Insect. Soc. (2011) 58:357–364
DOI 10.1007/s00040-011-0150-5

Insectes Sociaux

RESEARCH ARTICLE

High mating frequency and variation with lineage ratio in dependent-lineage harvester ants

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Received: 10 November 2010 / Revised: 7 January 2011 / Accepted: 11 January 2011 / Published online: 25 January 2011

Abstract Explaining the evolution of multiple mating is a challenge because of the associated costs. For social insects, mating frequency may have fitness consequences due to effects on social interactions or genetic diversity within colonies. Here, we investigated the evolution of mating frequency in a social insect species with a unique genetic system that requires multiple mating. In certain populations of *Pogonomyrmex* harvester ants, there are two interbreeding yet genetically distinct mitochondrial lineages. Queens must mate with males of the opposite lineage to produce workers and with males of the same lineage to produce reproductive females. We expected queens of the dependent-lineage system to exhibit high mating frequencies relative to other social insects. Furthermore, we expected queens from populations of highly asymmetric lineage ratios to exhibit even higher mating frequencies, to adequately sample the population and successfully mate with males of the less common lineage. To test these predictions, we estimated the mating frequency of 16 *P. barbatus* queens, and compared these mating frequencies between two populations, one with relatively equal lineage ratio (60:40) and a second with a highly asymmetrical lineage ratio (96:4). Overall, observed mating frequency exceeded 10, which is high in comparison to other social insects, and our estimates of effective mating frequency were among the highest of *Pogonomyrmex* species. Mating frequency at the site with the asymmetrical lineage ratio was also significantly higher than the site with the more even ratio. Our results suggest that obligate multiple mating as well as lineage ratio contribute to the evolution of high mating frequency in dependent-lineage populations.

Keywords Mating frequency · Dependent-lineage · *Pogonomyrmex* · Harvester ants · Gene flow

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Introduction

The evolution of multiple mating (polyandry) by female insects is paradoxical because of the associated costs. Higher mating frequency may deplete energy reserves (Daly, 1978), increase predation risks (Wing, 1988; Fairbairn, 1993; Rowe et al., 1994), and increase physical damage by males (see reviews of Chapman et al., 2003; Arnqvist and Rowe, 2005; Lessells, 2006). Despite these costs, polyandry seems to be relatively common among non-social insects and is associated with higher offspring production relative to monoandry in many taxa (Arnqvist and Nillson, 2000). Multiple mating is less common among the social insects, whose ancestral state is thought to have been monoandry (Hughes et al., 2008), although some species have evolved extremely high mating frequencies, including honeybees (Tarpy and Nielsen, 2002), vespine wasps (Foster and Ratnieks, 2001), army ants (Kronauer et al., 2004, 2007), leaf cutter ants (Boomsma et al., 1999), and some seed-harvesting ants (Wiernasz et al., 2004).

For social insects, the evolution of mating frequency is a topic of considerable interest because of its complex effects on colonies. Increased mating frequency of queens can have positive effects on a colony due to increased genetic diversity in the workforce (reviewed in Oldroyd and Fewell, 2007), which may improve colony performance via task

specialization of ants of specific patrilines (Waibel et al., 2006; Wiernasz et al., 2008) or increase disease resistance (Brown and Schmid-Hempel, 2003). Benefits of multiple mating may also include buffering against insufficient sperm (Cole, 1983), or reducing the chance of diploid males (Page, 1980). On the contrary, increased mating frequency may have negative effects due to reduced relatedness among nestmates and associated increases in within-colony conflict (Ratnieks et al., 2006). In addition, when coupled with a decrease in relatedness between workers, increased mating frequency can also decrease the effective strength of selection on worker expressed genes that have indirect fitness effects, thereby decreasing the response to selection in polyandrous relative to monoandrous populations (Linksvayer and Wade, 2009).

