polyandry) in animals, to compensate for costs associated with obtaining additional mates. The most prominent hypotheses stress the benefits of increased genetic diversity. In social insects, queens of most species mate only once or have effective mating frequencies close to one. Yet, in a few species of ants, bees, and wasps, polyandry is the rule. In these species, colonies are usually headed by a single queen, whereas multiple queening adds diversity in several of the remaining species, especially in ants. Here we investigated mating frequency, inbreeding and relatedness between the queens and their mates in the polygynous ant *Plagiolepis pygmaea*, and the effect of polyandry on the genetic diversity as a function of the effective population size of individual colonies. Our results show that polyandry occurs frequently in the species. However, queens are frequently inseminated by close relatives, and additional sires add little genetic diversity among offspring of individual queens. In addition, the increase in diversity at the colony level is only marginal. Hence, contrary to established notions, polyandry in *P. pygmaea* seems not to be driven by substantial benefits of genetic diversity. Nonetheless, very small or as yet unidentified genetic benefits to one party (males, workers, queens) in conjunction with low costs of mating may favor polyandry. Alternatively, nongenetic factors, such as convenience polyandry, may be more important than genetic factors in promoting polyandry in *P. pygmaea*. *Key words:* convenience polyandry, genetic variability hypotheses, inbreeding, *Plagiolepis pygmaea*, polyandry, polygyny. [*Behav Ecol*]

Multiple mating is common in animals, but whereas the evolutionary advantages of having multiple partners easily can be seen for males, any such advantages are less evident for females (e.g., Jennions and Petrie 2000). Nevertheless, females of many animal species mate with multiple males (polyandry). To explain this evolutionary enigma, several nongenetic and genetic advantages have been proposed, including increased genetic heterogeneity of offspring, sperm selection through concealed sexual selection, additional resources through nutritional spermatophores, and avoiding sperm depletion (reviewed by Strassmann 2001). However, females may also mate with many males if avoiding extra copulations is more costly than conceding to mate (Alcock et al. 1978).

In social insects, social life adds complexity to mating systems, as the parties involved encompass not only the colony queen but also the inclusive fitness interests of her offspring workers. Thus, also colony-level selection enters the equation, rather than exclusively individual-level selection. Polyandry has evolved repeatedly in social Hymenoptera with some species being obligately polyandrous and others being facultatively polyandrous (Page 1986; Boomsma and Ratnieks 1996; Strassmann 2001). Although multiple mating occurs commonly across taxa, high levels of polyandry is restricted to a few species with strictly single-queen societies, such as the honey bees (*Apis*), seed harvester ants (*Pogonomyrmex*), army ants (*Dorylus* and *Eciton*), and the higher leaf-cutting ants (*Atta*

and *Acromyrmex*), where the queen may be inseminated by more than 15–20 males (e.g., Page 1980; Palmer and Oldroyd 2000; Denny et al. 2004; Kronauer et al. 2004; Rheindt et al. 2004; Sumner et al. 2004; Wiernasz et al. 2004).

Multiple paternity can have important genetic consequences in social insects because it adds genetic variation to colony offspring by increasing the effective population size through additional reproductively successful males. The benefits of genetic diversity may be expressed in 4 ways: 1) by improving colony-level resistance to pathogens (Hamilton 1987; Sherman et al. 1988; Schmid-Hempel 1998; Baer and Schmid-Hempel 1999), 2) by facilitating genetic polyethism by increasing phenotypic plasticity at the colony level (Crozier and Page 1985; Robinson and Page 1995), 3) by reducing within-colony relatedness asymmetries and, hence, alleviating reproductive conflicts among colony members (Woyciechowski and Lomnicki 1987; Ratnieks and Boomsma 1995), and 4) by reducing drift and counteracting inbreeding within local reproductive units (Page 1980; Zeh JA and Zeh DW 1997; Jennions and Petrie 2000; Crozier and Fjerdingstad 2001). Inbreeding may be particularly deleterious in social Hymenoptera owing to their haplodiploid sex-determining system because homozygosity at the sex-determining locus results in the production of sterile diploid males that contribute to neither work nor reproduction (Crozier 1971; Page 1980; for the impact of diploid male production in extinction proneness of hymenopteran populations, see also Zayed and Packer 2005). Indeed, in social insects, the genetically effective population size is small relative to the census population size owing to their reproductive division of labor. This may be important in particular when the risk of inbreeding (or outbreeding) depression in the population is high (Zeh JA and Zeh DW 1997; Jennions and Petrie 2000; Crozier and Fjerdingstad 2001).

