ORIGINAL PAPER

Low queen mating frequency in the seed-harvester ant *Pogonomyrmex* (*Ephebomyrmex*) *pima*: implications for the evolution of polyandry

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Abstract The evolution of polyandry is a central problem in the study of insect mating systems, and both material and genetic benefits have been proposed to offset the presumed costs of multiple mating. Although most eusocial Hymenoptera queens mate with just one or occasionally two males, high levels of polyandry are exhibited by several taxa, including seed-harvester ants of the genus *Pogonomyrmex*. Previous studies of queen mating frequency in *Pogonomyrmex* have focused on monogynous (one queen per colony) species in the subgenus *Pogonomyrmex*. We performed a genetic mother–offspring analysis of mating frequency in *Pogonomyrmex* (*Ephebomyrmex*) *pima*, a queen-dimorphic species with dealate and intermorph queens that differ in colony structure (intermorph colonies contain multiple queens). Our results demonstrate that both dealate and intermorph queens of *P.* (*E.*) *pima* are typically single maters, unlike their congeners analyzed thus far. Polyandry appears to be a derived trait in *Pogonomyrmex*, but comparative tests between *P.* (*E.*) *pima* queen morphs and across the genus provide no evidence that it evolved as an adaptation to increase genetic diversity within colonies or to obtain more sperm, respectively.

Keywords Genetic diversity · Multiple mating · Queen number · Social insects · Sperm limitation

Communicated by K. Ross

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Introduction

Why do females often mate with multiple males (polyandry)? While the selective advantage of multiple mating by males is obvious (fecundity is typically limited by the number of mates), the reproductive potential of females is generally constrained by higher investment in gamete production and parental care (Trivers 1972). Furthermore, polyandry can incur costs in terms of time, energy, and prolonged exposure to predators and pathogens (Daly 1978). In many insects, the costs of multiple mating appear to be offset by material benefits, such as increased sperm, gonadotropic accessory substances, and/or nutrients, which elevate female lifetime offspring production (Ridley 1988; Arnqvist and Nilsson 2000). Polyandry may also confer genetic advantages, including post-copulatory paternity biasing and increased genetic diversity among progeny (Eberhard 1996; Zeh and Zeh 1996, 1997; Jennions and Petrie 2000; Simmons 2001).

Whereas multiple mating is common among solitary insects (Arnqvist and Nilsson 2000), most queens of eusocial Hymenoptera (ants, bees, and wasps that live in societies marked by reproductive division of labor between queens and functionally sterile workers) mate with just one or occasionally two males; obligate polyandry (effective queen mating frequency ≥ 2) has been documented only in honey bees (*Apis* spp.), higher vespine wasps (*Vespula* spp.), and a handful of ant taxa (*Pogonomyrmex* seed-harvester ants; *Acromyrmex* and *Atta* leafcutter ants; *Aenictus*, *Dorylus*, *Eciton*, and *Neivamyrmex* army ants; and *Cataglyphis cursor*; reviewed by Strassmann 2001; Boomsma et al. 2007). The reproductive biology of eusocial Hymenoptera differs from that of many solitary insects—mating occurs during a brief period early in adulthood, females store a lifetime supply of sperm, and

males provide little nourishment. Moreover, social life influences the costs and benefits of multiple mating; for example, polyandry lowers relatedness among nestmates, which may decrease the inclusive fitness payoff for altruistic workers (Hamilton 1964; Bourke and Franks 1995; Crozier and Pamilo 1996).

Numerous hypotheses have been proposed to explain the adaptive significance of multiple mating in social insects (reviewed by Crozier and Page 1985; Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001). The leading candidate for a material benefit is an ample supply of sperm; the sperm limitation hypothesis suggests that queens in species with large, long-lived colonies need to obtain more sperm than provided by one male to maximize their fecundity (Cole 1983; Fjerdingstad and Boomsma 1998).