For some populations of *Pogonomyrmex* harvester ants, variation in mating frequency carries an additional set of consequences. In the monogynous species *P. barbatus* and *P. rugosus*, some populations operate under a dependent-lineage system with two interbreeding but genetically distinct mitochondrial lineages (Helms Cahan and Keller, 2003). This system involves an association of reproductive caste with genotype that requires multiple mating (Helms Cahan et al., 2002; Volny and Gordon, 2002a; Helms Cahan and Keller, 2003). In these populations, a queen must mate with males of the other lineage to produce sterile workers to feed and maintain the colony, and with males of the same lineage to produce reproductive females (gynes).

Previous studies on dependent-lineage populations demonstrated increased failure rates of colonies founded by queens that acquired low proportions of opposite lineage sperm (Anderson et al., 2006, 2008; Schwander et al., 2006). Therefore, the dependent-lineage system likely places selection pressure on queens to mate multiply in order to produce viable colonies. In addition, selection for multiple mating likely varies among dependent-lineage populations, as the potential aforementioned costs are predicted to increase with increasing asymmetry in lineage ratio (Andersen et al., 2006; Schwander et al., 2006). As lineage ratio increases in skew, so does the possibility of a female acquiring sperm only from a single lineage, resulting in greater colony failure rates in skewed populations than in more even-ratio populations (Helms Cahan et al., 2004). Furthermore, the proportions of queens that mate exclusively with males of their own lineage are generally low, except in populations of extreme lineage ratios (Schwander et al., 2006). Thus, the problem of acquiring same- and alternate-lineage sperm is much greater for queens in skewed populations. Although increased mating frequency will not change the proportion of opposite lineage sperm, it will increase the probability a given queen does not only acquire sperm from a single lineage, a considerably greater benefit in skewed lineage versus even lineage ratio populations.

Lineage ratios have previously been demonstrated to vary considerably among local natural populations over relatively small geographic areas (Schwander et al., 2006; Anderson et al., 2006), with limited dispersal among populations (Suni and Gordon, 2009). Given the potential for independent evolution among populations, the variation in lineage ratios may alter the benefits of multiple mating among local populations. In effect, the aforementioned benefits and necessity of multiple mating likely increase among queens in populations of skewed lineage ratios due to the increased sampling required to successfully mate with males of both the common and rare lineage.

High mating frequency in the dependent-lineage system may be an adaptive response linked to the additional requirements of queens to effectively sample males of both lineages (see Anderson et al., 2008). Although the evolution of high mating frequencies in the social insects likely predates the emergence of the dependent-lineage system, adaptive shifts in mating frequency may still occur in response to selection pressures imposed on the existing natural variation in mating frequency. To support our hypothesis on the link between the requirements of the dependent-lineage system and high mating frequency, we test two predictions. First, the mating frequencies of queens of the dependent-lineage system will exceed the mating frequencies typical of other *Pogonomyrmex* harvester ants, including those of queens from the conventional system of *P. rugosus*, which is one of the parental species from which dependent-lineage populations evolved. Second, when comparing mating frequency among two independent natural populations that vary significantly in their lineage ratios, we expect higher mating frequencies in the skewed versus the more even lineage ratio population. Although we do not propose the evolution of multiple mating as a de novo adaptation of the dependent-lineage system, demonstrating adaptive shifts in response to the requirements of the dependent-lineage system will further the understanding of the origins of the dependent-lineage system, benefits of multiple mating, as well as evolution in local populations.

Methods

We estimated mating frequency for dependent-lineage *P. barbatus* (the J lineages from Helms Cahan and Keller, 2003). The estimation of mating frequency in dependent-lineage populations poses a particular challenge. Gynes and workers within a colony are both offspring of the same queen, but have different fathers. Estimates to date have used only workers to estimate the mating frequency of their mother (Volny and Gordon, 2002a; Suni et al., 2007), which is not her true mating frequency because it disregards her mates of the same lineage, which produce gynes. Therefore,

this report is the first to estimate the true mating frequency of *P. barbatus* queens using both workers and gynes.

