

Research article

## Polyandry in two South American harvester ants

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**Abstract.** Although monandry (single mating) is the ancestral state in social hymenopteran insects, effective mating frequencies greater than 2 have been confirmed for a fair amount of ant species: *Cataglyphis cursor*, the leaf-cutters of the genera *Atta* and *Acromyrmex*, army ants of the genera *Eciton*, *Dorylus*, *Aenictus* and *Neivamyrmex*, and some North American seed harvester species of the genus *Pogonomyrmex*. This last genus spreads throughout open arid habitats from Patagonia to southwestern Canada. Whereas some North American *Pogonomyrmex* species are thoroughly studied, we know much less about these ants in South America. The objective of this study was to estimate the effective mating frequency of *Pogonomyrmex inermis* and *P. pronotalis*, two *Pogonomyrmex sensu stricto* species from the central Monte desert of Argentina. A total of 477 *P. pronotalis* workers from 24 colonies and 402 *P. inermis* workers from 20 colonies were analyzed using six and four highly polymorphic microsatellites, respectively. The multilocus analysis revealed that all colonies were monogynous and all queens multiply-mated. The effective mating frequency was 8.75 and 6.52 for queens of *P. pronotalis* and *P. inermis*, respectively; those values increased up to 15.66 and 9.78, respectively, when corrected for sampling errors. This is the first demonstration that queens in at least some members of the South American *Pogonomyrmex sensu stricto* are strictly polyandrous, with mating numbers per queen at least as high as those previously found for North American species. We suggest that multiple mating probably arose early in the evolution of the genus *Pogonomyrmex* and may be the basis of its ecological success and wide distribution.

**Keywords:** Ants, *Pogonomyrmex inermis*, *Pogonomyrmex pronotalis*, polyandry, mating frequency, microsatellites, Monte desert.

### Introduction

Monandry, or single mating by queens, is probably the ancestral state in the social hymenopteran insects (Hamilton, 1964; Strassmann, 2001; Brown and Schmid-Hempel, 2003). Strassmann (2001) suggested that single mating has facilitated the origins of sociality as it leads to high relatedness among nestmates. Indeed, phylogenetic studies of the attine ants and vespine wasps found that multiple mating is clearly a derived trait (Foster et al., 1999; Villesen et al., 2002). Still, multiple queen mating is a rare but taxonomically widespread phenomenon that has arisen convergently on several occasions in social hymenopterans (reviewed in Boomsma and Ratnieks, 1996; and Strassmann, 2001). In ants, high effective mating frequencies have now been confirmed for a fair amount of ant species: *Cataglyphis cursor* (Pearcy et al., 2004), leaf-cutter ants in the genera *Atta* and *Acromyrmex* (Bekkevold et al., 1999; Boomsma et al., 1999; Villesen et al., 1999; Fjerdingstad and Boomsma, 2000; Murakami et al., 2000), army ants of the genera *Eciton*, *Dorylus*, *Aenictus* and *Neivamyrmex* (Denny et al., 2004; Kronauer et al., 2004, 2007), and some seed harvesters of the genus *Pogonomyrmex* (Cole and Wiernasz, 1999, 2000; Volny and Gordon, 2002a; Gadau et al., 2003; Rheindt et al., 2004; Wiernasz et al., 2004).

The genus *Pogonomyrmex* is endemic to the Americas. It has two subgenera (*Ephebomyrmex* and *Pogono-*

**Table 1.** Microsatellite loci developed for *Pogonomyrmex pronotalis*.

Locus	Primer sequence (5' to 3')	Annealing temp. (°C)	Core repeat	Size range (bp)
Ppro1	F: CCTTCCAATATCACATTATTGC R: GTATTGTGGACAATTCTAGC	58	(TC) <sub>8</sub> T <sub>4</sub> (TC) <sub>12</sub>	218–248
Ppro2	F: CTCAGAAGACGCAGGAACG R: ATCCGTGAGCCAAACAACCTGC	58	(CG) <sub>7</sub> N <sub>38</sub> (GA) <sub>20</sub>	238–254
Ppro3	F: TGGCTAGGCACTTGCAGG R: ATCATCAATAATTCCTGAGAGC	52	(GA) <sub>27</sub>	313–351
Pbad2	F: CGGAATGTAGGAGCAACG R: ATATTGAAATTCGCATGTGG	55	(TC) <sub>17</sub> (TCCC) <sub>2</sub>	184–218