In contrast, polyandry may be favored because of its effects on the genetic structure of social insect colonies. In eusocial Hymenoptera, haplodiploid sex determination (diploid females develop from fertilized eggs, haploid males from unfertilized eggs) results in female workers that are more closely related to their full sisters, sons, and nephews than to their brothers, thereby generating divergent reproductive interests between queens and workers. Multiple mating may help to resolve potential queen–worker conflict over male production (Starr 1984; Woyciechowski and Lomnicki 1987; Ratnieks 1988; Pamilo 1991) and/or sex allocation (Queller 1993; Ratnieks and Boomsma 1995; but see Fjerdingstad et al. 2002) by diminishing these relatedness asymmetries.

Increased genetic diversity among workers could also enhance colony-level fitness by: (1) reducing variance in the production of sterile, non-functional diploid males, which occur when a paternal male shares a sex locus allele with the queen (Page 1980; Page and Metcalf 1982; Tarpy and Page 2002; Kronauer et al. 2007), (2) hindering disease transmission (Hamilton 1987; Sherman et al. 1988; Schmid-Hempel 1998; Baer and Schmid-Hempel 1999; Tarpy 2003; but see Kraus and Page 1998), (3) facilitating task differentiation and a more efficient division of labor (Page and Robinson 1991; Fewell and Page 1993; Fuchs and Moritz 1999; Myerscough and Oldroyd 2004), and/or (4) providing a buffer against environmental variability (Crozier and Page 1985; Page et al. 1995; Jones et al. 2004; but see Fjerdingstad 2004). We collectively refer to the queen–worker conflict and intracolonial genetic diversity hypotheses as the genetic benefits hypotheses, although conflict resolution would favor queens and genetic diversity would benefit entire colonies. Several of these hypotheses are not mutually exclusive, and the mechanisms underlying the evolution and maintenance of multiple mating may be pluralistic and/or taxon-specific (Crozier and Fjerdingstad 2001).

The proposed genetic benefits of polyandry may also be attained through the presence of multiple egg-laying queens per colony (polygyny). Polygyny, which is generally thought to be promoted by ecological constraints on independent colony founding (e.g., nest site limitation; Herbers 1993), increases intracolonial genetic diversity and, if queens are related, reduces relatedness asymmetry (Pamilo 1991), precluding the need for costly multiple mating. Thus, the genetic benefits hypotheses predict that polyandry should be less frequent among polygynous species than among monogynous species (one egg-laying queen per colony). Keller and Reeve (1994) reported a negative association between queen-mating frequency and number of queens per colony in ants, but reliable paternity data were lacking at that time (Boomsma and Ratnieks 1996). Over the past decade, genetic analyses of monogynous and polygynous ants, bees, and wasps have revealed obligate polyandry exclusively in monogynous species (reviewed by Boomsma et al. 2007). An important next step is to examine variation in mating frequency among closely related species that differ in queen number or within species that form both monogynous and polygynous colonies.

Seed-harvester ants of the genus *Pogonomyrmex* (subfamily Myrmicinae) are known to be highly polyandrous; queens mate with an average of 5 to 18 males among monogynous species in the subgenus *Pogonomyrmex* (Cole and Wiernasz 1999; Wiernasz et al. 2004; Gadau et al. 2003; Rheindt et al. 2004; Pol et al., unpublished data). In one of these species, *P. (P.) occidentalis*, a positive correlation between queen mating frequency and colony fitness suggests that multiple mating confers a colony-level genetic benefit (Cole and Wiernasz 1999; Wiernasz et al. 2004). However, mating frequency has not been analyzed in any polygynous *Pogonomyrmex* species or members of the subgenus *Ephebomyrmex*, which could shed light on when and why polyandry evolved.