Sampling

Pogonomyrmex barbatus colonies reproduce yearly when winged males and gynes fly to a mating aggregation each summer. We collected samples near the Arizona-New Mexico state line in July of 2006, before the mating aggregation, at two sites separated by 10 km. We collected on average 17 workers and 16 gynes from six colonies at site 1 (range 14–19 workers, 14–20 gynes) and ten colonies at site 2 (range 8–20 workers, 15–20 gynes), for a total of 280 workers and 284 gynes. We also collected 7 males from 25 colonies at site 1, and 26 colonies at site 2, for a total of 357 males. These males were used to determine genotypes of colony queens, and colony queen genotypes were then used to estimate the level of gene flow between the sites (see below). Samples were stored in 95% ethanol until DNA extraction and analysis. For site 1, we used the estimate of lineage ratio from a different study that surveyed over 300 colonies in 2006 and demonstrated a frequency of 40% for J1 (S. Suni, unpublished). For that study we determined lineages by amplifying a ~433 bp portion of the Cytochrome Oxidase I gene from one worker or gyne, and performing subsequent restriction digests that cut lineage-specific sequences, as in Schwander et al. (2006). We used DNA from one worker or gyne from each of the 26 colonies from site 2 to estimate the lineage ratio at that site. Mitochondrial lineages of colonies were determined either as above or by genotyping individuals at the microsatellite loci Myrt3 and Pr-1 (see below), which are diagnostic for lineage (Helms Cahan and Keller, 2003). Among the 26 colonies at site 2, there was only one J1 colony so we were unable to draw conclusions about variation of mating frequency with lineage. However, previous work suggests that mating is random with respect to lineage so queens of each lineage may not differ in mating frequency (Schwander et al., 2006; Suni et al., 2007).

Molecular analyses

We extracted DNA from all samples by boiling heads in 250 µl of a 5% Chelex (Bio-Rad) solution at 95°C for 20 min. This solution was then centrifuged and the supernatant used as the template for PCR amplification. Samples were genotyped at seven microsatellite loci using seven primer sets: L-18 (Foitzik et al., 1997), Myrt3 (Bourke et al., 1997), PO8 (Wiernasz et al., 2004), Pb5, Pb7, Pb8 (Volny and Gordon, 2002b), and Pr-1 (Gadau et al., 2003) according to the procedure described in Helms Cahan and Keller (2003). PCR products were run on an ABI 3100 automated DNA sequencer (Applied Biosystems) using fluorescent dyes, and analyzed using GENOTYPER software (Applied Biosystems). These loci are highly polymorphic, with an average of 12 alleles.

Mating frequency

Queen mating frequency was determined for each colony using the genetic software program COLONY (Wang, 2004). This program uses likelihood ratios based on multilocus genotypes to partition individuals into half sibling and full sibling groups. The number of half sibling groups is the number of patrilines, i.e. the number of times a queen mated. We also used this program to determine if there was overlap in the males that sire workers versus gynes. We considered workers and gynes to have the same father when they were placed in the same full sibling family by COLONY. We report both observed mating frequency, the number of fathers detected among our samples, and effective mating frequency, the mating frequency that would correspond to equal distribution of patrilines among offspring within a colony (Bourke and Franks, 1995). To account for the differing number of workers and gynes sampled from colonies, we adjusted observed mating frequency per colony to a sample-size corrected average—a minimum common sample size—as per Franck et al. (2000), using the formula $k_n = \sum_{i=1}^{k} (1| - C^n_{h-n_i}/C^n_h)$, where k_n is the number of patrilines adjusted for a minimum common sample size n, k is the total number of subfamilies, n_i is the number of workers or gynes in the ith subfamily, h is the sum of n_i and is the observed sample size, C^n_h is the number of different samples of n workers or gynes taken among h, and $C^n_{h-n_i}$ is the number of different samples not including the ith patriline.