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The social system is, however, likely to affect the relative costs and benefits of polyandry. In particular, single-queen (monogynous) colonies are vulnerable to reduced genetic diversity, whereas multi-queen (polygynous) colonies inherently have higher diversity owing to the presence of several reproductive queens. Therefore, polyandry is expected in species with monogynous colonies, whereas species with polygynous colonies are expected to be mostly monandrous (Keller and Reeve 1994). However, no study has measured the precise genetic impact of multiple mating in polygynous colonies.

Nongenetic explanations for polyandry in social insects may also apply, such as enhanced colony longevity due to an increased lifetime supply of sperm (Cole 1983; Fjerdingstad and Boomsma 1998; Schluns et al. 2005). Multiple mating may also evolve as a pure male strategy without benefits to queens or colony functions (convenience polyandry; Alcock et al. 1978; but see Crozier and Page 1985; Crozier and Fjerdingstad 2001). In convenience polyandry, males seek to maximize the number of copulations and females accept additional copulations because costs associated with resistance are higher than costs associated with conceding to mate. The costs of multiple mating probably vary among species depending on life-history traits, and, for instance, convenience polyandry may be more likely to occur when the risk of predation during mating is low. In polygynous ant species, females usually mate near or within their natal colony (Bourke and Franks 1995) so the costs of additional copulations are low compared with females of single-queen (monogynous) species that mate in swarms (Bourke and Franks 1995). Subsequently, queens of polygynous species may show high mating frequencies even if polyandry entails no benefits for the queens or for the colony as a whole. To date, studies conducted on polygynous social insects have shown moderate levels of female polyandry in ants (Pamilo 1993; Sundström 1993; Seppä 1994; Pedersen and Boomsma 1999). By contrast, polygyny is rare in bees and wasps; it has been reported in some stingless bees (e.g., Meliponine) and a few Epiponine bees where queens are usually singly mated and exceptionally in Vespinae in areas with warm climates (Ross 1986; Crozier and Pamilo 1996; Strassmann 2001; Toth et al. 2004). None of these studies examined precisely the degree of polyandry in these groups.

Here we analyze mating patterns in the polygynous ant *Plagiogilepis pygmaea*. In this species queens and males are strongly philopatric as new queens are recruited from colony daughters, new colonies are predominantly established by budding, and mating often takes place among members of the same or closely related colonies (Passera 1969; Passera et al. 2001; Trontti et al. 2005). Altogether, these lead to high inbreeding and significantly reduced effective population sizes of both sexes (Trontti et al. 2005). In particular, we investigate queen mating frequency and the relatedness between the queens and their mates. In addition, we study the extent to which the prevailing mating patterns enhance the effective population size under inbreeding and outbreeding.

## MATERIALS AND METHODS

### Field collection and sampling

*Plagiogilepis pygmaea* is widespread in Southern France, where it inhabits arid areas with low vegetation. Subterranean colonies of the species include typically 5–30 queens and several hundred workers. Nineteen colonies were collected in February 2004 from 3 locations near Toulouse (France): Tarabel ( $n = 15$ ), Fendeilles ( $n = 2$ ), and St Felix de Lauragais (SFL;  $n = 3$ ). Colonies contained on average 23.6 queens (range 0–56), workers, and larvae.

### Mating frequency analyses

Queen mating frequency ( $M_p$ ) was estimated in 2 complementary ways. We first reconstructed each paternal genotype based on mother–offspring allele combinations (see below); then, we confirmed the presence of the expected alleles from the sperm genotype. 1) Mother–offspring combination: Thirty-three experimental nests were made from the sampled colonies (above), each containing a single queen, and about 50 nestmate workers were set up in laboratory nests made of glass vials. All brood was carefully removed from the nests. The ants were fed honey and water and kept at  $27 \pm 1$  °C. After 40 days, worker larvae and pupae were removed from the 18 nests that had produced a minimum of 10 larvae and pupae (16 nests from Tarabel and one nest each from SFL and Fendeilles) and stored in ethanol for genetic analyses. In total, 224 offspring from 18 queens were obtained (range 8–17). Eggs laid in spring usually develop into workers under natural conditions (Passera 1969), but a small proportion of larvae may include also haploid male offspring (Aron et al. 2004). Therefore, larvae with a homozygous genotype at all loci were considered to be hemizygous males and were excluded from the paternity analysis. 2) Sperm typing: To isolate sperm DNA, all 33 queens from the experimental nests were dissected in DNA extraction medium and the spermatheca was removed, its envelope ruptured with forceps, the seminal fluid collected using a micropipette, resuspended in 30  $\mu$ l of extraction medium, and stored at  $-20$  °C for genetic analyses.

