Population-wide lineage frequencies predict genetic load in the seed-harvester ant *Pogonomyrmex*

Kirk E. Anderson^{*†}, Bert Hölldobler^{*†‡}, Jennifer H. Fewell^{*}, Brendon M. Mott^{*}, and Jürgen Gadau^{*}

^{*}School of Life Sciences and Center for Social Dynamics and Complexity, Arizona State University, Tempe, AZ 85287; and [‡]Department of Behavioral Physiology and Sociobiology, Biozentrum, University of Würzburg, Würzburg D-97074, Germany

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Many populations of the seed-harvester ant *Pogonomyrmex barbatus* exhibit genetic caste determination (GCD) generated by the interbreeding of two distinct yet interdependent lineages. Same-lineage matings are genetically predestined to become female reproductives (gynes) whereas alternate-lineage matings become workers. The perpetuation of this system requires that reproductives of both lineages are available for mating and are thus part of the effective population. We label these dependent lineage populations, because each lineage depends on the alternate lineage for worker production. Here we investigate the potential costs associated with GCD in a population with highly skewed lineage frequencies. We reared colonies using newly mated queens from a GCD population and an ecologically equivalent *Pogonomyrmex rugosus* population with environmental caste determination. GCD founding queens suffer a genetic load from mating randomly and produce fewer brood with advanced development compared with environmental caste determination queens. Our results indicate that GCD queens acquiring a high proportion of same-lineage sperm are unlikely to found a colony successfully. Given model parameters of random mating and founding queens mating with three males on average, there was a close fit between theoretical expectations of variation in colony worker production based on mating and lineage frequencies and empirical deficits in worker production. As expected, severely decreased worker production was specific to the common lineage, suggesting that negative frequency-dependent selection acts to stabilize a dependent lineage system.

genetic caste determination | dependent lineage system | frequency-dependent selection | mating frequency | obligate polyandry

The success of social insect societies is largely attributed to reproductive division of labor in which most colony members are sterile and provide for the reproductive caste, rather than producing their own progeny (1). The accepted mechanism for differentiation of colony members into reproductive and sterile castes is environmental caste determination (ECD), or caste polyphenism, a developmentally plastic process in which eggs or larvae of similar genotypes develop into discrete phenotypes depending on their environment (2–6). Genetic caste determination (GCD) occurs when different female genotypes are more likely to become either reproductives (gynes) or workers. Gynes become queens when they successfully mate and found a new colony. Although ECD is clearly predominant, there is growing evidence for a genetic influence on caste determination in some Hymenoptera (7–11).

Differentiation of gynes and workers is determined by genotype in many populations of the seed-harvester ants *Pogonomyrmex barbatus* and *Pogonomyrmex rugosus* (12–15). In GCD colonies, gynes are consistently homozygous at diagnostic loci whereas workers are heterozygous at the same loci (12, 16, 17). This genetic colony structure occurs because founding queens mate with males of two distinct and reproductively isolated lineages, producing heterozygous workers from alternate-lineage matings and homozygous gynes from same-lineage matings. We label these dependent lineage (DL) populations, because each lineage depends on the alternate lineage to produce functional colonies (17). The DLs

investigated in this study are labeled J1/J2 and have the morphology of *P. barbatus* (17, 18). The maintenance of a DL population relies on obligate polyandry because founding queens must mate with at least one male of each lineage to generate both workers and gynes (18). Assuming that queens mate randomly, GCD generates a population level constraint in which the probability that queens acquire alternate-lineage sperm and thus produce workers is a function of queen mating frequency and the relative frequency of each lineage in the population. The ratio of same-lineage to alternate-lineage sperm acquired by a newly mated queen determines her genetic load during colony founding. Because the availability of alternate-lineage (worker) sperm is determined by the relative frequency of each lineage in the population, the genetic load of a given colony is expected to be lineage-specific.