We calculated the effective mating frequency (m_e) for each colony as in Nielsen et al. (2003). We made calculations separately for workers and gynes, and then to obtain the total mating frequency for each queen added the resulting m_e. We calculated the chance that we failed to detect a patriline due to two fathers having the same multilocus genotype as $\prod(\sum q_i^2)_j$, which is the product over j loci of the sums of squares of q_i, the frequency of the ith allele at locus q (Starr, 1984). We used t tests to compare differences in our measures of mating frequency among sites. Separate t tests were used for observed mating frequency (K_{obs}), sample-size corrected mating frequency (K_{ssc}) and effective mating frequency (m_e). Although the number of workers and gynes sampled differed among colonies, the sites had similar average numbers of offspring sampled from each colony (34.7 for site 1, 35.6 for site 2). This justifies the use of observed mating frequency as an appropriate measure to compare mating frequency among sites; however, we also use the sample-size corrected mating frequencies as a more conservative estimate.

Genetic structure

We estimated gene flow between the sites using the inferred genotypes of the queens of the 25 J2 queens at site 2, and the queens of 25 J2 colonies at site 1. We inferred queen genotypes using the genotypes of 7 males as in Suni et al. (2007), or from the genotypes of workers and gynes, using the program COLONY (Wang, 2004). We used queen genotypes to calculate F_{st} between the sites, as well as expected heterozygosity in each site using the program Genalex (Peakall and Smouse, 2006). Because values of F_{st} depend on the level of genetic variation of the loci used, we also estimated genetic differentiation between sites using the standardized estimator G'_{st} (Hedrick, 2005), and the estimator D_{est} (Jost, 2008), which may more accurately represent differentiation among populations, and can be used for comparisons across species. To calculate both of these estimators, we used the online genetic software program SMOGD (Crawford, 2010).

Results

Our estimates of mating frequency for dependent-lineage *P. barbatus* are among the highest documented for *Pogonomyrmex* ants (Table 1). The average observed mating frequency for queens in this study was 10 ± 0.58 (SE), and the average effective mating frequency was 7.52 ± 0.39 (SE). The sites differed significantly in all of our measures of mating frequencies (see Table 2). Queens at site 1 (40% J1) mated an average of 8.2 ± 0.7 (SE) times, had a sample-size corrected average mating frequency of 7.1 ± 0.6 (SE), and an average effective mating frequency of 6.6 ± 0.62 (SE), while queens at site 2 (4% J1) mated an average of 11 ± 0.62 (SE) times, had a sample-size corrected average mating frequency of 8.8 ± 0.36 (SE), and an average effective mating frequency of 8.1 ± 0.43 (SE). The probability that we failed to detect a patriline was less than 0.001 at both sites. There was no significant difference in the sample-size corrected mating frequency with males of the same versus other lineage for queens at either site (paired *t* test, $P = 0.96$ for site 1; $P = 0.58$ for site 2; Table 3).

Results from the likelihood-based program COLONY revealed 2 out of 284 gynes (0.7% of all gynes sampled) were sired by males of the opposite lineage. One of these was from a colony at site 1 and the other from a colony at site 2. They were heterozygous at loci diagnostic for lineage and were placed into a full sibling family that besides them contained only workers from their respective colony. Heterozygous queens have been reported in dependent-lineage populations although they occur infrequently (Helms Cahan and Keller, 2003). All of the other gynes from these two colonies and from all other colonies were homozygous for loci diagnostic for lineage.

We found evidence of restricted gene flow between the sites. Expected heterozygosity averaged 0.62 in population 1 and 0.63 in population 2 (see Table 4 for observed and expected heterozygosity for each locus at each sample site). The F_{st} value calculated from the genotypes of 25 queens at each site was 0.11, and was significantly different from zero ($P = 0.01$). The standardized measure of genetic differentiation G'_{st} (Hedrick, 2005) was 0.31 (±0.0034 SE), and D_{est} was 0.16 (±0.0031 SE), indicating that the sites are significantly genetically differentiated.

