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Genetic caste determination in *Pogonomyrmex* harvester ants imposes costs during colony founding

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Abstract

Some populations of *Pogonomyrmex* harvester ants comprise genetically differentiated pairs of interbreeding lineages. Queens mate with males of their own and of the alternate lineage and produce pure-lineage offspring which develop into queens and inter-lineage offspring which develop into workers. Here we tested whether such genetic caste determination is associated with costs in terms of the ability to optimally allocate resources to the production of queens and workers. During the stage of colony founding, when only workers are produced, queens laid a high proportion of pure-lineage eggs but the large majority of these eggs failed to develop. As a consequence, the number of offspring produced by incipient colonies decreased linearly with the proportion of pure-lineage eggs laid by queens. Moreover, queens of the lineage most commonly represented in a given mating flight produced more pure-lineage eggs, in line with the view that they mate randomly with the two types of males and indiscriminately use their sperm. Altogether these results predict frequency-dependent selection on pairs of lineages because queens of the more common lineage will produce more pure-lineage eggs and their colonies be less successful during the stage of colony founding, which may be an important force maintaining the coexistence of pairs of lineages within populations.

Introduction

Division of labour between reproductive queens and sterile workers is a hallmark of insect societies and an important component of their ecological success (Hölldobler & Wilson, 1990). In ants and other social Hymenoptera forming large and complex societies, reproductive division of labour is generally associated with marked morphological differences between the queen and worker castes. These differences usually result from the combined effects of environmental and social factors such as diet, temperature and colony size inducing female larvae to engage into the queen or worker developmental pathway (Brian, 1957; Wheeler, 1994).

However, genetically determined caste differentiation has been recently discovered in two populations ('Hidalgo' and 'Junction') of *Pogonomyrmex* harvester ants (Helms Cahan *et al.*, 2002, 2004; Julian *et al.*, 2002; Volny & Gordon, 2002b; Helms Cahan & Keller, 2003). Each population consists of two genetically distinct and interbreeding lineages (H1 and H2 at Hidalgo and J1 and J2 at Junction). Queens mate multiply (Hölldobler, 1976; Gadau *et al.*, 2003) with males of their own lineage and males of the alternate, co-occurring lineage. Pure-lineage offspring develop into queens while inter-lineage females virtually always develop into workers (Helms Cahan *et al.*, 2002; Julian *et al.*, 2002; Volny & Gordon, 2002b; Helms Cahan & Keller, 2003). In both the Hidalgo and Junction populations, this mode of caste determination is obligate as pure-lineage females almost invariably fail to develop into workers (Helms Cahan

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et al., 2004). Interestingly, all four lineages have been shown to derive from historical hybridization between two well-studied species of harvester ants, *Pogonomyrmex rugosus* and *P. barbatus*. Both parental species have typical environmental caste determination, but the evolutionary trajectory which led from environmental caste determination in the parental species to genetic caste determination in the hybrid lineages remains speculative (see Helms Cahan *et al.*, 2002; Julian *et al.*, 2002; Volny & Gordon, 2002b; Helms Cahan & Keller, 2003).

The transition from an environmental to a genetic system of caste determination may have both positive and negative effects on colony efficiency. First, the two-lineage genetic composition of workers may provide benefits as a result of hybrid vigour (Fry *et al.*, 1998; Schmid-Hempel, 1998). Secondly, the differential genomic composition of queens and workers may also allow for caste-specific adapted genotypes in contrast to an environmental system of caste determination where queens and workers are constrained to share the same genome. Finally, genetic caste determination may also be associated with costs because of reduced flexibility in the ratio of queens and workers produced at different stages of the colony life cycle. In ants, colonies typically go through several distinct stages of growth and reproduction with exclusive investment in workers during the early stage of colony development and later an alternating production of reproductive individuals and workers when the colony has reached a given threshold size (Oster & Wilson, 1978; Hölldobler & Wilson, 1990). In harvester ants, new queens are generally produced in early summer once colonies have reached a size of about 10 000 to 12 000 workers, that is after about 4 years (Gordon, 1995). In such colonies, the window of time where male and/or female reproductives are produced lasts only a few weeks and during the remaining of the year colonies specialize on worker production. This implies that, if queens cannot adaptively modify the proportion of pure- and inter-lineage eggs laid, a system of genetic caste determination may be costly because it results in the production of a non-optimal ratio of queen- and worker-destined eggs.