The mating system of DL *Pogonomyrmex* is typical of many *Pogonomyrmex* in which caste is determined environmentally. After summer rains, colonies release males and gynes (reproductive virgin females) in highly synchronized nuptial flights (19–24). Sexuals mate at leks, after which the queen produces her first worker cohort using energetic reserves provisioned by her natal colony (25, 26). The colony-founding environment is highly desiccating, and mature colonies are overdispersed because of intraspecific competition (27–29). Founding colonies suffer severe selection at this stage of the life cycle, and the probability that a new colony will survive is <1% (25). Early colony growth depends on the production of workers; gynes produced at this stage of the colony life cycle represent a severe nutritional cost, and any resources diverted to the production of gynes can be considered genetic load.

This study quantifies genetic load in a DL population by determining the association between relative lineage frequency and brood development in founding colonies. The investigation considers queen mating frequency, which influences the ratio of gyne-destined to worker-destined eggs within GCD colonies. If queens mate randomly with multiple males of each lineage, some proportion of queens should mate primarily with males of the same lineage, resulting in a subset of new colonies in which mated queens lay few worker-destined eggs and many gyne-destined eggs. In the extreme case, queens mate with only same-lineage males, have maximal genetic load, produce exclusively gyne-destined eggs, and have no chance of colony establishment.

The level of genetic load is influenced by both mating frequency and the relative frequency of each lineage in the population, or “lineage skew.” The effective mating frequency of DL *P. barbatus* is estimated at 3.34 (30). With equal frequencies (0.5/0.5) of the two lineages, 12.5% of triply mated queens are expected to acquire only same-lineage sperm and hence be unable to produce workers. Similarly, 12.5% of queens are expected to acquire only alternate-lineage sperm, produce only workers, and have no genetic load during the colony-founding phase of the life cycle. The remaining

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Abbreviations: GCD, genetic caste determination; ECD, environmental caste determination; DL, dependent lineage; P/W, pupae and/or workers; PGI, phosphoglucose-isomerase.

[†]To whom correspondence may be addressed. E-mail: kirk.anderson@asu.edu or bert.hoelldobler@asu.edu.

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Fig. 5. PGI genotypes of larvae produced by queens from each lineage (from Figs. 3 and 4) within three distinct categories of P/W production (x axis). Black indicates the number of gynes producing only eggs as a result of nonfertilization or arrested egg development. Dark gray indicates queens producing at least one homozygous (gyne-destined) larva. Light gray indicates colonies in which all genotyped larvae were heterozygous (worker-destined).

among their brood. Eight colonies in the one- to two-P/W production class possessed homozygous larvae; one homozygous larva was detected in each of seven colonies, and one colony possessed two homozygous larvae (Fig. 5). Four of 25 (16%) rare lineage (J1) colonies produced one to two P/W; PGI genotypes revealed six worker-destined larvae and two gyne-destined larvae among their brood. One homozygous larva was detected in each of two colonies. Seven of 36 (19%) common lineage (J2) colonies produced at least four P/W, and all larvae ($n = 14$) genotyped from these colonies were worker-destined. Eighteen of 25 (72%) rare lineage (J1) colonies produced at least four P/W, and all but one larvae ($n = 36$) from these colonies were worker-destined (Fig. 5).

Discussion

Our results demonstrate that GCD in *P. barbatus* generates a genetic load beyond what is present in colonies with ECD (Figs. 1 and 2). Queens of DL populations are obligatorily polyandrous and must mate predominantly with alternate-lineage males to facilitate worker production and successfully found a colony. When males of the alternate lineage are at low frequencies, random mating increases the probability that common lineage gynes will acquire primarily same-lineage sperm. Gyne-biased sperm ratios constitute a genetic load for the common lineage, because workers are necessary to initiate colony growth and intralinear genotypes almost never become workers (14). Because queens found colonies independently, gyne-destined sperm borne by the common lineage represent a severe genetic load during colony founding (Fig. 2), and the frequency of the rare lineage should increase via negative frequency-dependent selection.