Both approaches will fail to detect multiple mating when 2 fathers have identical genotypes at all loci. Inbreeding further increases the probability of such nondetection errors. We estimated the average nondetection error of patriline from the combined offspring and sperm data, using the equation

$$P_{\text{nondetect}} = \frac{\sum_j \Pi_i f_{ij}}{n}, \quad (1)$$

where  $f_{ij}$  is the nest-level frequency of allele  $i$  at locus  $j$ , and  $n$  is the number of nests. We used nest-specific allele frequencies to allow for inbreeding (Trontti et al. 2005). Because males may contribute unequally to the offspring, we also estimated the effective mating frequency ( $M_{e,p}$ ) following Nielsen et al. (2003):

$$M_{e,p} = \frac{(n-1)^2}{\sum_{i=1}^k p_i^2 (n+1)(n-2) + 3 - n}, \quad (2)$$

where  $n$  is the total number of offspring of a queen,  $k$  is the number of males, and  $p$  is the proportional contribution to the brood of the  $i^{\text{th}}$  male. The effective number of patrilines equals the absolute mating frequency when all males contribute equally.

### Genetic analyses

Genotypes of 8–17 offspring of a given queen, 16 adult workers from her original colony, and the queens and the sperm in their spermathecae were scored at 6 microsatellite loci (P01, P06, P11, P20, P23, P25: Trontti et al. 2003). The DNA was extracted from fine ground samples or sperm suspension by incubating 90–120 min in 40  $\mu$ l of Chelex (Bio-Rad, Hercules, CA) at 85 °C. Samples were centrifuged for 30 s at  $10000 \times g$ , and 2  $\mu$ l of the supernatant was amplified by polymerase chain reaction (PCR) following the fluorescent analysis protocols described in Trontti et al. (2003), using a PTC-200 thermal cycler, and Taq Gold polymerase (Fermentas, St Leon-Rot, Germany). Negative controls containing no added DNA were prepared from each PCR reaction mix. The amplified products were separated in ABI capillary sequencer and sized against ROX-400 size standard (Applied Biosystems, Foster City, CA).

We analyzed population structure and colony kin structure based on worker genotype data from field colonies. In addition, we estimated the relatedness between queens and their mates, among the male mates of individual queens, and among offspring from single-queen laboratory colonies. These analyses encompassed only samples from the Tarabel population, as the number of colonies in the other 2 populations was too small to allow robust estimates. We estimated the inbreeding coefficient  $F_{IT}$  and gene diversities with FSTAT 2.9.3 (Goudet 1995), with colonies entered as populations. The 95% confidence intervals (CIs) were calculated by bootstrapping 15000 times over loci. We obtained all relatedness estimates with RELATEDNESS 5.0.8 (Queller and Goodnight 1989), always using the nests of the respective population as the reference group. The 95% CIs for each relatedness coefficient were obtained by jackknifing over groups.

### The effective population size

We modeled changes in the average effective population size ( $N_E$ ; the number of independent genomes) as the function of the mating frequency in the species. We calculated  $N_E$  for the first-generation offspring of individual queens and single colonies following Wright (1969):

$$N_E = (9N_m N_f) / (4N_m + 2N_f), \quad (3)$$

where  $N_m$  and  $N_f$  are, respectively, the number of reproductive males and females, assuming equal contribution of all males. Wright's equation of  $N_E$  takes into account the relative contribution of individuals to the next generation, but it assumes that these individuals are genetically independent samples, that is, unrelated. However, in our study species, both colony queens and colony fathers are related (Tronetti et al. 2005). Therefore, before applying Equation 3, we corrected the actual number of queens and males (separately) to take into account the average proportion of shared genes (i.e., relatedness) among the same-sex individuals within the reproductive units of colonies compared with the total population. This gives the added value for extra inseminations for a given set of mating frequencies under the observed relatedness patterns. We made the correction with the observed relatedness following