The aim of this study was to determine whether genetic caste determination is associated with productivity costs. We focused on the period of colony founding as colonies must produce only workers to maximize colony growth and because this is the stage during which there is highest selection and colony mortality (Gordon & Kulig, 1996). We first collected queens early during the mating flight to obtain queens that mated only to males of their own lineage or males of the other lineage. This allowed us to determine, by microsatellite genotyping, the probability of successful amplification of the two types of eggs which indicates the viability of eggs before hatching. Next, we estimated the proportion of pure-lineage (queen-destined) and inter-lineage (worker-destined) eggs laid by founding queens of three lineages (H1, J1 and J2) and tested whether the number of offspring produced during the first 6 weeks following the mating flight was negatively associated with the proportion of pure-lineage eggs produced. Finally, we tested whether the proportion of pure-lineage eggs laid by queens was positively associated with the frequency of queens of their own lineage in the mating flight. This would be the expected pattern if queens mate randomly with males of the two lineages and then use their sperm indiscriminately.

Methods

Test for differential egg viability

We estimated the viability of pure- and inter-lineage eggs by assessing the probability of successful amplification of the two types of eggs. As we sampled a mixture of eggs of different ages (approximately 24 h to 7 days), the estimated proportion of pure- and inter-lineage eggs laid by queens could be biased by a differential survival of the two egg types during early embryonic development. The proportion of the less viable egg type would be underestimated because only insufficient amounts of bad quality DNA may be extracted from older eggs, resulting in low amplification success. To account for such an effect and to generally compare viability of pure- and inter-lineage eggs, we extracted and amplified eggs of queens known to have mated with males of a single lineage (same or alternate lineage). To obtain such queens, we collected 110 queens in copula along with a male mate within the first 20 min of a mating aggregation at Hidalgo, New Mexico (containing the lineages H1 and H2) (Helms Cahan & Keller, 2003). The lineage of each queen was assessed by microsatellite genotyping using a piece of one mid-leg. All eggs laid by these queens during the weeks 7–9 after the mating flight were sampled and stored in 100% ethanol. The lineage of each queen's mates was then determined by dissecting out the spermatheca and genotyping its contents. By this method, we obtained 13 queens of the H2 lineage and 11 of H1 lineage which were mated with males of a single lineage. Five H1 queens and eight H2 queens were mated with males of the same lineage, while six H1 queens and five H2 queens were mated to males of the alternate lineage. Twenty eggs of each of these queens were used to estimate the probability of amplification of pure- and inter-lineage eggs.

Sampling and productivity measurements

To estimate the proportion of pure-lineage eggs laid by founding queens and to test whether this proportion was negatively associated with the number of offspring produced, we examined naturally mated, dealate queens from two mating aggregations. We collected 155 queens from a mating aggregation of the population at 'Junction',

New Mexico composed of the J1 and J2 lineages, and 93 from a mating aggregation of the H1 and H2 lineages of the 'Hidalgo' population (Helms Cahan & Keller, 2003). All queens were housed in 30-mL glass tubes with water-soaked cotton at one end and maintained in complete darkness at 60% humidity and 30 °C. After 6 weeks, the number of all advanced brood (last instar larvae, pupae and workers) were counted for each queen. Larvae and pupae were included in the brood count to increase the number of scorable offspring but were counted at 0.5 (larvae) and 0.75 (pupae) of the value of a worker. These weighting factors were determined by the length of time separating last instar larvae and pupae from the adult stage relative to the duration of the entire development. Using different weighting schemes did not qualitatively change the results.

We used a random subset of the naturally mated queens of each lineage for the analysis of egg genotypes (38 J2 and 28 J1 from the Junction population and 29 H1 from the Hidalgo population). Because only four founding queens of the H2 lineage were found at Hidalgo, this lineage was not included in the analyses. Eighteen to 24 eggs were sampled and genotyped from each queen after productivity measurements were completed.

Because the pure-lineage eggs had a very low probability of amplification (see Results), we estimated their proportion indirectly, using a simple correction factor. On average, 91% of inter-lineage eggs amplified, so that for each queen, the proportion of pure-lineage eggs was computed as $1 - (100/91 \times$ the proportion of inter-lineage eggs).