Our results agree with previous assertions that queens mate and use sperm randomly (14, 15). Lineage frequencies of reproductive colonies in the field, as measured by genotypes of male reproductives, predict lineage-specific variation in brood production and larval genotypes among colonies of both DLs (Figs. 3 and 4). Worker production by colonies of the common lineage J2 was much lower than that of either lineage J1 or ECD colonies, primarily because the frequency of gyne-destined eggs (genetic load) was much greater in lineage J2 (Fig. 5). Worker production by the rare lineage J1 was similar to that of ECD colonies, and correspondingly J1 possessed few gyne-destined genotypes among the first brood cohort (Fig. 5). Variation in the early production of gyne genotypes across all colonies did not differ from the expected categories of

brood production based on both rare and common lineage queens mating randomly with three males (Fig. 3).

In a DL population, producing the first worker cohort is a direct fitness measure that depends on the acquisition of alternate-lineage sperm by the founding queen. However, colony fitness and the maintenance of the rare lineage depend on both successful colony founding and the acquisition of same-lineage sperm needed for gyne production at colony maturity. With extremely skewed lineage frequencies and low mating frequencies, frequency-dependent selection on colony founding results in the rare lineage producing progressively fewer colonies capable of gyne production. Because selection on colony founding is already severe due to extrinsic causes, the lack of rare lineage gynes in the effective population may result in the loss of the rare lineage and drive a DL population to extinction. Alternatively, as queen mating frequency increases, the average sperm ratios acquired by queens of each lineage approach relative lineage frequencies. As a lineage becomes increasingly common, a higher mating frequency increases the selective pressure on the common lineage because the average sperm ratios of common lineage queens become increasingly gyne-biased. Thus, a higher mating frequency would increase the genetic load on common lineage queens and hasten the rate of rare lineage recovery.

The reported effective queen mating frequency of DL *P. barbatus* is likely higher than the published 3.34 (30). This value was generated by using only workers from GCD colonies and thus reflects only alternate-lineage matings. Because gynes are the result of same-lineage matings, determining the actual mating frequency in a DL population requires genotyping both gynes and workers. Also, the mature GCD colonies used to determine mating frequency (30) represent a biased subset of colonies that have survived the genetic load on colony founding. The fact that the majority of these colonies produce gynes on an annual basis (34) suggests they have also mated with at least one same-lineage male, and therefore the actual mating frequency is at least 1 greater than the 3.34 estimate. This conservative estimate places the mating frequency of DL *P. barbatus* close to the mating frequency of 4.71 established for ECD *P. rugosus* (35).

Our binomial model tested indirectly the nested hypotheses of mate choice, sperm competition, and sperm precedence. Although the model suggests that gynes mate randomly with an average of three males (Fig. 3), the associated *P* value (0.07) has marginal significance, indicating a higher mating frequency, sperm precedence, or extrinsic influences on colony founding success. Other harvester ant species, *P. rugosus*, *Pogonomyrmex occidentalis*, and *Pogonomyrmex badius*, show considerable variability in patriline numbers and an unequal patriline representation within colonies (11, 35, 36). Thus, even if a queen mates with multiple males, the sperm of a single male may be overrepresented during initial brood production. These genetic data correspond to the significant time difference for the first vs. subsequent copulations observed in both DL *P. barbatus* and DL *P. rugosus* (20). The first male spends more time in copula and apparently transfers more sperm than subsequent males. Thus, some of the variation in brood production among lineages may be due to differences in the amount of sperm contributed by each male. Additionally, some *Pogonomyrmex* males are suspected to mate with multiple females (20, 37), which may also influence our results. Given the severe mortality among founding queens and the increased probability of inseminating a same-lineage female, males that mate multiply would be favored by selection.

Precopulatory mate choice has been suggested as a mechanism to account for DL populations having a low mating frequency (38). In an early series of behavioral experiments Hölldobler (20) determined that antennal contact was necessary for males to recognize species-specific queen surface pheromones (likely cuticular hydrocarbons). In the J lineages, males possess significantly different cuticular hydrocarbon patterns (39), suggesting that gynes from

each lineage also differ and that the potential exists for males to discriminate between lineages. However, in a population composed of both DL *P. rugosus* and DL *P. barbatus*, both morphotypes frequently attempted copulation with heterospecific gynés (20), suggesting that mate recognition may break down between sister species and that each lineage is unable to distinguish between a same-lineage and alternate-lineage hydrocarbon profile.

