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# Polyandry and fitness in the western harvester ant, *Pogonomyrmex occidentalis*

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## Abstract

**Using four highly polymorphic microsatellite markers (12–28 alleles), we gentoyped workers from 63 colonies of *Pogonomyrmex occidentalis*. Colonies have a single, multiply mated queen, and an average number of 6.3 patrilines per colony. Colony growth was measured over an 8-year period in the study population. Intracolonial relatedness and colony growth are correlated negatively, indicating a substantial fitness benefit to multiple mating.**

*Keywords*: fitness, growth, mating frequency, microsatellites, relatedness

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## Introduction

Multiple mating is common in insects (reviews of various orders in Choe & Crespi 1997; Simmons & Siva-Jothy 1998; Arnqvist & Nilsson 2000), but it is relatively uncommon among the social insects. Polymorphic microsatellite loci are common in social insects (e.g. Evans 1993); however, the availability of molecular markers has not changed the picture of mating in this group. Although in a large number of species a fraction of queens mate twice (reviewed in Boomsma & Ratnieks 1996; Crozier & Pamilo 1996), there are few species where queens routinely mate a moderate to a large number of times (Strassmann 2001). Honeybees and other members of the genus *Apis* are the pre-eminent example of high mating frequency with species where queens may mate more than 20 times (Éstoup *et al*. 1994; Moritz *et al*. 1995; Oldroyd *et al*. 1995, 1996). Several species of *Vespula* (hornets) (Ross & Visscher 1983; Ross 1986), as well as fungus growing ants of the genus *Acromyrmex* (Reichardt & Wheeler 1996; Fjerdingstad & Boomsma 1998; Fjerdingstad *et al*. 1998; Boomsma *et al*. 1999), mate about seven times per female.

The evolution of multiple mating in social insects is of interest because reduced relatedness due to polyandry eliminates the advantage to reproductive altruism that results from high relatedness. Several hypotheses have been proposed to explain how increased genetic variance within the worker population of the colony improves colony performance (reviewed in Crozier & Fjerdingstad 2001). We will discuss the ones most relevant to high levels of multiple mating. Increased genetic diversity may reduce pathogen load by producing a worker force that is more resistant to diseases and less likely to transmit them to colony mates than one that is genetically uniform (Hamilton 1987; Sherman *et al*. 1988; 1998; Schmid-Hempel 1994; reviewed in Schmid-Hempel 1998; Tarpy 2003). Queens which mate multiply reduce the variance in diploid male production and thus the costs of producing individuals that lower colony fitness (Page 1980; Ross & Fletcher 1986; Ross *et al*. 1993; Cook & Crozier 1995; Keller & Reeve 1995; Tarpy & Page 2002). Colonies in which the queen mates a large number of times may have higher fitness because workers differ genetically in their propensity to perform certain behaviours. As a consequence, a genetically diverse colony will contain a large array of 'specialist' workers which will maximize colony efficiency (Crozier & Page 1985; Calderone & Page 1988, 1991, 1992; Robinson & Page 1988; Robinson 1992; Fewell & Page 1993; Page *et al*. 1995; Fuchs & Moritz 1998; Julian & Cahan 1999; Beshers & Fewell 2001).

Harvester ants of the genus *Pogonomyrmex* are another group of social insects with queens that mate multiple times. Both behavioural observations (e.g. Nagel & Rettenmeyer 1973; Hölldobler 1976) and molecular data (Cahan *et al*. 2002; Julian *et al*. 2002; Volny & Gordon 2002a, 2002b; Gadau *et al*. 2003) show that multiple mating is typical. In an allozyme study of the western harvester ant, *P. occidentalis* Cresson, analysis of a large population indicated that queens mated an average of six times. Colonies with high levels of polyandry enjoyed a substantial fitness advantage

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relative to those with low levels of polyandry, because of differences in growth rate (Cole & Wiernasz 1999). Colony growth was significantly negatively correlated with colony relatedness in this population and consequently the probability of survival during the early stages of colony founding and the time to attain reproductive size. However, in the previous study we could not measure the relatedness of individual colonies with any accuracy, because of insufficient genetic power.

In this study, we use highly polymorphic microsatellites to measure genetic diversity and relatedness in individual colonies of *P. occidentalis*. We combine these data with information on colony growth rates to re-examine our earlier results on the relationship between genetic diversity and colony fitness.