$$N = [1 - (1 - r)^n] / r, \quad (4)$$

where  $n$  is the number and  $r$  is the relatedness among same-sex individuals, respectively (see also Appendix). As an estimate of relatedness among queens, we used the relatedness among colony workers owing to the low number of queens obtained per colony. This is justified because the relatedness among workers and queens are indistinguishable (Tronetti et al. 2005). The relatedness among males was calculated from the male genotypes obtained from sperm typing and mother-offspring allele combinations. Finally, we corrected the  $N_E$  with the average inbreeding, that is, the average proportion of genes shared by females and males (e.g., Hedrick 2005):

$$N_e = N_E / (1 + F) \quad (5)$$

where  $F$  equals the inbreeding coefficient of the population. The calculations for  $N_E$  assume equal contribution of males and females to the offspring and thus provide the conservative maximum estimate.

## RESULTS

The number of alleles scored at the 6 microsatellite loci ranged from 3 to 8, with a mean expected heterozygosity

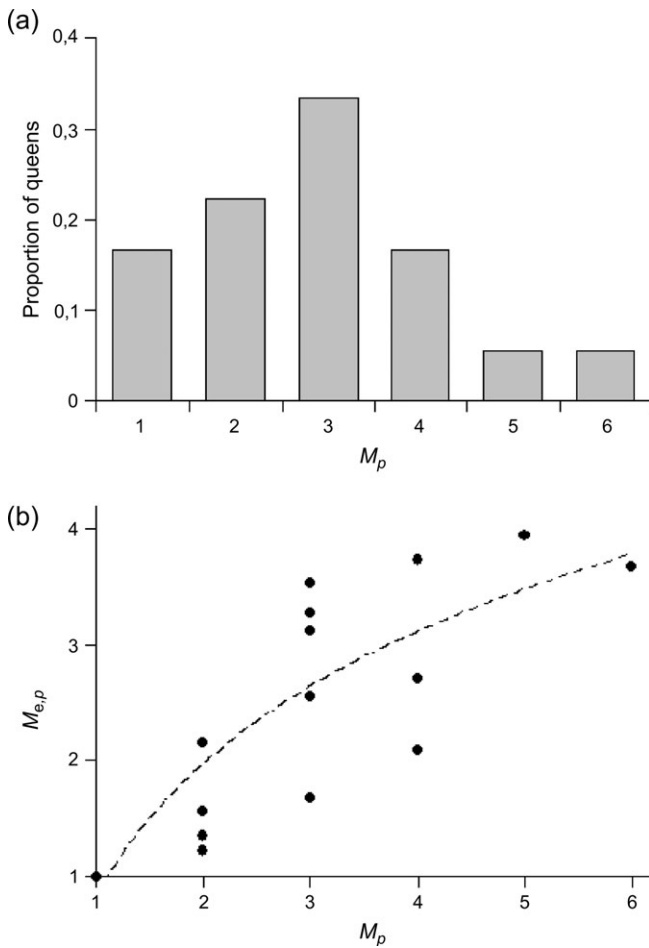
$H_E = 0.624$  (0.398–0.801). The proportion of males among the offspring in the experimental nests was less than 1% (2 of 224 larvae or pupae) of all brood analyzed. As expected, based on earlier studies (Tronetti et al. 2005), also the Tarabel population was significantly inbred ( $F_{IT} = 0.39$ ; 95% CI 0.25–0.62); consistent with this result, queens were closely related to their male mates ( $r = 0.30 \pm 0.16$ ). The different male mates of single queens were also closely related ( $r = 0.46 \pm 0.18$ ). These relatedness values coincide with those expected between brothers and sisters ( $r = 0.25$ ) and among brothers ( $r = 0.50$ ) under haplodiploidy (Bourke and Franks 1995). Consequently, the diploid offspring of single queens were closely related ( $r = 0.75 \pm 0.09$ ), which equals the expectation under single mating ( $r = 0.75$ ). By contrast, workers were more distantly related in the corresponding field colonies ( $r = 0.385 \pm 0.182$ ), which contained several queens.