*myrmex sensu stricto*; see Taber, 1998 for taxonomic grouping) comprising about 60 species (MacMahon et al., 2000). They occur principally throughout open arid habitats from Patagonia to southwestern Canada. They have been extensively studied in North American deserts, where they are a dominant feature (MacKay, 1991; Johnson, 2000, 2001; MacMahon et al., 2000). To date, members of all four major Nearctic species groups of *Pogonomyrmex sensu stricto* have been genetically investigated: *Pogonomyrmex occidentalis* (Cole and Wiernasz, 1999, 2000; Wiernasz et al., 2004), *P. barbatus* (Cahan et al., 2002; Volny and Gordon, 2002a), *P. rugosus* (Gadau et al., 2003), and *P. badius* (Rheindt et al., 2004). They are all highly polyandrous, suggesting that multiple mating by queens is a plesiomorphic character in this genus, or at least in the North American subgenus *Pogonomyrmex sensu stricto* (Strehl, 2005). The hypothesis that multiple mating is a default trait in *Pogonomyrmex* has been challenged recently, when *Pogonomyrmex (Epebomyrmex) pima* was shown to be singly mated (Holbrook et al., 2007). Unfortunately, very little is known about other *Pogonomyrmex* species, particularly of those in South America. Only three species from Argentina (*P. pronotalis*, *P. inermis* and *P. rastratus*) have been the focus of recent ecological studies (e.g., Pirk et al., 2004, 2007; Pol and Lopez de Casenave, 2004; Pirk and Lopez de Casenave, 2006), but their mating systems remain unknown. Field observations of mating flights from *P. pronotalis* in the central Monte desert suggest that virgin queens mate with multiple males like their North American congeners (pers. obs.). If multiple mating by queens is present in both North and South American species, the plausibility of the hypothesis of it being an ancestral trait in *Pogonomyrmex sensu stricto* genus would be strongly reinforced.

The aim of this study was to estimate the effective mating frequency of *Pogonomyrmex inermis* and *P. pronotalis* populations in the central Monte desert, Argentina, using polymorphic microsatellite markers, in order to investigate whether multiple mating is also present in the South American species of the genus. This study may contribute to further comparative studies testing evolutionary hypotheses about potential selective factors favoring multiple mating by queens and its sociogenetic consequences for ants.

## Materials and methods

### Sample collection

We collected 20–30 workers from each of 35 *P. pronotalis* colonies and 45 *P. inermis* colonies during the 2003–2004 austral spring-summer season in Nacuñán (34°03'S–67°54'W, Province of Mendoza, Argentina), in the central Monte desert. Individuals were collected directly from nest entrances and were immediately preserved in 96% ethanol, keeping record of source colony.

### DNA isolation

We isolated DNA from 477 *P. pronotalis* workers from 24 colonies (mean number of workers ± SD per colony: 19.9 ± 2.9) and 402 *P. inermis* workers from 20 colonies (20.1 ± 0.4).