## Materials and methods

We collected workers from colonies that comprise part of a large, well-studied population of *P. occidentalis* in western Colorado (see Wiernasz & Cole 1995 for a description of the study site). These colonies are part of an ongoing study of reproductive allocation in this species (Cole & Wiernasz 2000b) and differ from the colonies used in the previous study of growth and relatedness (Cole & Wiernasz 1999). Ants were collected by agitating the colony until a larger number of workers were outside the nest and then collecting 20–40 individuals at random. Live ants were shipped by overnight freight to the University of Houston, where they were stored at −80 °C until DNA extraction.

DNA was extracted using the PureGene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). We removed the gaster ground ants individually in 75 µL of cold cell lysis solution, and then incubated at 65 °C for 1 h. We then added 25.5 µL of 7.5 M ammonium acetate to each tube, incubated for 10 min on ice, and then centrifuged at 14 000 *g* for 5 min. The supernatant was placed in 75 µL of cold 99% isopropanol (Sigma), and the tubes inverted gently several times to mix. The samples were held at −20 °C for 3.5 h and then centrifuged at 14 000 *g* for 5 min. The isopropanol was poured off, the sample was allowed to dry, and then washed in 80% ethanol for 1.5 h. After another centrifugation step (14 000 g for 5 min) the ethanol was poured off, the tubes allowed to thoroughly air-dry and the DNA resuspended in 60 µL of Tris EDTA (pH 8.0). Extracted DNA was stored at 4 °C.

Microsatellite loci were developed for *P. occidentalis* from libraries constructed by Genetic Identification Services (GIS, Chatsworth, CA, USA). GIS sequenced eight ($[\mathrm{CA}]_n$ library), nine ($[\mathrm{GA}]_n$ library), 45 ($[\mathrm{ATG}]_n$ library) and nine ($[\mathrm{TAGA}]_n$ library) clones; 41 of these (six, six, 24 and five, respectively) contained the repeat motif. In collaboration with Joan Herbers (Ohio State University), primers were constructed for 11 putative microsatellite loci, which were then screened for polymorphism in both Great Plains and western Colorado populations of *P. occidentalis*. We present data based on four of these loci: Po01, Po03, Po07 and Po08 (see Table 1) because they were the most variable in the initial screen.

We used the polymerase chain reaction (PCR) to amplify these loci in 20 workers from each of 63 colonies. All four loci amplify using the same programme. We use 0.6 µL of genomic DNA and 0.11 µL (0.5 unit) *Taq* polymerase (Promega) in a total reaction volume of 18 µL; $\mathrm{MgCl_2}$ concentration was 2.0 mM, primer concentration was 25 mM and dNTP concentration was 2 mM. PCR was performed on either a Techne Genius or an MJResearch DNA Engine using the following programme for all loci: 3 min at 95 °C; 30 cycles of denaturation at 95 °C (30 s), annealing at 52 °C (40 s) and extension at 72 °C (40 s); 3 min at 72 °C.

PCR products were labelled internally using a fluorescent marker (6-Fam, Hex, or Ned®, and separated on an ABI 3100 genetic analyser in the laboratory of Joan Strassmann (Rice University). We used the genographer software program (Benham 2001) to determine each worker's multilocus genotype. The queen's genotype and patriline identities

**Table 1** Summary of the characteristics of the used microsatellite loci

| Locus | F primer/R primer (5′→ 3′) | Core repeat | GenBank Accession no. | No. of alleles (bp) | $H_O$ | $H_E$ | Individuals | Colonies |
|---|---|---|---|---|---|---|---|---|
| Po01 | CAACCCACCCATCACCATC<br>CCGTGTGTCTTTCTATGGATG | $(\mathrm{AC})_{23}$ | AY301006 | 28 (151–205) | 0.974 | 0.930 | 1210 | 63 |
| Po03 | ATTGCTTTCCCACTTGACC<br>TGAAGAGCGATAGGCAGAGT | $(\mathrm{TC})_{24}$ | AY301007 | 19 (160–194) | 0.935 | 0.905 | 1176 | 61 |
| Po07 | TATCCGTGATTTCTGCCTAG<br>GACCGCTAATTTGTCTCTCA | $(\mathrm{TCG})_9(\mathrm{TCA})_4$ | AY301008 | 12 (270–306) | 0.827 | 0.819 | 1193 | 62 |
| Po08 | ACCACCAACCTCATTACGA<br>GCTCAGCATACTGTTCTCCA | $(\mathrm{ATC})_6(\mathrm{CAG})_{16}$ | AY301009 | 15 (223–267) | 0.809 | 0.824 | 1213 | 63 |

