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Male parentage in dependent-lineage populations of the harvester ant *Pogonomyrmex barbatus*

SEVAN S. SUNI,* CHRISTOPHER GIGNOUX† and DEBORAH M. GORDON*

**Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020, USA, †Department of Anthropological Sciences, Stanford University, Stanford, CA 94305-2117, USA*

Abstract

We investigated the extent to which workers reproduce in a dependent-lineage population of the monogynous harvester ant *Pogonomyrmex barbatus*. Dependent-lineage populations contain two interbreeding, yet genetically distinct mitochondrial lineages, each associated with specific alleles at nuclear loci. Workers develop from matings between lineages, and queens develop from matings within lineages, so queens must mate with males of both lineages to produce daughter queens and workers. Males develop from unfertilized eggs and are haploid. Worker production of males could lead to male-mediated gene flow between the lineages if worker-produced males were reproductively capable. This could result in the loss of the dependent-lineage system, because its persistence depends on the maintenance of allelic differences between the lineages. To investigate the extent of worker reproduction in *P. barbatus*, we genotyped 19–20 males and workers from seven colonies, at seven microsatellite loci, and 1239 additional males at two microsatellite loci. Our methods were powerful enough to detect worker reproduction if workers produced more than 0.39% of males in the population. We detected no worker-produced males; all males appeared to be produced by queens. Thus, worker reproduction is sufficiently infrequent to have little impact on the dependent-lineage system. These results are consistent with predictions based on inclusive fitness theory because the effective queen mating frequency calculated from worker genotypes was 4.26, which is sufficiently high for workers to police those that attempt to reproduce.

Keywords: inclusive fitness, parentage, social insect, worker reproduction

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Introduction

Social insect societies are characterized by reproductive division of labour between queens and workers (Oster & Wilson 1978). However, workers of some species can produce unfertilized eggs that develop into haploid males (Charnov 1978). The lack of worker reproduction in colonies with a living queen has been explained by invoking inclusive fitness theory (Villesen & Boomsma 2003; Kronauer *et al*. 2006). However, the degree to which worker reproduction depends on nestmate relatedness remains controversial (Hammond & Keller 2004; Ratnieks *et al*. 2006). Worker reproduction is predicted not to occur when the population-wide effective mating frequency of queens is sufficiently high that workers are more related to the queen's sons than they would be to the sons of other workers (Hamilton 1972; Starr 1984; Ratnieks 1988). These circumstances could select for worker policing, worker aggression towards workers with developing ovaries (Monnin & Ratnieks 2001; Iwanishi *et al*. 2003) or selective removal of eggs laid by other workers (Ratnieks 1989; Foster & Ratnieks 2000, 2001; Halling *et al*. 2001; Oldroyd *et al*. 2001; Helanterä & Sundström 2005). Policing may also occur in the absence of relatedness-based conflict among colony members (Ratnieks 1988; Arevalo *et al*. 1998; Hartmann *et al*. 2003; Hammond & Keller 2004).

Inclusive fitness theory predicts that worker reproduction is absent or suppressed in harvester ants (genus *Pogonomyrmex*). Queens mate with many males in North American populations of this genus (Cole & Wiernasz 1999; Volny & Gordon 2002a; Gadau *et al*. 2003), which should result in low relatedness among nestmates, and no worker-produced

Correspondence: Sevan Suni, Fax: (650) 723 1826; E-mail: sssuni@stanford.edu

males. However, workers do have developed ovaries, and males have been observed in queenless laboratory colonies of *Pogonomyrmex badius* (Smith & Tschinkel 2006), and *Pogonomyrmex barbatus* (D.M. Gordon, personal observation). The extent to which workers realize their reproductive potential in natural populations remains unknown.

The evolution of worker reproduction within the harvester ant species complex that includes *P. barbatus* and its sister species *Pogonomyrmex rugosus* may be complicated by a newly discovered system of reproductive caste determination in some populations. In most ant species, the development of female eggs into either queens or workers is governed by environmental factors (Wheeler 1986, 1994). However, recent work has uncovered populations of *P. barbatus* in which reproductive caste is strongly associated with genotype. These dependent-lineage populations are widespread throughout the southwestern USA (Anderson *et al*. 2006; Schwander *et al*. 2007) and contain sets of two interbreeding but genetically distinct lineages (J1 and J2; Helms Cahan *et al*. 2002, 2004; Julian *et al*. 2002; Volny & Gordon 2002a; Helms Cahan & Keller 2003; Schwander *et al*. 2007). Lineages are cryptic and are characterized by distinct mitochondrial haplotypes, each associated with specific alleles at nuclear microsatellite loci (Helms Cahan & Keller 2003). Between-lineage matings result in the production of workers, and within-lineage matings result in the production of gynes; each lineage is unable to produce workers independently of the other (Helms Cahan *et al*. 2004). Thus, workers are heterozygous at the nuclear loci at which alleles are fixed within the lineages (Julian *et al*. 2002; Volny & Gordon 2002a; Helms Cahan & Keller 2003). Males develop from unfertilized eggs and are haploid.