Discussion

The mating frequencies reported in this study are among the highest for a hymenopteran species, rivaled only by *Apis* bees (Estoup et al., 1994; Moritz et al., 1995; Strassmann, 2001; Tarpy and Nielsen, 2002), army ants (Kronauer et al.,

Table 1 Reported average effective mating frequencies m_e (±95% CI) of *Pogonomyrmex* spp., and the number of colonies sampled in each study (*N*)

Pogonomyrmex spp.	K_{obs}	m_e	*N*	Reference
DL *P. barbatus* (4% J1)	11.0 ± 1.22	8.10 ± 1.11	6	Current
DL *P. barbatus* (40% J1)	8.20 ± 1.37	6.60 ± 1.40	10	Current
P. badius	20.4 ± 5.73	7.70 ± 1.48	8	Smith et al., 2008
P. badius	11.0 ± 3.10	6.66 ± 1.61	15	Rheindt et al., 2004
P. occidentalis	6.29 ± 0.37	4.62 ± 0.29	63	Wiernasz et al., 2004
P. rugosus	6.00 ± 0.10	4.71 ± 0.88	20	Gadau et al., 2003
DL *P. rugosus*	6.20 ± 0.47	4.93 ± 0.40[a]	45	Helms Cahan and Julian, 2010
P. (*Ephebomyrmex*) *pima*	1.30 ± 0.26	1.15 ± 0.18	20	Holbrook et al., 2007

DL dependent-lineage

[a] m_e calculated from raw data used in Helms Cahan and Julian (2010), from colonies in which workers and gynes were sampled, calculated separately for each lineage and summed to give total m_e

Table 2 The number of colonies sampled, the frequency of lineage 1 in the population, the differences among sites in the observed number of patrilines ± SE found among gynes and workers (K_{obs}), sample-size corrected (K_{ssc}), and effective mating frequencies (m_e), and the associated *t* test statistics and *P* values

Site	*N* colonies (J1, J2)	J1 (%)	K_{obs}	*t* test statistic, *P* value	K_{ssc}	*t* test statistic, *P* value	m_e	*t* test statistic, *P* value
1	2, 4	40	8.2 ± 0.7	3.1, 0.0045	7.1 ± 0.6	2.5, 0.017	6.6 ± 0.62	1.9, 0.046
2	0, 10	4	11 ± 0.62	3.1, 0.0045	8.8 ± 0.36	2.5, 0.017	8.1 ± 0.43	1.9, 0.046

Table 3 For each colony, its lineage, the number of gynes sampled, the number of patrilines found among gynes (same-lineage matings), the number of workers sampled, the number of patrilines found among workers (other lineage matings), and the total number of patrilines

Colony	Lineage	*N* gynes	Gyne patrilines	*N* workers	Worker patrilines	Total patrilines
2_1	2	18	5	20	4	9
2_2	2	20	7	20	3	10
2_3	2	19	6	17	4	10
2_4	2	10	4	20	6	10
2_5	2	19	3	8	5	10
2_6	2	20	5	20	5	10
2_7	2	15	6	18	5	11
2_8	2	19	6	18	6	12
2_9	2	16	6	19	8	14
2_10	2	20	7	20	8	15
Average	–	**18**	**5.5**	**18**	**5.4**	**11**
1_1	2	20	4	16	3	7
1_2	2	14	4	16	3	7
1_3	1	19	2	14	5	7
1_4	1	20	1	16	7	8
1_5	2	16	6	19	3	9
1_6	2	19	6	19	5	11
Average	–	**18**	**3.8**	**17**	**4.3**	**8.2**