The genotype distributions in the parent-offspring combinations were consistent with Mendelian inheritance. Mother-offspring combinations indicated that 15 out of 18 queens had mated with 1–6 males, with an average mating frequency of  $2.89 \pm 1.37$ ,  $n = 18$  (Figure 1a). The same paternal genotypes, and no additional ones, were found in the genotyped spermathecal contents. This indicates that all males who inseminate queens contribute to the offspring. The detected number of fathers did not correlate with the number of offspring analyzed (Pearson's correlation;  $r^2 = 0.134$ ;  $P = 0.135$ ;  $n = 18$ ), indicating that our sample sizes were sufficient to detect all patrilines. However, owing to the high relatedness between the male mates of individual queens, the probability of nondetection due to identical paternal genotypes ( $P_{\text{nondetect}}$ ) was as high as 0.23. This corresponds to a corrected average absolute mating frequency of  $M_p = 3.55$ . In turn, the average effective number of fathers was  $M_{e,p} = 2.37 \pm 1.05$ ,  $n = 18$  (range 1–3.94), which is slightly lower than the uncorrected  $M_p$  (see above; Figure 1b).

When the within-colony and within-queen effective population size for sets of 10 queens and single queens, respectively, is plotted against the number of fathers (range 1–10), polyandry tends to increase the effective population size under outbreeding. For multiple queens, the shape of the function for the total effective population size under outbreeding equals that of single-queen offspring, except that the values are higher than those for single queens (Figure 2). However, under inbreeding, the increase of the effective population with the mating frequency is negligible. Among single-queen offspring, the advantage rapidly declines after the second mating (Figure 2). In addition, the effective population size for inbred single-queen offspring remains lower than that for singly mated outbred queens. Furthermore, mating frequency has no notable impact on the effective population size in multiqueen colonies under the empirically observed level of inbreeding. This is due to the fact that the additional colony fathers are highly related to the queen and to each other.

## DISCUSSION

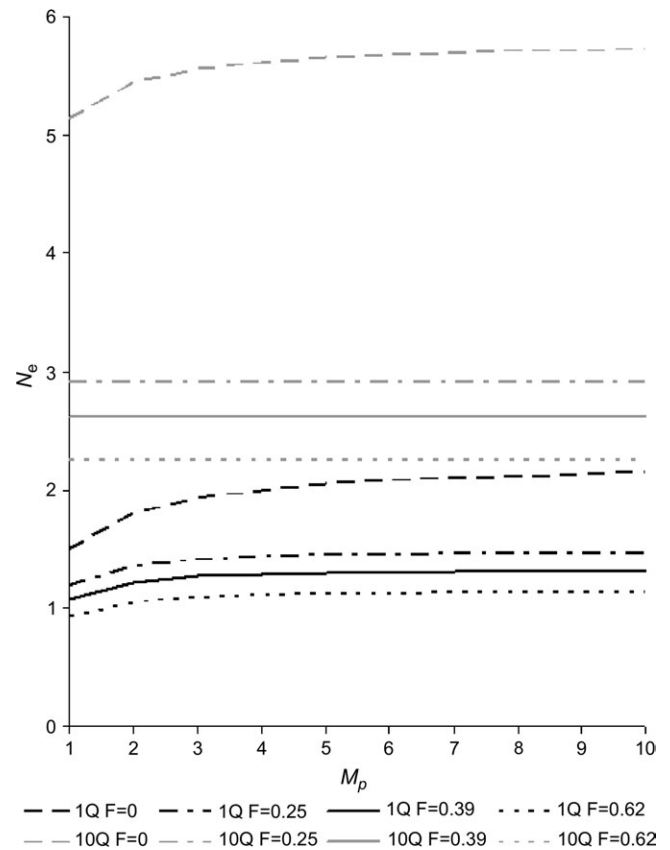
Our data show that over 80% of the queens in the polygynous ant *P. pygmaea* mate with more than one male. The observed average mating frequency of 2.89 is an exceptionally high mating frequency for a polygynous species. It is also a minimum estimate because mating commonly takes place between relatives, and some patrilines may therefore remain undetected. As expected under high levels of inbreeding, additional sires have little effect on the within-colony effective population size and the increase in the effective population size of colonies levels out after 2 inseminations. Thus, polyandry in *P. pygmaea* does not alleviate the loss of genetic variation caused by fixation of alleles owing to inbreeding and



**Figure 1**  
(a) Distribution of queen mating frequency ( $M_p$ ) and (b) the effective paternity ( $M_{e,p}$ ) as a function of  $M_p$ .

genetic drift. Interestingly, the increase in diversity attributable to polyandry is exactly canceled out by the observed level of inbreeding in the study species. Hence, polyandry is unlikely to be selectively favored due to substantial colony-level benefits attributable to enhanced genetic diversity. Instead, our results suggest that a combination of subtle genetic benefits, low mating costs, and male-driven benefits may be more important in promoting polyandry in *P. pygmaea*.