Models that explain the maintenance of the dependent-lineage system must explain both the persistence of genetically distinct lineages within populations, and the association of heterozygosity with workers and homozygosity with gynes. Helms Cahan & Keller (2003) proposed that the two lineages arose from historical hybridization events and in the process became fixed for incompatible alleles at interacting nuclear loci, so an interlineage combination of nuclear alleles is necessary to initiate worker development. Linksvayer *et al*. (2006) proposed that the system is the result of deleterious incompatibilities between nuclear and mitochondrial genomes in interlineage individuals that result in the development of workers. Under both models, worker reproduction could affect the stability of the dependent-lineage system (Linksvayer *et al*. 2006), because worker-produced males could have nuclear genomes with alleles of both lineages at different nuclear loci. If recombination occurs within workers, worker production of males could lead to male-mediated gene flow between the lineages. Moreover, if worker offspring were plentiful and reproductively capable, this could cause the dependent-lineage system to collapse, because its persistence depends on the maintenance of allelic differences between the lineages. However, evidence suggests that little to no gene flow between the lineages has occurred (Helms Cahan & Keller 2003; Anderson *et al*. 2006), and the dependent-lineage system seems to be relatively ancient (Anderson *et al*. 2006). To investigate the maintenance of this system in harvester ants, we examined the extent to which workers produce males in a natural, dependent-lineage population of *P. barbatus*.

Materials and methods

Field site and colony collection

Specimens were collected from colonies within 8 km of our long-term study site in southeastern Arizona (Gordon & Kulig 1996). *Pogonomyrmex barbatus* colonies reproduce yearly, by producing winged males and females that fly to a mating aggregation. Male ants and workers were collected in July 2005 and 2006, before male ants had flown to the mating aggregation. One hundred and eighty-four nests were excavated with shovels. Twenty males and 19 or 20 workers were collected from seven nests, for a total of 279 individuals. Seven males each were collected from an additional 177 nests, for a total of 1239 individuals. All ants were stored in 95% ethanol until DNA extraction and analysis.

DNA extraction and microsatellite analysis

DNA was extracted from all males and workers by boiling heads in 250 μL of a 5% Chelex (Bio-Rad) solution at 95 °C for 20 min. Samples were centrifuged and the supernatant was used as the template for polymerase chain reaction (PCR) amplification. Twenty males and 19 or 20 workers from seven colonies 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 & Gordon 2002b), and Pr-1 (Gadau *et al*. 2003), according to the procedure described in Helms Cahan & Keller (2003). The remaining 1239 males were genotyped at the microsatellite loci Myrt3 and Pr-1. PCR products were run on an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems) using fluorescent dyes, and analysed using GENOTYPER software (Applied Biosystems).

Genetic analysis

The colonies we sampled are within the geographical range of the dependent-lineage system as documented in Anderson *et al*. (2006) and Schwander *et al*. (2006). We confirmed this by amplifying a 433-bp portion of the *cox*1 gene of 1 worker from 300 colonies on our long-term study site, as in Helms Cahan & Keller (2003), and performing restriction digests using enzymes that cut only one lineage, as in Helms

Cahan *et al*. (2006). This revealed two cryptic lineages in the geographical area sampled and allowed us to estimate the lineage ratio on the study site.

We assessed male parentage in *P. barbatus* in two ways. As is common in studies of worker reproduction (Dijkstra & Boomsma 2006; Kronauer *et al*. 2006), we reconstructed queen genotypes from worker genotypes and then compared male genotypes to queen genotypes, using the 20 males and 19–20 workers from seven colonies. Workers from the seven colonies were also used to estimate mating frequency. For the remaining 177 colonies, we also took advantage of allelic differences between the two lineages and assessed male parentage based only on male genotypes (see below). Additionally, we tested for linkage between loci using the 140 males sampled with workers, and the program GENEPOP (Raymond & Rousset 1995).