Genotyping of adults, spermatheca contents and eggs

We used two different methods to assign individuals to lineages in each population. For queens that needed to be kept alive, we identified the maternal lineage of one worker offspring based on the 433-bp portion of the *cox1* mitochondrial gene described in Helms Cahan & Keller (2003). *Cox1* of J1 and J2 and of H1 and H2 can be distinguished from one another at two restriction sites for each pair (Fig. 1). To prevent misidentification because of low enzyme activity, two independent reactions were performed for each worker from a single PCR, each with a different enzyme. To distinguish individuals of the J1 and J2 lineages, we used the enzyme *Pvu*II that has a restriction site for the *cox1* portion of J1 but not of J2 and *Bsa*I that has a restriction site for J2 but not for J1 (Fig. 1). Similarly, to distinguish individuals of the H1 and the H2 lineages, we used the enzymes Mfe1 and *Bsp*CNI cutting respectively, the *cox1* portion of H1 and H2 (Fig. 1). For queens that could be killed, we used microsatellite genotyping at loci with fixed differences between lineages (Table 1). We used the microsatellite markers Pb-5, Pb-7 (Volny & Gordon, 2002a) or PR1 (Gadau *et al.*, 2003) to assign individuals to lineages in the Hidalgo population and we used Myrt-3 (Evans, 1993) or PR1 (Gadau *et al.*, 2003) to assign individuals to lineages in the Junction population (Table 1).

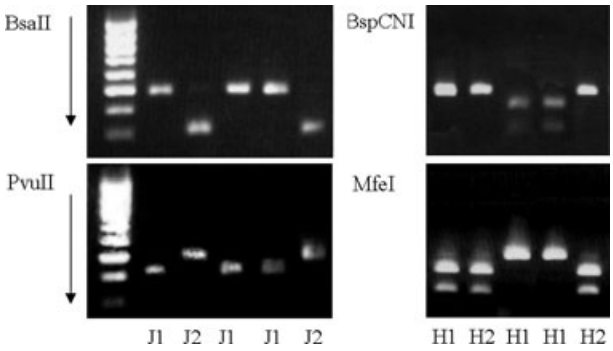

Fig. 1 Restriction digests of the 433-bp portion of the *cox1* mitochondrial sequence. To assign individuals to the lineages J1 and J2, the *cox1* sequences are digested independently with *Bsa*II and with *Pvu*II, to assign individuals to the lineages H1 and H2, the sequences are digested with *Bsp*CNI and with *Mfe*I.

DNA was extracted in 250 μL of 5% Chelex (Sigma-Aldrich, Steinheim, Germany) at 95 °C for 20 min. PCR reactions were performed as described in Helms Cahan & Keller (2003) except for PR1 where the hybridization temperature in the PCR cycles was set to 54 °C and with 4 μL DNA in a 20-μL reaction volume for the PCR of *cox1*. Amplified fluorescent fragments were visualized on 5% polyacrylamide/6 M urea sequencing gels using an automated 377 ABI sequencer. Gels were analysed with GENESCAN V.3.1.2. Software (Applied Biosystems, Foster City, CA, USA).

DNA from eggs was extracted by digesting each egg during at least 4 h at 55 °C in 50 μL of extraction buffer (containing 100 mM NaCl, 50 mM Tris, 1 mM EDTA, 0.5% SDS, and 200 μg mL^{-1} proteinase K). After a single phenol–chloroform extraction, DNA was precipitated with 1/10 volume of 3 M sodium acetate and 2.5 volumes of ethanol (100%), with 2 μL glycogen added as a DNA carrier. The pellets were rinsed with 70% ethanol, dried at 50 °C and resuspended in 30 μL of distilled water. Eggs from queens sampled at Junction were genotyped at the microsatellite locus Myrt-3 (Evans, 1993), eggs from individuals sampled at Hidalgo at PR1 (Gadau *et al.*, 2003) (Table 1). Amplification and visualization of DNA fragments was performed as described before with 4 μL DNA extraction in a 10 μL reaction volume.