DNA of each worker was extracted using the PureGene® DNA Isolation Kit (Gentra Systems), following a modified protocol. Individuals were homogenized in liquid nitrogen and incubated for 1 h at 65°C in 100 µL Cell Lysis Solution. Samples were subsequently put on ice and treated with 34 µL Protein Precipitation Solution, left on ice for 5 min, and finally centrifuged at 14000 rpm for 5 min. Supernatants were transferred into 130 µL of cold 100% isopropanol (2-propanol). Tubes were gently inverted several times for proper mixing. Samples were kept at -20°C for 1–2 h and then centrifuged at 14000 rpm for 10 min. After pouring off the isopropanol, the pellet was washed twice in 100 µL of cold 70% ethanol and then dried in a vacuum centrifuge. The DNA was then resuspended in 50 µL of low TE (1 mM Tris pH 8.0, 0.1 mM EDTA). Prior to storage of the purified samples of DNA at -20°C, 5 µL per sample were run on a 0.8% agarose minigel at 130 V for 30 min, stained in an aqueous ethidium bromide solution for 15 min and inspected under ultraviolet light. A picture was taken of each gel for later reference to the amount of DNA yielded.

### Microsatellites and PCR reaction

Of the sixteen microsatellite primer sets tested in *Pogonomyrmex pronotalis* and *P. inermis*, only *LxAGT1* (developed for *Leptothorax acervorum*; Bourke et al., 1997) worked reliably in both species. Additionally *Pb5* (developed for *P. barbatus*; Volny and Gordon, 2002b) amplified consistently in *P. inermis*. Since these were too few markers to get an accurate mating frequency estimate, we developed three new microsatellite markers for *P. pronotalis* (*Ppro1*, *Ppro2* and *Ppro3*; see Table 1), that also worked in *P. inermis*, using an enrichment procedure introduced by Fischer and Bachmann (1998), and modified after Rütten et al. (2001) and Kronauer and Gadau (2002). We used also the primer *Pbad2* which was developed by F. Rheindt for *P. badius* (unpublished) following the same protocol.

PCR was performed in a reaction volume of 12 µL containing 0.5 units of Taq DNA polymerase (Fermentas), 1 µL of 10× PCR buffer [750 mM Tris-HCl (pH 8.8), 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% Tween 20] (Fermentas), 2.1 mM of MgCl<sub>2</sub>, 170 µM of deoxynucleoside triphos-

phates (dNTPs), 42 nm each of the IRDye™-labeled forward primer and the unmodified reverse primer, and 2 µL of template DNA diluted 1:10, or 1:3 if the DNA concentration of the amplification appeared rather low on a minigel. PCRs were conducted in an Eppendorf Mastercycler® (3°C/s). The cycle parameters were as follows: 3 min at 94°C, followed by 30–35 cycles at 94°C for 40 s, 40 s at the primer-specific annealing temperature (Table 1) and 40 s at 72°C. PCR products were run on 6% polyacrylamide gels on a LI-COR 4300 DNA Analyzer. Alleles were analyzed using SAGA GENERATION 2 software and checked visually.

*Queen genotype, mating frequency and worker relatedness*

DNA from 20–22 workers from each of 20 *P. inermis* colonies was analyzed using six loci, 13–22 workers from each of 19 colonies of *P. pronotalis* using four loci, and 14–20 workers from each of five *P. pronotalis* colonies with only three loci due to low amplification success (see Appendix).

Queen genotypes were inferred from the worker genotypes using the “narrow approach” implemented in the software MateSoft v.1.0 (Moilanen et al., 2004). Furthermore, based on either worker or inferred queen genotypes, there was no evidence of linkage disequilibrium among pairs of loci in any species (Fisher’s exact probability tests,  $p > 0.05$ , implemented by GENEPOP V3.4 software; Raymond and Rousset, 1995). The few individuals whose genotypic array could only be explained by polygyny were omitted from further analyses (see Discussion).

The absolute number of mates per queen was determined by interpreting the band-sharing patterns exhibited by all workers from the same colony in the most parsimonious way under the assumption of monogyny. We calculated three measures of mating frequency: (1) the effective number of patriline, as  $m_e = 1/(\sum q_i^2)$ , where  $q_i$  is the proportion of workers sired by the  $i$ th male (Starr, 1984), (2) the effective number of matings after correcting for sampling error, using the equation from Pamilo (1993):  $m_{ep} = (n-1)/(n \sum q_i^2 - 1)$ , where  $n$  is sample size, and (3) the improved equation from Nielsen et al. (2003):  $m_{ep2} = (n-1)^2 / [\sum q_i^2 (n+1)(n-2) + 3 - n]$ , that is approximately unbiased at relative small sample sizes and high paternity frequencies. Also we estimated the probability of two paternal males bearing the same allele at each locus using data from workers, as  $P_{non-det} = (1 - \text{Het}_{exp/locus})^n$ , where  $n$  is the number of genotyped loci (Pamilo, 1993).