Genotypes of colony queens were inferred from the genotypes of workers using the program MATESOFT, version 1.0 (Moilanen *et al*. 2003), which lists all possible queen genotypes and their respective probabilities. In cases where more than one queen genotype was possible, the most likely queen genotype was used in subsequent analyses. We used the program FSTAT to calculate background allele frequencies from worker genotypes. These allele frequencies were also used to estimate relatedness among workers within colonies as in Queller & Goodnight (1989), using the program SPAGeDi (Hardy & Vekemans 2002). Mating frequency statistics were calculated using MATESOFT, and the estimated effective number of matings was corrected for sample size following Nielsen *et al*. (2003). The effective number of matings is the number of equally contributing mates that would result in the same average relatedness among the worker offspring. Lineage-specific nondetection errors for patrilines were calculated from allele frequencies generated from workers' fathers, as $\Pi_1^n(1 - H_i)$, where H_i is the expected heterozygosity at the i^{th} locus of n loci (Foster & Ratnieks 2001).

For the seven colonies from which we sampled both males and workers, the parentage of males was determined by comparing the multilocus genotypes of males with that of the inferred queen. All loci used were informative; the worker's maternal and paternal alleles differed. At an informative locus, there is a 50% chance that a worker-produced male receives an allele from his worker mother that is not shared by the queen (Foster & Ratnieks 2001). Males were considered to have been produced by workers if they carried an allele at any locus not compatible with the queen's genotype. The probability of detecting a worker-produced male (P_j), the total number of assignable males (N_a), and the probability of not sampling any worker-produced males were calculated as in Foster & Ratnieks (2001): the probability of detecting a worker-produced male was calculated for each nest as $\sum_1^n P_i\,(1 - 0.5^{l_i})$ where n is the number of patrilines, P_i is the proportional representation of the ith patriline, and l_i is the number of informative loci analysed at the ith patriline. The total number of assignable males is (P_jN_j) where N_j is the number of males analysed for the j^{th} nest. The probability of not sampling any worker-produced males was $(1 - x)^{Na}$ where x is the hypothetical proportion of males that are produced by workers.

For the 177 colonies from which only males were sampled, we established parentage based on genotypes at two microsatellite loci diagnostic for lineage (Myrt3 and Pr-1). Workers are almost invariably heterozygous at these loci (Volny & Gordon 2002a; Helms Cahan & Keller 2003; Helms Cahan *et al*. 2006; Schwander *et al*. 2006). Queen-produced males have alleles corresponding to their mitochondrial lineage at all loci. However, worker-produced males could have a lineage-1 allele at one locus and a lineage-2 allele at the other locus. Also, if queens produce all males, at most two alleles should be present among males from a given colony. Thus, we classified males as worker-produced if they had an interlineage genotype across nuclear loci, or more than two alleles were present at one locus among all the males from a given colony. For such males, the probability of detecting a worker-produced male as such is 0.5.

Results

We found no evidence of worker reproduction in *Pogonomyrmex barbatus*. For each colony, we detected no more than two alleles per locus among the males sampled. We detected only one allele at each locus for all males. All loci were unlinked and all males had multilocus genotypes with alleles from only one lineage. Thus, it appears that each male was queen-produced.

Queen genotypes inferred from males were in all cases consistent with queen genotypes inferred from workers. The power of detecting the correct queen genotype, calculated by MATESOFT, was greater than 0.99 for all colonies. Nondetection errors for patrilines were $5e10^{-4}$ for lineage 1 and $7e10^{-4}$ for lineage 2. Queen genotypes inferred from males were identical to the most probable queen genotype inferred from workers for six colonies. In one case (colony 5), worker genotypes indicated that the queen was heterozygous at locus PO8, although we found only one allele among all males from this colony. The probability of 20 males receiving only one of two queen alleles is very low (0.5^{20}). The five workers that possessed the allele not found among males could have been foreign, but we included these individuals because doing so did not change the outcome of our analysis. The probabilities of detecting a worker-produced male (P_j) for each colony from which we sampled 20 males and 19 or 20 workers ranged from 0.942 to 0.992 and the mean, weighted by the number of males analysed for each colony, was 0.985 (Table 1). P_j is 0.992 for