Results

Genotyping of eggs laid by queens mated to a male of the same lineage or a male of the alternate lineage revealed a strong difference in the survival of pure-lineage and inter-lineage eggs (Fisher's exact test, $P < 0.0001$). Almost all eggs produced by queens mated with a male of the other lineage could be amplified (91%, $n = 220$)

Table 1 Allele frequencies of the used microsatellite markers with fixed differences between H1 and H2, respectively J1 and J2. All markers except PR1 have been shown to be discriminant in earlier studies (Volny & Gordon, 2002b; Helms Cahan & Keller, 2003).

Locus	Allele	H1	H2
PR1	*N*	80	132
	394	0.724	–
	400	0.276	–
	406	–	0.921
	412	–	0.005
	436	–	0.074
Pb-5	*N*	79	129
	214	–	0.031
	216	–	0.042
	224	–	0.069
	226	–	0.85
	228	–	0.008
	230	0.006	–
	232	0.988	–
	234	0.006	–
Pb-7	*N*	82	132
	141	–	0.008
	143	–	0.977
	145	–	0.004
	148	–	0.011
	150	0.006	–
	152	0.591	–
	154	0.037	–
	156	0.360	–
	158	0.006	–
		J1	J2
Pr-1	*N*	57	108
	388	0.851	–
	394	0.079	–
	399	–	1.000
	400	0.07	–
Myrt-3	*N*	57	108
	181	0.462	–
	183	–	0.993
	185	–	0.007
	189	0.241	–
	191	0.296	–

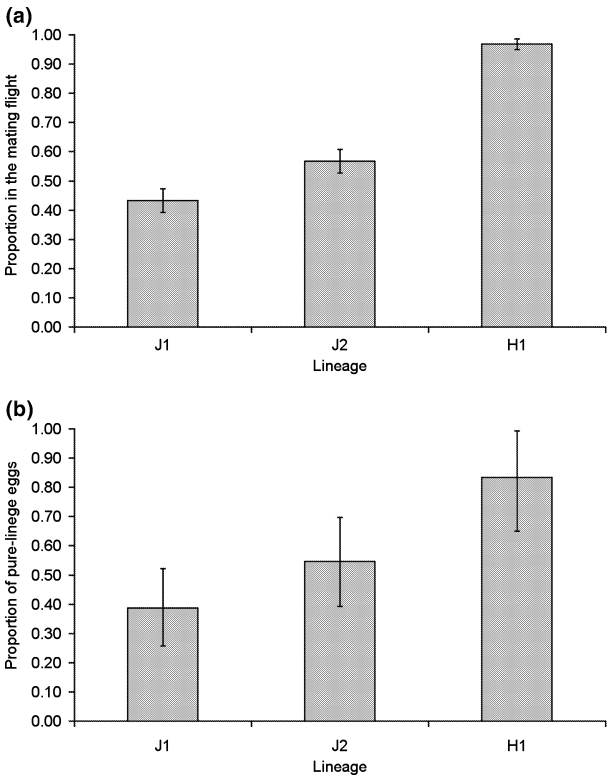

Fig. 2 (a) The proportion of analysed lineages in the mating swarms. The mating swarm at Junction had relatively even proportions of J1 (43%; $N_{tot} = 140$) and J2 (57%) while the mating flight at Hidalgo (lineages H1 and H2) was highly biased towards H1 (97%, $N_{tot} = 93$). (b) The proportion of pure-lineage eggs laid by naturally mated queens of each analysed lineage. The more frequent each lineage was in the mating flight, the more pure-lineage eggs the queens laid ($F_{2,92} = 55.42$, $P < 0.0001$, Tukey *post-hoc*: all $P < 0.05$).

while only 22% of the eggs produced by queens mated with a male of the same lineage amplified ($n = 260$). These data indicate that, in contrast to inter-lineage eggs, a very high proportion of pure-lineage eggs failed to undergo a normal development. Interestingly, the proportion of amplifiable pure-lineage eggs differed among queens ($\chi^2_{12} = 71.0$, $P < 0.0001$), as over 25% of these eggs (15 out of 58) were laid by a single H1 queen mated to a H1 male. Measuring the amplification success of eggs further allowed us to validate the high efficiency of the molecular methods applied, as 91% of inter-lineage eggs amplified. Failed extractions due to technical reasons are thus rare (<10%) and should occur randomly for pure- and inter-lineage eggs. An inspection of egg genotypes ($n = 201$) laid by queens mated with males of the alternate lineage also revealed that all the egg genotypes had paternal alleles hence indicating that they were diploid. The upper 95% confidence estimate of haploid male eggs (binomial distribution) was lower than 1.5%.