We performed a genetic mother–offspring analysis of pedigree-effective mating frequency in *Pogonomyrmex (Ephebomyrmex) pima*, a queen-dimorphic species with larger, winged (alate) queens and smaller, wingless (intermorph) queens, which do not co-occur within colonies (Johnson et al. 2007). Alate queens fly to swarms to mate, then remove their wings (now referred to as dealates) and found colonies independently (Johnson et al. 2007; RAJ, unpublished data). In contrast, intermorph queens appear to mate at their natal nest and are then readopted, with colonies later reproducing by budding—a process that occurs in other ant species with wingless queens (Johnson et al. 2007; RAJ, unpublished data; Heinze and Tsuji 1995). Colony structure also varies between queen morphs; censused intermorph queen colonies contained multiple inseminated queens (at least 2–14 per colony), whereas

partial and complete excavations of mature and incipient dealate colonies, respectively, never revealed more than one mated queen (Johnson et al. 2007). Because polygyny ensures genetic diversity in intermorph colonies, a higher mating frequency of dealate queens would support the genetic benefits hypotheses. In addition, we used comparative data on mating frequency and colony size across *Pogonomyrmex* to infer the phylogenetic origin of multiple mating in the genus and to evaluate the sperm limitation hypothesis.

Materials and methods

Collections and laboratory rearing

Pogonomyrmex (E.) *pima* is restricted to the Sonoran Desert of North America (Johnson 2000). We studied a population approximately 2 km south of the Sacaton Mountains, Pinal County, Arizona (32°56′N, 111°42′W, elevation 430 m). Newly mated dealate queens were collected after mating flights in September 2002 and 2004. We also excavated mature dealate and intermorph queen colonies from March–May 2003 and May–Aug 2005. Excavated colonies were transferred to the laboratory, where each individual was identified as dealate queen, intermorph queen, or worker (Johnson et al. 2007). We reared brood from newly mated and excavated queens using plaster nest containers that were maintained at 30°C. Single newly mated dealate queens were collected before colony founding and thus kept in isolation, whereas each queen from a mature colony was isolated with workers from that colony. This procedure facilitated paternity analysis without the confounding effects of polygyny. Frozen fruit flies and Kentucky bluegrass seeds were provided ad libitum. Larvae, pupae, and callow workers were removed from colonies as they became available and stored in 95% ethanol at −20°C for later analysis.

Genetic analyses

In 2003, DNA was extracted following a modified version of the Puregene® DNA Purification Kit protocol (Gentra Systems) used by Gadau et al. (2003). In 2006, DNA was extracted using a standard Chelex protocol. Preserved individuals were dried and crushed in liquid nitrogen. We added 50 μl of 5% Chelex and 25 mg Protease K. Samples were then incubated at 57°C for 1 h, boiled at 95°C for 5 min, and centrifuged at 14,000 rpm for 10 min. The supernatant was removed and stored at −20°C.

We amplified four polymorphic microsatellite loci: L18 (Foitzik et al. 1997), LxAGT1 (Bourke et al. 1997), Myrt3 (Evans 1995), and Pb8 (Volny and Gordon 2002a). Myrt3

was used only in 2006. Polymerase chain reactions (PCR) were performed in 25.0 μl volumes containing 1.0 μl diluted (1:10) template DNA, 2.5 mM MgCl$_2$, 0.1 mM of each deoxyribonucleotide triphosphate (dNTP), 0.4 μM of each primer (labeled with either IRDye 700 or IRDye 800; Li-Cor), 5.0 μl of 5× *GoTaq* buffer, and 0.5 U *Taq* DNA polymerase (Promega). Loci were amplified using the following PCR touchdown program: an initial denaturation step of 5 min at 94°C; 15 cycles of 45 s at 94°C, 45 s at 60–45°C (−1°C per cycle), and 45 s at 72°C; 20 cycles of 45 s at 94°C, 45 s at 55°C (L18: 53°C), and 45 s at 72°C; and a final elongation step of 10 min at 72°C. PCR products were genotyped using a Li-Cor 4300 DNA analyzer. Alleles were scored using the program SAGA (Li-Cor) and homologized across colonies by running them all on one gel.