Table 1 Worker production of males was zero. Shown are measures demonstrating confidence in our ability to identify worker-produced males (WPM) for males from the seven colonies from which workers were sampled: the number (n) of males and workers analysed, the probability of detecting a worker's son (P_j), and the percentage of WPM. The overall probability of detecting a worker-produced male (P_j) is the average over colonies weighted by the number of males analysed per colony

Colony	n males	n workers	P_j	Percentage of WPM
1	20	20	0.992	0
2	20	20	0.992	0
3	20	20	0.992	0
4	20	20	0.942	0
5	20	19	0.992	0
6	20	20	0.992	0
7	20	20	0.992	0
Overall	140	139	0.985	0

Table 2 Mating frequency statistics for matings between lineages in seven colonies. Shown are the lineages of colony queens, number of worker offspring analysed (n), the observed number of patrilines among workers (K_{obs}), and the sample size-corrected estimate of queen effective mating frequency (M_e). Mean number of patrilines (K_{obs}), and mating frequency (M_e) ± SE were calculated by jackknifing over colonies

Colony	Lineage	n workers	K_{obs}	M_e
1	1	20	6	3.13
2	2	20	4	3.08
3	1	20	7	5.03
4	1	20	4	3.64
5	2	19	3	1.79
6	1	20	6	5.62
7	2	20	3	2.77
Overall		139	4.71 ± 0.5	3.58 ± 0.61

six colonies because all seven loci were informative in all patrilines. In colony 4, P_j is lower because one workers' father had the same allele as the queen at one locus. For colonies from which we sampled both males and workers, the number of assignable males (N_a) was 138. For the remaining 177 colonies from which we sampled only males, the number of assignable males was 620, so the total number of assignable males was 758. We calculated the probability of not sampling any worker-produced males for $(1 - x)^{Na} = 0.05$, and as in Kronauer *et al.* (2006). Therefore, for $N_a = 758$, the probability that we failed to detect a worker contribution more than 0.39% is less than 0.05.

The mean ± standard error (SE) relatedness among workers was 0.35 ± 0.01. Mating frequencies are given in Table 2. The mean ± SE observed number of patrilines (K_{obs}) and sample size-corrected estimate of effective mating frequency (M_e) estimated by jackknifing over colonies were 4.71 ± 0.61 (K_{obs}) and 3.58 ± 0.5 (M_e). K_{obs} is lower than the total number of males with whom a queen mates, as it is an estimate only of the number of times a queen mates with a male of the opposite lineage. Also, it is possible that mating frequencies differ between the lineages. To test this, we computed average observed mating frequencies for each lineage and compared the number of patrilines of lineage 1 queens to lineage 2 queens for the seven colonies from which we sampled workers. The average number of patrilines found among lineage 1 is significantly higher than the number found among workers of lineage 2 (Tables 2, t-test, $P < 0.03$). This difference in patrilines among the lineages reflects the asymmetry in the lineage ratio. From the sample of 300 colonies, the ratio of lineage 1 to lineage 2 was 42:58, which is significantly different from 1:1 (chi-squared test; $P < 0.05$).

Discussion

Our results demonstrate that queens dominate male production in *Pogonomyrmex barbatus.* We detected no worker-produced males out of the 1379 analysed. The mechanisms underlying the absence of worker-produced males vary within the social insects, including direct policing by the queen in some species (Bego 1990; Saigo & Tsuchida 2004; Wenseleers *et al.* 2005). However, mature colonies of *P. barbatus* can contain over 10 000 workers (Gordon 1992), making it unlikely that direct restraint by the queen prevents the production or development of worker-produced males. Another possibility is that the lack of worker-produced males could be a result of the dependent-lineage system. Linksvayer *et al.* (2006) suggested that viability of males may depend on interactions between lineage-specific nuclear and mitochondrial gene products. Thus, the lack of worker-produced males could be the result of low viability of worker offspring. Polyandry is widespread throughout the genus *Pogonomyrmex*, so inclusive fitness theory predicts that worker-produced males are unlikely regardless of the mode of caste determination. It is also possible that workers do reproduce but no recombination occurs, or they discard the genome of one lineage. In this case, worker-produced males would be genetically identical to queen-produced males and would be undetectable by our molecular methods, but would not interfere with dependent-lineage dynamics.