F/R primer = sequences of forward/reverse primers used to amplify the loci (5′→3′ direction); no. alleles = number of detected alleles among the genotyped individuals (range of allele sizes); $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity.

were determined by inspection. Allele frequencies ($q_i$) at each locus were calculated from the male genotypes (patrilines) in the sample ($n = 384-401$). Expected heterozygosity was calculated from the allele frequencies at each locus as: $1 - \sum q_i^2$; observed heterozygosity was calculated as the proportion of workers that were heterozygous at each locus.

We calculated three measures of mating frequency. The total number of patrilines is the number that was observed in the sample from each colony. The effective number of patrilines is: $p_e = 1/(\sum q_i^2)$, where $q_i$ is the proportion of workers sired by the $i$th male (Starr 1984). This measure is exact for a large sample, but underestimates $p_e$ when sample sizes are small, because it is possible to miss workers from patrilines that are actually present. The effective number of matings after correcting for this bias has been calculated by Pamilo (1993) as:

$$p_e = (N-1)\Big/\left[\left(N\sum q_i^2\right)-1\right],$$

where $N$ is the sample size, and will always be greater than or equal to $p_e$. The extent of Pamilo's correction is a function of the sample size and the number of patrilines. Relatedness was estimated as $[0.25 + 0.5(1/p_e)]$ (Hamilton 1964), using either the effective number of patrilines or the corrected effective number of patrilines.

To estimate the correlation between the calculated relatedness and the true, but unknown, relatedness in the colony, we performed a randomization procedure. We constructed 63 simulated colonies by randomly sampling the observed distribution of patrilines (Fig. 1A). For each simulated colony we randomly sampled 20 workers from the patrilines of that colony. For each colony we calculated the 'true' relatedness from the patriline number and the estimated relatedness using the random sample of workers. We calculated the correlation for the sample of 63 colonies and we performed 1000 iterations of this procedure to calculate the mean correlation.

We measured the size of the external nest cone to determine colony growth. Nest cone volume [Ln(length) × Ln(width) × Ln(height + 1)] correlates highly ($r \sim 0.9$) with the estimated number of foragers (Wiernasz & Cole 1995). All colonies that are part of our study population are measured annually in July. We calculated growth as the difference in size between 1994 (the year our allocation study began) and 2002. Because small colonies grow more than large colonies, we standardize this measure by using the residuals of size change on the initial size (Cole & Wiernasz 1999). Of the 63 colonies that are in the genetic data set, 44 of the colonies had size measures for both 1994 and 2002 (some colonies died; others were added to the study in 1996). We calculated the relationship between growth and the measures of colony genetic diversity using regression analysis.

## Results

The microsatellite loci are variable (Table 1). Both queens and workers can be heterozygous at any locus, unlike the hybrid forms of other *Pogonomyrmex* (Volny & Gordon 2002a; Cahan *et al*. 2002; Julian *et al*. 2002). Our data are consistent with a colony structure of single queens that all mate multiply.

The average number of patrilines detected in colonies was 6.29 ± 0.19 (SE, $n = 63$ colonies). The average effective patriline number, $p_e$, was 4.62 ± 0.15; with the sample size correction (Pamilo 1993) this becomes 5.98 ± 0.27. There is considerable variability in the patriline numbers, with a range of 2–11 patrilines in our samples of 20 workers (variance = 2.3, Fig. 1a). Approximately 30% of the colonies have eight or more or four or fewer patrilines. Applying Pamilo's (1993) correction it is expected that the range of effective patriline numbers is from 2.0 to 14.6 (Fig. 1B). Mean intracolonial relatedness is low ($r = 0.366 \pm 0.004$, $r_{\text{corrected}} = 0.345 \pm 0.005$). Colonies with the same number of patrilines often have different relatedness due to differences in the distribution of workers among patrilines. The total variance in relatedness is 0.00130. The variance in relatedness that is due to variation in patriline numbers is 0.00081 (62% of the total), while the remainder is a consequence of the unequal distribution of workers among patrilines (because the covariance between the two quantities is very low). The