An average worker–worker relatedness value for each colony was determined under the assumption that the males that mated with the queen of a colony were unrelated, using  $G = 1/4 + [0.5(1/m_e)]$  (Pamilo, 1993).

**Results**

The loci employed in this study proved to be useful genetic markers: they were highly polymorphic and showed high levels of observed heterozygosity, with a minimum of 0.59 and of 0.89 for *P. pronotalis* and *P. inermis*, respectively (Table 2).

Overall, queen genotypes could be inferred unambiguously in all colonies of both species. However, we found one worker in each of two and three colonies of *P. inermis* and *P. pronotalis*, respectively, whose genotype could not be explained by monogyny (i.e., 0.57% of all workers analyzed). These individuals were omitted from the estimation of mating frequency and intracolony worker–worker relatedness.

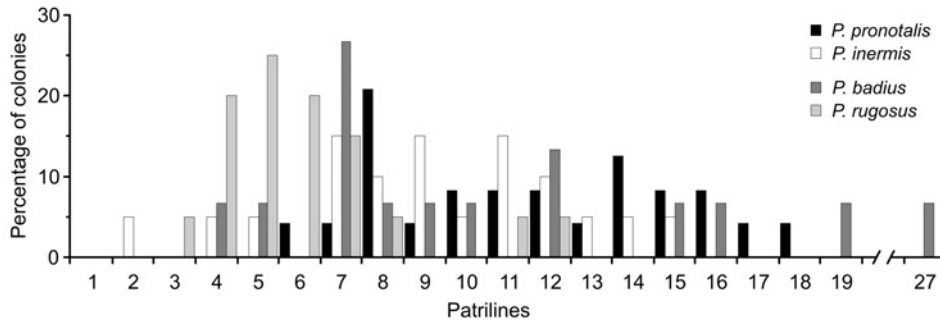
Absolute number of mates per queen observed in *P. pronotalis* and *P. inermis* colonies are as high as those reported for other highly polyandrous species of the genus *Pogonomyrmex* (Fig. 1). The lowest values detected were 6 and 2 in *P. pronotalis* and *P. inermis*, respectively.

The average ( $\pm$ SD) effective mating frequency observed ( $m_e$ ) was  $8.75 \pm 3.26$  (range: 4–16,  $n = 24$ ) for *P. pronotalis* and  $6.52 \pm 2.77$  (range: 2–12,  $n = 20$ ) for *P. inermis* (Table 3). Average values of effective mating frequency increased considerably when corrected for sample size effects using Pamilo’s (1993) equation (*P. pronotalis*:  $m_{ep} = 17.50$ ; *P. inermis*:  $m_{ep} = 10.46$ ), and also increased quite much with Nielsen et al. (2003) equation (*P. pronotalis*:  $m_{ep2} = 15.66$ ; *P. inermis*:  $m_{ep2} = 9.78$ ; Table 3).

The average ( $\pm$ SD) intracolony worker relatedness was  $0.31 \pm 0.02$  for *P. pronotalis* and  $0.35 \pm 0.06$  for *P. inermis* (Table 3). The probability that a second paternal male would not be detected (due to carrying the same alleles at all marker loci as the first male) was estimated to be  $< 1\%$ .

**Table 2.** Characteristics of microsatellite loci analyzed from *P. pronotalis* and *P. inermis*.  $H_O$ : observed heterozygosity;  $H_E$ : expected heterozygosity; A: number of alleles;  $n$ : number of workers/colonies analyzed; “–”: loci that were not amplified for the species because they showed no or very low variability.