Worker removal of worker-produced male eggs or aggression towards reproducing individuals may contribute to the observed lack of worker-derived offspring in *P. barbatus*. Workers often differ in their relatedness to queen-produced and worker-produced males (Wenseleers & Ratnieks 2006). An average effective mating frequency of 3.58, and low value of relatedness among workers within colonies (0.35 ± 0.01), is sufficient for workers to be more related to males produced by the queen than by their sisters, and to police one another. Mating frequencies may be similar throughout the genus *Pogonomyrmex*. Frequencies above 2.0, which would lead to low relatedness within colonies, have also been found in the harvester ants *P. rugosus* (Gadau *et al*. 2003), *P. badius* (Rheindt *et al*. 2004) and *P. occidentalis* (Wiernasz *et al*. 2004).

Our estimate of effective mating frequency is close to the previous estimate for *P. barbatus* (Volny & Gordon 2002a). Both estimates are based on data from worker genotypes, which provide an estimate only of the number of times the queen mated with males of the opposite lineage. Reproductive females in dependent-lineage populations of *P. barbatus* have fathers of the same lineage. If both lineages were equally represented in the population and queens mated randomly, or chose mates of both lineages based on their cuticular hydrocarbon profiles (Volny *et al*. 2006), the true average observed mating frequency would be doubled, or 9.42. However, an asymmetrical lineage ratio (Schwander *et al*. 2005) may result in differences in the number of patrilines represented among workers of each lineage. Lineage 2 is more frequent in the geographical area around our study site. For the seven colonies from which we sampled workers, the number of patrilines found among workers of lineage 1 was significantly higher than the number found among workers of lineage 2. Thus, the average observed mating frequency is between 4.71 and 9.42, and may be best estimated by adding the averages for each lineage: 9.08. Also, the ratio of patriline number for lineage 2 to lineage 1, 37:63, is not significantly different from the ratio of lineage 1:2 in our population (*z*-test for difference between population proportions; $P = 0.47$). These lineage-specific differences in patriline number among workers are consistent with random mating and indiscriminate use of sperm by queens (Schwander *et al*. 2005).

Our estimates of mating frequency in combination with the observed lack of worker-produced males have important implications for the maintenance of the dependent-lineage system. Schwander *et al*. (2006) and Anderson *et al*. (2006) suggested that negative frequency-dependent selection acts on queens of the more frequent lineage at the mating flight; queens of the more frequent lineage have a lower change of mating with a male of the opposite lineage, producing workers, and establishing a colony. Thus, the factors most important to the persistence of the system are mating frequency and the lineage ratio. The probability that queens will mate only with males of the same lineage is the first term of the binomial expansion $[f(a) + f(b)]^n$, where f is lineage frequency, a and b represent both lineages, and n is the number of matings (Anderson *et al*. 2006). With an effective mating frequency of 6.9, and a lineage ratio of 42:58 in our population, 0.3% of lineage 1 queens and 2% of lineage 2 queens are be expected to be unable to produce workers. Given the assumption that both lineages produce an equal amount of reproductives, this sevenfold difference between the lineages in the probability of successful colony establishment could contribute to the maintenance of an asymmetrical lineage ratio through time.

To our knowledge, this is the first study documenting the lack of worker-produced males in the genus *Pogonomyrmex*. Inclusive fitness theory predicts their absence in other *Pogonomyrmex* species as well, given high mating frequencies within the genus. Indeed, the only documented evidence of worker reproduction in *Pogonomyrmex* is in queenless colonies of the Florida harvester ant *P. badius* (Smith & Tschinkel 2006) and *P. barbatus* (D.M. Gordon, personal observations). More work is needed to determine the mechanism underlying the lack of worker-produced males in *P. barbatus*, since workers have developed ovaries and laboratory colonies of *P. barbatus* have been observed to produce males after queen death. The extent to which workers produce males in queenless colonies in natural populations remains unknown. Our study was robust enough to detect worker-produced males if workers produced greater than 0.39% of the males among all colonies sampled. Thus, if it occurs, worker reproduction is sufficiently infrequent that it probably has little impact on colony or population-level processes in *P. barbatus*, particularly on the maintenance of the dependent-lineage system.

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References

Anderson KE, Gadau J, Mott B *et al*. (2006) Distribution and evolution of genetic caste determination in *Pogonomyrmex* seed harvester ants. *Ecology*, **87**, 2171–2184.

Arevalo E, Strassmann J, Queller D (1998) Conflicts of interest in social insects: male reproduction in two species of *Polistes*. *Evolution*, **52**, 797–805.