Estimating mating frequency

We assigned patrilines and estimated mating frequency using queen and offspring genotypes from colonies that had at least seven diploid offspring. Several queen genotypes were inferred from the multilocus genotypes of offspring. We performed calculations manually in 2003 and used the program MateSoft 1.0 (Moilanen et al. 2004) in 2006. Overall, we analyzed 299 diploid offspring from 11 dealate and 9 intermorph queens. All 16 offspring of intermorph queen PpI6 were homozygous at LxAGT1 for either of the two observed maternal alleles, suggesting double mating. However, the likelihood of all 16 offspring being homozygous for either of two alleles is very low (we would expect eight heterozygotes), and the genetic structure observed across the other three loci was most parsimoniously explained by monandry. Therefore, we deduced the presence of a null (i.e., non-amplifying) paternal allele at LxAGT1 and scored offspring using a mock allele that assumed single mating (Dakin and Avise 2004).

We calculated heterozygosity and inbreeding coefficients (F_{IS}) and conducted χ^2 tests for Hardy–Weinberg (H–W) and linkage equilibrium at each locus using a larger sample of observed and inferred queen genotypes (one individual per colony; $n = 27$) and the program GDA 1.1 (Lewis and Zaykin 2002). Two sources of error can occur when estimating mating frequency: (1) nondetection due to limited genetic variability (i.e., failing to separate two fathers that have the same genotype), and (2) excluding rare patrilines due to low sample size (Boomsma and Ratnieks 1996). We computed the average weighted non-identification error (f') due to nondetection and sampling error (see Pedersen and Boomsma 1999a).

Numerical mating frequency (k_{obs}) is the minimum number of mates required to explain observed offspring genotypes. Pedigree-effective mating frequency accounts

for the relative contributions of multiple fathers to colony offspring and is defined as

$$m_e = \frac{1}{\sum p_i^2}$$

where p_i is the proportional paternity contribution of the ith male (Starr 1984). Nielsen et al. (2003) derived the following unbiased estimator of effective mate number corrected for sampling error:

$$m_{e3} = \frac{(n-1)^2}{\sum p_i^2 (n+1)(n-2) + 3 - n}.$$

To test whether dealate queens mate with more males than intermorph queens (genetic benefits hypotheses), we compared sample-size corrected effective mating frequencies (m_{e3}) of the two queen morphs using a Mann–Whitney U test. Under certain conditions (discussed below), the sperm limitation hypothesis predicts that mating frequency should increase with colony size across species. We tested for an association between effective mating frequency and maximum colony size across *Pogonomyrmex* species for which data are available using a Spearman rank correlation. All values are reported as mean±1 SE.

Results

Microsatellite loci were highly variable, with 2 to 23 alleles per locus and expected heterozygosities (H_E) that ranged from 0.41 to 0.95 (Johnson et al. 2007). Allele frequencies at each locus conformed to H–W equilibrium ($P > 0.2$), and there was no evidence of linkage disequilibrium between loci ($P > 0.3$). The 95% confidence interval of F_{IS} bootstrapped across loci (−0.093–0.123) overlapped with zero, indicating a lack of inbreeding.

Sample-size corrected effective mating frequency (m_{e3}) did not differ between dealate (1.21±0.16) and intermorph queens (1.07±0.06) of *P. (E.) pima* ($n_{dealate} = 11$, $n_{intermorph} = 9$, $U = 53$, $P = 0.7$; Table 1). Data were pooled across queen morphs for further analysis. Mean numerical mating frequency (k_{obs}) was 1.30±0.13; offspring genotypes revealed single mating in 15 queens, double mating in four queens, and triple mating in one queen (Fig. 1). Skewed paternity contributions among offspring of multiply-mated queens resulted in a population-wide pedigree-effective mating frequency (m_{e3}) of 1.18±0.12 when corrected for sampling error (Table 1; uncorrected $m_e = 1.15±0.09$). Mate number was not underestimated because of low genetic variation or sample size (average weighted non-identification error $f' = 0.04$; i.e., 4% probability of failing to identify an additional patriline). Across *Pogonomyrmex* species, effective mating frequency was not correlated with maximum colony size ($n = 6$, $r_s = -0.116$, $P = 0.8$; Table 2).