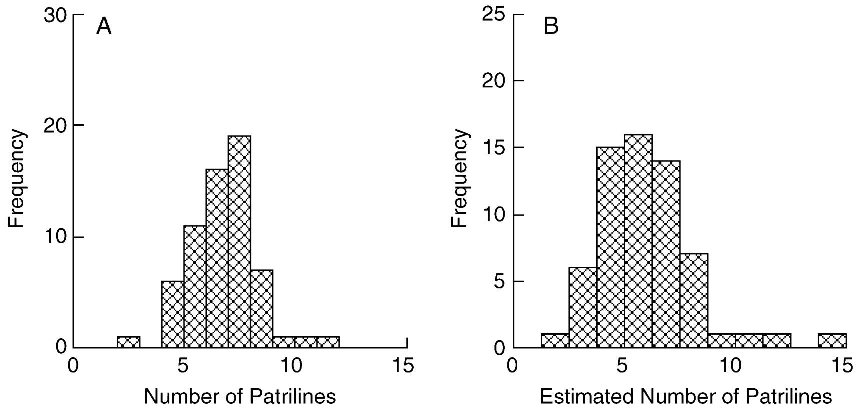

**Fig. 1** (A) The frequency distribution of the number of patrilines per colony. (B) The frequency distribution of the number of patrilines per colony applying the correction of Pamilo (1993).

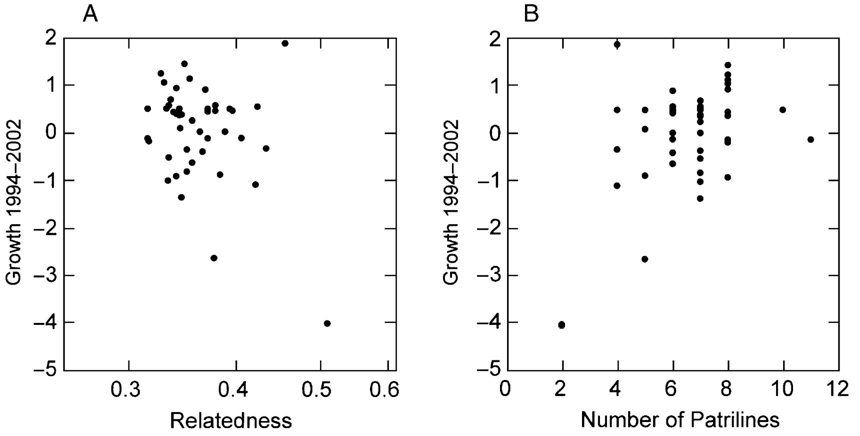

**Fig. 2** (A) The relation between colony growth from 1994 to 2002 and colony relatedness. Relatedness is shown on a log axis to maximize the spread among relatedness measurements (B) The relation between colony growth and the number of patrilines in a colony.

mean correlation between observed and expected relatedness in the randomization is 0.89. This estimate is based on a sample size of 20 workers per colony; the mean sample size of ~19.5 workers negligibly lowered the correlation.

The colonies in this study fall into two distinct geographical groups separated by approximately 500 m. There are no differences in the distribution of patrilines in the two localities (Kolmogorov–Smirnov two-sample test statistic = 0.21, $P > 0.45$).

All the measures of genetic diversity (patrilines, relatedness and corrected relatedness) are significantly correlated with growth over the 8-year interval 1994–2002. Relatedness ($r = -0.35$, $P = 0.019$, $n = 44$ colonies in all cases, Fig. 2A) and corrected relatedness ($r = -0.35$, $P = 0.020$) were virtually identical, while the number of patrilines was slightly more correlated ($r = 0.38$, $P = 0.01$, Fig. 2B). By any measure increasing amounts of genetic diversity are associated with more rapid colony growth.