Bego LR (1990) On social regulation in *Nannotrigona* (*Scaptotrigona*) *postica* Latreille with special reference to productivity of colonies (Hymenoptera, Apidae, Meliponinae). *Revista Brasileira de Entomologia*, **34**, 721–738.

Bourke AFG, Green HAA, Bruford MW (1997) Parentage, reproductive skew and queen turnover in a multiple-queen ant analysed with microsatellites. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 277–283.

Charnov EL (1978) Evolution of eusocial behavior: offspring choice or parental parasitism? *Journal of Theoretical Biology*, **75**, 451–465.

Cole BJ, Wiernasz DC (1999) The selective advantage or low relatedness. *Science*, **285**, 891–893.

Dijkstra MB, Boomsma JJ (2006) Are workers of *Atta* leafcutter ants capable of reproduction? *Insectes Sociaux*, **53**, 136–140.

Foitzik S, Haberl M, Gadau J, Heinze J (1997) Mating frequency of *Leptothorax nylanderi* ant queens determined by microsatellite analysis. *Insectes Sociaux*, **44**, 219–227.

Foster KR, Ratnieks FLW (2000) Facultative worker policing in a wasp. *Nature*, **407**, 692–693.

Foster KR, Ratnieks FLW (2001) Convergent evolution of worker policing by egg eating in the honeybee and common wasp. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **268**, 169–174.

Gadau J, Strehl CP, Oettler J, Hölldobler B (2003) Determinants of intracolonial relatedness in *Pogonomyrmex rugosus* (Hymenoptera; Formicidae): mating frequency and brood raids. *Molecular Ecology*, **12**, 1931–1938.

Gordon DM (1992) How colony growth affects forager intrusion between neighboring harvester ant colonies. *Behavioral Ecology and Sociobiology*, **31**, 417–427.

Gordon DM, Kulig AW (1996) Founding, foraging, and fighting: colony size and the spatial distribution of harvester ant nests. *Ecology*, **77**, 2293–2409.

Halling LA, Oldroyd BP, Wattanachaiyingcharoen W *et al*. (2001) Worker policing in the bee *Apis florea*. *Behavioral Ecology and Sociobiology*, **49**, 509–513.

Hamilton WD (1972) Altruism and related phenomena, mainly in social insects. *Annual Review of Ecology and Systematics*, **3**, 193–232.

Hammond RL, Keller L (2004) Conflict over male parentage in social insects. *PLoS Biology*, **2**, 1–11.

Hardy OJ, Vekemans X (2002) SPAGEDi: a versitle computer program to analyze spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Hartmann A, Wantia J, Torres JA, Heinze J (2003) Worker policing without genetic conflicts in a clonal ant. *Proceedings of the National Academy of Sciences, USA*, **100**, 12836–12840.

Helanterä H, Sundström L (2005) Worker reproduction in the ant *Formica fusca*. *Journal of Evolutionary Biology*, **18**, 162–171.

Helms Cahan S, Keller L (2003) Complex hybrid origin of genetic caste determination in harvester ants. *Nature*, **424**, 306–309.

Helms Cahan S, Parker JD, Rissing SW *et al*. (2002) Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 1471–2954.

Helms Cahan S, Julian GE, Rissing SW *et al*. (2004) Loss of phenotypic plasticity generates genotype–caste association in harvester ants. *Current Biology*, **14**, 2277–2282.

Helms Cahan S, Julian GE, Schwander TS, Keller L (2006) Reproductive isolation between *Pogonomyrmex rugosus* and two lineages with genetic caste determination. *Ecology*, **87**, 2160–2170.

Iwanishi S, Hasegawa E, Ohkawara K (2003) Worker oviposition and policing behaviour in the myrmicine ant *Aphaenogaster smythiesi japonica* Forel. *Animal Behavior*, **66**, 513–519.

Julian GE, Fewell JH, Gadau J, Johnson RA, Larrabee D (2002) Genetic determination of the queen caste in an ant hybrid zone. *Proceedings of the National Academy of Sciences, USA*, **99**, 8157–8160.

Kronauer DJC, Schöning JS, Boomsma JJ (2006) Male parentage in army ants. *Molecular Ecology*, **15**, 1147–1151.

Linksvayer T, Wade M, Gordon DM (2006) Genetic caste determination in harvester ants: possible origin and maintenance by cyto-nuclear epistasis. *Ecology*, **87**, 2185–2193.