Locus	<i>P. pronotalis</i>				<i>P. inermis</i>			
	$H_O$	$H_E$	A	$n$	$H_O$	$H_E$	A	$n$
LxAGT1	0.89	0.90	17	307/18	0.79	0.87	17	387/20
Pbad2	0.95	0.86	14	395/24	0.91	0.86	13	387/20
Pb5	–	–	–	–	0.72	0.74	7	387/20
Ppro1	0.93	0.88	14	459/24	0.78	0.84	18	391/20
Ppro2	–	–	–	–	0.59	0.66	8	395/20
Ppro3	0.92	0.91	18	409/24	0.91	0.91	26	368/20



**Figure 1.** Percentage of studied colonies with absolute number of mates per queen for *P. pronotalis* and *P. inermis* (this study) compared to those of the two *Pogonomyrmex* species with the highest effective mating frequencies reported so far: *P. badius* (Rheindt et al., 2004) and *P. rugosus* (Gadau et al., 2003), both from North America.

**Table 3.** Individual mating frequency statistics of *P. pronotalis* and *P. inermis* queens inferred from workers' genotypes. Observed ( $K_{obs}$ ) and effective paternity are given per colony, as well as the intracolony worker relatedness value ( $G$ ). Effective paternity calculated according to Starr (1984;  $m_e$ ), Pamilo (1993;  $m_{ep}$ ), and Nielsen et al. (2003;  $m_{ep2}$ ). Means and standard deviations per species are also indicated.

Colonies	<i>P. pronotalis</i>					<i>P. inermis</i>				
	$K_{obs}$	$m_e$	$m_{ep}$	$m_{ep2}$	$G$	$K_{obs}$	$m_e$	$m_{ep}$	$m_{ep2}$	$G$
A	12	7.56	10.99	10.54	0.32	4	3.51	4.04	3.98	0.39
B	14	10.26	19.11	17.59	0.30	7	5.13	6.55	6.37	0.35
C	15	13.36	34.97	30.10	0.29	10	4.76	5.94	5.79	0.36
D	17	14.23	38.46	33.09	0.29	14	11.76	27.14	23.86	0.29
E	18	16.13	57.71	46.33	0.28	11	9.09	15.83	14.69	0.31
F	16	11.52	23.08	21.07	0.29	5	2.41	2.60	2.58	0.46
G	14	8.34	12.82	12.20	0.31	9	7.14	10.56	10.05	0.32
H	8	5.90	7.70	7.48	0.33	8	5.41	7.04	6.82	0.34
I	7	4.84	5.92	5.80	0.35	7	4.88	6.13	5.97	0.35
J	8	6.37	8.56	8.29	0.33	9	5.41	7.04	6.82	0.34
K	15	10.08	17.76	16.56	0.30	12	6.90	10.00	9.55	0.32
L	10	6.06	8.26	7.96	0.33	2	1.98	2.09	2.08	0.50
M	13	9.80	17.50	16.23	0.30	8	5.71	7.60	7.34	0.34
N	16	13.36	34.97	30.10	0.29	9	7.41	11.18	10.61	0.32
O	6	4.33	5.99	5.63	0.37	11	7.69	11.88	11.23	0.32
P	14	11.30	23.30	21.06	0.29	15	11.11	23.75	21.21	0.30
Q	11	6.66	9.49	9.08	0.33	7	3.23	3.65	3.60	0.41
R	9	6.53	11.36	10.20	0.33	13	8.64	13.59	12.89	0.31
S	12	10.00	19.00	17.36	0.30	11	8.33	13.57	12.73	0.31
T	10	8.00	12.67	11.93	0.31	12	10.00	19.00	17.36	0.30
U	8	4.74	6.31	6.05	0.36	–	–	–	–	–
V	8	6.26	11.15	9.86	0.33	–	–	–	–	–
W	11	8.10	13.91	12.83	0.31	–	–	–	–	–
Y	8	6.33	8.99	8.59	0.33	–	–	–	–	–
Mean	11.67	8.75	17.50	15.66	0.31	9.20	6.52	10.46	9.78	0.35
SD	3.28	3.26	12.69	10.25	0.02	3.25	2.77	6.82	5.98	0.06