Table 1 Samples used for genetic mother-offspring analysis of mating frequency in *Pogonomyrmex* (*Ephebomyrmex*) *pima*

Dealate queens				Intermorph queens			
Queen	n[a]	k_{obs}[b]	m_{e3}[c]	Queen	n[a]	k_{obs}[b]	m_{e3}[c]
PpD1	46	1	1.00	PpI1	19	1	1.00
PpD2	7	2	1.19	PpI2	14	1	1.00
PpD3	16	1	1.00	PpI3	14	2	1.16
PpD4	18	1	1.00	PpI4	28	1	1.00
PpD5	14	1	1.00	PpI5	9	1	1.00
PpD6	11	1	1.00	PpI6	16	1	1.00
PpD7	11	1	1.00	PpI7	8	1	1.00
PpD8	12	1	1.00	PpI8	16	2	1.54
PpD9	16	1	1.00	PpI9	7	1	1.00
PpD10	9	3	3.36				
PpD11	8	2	1.44				
Mean	15.3	1.4	1.27	Mean	14.6	1.2	1.08

[a] Number of genotyped diploid offspring
[b] Observed number of patrilines
[c] Sample-size corrected pedigree-effective mating frequency (Nielsen et al. 2003)

Discussion

The origin of multiple mating in *Pogonomyrmex*

Pogonomyrmex (*E.*) *pima* is the first predominantly monandrous species to be analyzed in the otherwise polyandrous genus *Pogonomyrmex* (Table 2). A minority of *P. (E.) pima* queens mated two or three times, which may result from multiple males clustering around females in mating swarms. Because *Ephebomyrmex* is the sister taxon
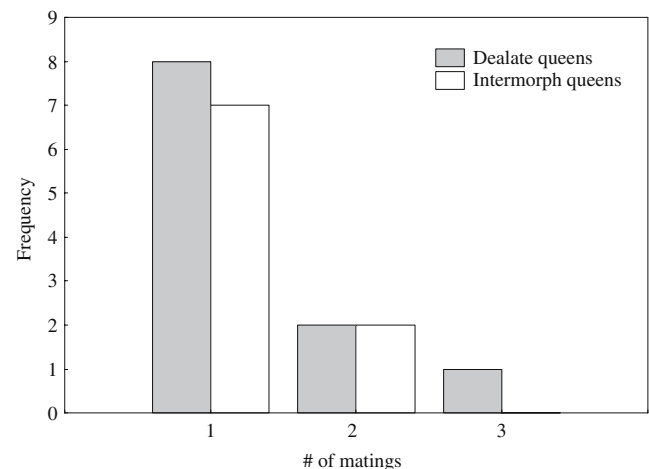
Fig. 1 Frequency distribution of number of matings by dealate and intermorph queens of *Pogonomyrmex* (*Ephebomyrmex*) *pima*. Values represent numerical mating frequencies (k_{obs}) uncorrected for paternity skew or sample size. Estimates were obtained through genetic mother–offspring analysis using microsatellite markers. Sample-size corrected effective mating frequency did not differ between dealate and intermorph queens (see text for statistical details)

Table 2 Queen mating frequencies, number of queens per colony, and colony size in *Pogonomyrmex* seed-harvester ants

Species	m_e[a]	No. queens[b]	Colony size[c]
P. (E.) pima	1.18 ± 0.12[d]	m-p	400[i]
P. (P.) rugosus	4.71 ± 2.02[e]	m	14,700[j]
P. (P.) occidentalis	5.98 ± 0.27[f]	m	8,800[k]
P. (P.) badius	9.16 ± 2.95[g]	m	8,100[l]
P. (P.) inermis	10.46 ± 1.52[h]	m	700[m]
P. (P.) n. sp. ARG-01	18.00 ± 2.53[h]	m	700[m]

P (P.) barbatus is also polyandrous ($m_e > 3$; Volny and Gordon 2002b; Helms Cahan et al. 2002), but available estimates of mating frequency are confounded by genetic caste determination