Although polyandry does not enhance overall genetic variation among offspring in *P. pygmaea*, each additional mate increases the chance of obtaining an unrelated mate and may therefore increase the variance in inbreeding among offspring. This could be selectively favored if even a small fraction of outbred offspring confers a fitness advantage either to individual queens or to entire colonies, especially if outbreeding is rare. For example, deleterious effects caused by genetic incompatibility or matched matings leading to the production of diploid males may be alleviated if some fraction of the brood remains unaffected (Crozier and Page 1985). Therefore, the possibility remains that minor effects mediated by additional patrines may increase the variance and thus enhance the quality of some offspring. This could favor polyandry in *P. pygmaea*, especially because the costs of polyandry are likely to be small in the species. All studied populations of the species are highly inbred, and no diploid males were found among the 160 individuals that were analyzed from one of the study populations (Trontti et al. 2005). Taken together, this



**Figure 2**  
The effective population size ( $N_e$ ) among offspring of a single queen (1 Q) and a colony of 10 queens (10 Q) as a function of queen mating frequency ( $M_p$ ), both under outbreeding ( $F = 0$ ) and when inbreeding equals the observed value for the species ( $F = 0.39$ ) and its upper and lower 95% CIs ( $F = 0.25$  and  $F = 0.62$ ) (for details see Equations 3, 4, and 5).

suggests that inbreeding does not entail significant costs, so that even minor advantages may come into play.

Two nongenetic hypotheses are also consistent with polyandry in *P. pygmaea*. First, queens may have to mate multiply to ensure their lifetime supply of sperm (Cole 1983). Consistent with this hypothesis, previous studies on leaf-cutting ants and the honeybee have shown that multiple mating allows queens to effectively increase their sperm store, suggesting that polyandry could be an adaptive strategy to avoid sperm depletion (Fjerdingstad and Boomsma 1998; Kraus et al. 2004; Schluns et al. 2005). However, sperm supply is usually not considered to be a driving force of polyandry in species that permanently have more than one colony queen, as the future of the colony is not dependent on a single individual. Nevertheless, males may distribute their sperm across several females, instead of spending all on one female. This would increase the chance of inseminating a female, which eventually will produce daughter queens (paternal genes are only transferred to female offspring under haplodiploidy). This would further promote polyandry if the queens do not obtain enough sperm from single copulations (for possible bet hedging in *Drosophila*, see also Pitnick and Markow 1994). In the same vein, multiple mating of queens may be male driven in the ant *Cardiocondyla batesii*, given that the genetic benefits and sperm-limitation hypotheses are unlikely to apply in this species. Unlike most ant males, males of *C. batesii* are, however, able to replenish sperm storages after ejaculation

(cf., Schrempf et al. 2005). We have no data on sperm counts in queens and males in *P. pygmaea*, but in the closely related parasite *Plagiolepis xene*, one male may inseminate up to 11 females (Aron S, personal observation). Multiple mating by males is also common in several species of *Formica* (Fortelius 2005). The possibility of bet hedging by males will thus require further study.

Second, queens may mate multiply for convenience (Alcock et al. 1978) as the costs of mating probably are low owing to intranidal mating and natal philopatry of females (Passera et al. 2001; Trontti et al. 2005; this study). Given that population-wide sex ratios are highly male biased (over 90% males; Aron S, in preparation), it is also highly likely that each female is attended simultaneously by multiple males while in their natal nest. This may provide a strong incentive for convenience polyandry, as young females would have to leave the nest in order to avoid copulations, and low costs of mating as males are readily available and females need not disperse. Mating for convenience was also suggested as a possible explanation to account for multiple mating in a number of monogynous (*Nothomyrmecia macrops*, Sanetra and Crozier 2001) and polygynous (*Formica paralugubris*, Chapuisat 1998; *Myrmica* ants, Pedersen and Boomsma 1999; *Proformica longisetata*, Fernández-Escudero et al. 2002) ant species.