## Discussion

*Pogonomyrmex inermis* and *P. pronotalis* exhibited high levels of effective mating frequency. Indeed, values higher than 8 (found in at least 30% of the *P. inermis* and 50% of the *P. pronotalis* colonies analyzed) are similar or slightly higher than those found in the three *Pogonomyrmex* species with the highest effective mating frequencies reported so far (6.7 for *P. badius*, 6.3 for *P. occidentalis*, and 4.7 for *P. rugosus*; also see Fig. 1), which were calculated using the same methods (Gadau et al., 2003; Rheindt et al., 2004; Wiernasz et al., 2004).

Tarpy and Nielsen (2002) showed that some estimates of effective mating frequency that correct for sampling error (e.g., using Pamilo's equation) can be inflated at small sample sizes, particularly when paternity number is high. This is because the confidence intervals of effective mating frequency are expected to be asymmetrical around the mean in these conditions, with most uncertainty lying above the true  $m_e$  (because of compensation for errors of omission, or the non-detection of rare patrines). Thus, a possible overestimation of the effective mating frequency may result from the low ratio of number of workers sampled over the number of patrines found per colony. However, estimates of effective mating frequency using the Nielsen et al. (2003) equation were not that different in this study, suggesting that sampling error had not a big influence on the results (see Tarpy and Nielsen, 2002; Nielsen et al., 2003). Moreover, the observed number of patrines with only 20–22 workers per colony (a parameter that usually underestimates the effective mating number at small sample sizes; see Tarpy and Nielsen, 2002) was  $>6$  in 90% of the colonies sampled (Table 3), so we can be confident that these two South American harvester ants have a high level of polyandry.

Our estimates of effective mating frequency are based on the assumption of monogyny, so any incidence of polygyny would be another possible source of inflation. The genotype of one worker in each of two colonies of *P. inermis* and in each of three colonies of *P. pronotalis* could not be explained by a single mother, which may arise either from collecting error, polygyny, or brood raiding. All workers were carefully collected at reasonably isolated nest entrances, so collecting errors are unlikely. Polygyny seems to be an exception or an ancestral state within *Pogonomyrmex* genus (Cole and Wiernasz, 2000; Gadau et al., 2003). Multiple queens have been observed several times in the subgenus *Ephebomyrmex* (Kusnezov, 1951; Kugler, 1978; Heinze et al., 1992; Heinze, 1998), whereas only one record of polygyny and pleometrosis (multiple founding queens) has been reported in the subgenus *Pogonomyrmex sensu stricto* (in a single population of *Pogonomyrmex californicus*: Johnson, 2004). None of the South American species have been studied in this respect, so there might be exceptional incidents of pleometrosis or secondary polygyny in our study colonies. Even though brood raiding is also believed to be rare in

these ants, we lack enough natural history information of these species to distinguish between these alternative hypotheses. Their incidence in these species, anyway, seems small (5 out of 879 workers analyzed).