## Discussion

*P. occidentalis* colonies are the product of a single, multiply mated queen. In contrast to a previous suggestion (Fjerdingstad & Keller 2000), we found no evidence of polygyny in our population. While all queens mate more than a single time, there is a great deal of variability within the population, both in the mating frequency of queens and in the genetic diversity of colonies. As in other polyandrous species (Fernández-Escudero *et al*. 2002; Gadau *et al*. 2003), both variation in the number of patrilines and differences in the apportionment of offspring among patrilines contribute to variation in genetic diversity. At the population level we observe that most of the variation in relatedness among colonies is due to differences in the mating frequency but that an appreciable fraction is due to differences in sperm usage or to the amount of sperm contributed by each male.

Increased genetic diversity in colonies of *P. occidentalis* is due solely to increased mating frequency rather than to differences in the number of queens or the possibility of intraspecific brood raiding. In the 1213 ants genotyped in this study, all workers could be assigned to the single resident queen. Gadau *et al*. (2003) showed that some workers from their collections of *P. rugosus* were apparently not from the home colony. They interpreted this result as a probable instance of intraspecific brood raiding. It is also well known that worker 'drifting', the observation that foreign forager bees move among hives, occurs frequently in colonies of honeybees (see, e.g. Neumann *et al*. 2000). Gadau *et al*. (2003) collected workers from 14 colonies in the field. Of those, collections were made of foraging workers on trunk trails in three colonies. Each of these collections showed multiple matrilines. It is possible that occasional workers from multiple colonies may share the same foraging trunk trail. In the remaining 11 colonies, there were foreign workers among the sample in three of them. Whether worker drifting or intraspecific brood raiding is occurring in *P. rugosus*, a similar phenomenon is not occurring in *P. occidentalis.*

Cole & Wiernasz (1999, 2000a) showed that colony growth was negatively correlated with colony relatedness. Although the correlation was low, $r = -0.102$, it was highly significant due to the large number of colonies measured ($n = 1492$). In the previous study, because we could not measure the relatedness of individual colonies accurately, we used path analysis to estimate that the true correlation between relatedness and growth would be −0.86. In this study we can measure relatedness more accurately within a single colony and growth has been measured over 8 years. The correlation in the current study (−0.35) is substantially stronger than the previously reported correlation. Given the correlation between estimated colony growth and actual colony growth ($r = 0.89$, Cole & Wiernasz 1999) and the correlation between measured relatedness and actual relatedness ($r = 0.89$) in this study, we estimate that the true correlation is approximately −0.56. These estimates (−0.86 and −0.56) are broadly similar; both reflect a substantial advantage when queens mate multiple times. The smaller value in the current study is probably a consequence of the size of the colonies in this study. Because growth rates decrease when colonies reach reproductive size, differences in colony growth rate decline (Wiernasz & Cole 1995).

The effect of multiple mating on the colony phenotype has the effect of a material benefit to the queen, in that increased genetic variation among workers increases the probability of colony (queen) survival, longevity or reproductive effort, ultimately yielding higher lifetime reproductive success. In this species, the mechanism by which genetic diversity benefits the colony is likely to operate in one of two ways. A growing body of evidence suggests that pathogens and parasites are important selective agents in the survival and growth of social insect colonies, and that increased genetic diversity reduces parasite load (Schmid-Hempel 1998; Baer & Schmid-Hempel 1999). Alternatively, a genetically diverse worker force may result in higher colony efficiency (reviewed in Beshers & Fewell 2001). While a number of studies have demonstrated that worker patrilines differ in task performance (e.g. Hughes *et al*. 2003), there is little evidence that these differences lead to increased colony performance or fitness.

Both mechanisms may contribute to selection for multiple mating in harvester ants. In long-lived species, such as *P. occidentalis* (Wiernasz & Cole 1995), the pathogens are expected to coevolve with their host. Coevolution by pathogens is slowed in highly polyandrous colonies, because each genotype will be relatively uncommon; reproductive daughters of a polyandrous female may enjoy a frequency-dependent advantage in survival (Hamilton 1987). Maximizing colony performance through division of labour may be especially important in a genus such as *Pogonomyrmex*, where mature colonies contain tens of thousands of workers (Lavigne 1969; Gordon 1999) and are structurally complex (Tschinkel 2001). The current data do not favour one hypothesis — increased growth could result from a lower pathogen load or from increased foraging success or more efficient brood care. We suggest that the two mechanisms need not be mutually exclusive: colony survival may be enhanced if workers differ in their propensity to groom nestmates or in their ability to detect pathogens.

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