[a] Mean sample-size corrected effective mating frequency ($\pm$SE)
[b] Monogyny (m), polygyny (p), or mixed (m-p)
[c] Approximate maximum number of workers per colony
[d] This study
[e] Gadau et al. 2003
[f] Wiernasz et al. 2004
[g] Rheindt et al. 2004
[h] Pol et al., unpublished data
[i] Johnson et al. 2007
[j] MacKay 1981
[k] Lavigne 1969
[l] W.R. Tschinkel, personal communication
[m] RAJ, unpublished data

to the subgenus *Pogonomyrmex* (Taber 1998; Strehl 2005; J. Gadau, unpublished data), the difference in mating frequency between *P. (E.) pima* and its multiply-mated congeners in the subgenus *Pogonomyrmex* implies that high levels of polyandry either evolved in the subgenus *Pogonomyrmex* or were secondarily reduced to near-single mating in *Ephebomyrmex*. Both scenarios are equally parsimonious; however, low mating frequencies in the closely related genus *Myrmica* ($1.0 < m_e < 1.2$; Boomsma et al. 2007; Brady et al. 2006) favor the hypothesis that polyandry is derived, as has been inferred for other ants, bees, and wasps (Foster et al. 1999; Strassmann 2001; Villesen et al. 2002; Kronauer et al. 2007). Data are needed from additional species in both subgenera to determine the precise transition from monandry to polyandry.

If polyandry is derived in *Pogonomyrmex*, then what triggered the shift from single to multiple mating? Research on fungus-growing ants (tribe Attini) has linked the evolution of polyandry to specialization for leafcutting behavior and increased colony size and longevity (Villesen et al. 2002). Similarly, the origin of multiple mating in seed-harvester ants may have coincided with changes in diet, disease susceptibility, and/or social organization. At this time, we are unable to identify specific selective pressures for polyandry and/or intracolonial genetic diversity that have operated in the subgenus *Pogonomyrmex* but not in *Ephebomyrmex*.

Genetic benefits

Although intermorph colonies of *P. (E.) pima* contain more queens than dealate colonies, both queen morphs typically mated with a single male, failing to support the genetic benefits hypotheses. The costs of multiple mating for dealate queens may outweigh any benefits of increased intracolonial genetic diversity. Likewise, low mating frequencies are exhibited by both dispersing and non-dispersing queens of *Formica paralugubris* (Chapuisat 1998) and monogynous and polygynous queens of *Myrmica sulcinodis* (Pedersen and Boomsma 1999b) and *Solenopsis invicta* (Ross and Fletcher 1985), despite differences in colony structure that would favor alternative mating strategies. On the other hand, these findings could reflect constraints against the evolution of dimorphic mating strategies within species, suggesting that inter-specific comparisons of mating frequency and queen number may be more fruitful.

At the species level, single mating by *P. (E.) pima* may be consistent with the genetic benefits hypotheses if dealate and intermorph queens exhibit a common response to selection on mating frequency. Intermorph colonies are relatively frequent in the two populations of *P. (E.) pima* studied intensively to date (61–82%; RAJ, unpublished data), and there is no evidence of nuclear or mitochondrial genetic divergence between dealate and intermorph queens (Johnson et al. 2007). Even if an allele for multiple mating increased the fitness of dealate queens, it could have difficulty spreading in a population where polygyny ensures genetic diversity in the majority of colonies.

Pogonomyrmex (E.) pima is the only *Pogonomyrmex* species exhibiting any degree of polygyny for which mating frequency has been determined (Table 2). Evaluating the relationship between mating frequency and queen number across *Pogonomyrmex* would require more information on the mating strategies of polygynous congeners such as *P. (E.) imberbiculus* (Heinze et al. 1992) and one population of *P. (P.) californicus* (Johnson 2004).