2004, 2007), and the harvester ant *P. badius* (Rheindt et al., 2004; Smith et al., 2008), and may be partially explained by the specifics of the dependent-lineage system. The dependent-lineage system differs from the other more classic social systems of the hymenoptera in that multiple mating is fundamentally required to produce viable colonies that can produce gynes. Previous research has demonstrated that colonies founded by queens with low proportions of opposite lineage sperm are more prone to colony failure (Anderson et al., 2006, 2008; Schwander et al., 2006). Specifically, early colony founding is heavily reliant on the initial production of workers, thus production of gynes at this time can cripple early productivity and lead to colony failure. It is likely that selection strongly favors high mating frequency among queens to adequately acquire sperm from both lineages, and therefore, as expected, the mating frequencies reported here are among the highest observed among *Pogonomyrmex* harvester ants, including one parental species (Table 1). However, although colonies of another set of dependent lineages (the *P. rugosus*-like "H" pair from Helms Cahan and Keller, 2003) had a higher average effective mating frequency than parental *P. rugosus* (Table 1), the 95% confidence intervals overlapped indicating that the difference between parental and dependent-lineage is not significant for this lineage pair.

In addition to finding that mating frequencies of *P. barbatus* queens are high overall, we found population-specific differences that may also be explained by the specifics of the dependent-lineage system. Previous reports on the consequences of low proportions of other lineage sperm suggest that the cost of mating two few times with males of the opposite lineage should increase dramatically as lineage ratios stray from 1:1. If queens mate randomly in mating aggregations, then as lineage ratios increase in skew, so should the proportion of queens only acquiring sperm from the common lineage, thus providing a testable hypothesis that selection should favor higher mating frequencies in populations of greater lineage ratio skew relative to more symmetrical populations. Consistent with this prediction, in our sample of two neighboring yet somewhat genetically

Table 4 The number of alleles (N_a), observed heterozygosity (H_{obs}), and expected heterozygosity (H_{exp}) for each locus at each sampling location

Site	Locus	N_a	H_{obs}	H_{exp}
1	L-18	15	0.84	0.88
	Myrt3	6	0.61	0.61
	PO8	7	0.63	0.79
	Pb7	11	0.45	0.76
	Pb5	14	0.53	0.8
	Pb8	18	0.83	0.86
	Pr-1	2	0.48	0.49
	Average	**10.4**	**0.62**	**0.74**
2	L-18	19	0.75	0.87
	Myrt3	8	0.51	0.42
	PO8	9	0.67	0.73
	Pb7	21	0.82	0.89
	Pb5	11	0.49	0.65
	Pb8	19	0.66	0.84
	Pr-1	7	0.53	0.40
	Average	**13.4**	**0.63**	**0.69**

distinct local populations varying significantly in their lineage ratio, queens of the highly skewed population exhibited higher mating frequencies compared to their counterparts from the more equal lineage population, further supporting the notion of the adaptive value of high mating frequency in the dependent-lineage system.

We suggest that shifts in mating frequency occurred in response to local lineage ratios. It is possible that mating frequency is plastic and the differences between the sites are due to environmental conditions. However, it is also possible that the mating frequency differences reflect adaptive responses to selection on the existing variation in mating frequency. If there is a genetic basis to mating frequency, restricted gene flow between sites could have facilitated local adaptation at each site. This would require that (1) there was little dispersal between sites, and (2) that the conditions that promote evolution of high mating frequency remained similar over several generations. The first condition is met for this species; dispersal is restricted over an area smaller than the geographic area sampled (Suni and Gordon, 2009). Our estimates of F_{st}, G'_{st}, and D_{st} between the sites were 0.11, 0.31 and 0.16, indicating that gene flow is restricted between the sites. The second condition is also likely met; the lineage ratio near site 1 remained relatively stable for the past 15 years (S. Suni, unpublished). While this is shorter than the lifetimes of some *Pogonomyrmex* colonies, mating flights occur yearly, and many new colonies become established within a 15-year period. Thus, it is conceivable that the lineage ratio remained relatively symmetrical over time at site 1. Likewise, the ratio at site 2 may have remained highly asymmetrical over time and may have promoted the evolution of a higher average mating frequency at that site.