Of the 1995 analysed eggs laid by naturally mated queens only 884 (44%) amplified successfully indicating that queens laid a large number of nonviable pure-lineage eggs during colony founding. Indeed, there were only 86 pure-lineage eggs (9.7%) among the 884 eggs successfully genotyped. Correcting for the probability of amplification of the two types of eggs (see methods) revealed that, overall, the proportion of pure-lineage eggs was 54%.

The relative frequency of J1 and J2 queens in the mating swarm at Junction was 47% and 53% respectively ($n = 140$) and 97% and 3% ($n = 93$) for H1 and H2 queens at Hidalgo respectively (Fig. 2a). The proportions of pure-lineage eggs laid by queens of different lineages were qualitatively in line with the pattern predicted if queens mate randomly with the two types of males and

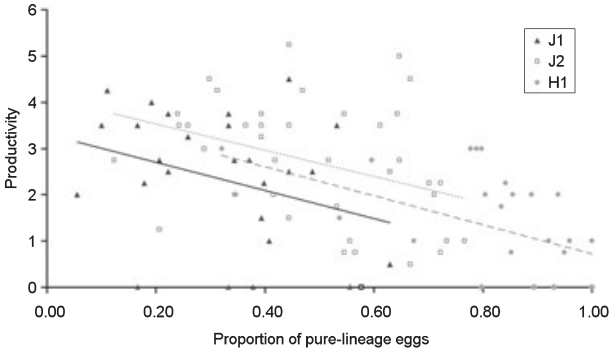

Fig. 3 The productivity of incipient colonies (weighted number of offspring produced during the first six weeks of colony founding) in relation to the proportion of pure lineage eggs laid by the naturally mated queens.

indiscriminately use their sperm during colony founding, i.e. the more frequent queens of a given lineage were in their mating flights, the more pure-lineage eggs they laid ($F_{2,92} = 55.42$, $P < 0.0001$; Tukey *post-hoc*, all $P < 0.05$; Fig. 2b).

Colony productivity was significantly decreased by the proportion of pure-lineage eggs laid and also depended on the lineage of queens (ANCOVA on arcsin square root-transformed data: effect of lineage: $F_{2,89} = 11.27$, $P < 0.0001$; effect of proportion of pure-lineage eggs: $F_{1,89} = 19.75$, $P < 0.0001$; interaction: $F_{2,89} = 0.01$, $P = 0.99$, Fig. 3). Colonies headed by queens of the J2 lineage were more productive than colonies headed by J1 queens (Tukey *post-hoc*: $P < 0.05$, Fig. 3), while there was no significant difference between colonies headed by queens of the H1 lineage and queens of the two other lineages (Tukey *post-hoc* $P > 0.05$). Importantly, the negative effect of production of pure-lineage eggs was observed over the whole range of the estimated proportions of pure-lineage eggs (Fig. 3). During the first 6 weeks of colony founding, colonies produced overall about 1.17 (range: 0–4) workers, 0.78 (0–3) pupae and 0.82 (0–6) last instar larvae (average ± SD of workers, pupae and larvae, respectively for J1: 1.50 ± 1.04, 0.93 ± 0.90, 0.29 ± 0.46; J2: 1.71 ± 1.18, 1.13 ± 0.84, 0.26 ± 0.50; H1: 0.14 ± 0.35, 0.17 ± 0.38, 2.07 ± 2.22) and the number of offspring produced was decreased by one unit for 37% more pure-lineage eggs.

Discussion

Our results demonstrate that genetic caste determination in *Pogonomyrmex* is associated with an intrinsic cost in terms of productivity during colony founding. Queens must mate with both same-lineage and alternate-lineage males in order to found a colony and produce fertile reproductives, but only the progeny of alternate-lineage males can be used to produce the initial workforce. Queens laid a ratio of pure- and inter-lineage eggs that matched the relative frequencies of females of the two lineages in the population, resulting in a high proportion of genetically inappropriate (queen-destined) eggs that imposed a substantial cost on initial productivity. The greater the proportion of pure-lineage eggs laid by queens was, the fewer offspring were produced in the first cohort of workers (Fig. 3). This is presumably a strong disadvantage, as the number of workers determines the competitive ability of incipient colonies and thus directly influences the probability of successful colony establishment (Gordon & Kulig, 1996). Moreover, rapidly growing colonies survive longer than slowly growing colonies and attain reproductive maturity at younger ages (Cole & Wiernasz, 1999). Thus, the negative effect of genetic caste determination on colony success might be even stronger when the whole life cycle is considered.