Moilanen A, Sundström L, Pedersen JS (2003) MATESOFT: a program for genetic analysis of mating systems 1.0b. *Molecular Ecology Notes*, **4?**, 795–797.

Monnin T, Ratnieks FLW (2001) Policing in queenless ponerine ants. *Behavioral Ecology and Sociobiology*, **50**, 97–108.

Nielsen R, Tarpy DR, Reeve HK (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. *Molecular Ecology*, **12**, 3157–3164.

Oldroyd BP, Halling LA, Good G *et al*. (2001) Worker policing and worker reproduction in *Apis cerana*. *Behavioral Ecology and Sociobiology*, **50**, 371–377.

Oster GF, Wilson EO (1978) *Caste and Ecology in the Social Insects*. Princeton University Press, New Jersey.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ratnieks F (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *American Naturalist*, **132**, 217–236.

Ratnieks F (1989) Worker policing in the honeybee. *Nature*, **342**, 796–797.

Ratnieks F, Foster K, Wenseleers T (2006) Conflict resolution in insect societies. *Annual Review of Entomology*, **51**, 581–608.

Rheindt FE, Gadau J, Strehl C, Hölldobler B (2004) Extremely high mating frequency in the Florida harvester ant (*Pogonomyrmex badius*). *Behavioral Ecology and Sociobiology*, **56**, 472–481.

Saigo T, Tsuchida K (2004) Queen and worker policing in monogynous and monandrous colonies of a primitively eusocial wasp. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **271**, 509–512.

Schwander T, Rosset H, Chapuisat M (2005) Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. *Behavioral Ecology and Sociobiology*, **59**, 215–221.

Schwander T, Helms Cahan S, Keller L (2006) Characterization and distribution of *Pogonomyrme*x harvester ant lineages with genetic caste determination. *Molecular Ecology*, **16**, 367–387.

Schwander T, Helms Cahan S, Keller L (2007) Genetic caste determination imposes costs during colony founding. *Journal of Evolutionary Biology*, **19**, 402–409.

Smith CR, Tschinkel WR (2006) The sociometry and sociogenesis of reproduction in the Florida harvester ant (*Pogonomyrmex badius*). *Journal of Insect Science*, **6**, 1536–2442.

Starr CK (1984) Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In: *Sperm Competition and the Evolution of Animal Mating Systems* (ed. Smith RL), pp. 428–459. Academic Press, London, UK.

Villesen P, Boomsma JJ (2003) Patterns of male parentage in the fungus-growing ants. *Behavioral Ecology and Sociobiology*, **53**, 246–253.

Volny V, Gordon DM (2002a) Genetic basis for queen-worker dimorphism in a social insect. *Proceedings of the National Academy of Sciences, USA*, **99**, 6108–6111.

Volny VP, Gordon DM (2002b) Characterization of polymorphic microsatellite loci in the red harvester ant, *Pogonomyrmex barbatus*. *Molecular Ecology Notes*, **2**, 302–303.

Volny VP, Greene MJ, Gordon DM (2006) Brood production and lineage discrimination in the harvester ant *Pogonomyrmex barbatus*. *Ecology*, **87**, 2194–2220.

Wenseleers T, Ratnieks FLW (2006) Comparative analysis of worker reproduction and policing in eusocial hymenoptera supports relatedness theory. *American Naturalist*, **168**, 163–179.

Wenseleers T, Tofilski A, Ratnieks FLW (2005) Queen and worker policing in the tree wasp *Dolichovespula sylvestris*. *Behavioral Ecology and Sociobiology*, **58**, 80–86.

Wheeler DE (1986) Developmental and physiological determinants of caste in social hymenoptera: evolutionary implications. *American Naturalist*, **128**, 13–34.

Wheeler DE (1994) Nourishment in ant societies; patterns in individuals and societies. In: *Nourishment and the Evolution in Insect Societies* (eds Hunt JH, Nalepa CA), Westview Press, Boulder Colorado.

Wiernasz D, Perroni C, Cole B (2004) Polyandry and fitness in the western harvester ant, *Pogonomyrmex occidentalis*. *Molecular Ecology*, **13**, 1601–1606.

This work is part of Sevan Suni's doctoral research at Stanford University. Christopher Gignoux received his bachelor's degree from Stanford University and now works for the personal genetics company 23andme, Inc. Deloorah Gordon is a professor of behavioural ecology at Stanford University.