Interestingly, the aforementioned studies on the dependent-lineage system assume mating to be random; however, here we report no differences in the number of times queens mate with males of each lineage. This may initially seem surprising for the site with the highly asymmetrical lineage ratio. With random mating, and lineage frequencies at mating aggregations corresponding to frequencies in the population, queens are expected to mate with many more males of the common lineage. Although this may suggest non-random mating in our sample, our sample included only colonies that produced gynes, meaning they were at least 5 years old and had survived the colony founding stage, when 99% of colonies fail. Therefore, it is still possible that mating is random, yet our sample only includes the minority (<1%) of queens successful at acquiring enough opposite lineage sperm to produce the workforce necessary to establish and sustain a colony (i.e. not representative of the initial sample of queens at mating aggregations). Had we sampled rare lineage colonies at the site with the asymmetrical lineage ratio we might have found that they showed a more skewed ratio of same to opposite lineage mates, because the costs of mating with few rare lineage males may not be as great for the rare lineage as it would be for the common lineage. A study in which queens were collected from mating aggregations and allowed to initiate colonies in the laboratory revealed that the worker–gyne ratio mirrored those of the corresponding lineage ratios in mating aggregations (Schwander et al., 2006). This suggests random mating in the initial sample of queens before selection, yet the appearance of non-random mating when considering only those colonies surviving the initial selection.

The lack of differences in mating frequency with same and opposite lineage males could also be explained by differences in sex ratio among males and gynes within colonies, which could increase the number of males of the rare lineage at mating aggregations. This would occur if queens of the rare lineage that fail to mate with males of the same lineage, and cannot produce gynes, produce males instead. Previous measures of reproductive output showed that 17% of colonies tended to produce only males at a site with about 40% J1 (Gordon and Wagner, 1997). At the site with 4% J1, it is likely that even more queens of the rare lineage fail to mate with males of their lineage and are able to produce only males. Recent theoretical work showed that asymmetry in the lineage ratio can lead to biased sex ratios (Anderson et al., 2009). How would increasing asymmetry in colony sex ratios affect selection on mating frequency in dependent-lineage populations? The effects should depend on the relative fitness of colonies that produce only males with respect to colonies that produce a more equal number

of males and gynes. To our knowledge this is an unexplored area that would be an interesting avenue for future research.

Overall, several hypotheses have been proposed to explain the evolution of multiple mating in social insects, such as increased disease resistance (Sherman, 1988), reduced risk of diploid males (Page 1980), increased potential to detoxify the secondary compounds (Gadau et al., 2003), and increased genetic diversity of workers (reviewed in Oldroyd and Fewell, 2007), particularly if workers of some patrilines are more effective at performing specific tasks (Hughes et al., 2003; Julian and Fewell, 2004; Rheindt et al., 2005; but see Fournier et al., 2008). Our direct comparison of mating frequencies to other *Pogonomyrmex* species, including one of the parental species, as well as the comparisons among local populations, likely controlled for differences in mating frequency arising from these alternative possibilities. The mechanism responsible for the high mating frequency at the site with the asymmetrical lineage ratio is unknown. It could be that differences in mating behavior evolved in that population, that mating frequency is plastic, or that the variation in mating frequency is similar between the sites but selection favors queens that mate more times at the site with the asymmetrical lineage ratio. Regardless of the mechanism, the difference in average mating frequency between the sites suggests that high mating frequency may be favored by selection when lineage ratios are asymmetrical. In summary, we suggest that the specifics of the dependent-lineage system contributed to the maintenance and further evolution of high mating frequencies over time.

Acknowledgments We thank the Center for Evolutionary Biology at Stanford University for its support, the Southwest Research Station in Portal, AZ, for their facilities, and J. Van Cleve and B. Brosi for helpful comments. We also thank the Center for Insect Science, University of Arizona, for their support (NIH Grant 5 K12 GM000708).

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