We found that the vast majority of pure-lineage eggs failed to develop during colony founding. Only 22% of the eggs of queens that mated only to same-lineage males reached a stage at which they could be successfully genotyped, compared with over 90% success for eggs laid by queens mated only to alternate lineage males. This is in line with previous results suggesting that pure-lineage progeny terminate development during the egg stage (Helms Cahan *et al.*, 2004). Such early termination of development would tend to minimize the costs of a genetic caste determination system, as only few resources are invested into genetically inappropriate offspring during colony founding. This high mortality of pure-lineage eggs is especially intriguing as these individuals appear to develop into sexual females at more advanced colony life-history stages. The proximate reason for this early abortion of pure-lineage eggs in founding colonies remains entirely enigmatic, although it might be triggered by incompatibility between loci during initiation of worker development (Helms Cahan & Keller, 2003).

The proportions of pure-lineage eggs across lineages were in accordance with random mating, as they increased with the relative frequency of females of this lineage in the mating flight (Fig. 2a,b). We also found no evidence that queens preferentially select sperm to fit colony needs. Because only workers are produced in founding colonies, queens capable of selecting sperm should lay few if any pure-lineage eggs regardless of the proportions of same-lineage males. Yet, we found up to over 80% pure-lineage eggs in the most frequent lineage (H1).

Because queens do not control the genotypes of the female eggs they lay, a significant proportion of the initial brood in founding colonies cannot be used directly for worker production. In the most extreme case, queens that mate exclusively with same-lineage males suffer significant fitness costs because they largely fail to produce workers (Helms Cahan *et al.*, 2004). As extremely high mating frequencies are typical for the genus, however (Hölldobler, 1976; Helms Cahan *et al.*, 2002;

Julian *et al.*, 2002; Volny & Gordon, 2002a,b; Gadau *et al.*, 2003; Wiernasz *et al.*, 2004), the proportion of queens mating exclusively with males of their own lineage by chance is generally low except in populations with extremely unbalanced relative proportions (Helms Cahan *et al.*, 2004). We sampled queens from a highly skewed mating aggregation (97% H1 queens) and from a relatively balanced aggregation (47% and 53% of J1 and J2 queens) and found that costs are not relevant under high lineage skew. Colony productivity decreased proportionally over the whole range of the measured pure-lineage egg proportions, with nearly identical slopes for all three lineages despite being located at different points along the distribution (Fig. 3). Thus, even in a population with balanced proportions of the two lineages, where the risk of mating exclusively with males of a single lineage would be minimal, production of genetically inappropriate eggs is costly and can significantly reduce productivity.

Evidence for a cost of excess egg production may have implications for studies of other evolutionary phenomena in social insects. In queen–worker conflict over the sex ratio, for example, workers can manipulate the sex ratio by selectively rearing female over male eggs. Colonies may eventually profit from raising inappropriate offspring to store resources for later use (see Mock & Forbes, 1995; Chapuisat *et al.*, 1997); however, it has been largely neglected that a secondary modification of the sex ratio may also be associated with costs (for reviews see Bourke & Franks, 1995; Crozier & Pamilo, 1996). When such costs are included, they strongly affect the predictions of optimal sex ratio allocation and plausibly account for part of the variance observed across empirical studies (Reuter *et al.*, 2004). Egg costs may also play a role in the evolution of queen control over fertilization during egg laying. In the polygynous species *Linepithema humile* (Aron *et al.*, 1994), and *Pheidole pallidula* (Keller *et al.*, 1996a) and in the monogynous species *Colobopsis nipponicus* (Hasegawa, 1992) and the monogynous form of *Solenopsis invicta* (Aron *et al.*, 1995), the percentage of haploid eggs laid by queens never falls below 10% throughout the year, although no males are reared to adulthood outside the reproductive season. While a continuously high proportion of male eggs may be part of an adaptive reproductive strategy in *L. humile* (Aron *et al.*, 1994; Keller *et al.*, 1996b; Aron & Passera, 1999), the authors suggested that continual production of haploid eggs might reflect constraints on the efficiency in egg fertilization in the three other species. In contrast, queens of two monogynous species, the honeybee *Apis mellifera* (Ratnieks & Keller, 1998; Sasaki & Obara, 2001) and *Lasius niger* (Aron & Passera, 1999), have been shown to efficiently decrease the proportion of male eggs to 1–5% outside the reproductive season. This variation in the level of fertilization control may reflect different selection intensities related to the costs of male egg production. Honey bees use different cells to raise males and workers, so that queens are probably selected to adjust fertilization on a frequent basis (Ratnieks & Keller, 1998). *Lasius niger* queens found colonies independently and should thus be strongly selected to minimize costs during colony founding (Aron & Passera, 1999). Interestingly, *Pogonomyrmex* queens, which also found independently, seem to have perfect control over the sex of their offspring, as all eggs from queens mated exclusively with males of the alternate lineage had an inter-lineage hybrid genotype and were thus diploid, female eggs.