Overall, our results add new insights to earlier results on mating frequency in other species. First, *P. pygmaea* is unusual in that colonies are polygynous, yet the queens show mating frequencies well above the average for both monogynous and polygynous ants (Boomsma and Ratnieks 1996). Indeed, our results run counter to the expected association between mating strategy and social organization (Keller and Reeve 1994) and thus add fuel to the debate on this topic (Boomsma and Ratnieks 1996; Pedersen and Boomsma 1999). Second, the effect of polyandry on within-colony genetic diversity was negligible, given the observed levels of inbreeding in the study population. However, in populations with lower levels of inbreeding, the effects on diversity may be more substantial. Thus, minor or occasional colony-level benefits due to polyandry cannot be ruled out, but given that mating costs are comparatively low, a major selective force is likely to act through male interests in conjunction with convenience or forced polyandry of females.

Whereas genetic benefits from polyandry have rarely been studied in polygynous species, several studies on monogynous species suggest that enhanced genetic diversity indeed benefits queens and their colonies (e.g., Schmid-Hempel 1998; Baer and Schmid-Hempel 1999; Cole and Wiernasz 1999; Hughes and Boomsma 2004; Wiernasz et al. 2004). For instance, genetic variability enhances parasite resistance of leaf-cutting ant colonies (Hughes and Boomsma 2004; see also Sumner et al. 2004) and colony growth rate in seed harvester ants (Cole and Wiernasz 1999; Wiernasz et al. 2004). Similarly, in the ant *Cataglyphis cursor*, the queens are produced by parthenogenesis but workers arise from fertilized eggs, suggesting that genetic variation is vital for the worker caste (Percy et al. 2004). However, fewer studies have reported a correlation between genetic variation, phenotypic plasticity, and the actual colony performance (for an ant, see Hughes et al. 2003; for honey bee, see Jones et al. 2004).

Altogether, *P. pygmaea* provides an example where genetic benefits are unlikely to explain female polyandry, owing to high inbreeding. In turn, nongenetic factors such as convenience polyandry are more plausible in explaining polyandry in this species than in those having mating flights, which exposes queens to predation. Social insects differ from other study organisms in that they mate only during a short period before colony founding, but the queens and their colonies live over several years. Consequently, the mating strategies adop-

ted by the queens have long-lasting fitness consequences and are expected to be better refined than in organisms that mate newly at each reproductive season. Social insects therefore provide an important example for the general evolution of mating strategies. Separating the contribution of different genetic and nongenetic factors to the prevailing mating frequency is however not straightforward. The roles of nongenetic factors as the driving force of female polyandry are generally recognized outside the social insects, such as birds and mammals; however, even then polyandry for purely nongenetic benefits is considered unlikely as genetic variability invariably follows from polyandry (Jennions and Petrie 2000). Few polygynous social insects are shown to have high queen mating frequencies such as observed in *P. pygmaea*, which might result from difficulty in observing polyandry from worker data of natural multiple queen colonies. Thus, it may be premature to generalize the role of genetic benefits as the prominent driving force of polyandry in social Hymenoptera.

#### APPENDIX: DERIVATION OF EQUATION 4

When additional queens or their mates share genes by common descent, their genetic contribution to the female or male population equals the probability that the given individual carries genes that are not yet represented by the already established individuals. Thus, to calculate the genetically effective population size, the proportion of common genes needs to be subtracted from the default value of each individual, which is 1. The average proportion of genes in the female or male population is indicated by relatedness  $r$  and the proportion of not-common genes by  $1 - r$ . Therefore, the queens and males have the following genetic contributions in the order of their appearance:

$$\begin{aligned} 1. &= 1 \text{ or alternatively } 1. = (1 - r)^0, \\ 2. &= 1 - r \text{ or alternatively } 2. = (1 - r)^1, \\ 3. &= (1 - r)^2, \\ 4. &= (1 - r)^3, \\ n. &= (1 - r)^{n-1}. \end{aligned}$$

To calculate the total genetically effective queen or male population size  $N$ , the contribution of all  $n$  queens or males is summed  $N = \sum_{i=1}^n (1 - r)^{i-1}$ , which can be rearranged to  $N = [1 - (1 - r)^n]/r$ .

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