Sperm limitation

Comparative data refute the hypothesis that queens of *Pogonomyrmex* species with larger colonies mate with more males (Table 2), a trend which occurs more broadly across ant genera and has been cited as general support for sperm limitation (Cole 1983; but see Boomsma and Ratnieks 1996). Our analysis was not adjusted for phylogenetic non-independence because there is no re-solved phylogeny within the genus and a single origin of polyandry cannot be ruled out. However, the six species examined thus far span a wide taxonomic distribution. The four North American species (*P. pima*, *P. rugosus*, *P.*

occidentalis, and *P. badius*) include one member of the subgenus *Ephebomyrmex* and three species from the subgenus *Pogonomyrmex* representing each of the three species complexes (Cole 1968). The two South American species (*P. inermis*, *P.* n. sp. ARG-01) represent two additional species complexes that are restricted to that continent (Kusnezov 1951).

Sperm limitation may depend on several factors in addition to colony size: number of queens per colony, longevity of colonies and individual workers, reproductive allocation, and amount of sperm provided by males relative to the requirements of queens (Boomsma and Ratnieks 1996; Fjerdingstad and Boomsma 1998). *Pogonomyrmex* (*E.*) *pima* is the only focal species in which polygyny may reduce selection on individual queens for high fecundity; the others are exclusively monogynous (Table 2). However, the high mating frequencies of *P.* (*P.*) *inermis* and *P.* (*P.*) n. sp. ARG-01, which have relatively small colonies, are unlikely to be explained by differences in longevity and/or reproductive allocation between these species and the other (large-colony) polyandrous *Pogonomyrmex* species (Pol et al., unpublished data; Table 2). Finally, a survey across the genus indicated that virgin males in all of 12 species, including *P.* (*E.*) *pima*, contained several times the number of sperm as fully inseminated queens (RAJ, unpublished data). Thus, a single male is able to completely fill a female's sperm storage organ, or spermatheca, although it is unknown whether *Pogonomyrmex* males mate multiply as in the leafcutter ant *Acromyrmex versicolor* (Reichardt and Wheeler 1996).

In ants and other taxa, polyandry may confer material benefits, including an increased sperm supply. Queens of *Atta colombica* and *Lasius niger* obtain more sperm when they mate with more males (Fjerdingstad and Boomsma 1998; Fjerdingstad and Keller 2004), although this could result from multiply-mating males dividing their sperm among females after the origin of polyandry. Mating itself has been shown to increase female fitness (longer lifespan, earlier oviposition) in *Cardiocondyla obscurior*, and multiple mating could have a similar positive effect in other species (Schrempf et al. 2005). However, the act of remating and/or maintaining larger, genetically diverse sperm stores may also carry metabolic costs, as evidenced by reduced immune response and apparent lower survival during colony founding in *A. colombica* queens with more mates and sperm (Baer et al. 2006).

In solitary insects where males do not provide nuptial gifts, positive effects of remating on female fertility and egg production may be mediated by replenishment of depleted sperm supplies (Thornhill and Alcock 1983; Gromko et al. 1984) and/or transfer of gonadotropic accessory substances with the ejaculate (Eberhard and Cordero 1995; Arnqvist and Nilsson 2000; Colonello and Hartfelder 2005). Little is known about the function of male accessory gland products in social insects (Boomsma et al. 2005), but Colonello and Hartfelder (2005) found no effect of peptide or lipid extracts on oogenesis in *Apis mellifera*.

Conclusions

Pogonomyrmex (*E.*) *pima* provides the first documented case of mainly single mating in a genus otherwise known to be highly polyandrous. We infer that multiple mating is a derived trait in *Pogonomyrmex*, but intra- and interspecific comparisons fail to support either the genetic benefits or sperm limitation hypotheses as an explanation for its evolution. *Pogonomyrmex* is an ideal system for examining the evolution and maintenance of multiple mating in social insects. While other genera tend to be either predominantly monandrous (with occasional, or facultative, multiple mating) or exclusively obligately polyandrous (Boomsma et al. 2007; but see Sumner et al. 2004), *Pogonomyrmex* exhibits wide variability in mating frequency that may correspond with differences in social organization, life history, and/or ecology. Further genetic analyses of reproductive behavior, experimental investigation of the fitness consequences of intracolonial genetic diversity, and a molecular phylogeny should provide insight into the selective forces shaping mating strategies in seed-harvester ants and perhaps other social insects.

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