The combination of random mating and reduced productivity of queens with higher proportions of pure-lineage eggs results in frequency-dependent founding success. Under random mating, queens from the more frequent lineage are more likely to mate with males of their own lineage. Queens of the more frequent lineage will thus lay a higher proportion of pure-lineage eggs, with lower productivity during colony founding. As a consequence, the relative frequencies of the lineages in the new cohort of established colonies should become more even. This balancing effect at colony founding may be an important force maintaining the coexistence of the two lineages. It should be noted, however, that genetic caste determination may also impose costs at reproduction, when inter-lineage eggs are laid but are not used to produce queens. Such a cost would disproportionately affect the rarer lineage and may moderate the balancing effect of frequency-dependent founding success.

Although interbreeding lineages are dependent on one another for worker production, they are also competitors for territory and resources. We found a significant difference in productivity between two interbreeding lineages, J1 and J2, which may be important in determining their relative abundances in the field. For any given level of pure-lineage egg production, incipient colonies headed by queens of the J2 lineage were more productive than colonies headed by queens of the J1 lineage. This difference in productivity may be due to variation in queen fecundity, brood survival or developmental speed. Given the strong competition among incipient colonies, such an advantage should lead to higher colony founding success for J2 queens. Thus, despite the negative frequency-dependent founding success for queens of both lineages, the overall advantage of J2 queens should bias the equilibrium value towards J2. In line with this prediction, five additional populations exhaustively genotyped for another study all revealed proportions skewed in favour of the J2 lineage (Schwarder et al., unpublished). This difference in productivity between the co-occurring lineages adds additional support for the existence of balancing selection on lineage coexistence, as without some negatively frequency-dependent mechanism the competitive advantage of J2 queens should lead to loss of the J1 lineage and drive the system to extinction.

The four lineages that display genetic caste determination are historically derived from species with

environmental caste determination (Helms Cahan & Keller, 2003). We can infer the likely productivity of an environmental caste mechanism from queens with no pure-lineage eggs, as all of their progeny would be genetically appropriate for the worker caste and could potentially develop. Based on the regression slopes, such queens are predicted to have nearly twice the productivity of those with genetic caste determination in a population with equal lineage proportions (Fig. 3). This suggests that, all else being equal, lineages with genetic caste determination should be invaded either by extant populations of the parental species or by an environmental-caste mutant. However, two distinct cases of the phenomenon exist (Hidalgo and Junction), both of which are probably widely distributed (Helms Cahan *et al.*, 2002) and they do not appear to be of recent origin (Helms Cahan & Keller, 2003). This suggests that there may be other phenotypic benefits that outweigh these costs. For example, high heterozygosity of inter-lineage offspring may confer advantages to incipient colonies that compensate for their reduced numbers, or the differing genomic composition of workers from queens may allow for caste-specific adapted genotypes.

In conclusion, this study shows that genetic caste determination imposes direct costs due to the waste of queen-destined eggs in incipient colonies. This cost increases proportionally with the number of pure-lineage eggs and is thus not relevant for queens that fail to mate with males of the alternate lineage. The proportion of same- vs. alternate-lineage matings depends on the relative frequency of each lineage in the population. As a result, the founding success of queens becomes negatively frequency dependent, which may be an important force maintaining the coexistence of the two lineages.

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