Multiple mating of queens in social insects has been associated with large colony size and advanced social organization (Cole, 1983). Across monogynous ant species, mature colony size and queen mating frequency correlate positively (Boomsma and Ratnieks, 1996; but see below). One mechanism proposed to explain this pattern is that queens need to mate multiply in order to build up large sperm stores, enabling them to produce many workers and create a large colony before starting to produce sexual individuals (Fjerdingstad and Boomsma, 1998). Although a significant increase in the amount of sperm stored by the queen with mating number has been shown (*Atta colombica*: Fjerdingstad and Boomsma, 1998; *Lasius niger*: Fjerdingstad and Keller, 2004), the storage of large numbers of sperm has a considerable metabolic cost, increasing the risk of early failure of colonies (*Atta colombica*: Baer et al., 2006). Thus, multiple mating by queens should be selected for only if the cost of sperm storage increases less sharply with the number of sperm (and of matings) than do the benefits of multiple mating (e.g., being able to produce a bigger colony and more sexual offspring). However, some comparative studies do not support the hypothesis that multiple mating is a function of increasing colony size (e.g., Boomsma et al., 1999; Fjerdingstad and Boomsma, 2000). The results of this study also seem to disagree with that hypothetical correlation, since *P. pronotalis* and *P. inermis* exhibit high levels of polyandry even though they have smaller colonies and lower population densities than several North American species (Holbrook et al., 2007; Pol, Pirk and Lopez de Casenave, unpubl.; Nobúa Behrmann et al., unpubl.). Comparative studies of mating frequency in potentially polyandrous species with different colony sizes, controlling for a phylogenetic effect (Felsenstein, 1985; Harvey and Pagel, 1991), are needed to clarify this issue.

The fitness advantage of multiple mating in social insects has been suggested to come from an increase in genetic diversity within the colony, which has been suggested to benefit social Hymenoptera colonies in two, not necessarily exclusive, ways: (1) it could reduce pathogen load, producing a worker force more resistant to diseases and less likely to transmit them to nestmates than in genetically uniform colonies (Hamilton, 1987; Schmid-Hempel, 1994; Baer and Schmid-Hempel, 1999; Schmid-Hempel and Crozier, 1999; but see Baer et al., 2006); and (2) high genetic diversity within the colony may lead to higher efficiency and colony fitness by enhancing division of labor via a larger array of workers behaviors, tasks or castes (Crozier and Page, 1985; Page et al., 1989, 1995; Fewell and Page, 1993; Fuchs and Moritz, 1998; Julian and Cahan, 1999; Beshers and Fewell, 2001; Hughes et al., 2003; Fjerdingstad and Crozier, 2006). Pathogen resistance seems to play a crucial role in leaf-cutter ants, which depend exclusively on a vulnerable fungal symbiosis to obtain their food (Weber, 1972; Mueller et al., 2001), but it is not so clear in harvester ants, which collect

seeds as their main food source (e.g., MacMahon et al., 2000; Pirk and Lopez de Casenave, 2006). On the other hand, improved resource utilization or competitive ability through a better division of labor may be especially important in a genus such as *Pogonomyrmex*, which lives in desert regions where seed availability as well as environmental conditions change continuously (Noy-Meir, 1973; Goldberg and Novoplansky, 1997; Wilby and Shachak, 2000; MacMahon, 2001), demanding a fine-tuned regulation of the foraging system. Current data do not allow a test of these hypotheses, and both mechanisms may contribute to selection for multiple mating in *Pogonomyrmex* harvester ants.

In summary, our study demonstrates that queens in at least some members of the South American *Pogonomyrmex sensu stricto* species group are highly polyandrous. Therefore, multiple mating appears to have arisen early in the evolution of this group, maybe at the origin of the *Pogonomyrmex sensu stricto* group or during the spread of this subgenus. Indeed, it might have been linked to the development of seed harvesting in desert habitats (Gadau et al., 2003). Notwithstanding, studies on more basal species of *Pogonomyrmex sensu stricto* from North, Central and South America should be conducted in order to corroborate this conclusion, and provide a stronger context to evaluate the single-mating status of *Pogonomyrmex (Ephedomyrmex) pima* (Holbrook et al., 2007) as a reversal along the evolution of polygyny in this species or the retention of an ancestral status in this other subgenus of *Pogonomyrmex*.

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### Supplementary online material

Appendix (a fully detailed Appendix is provided as supplementary online material). Scoring tables with allele inferences of all *P. pronotalis* and *P. inermis* workers is freely available to download from: <http://www.ege.fcen.uba.ar/Ecodes/>.

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