Because a founding *P. barbatus* queen produces her first worker cohort without foraging (26), her energetic reserves are vital to early colony survival. It was speculated that gyne-destined eggs may fail to hatch because of nutritional or hormonal variation experienced by the egg (14, 15). Given that founding queens lay 40–70 eggs that result in four to eight workers, a mechanism that stops gyne-destined egg development would translate to a much lower energetic loss for the queen (14). Thus, gynés with suboptimal sperm ratios could successfully found a colony. However, our results indicate that successful colony founding depends on acquiring primarily alternate-lineage sperm to produce a viable first-worker cohort. In addition to colonies producing no pupae or workers, the common lineage (J2) produced many colonies in an intermediate growth category that contained both gyne-destined and worker-destined larvae (Fig. 5). Even though these gynés had mated with both lineages, they likely possessed sperm ratios biased toward gyne production. Additionally, we demonstrate that many gyne-destined eggs hatch and are then nurtured into larval development, as evidenced by the production of gyne-destined larvae in numbers that agree with predictions from our model (Fig. 5). The inability of founding queens to discriminate between gyne-destined and worker-destined genotypes decreases the likelihood that common lineage queens will produce a strong initial work force capable of surviving the competitive environment of colony founding. Collectively these data suggest that the genetic load of gyne-biased genotypes in GCD founding colonies translates into the severe nutritional load of gyne-destined larvae and that the production of functional adult gynés during early colony establishment is limited by inadequate nutrition. The cost of producing gyne-destined genotypes during the ergonomic phase of colony development (33, 34) may be offset if heterozygous workers develop more rapidly or require less investment. Although distinct behavioral differences have been documented between workers of ECD and GCD colonies, there is no clear ecological advantage to having an interspecific workforce (40). However, it remains possible that worker heterosis may give GCD colonies a competitive advantage in some environments (40, 41).

Our data indicate that queens of the less frequent lineage produced fewer same-lineage (gyne-destined) eggs, and had greater colony founding success (14, 15). The genetic load of gyne-destined genotypes carried by common lineage should act to equalize the genetic load between lineages via negative frequency-dependent selection on colony founding success. The result should be relatively stable frequencies that fluctuate around an equilibrium of 0.5/0.5. However, even when lineages are present at relatively equal proportions, an estimated 54% of the eggs laid by founding queens cannot be used for worker production (15), resulting in a severe genetic load for both lineages relative to populations without GCD. If one lineage were to evolve a fitness advantage independent of lineage frequencies, the population may quickly shift to a different lineage equilibrium. Lineage frequencies may then reflect an equilibrium between the fitness gained by the rare lineage from worker-biased sperm ratios and any fitness gained by the common lineage independent of balancing selection.

In this study lineage frequencies were highly skewed toward lineage J2, as has been revealed for many DL *P. barbatus* populations investigated (K.E.A., unpublished observations) (15). Our data revealed greater than expected brood production by the J2 lineage, suggesting that J2 may survive early colony founding better than J1 regardless of lineage frequencies (Fig. 3). Because lineage J1 is the product of recent hybridization (17, 18), it may retain

hybrid-derived genome incompatibilities that limit its survival. Existing data suggest that lineage J2 is not of hybrid origin (17, 18) and may thus retain a highly coadapted genome that counteracts the genetic load associated with the common lineage.

Sympatry between ECD and GCD colonies (17, 31) is expected to result in replacement of the DL system unless other factors extrinsic to the system (i.e., environmental selection) compensate for the genetic load generated by GCD. Under extreme lineage skew, however, worker productivity of the common lineage is greatly reduced whereas that of the rare lineage is similar to ECD colonies. Thus, the rare lineage can compete with sympatric ECD colonies whereas the costs to the common lineage are greatly increased. Competition with ECD colonies in combination with genetic load of the common lineage may increase the rate of rare lineage colony establishment. Alternatively, when frequencies are relatively equal, both lineages will bear the genetic load equally and may be outcompeted by ECD colonies, promoting lineage skew. Other selective forces may then act to maintain skewed but stable lineage frequencies.

We conclude that the excess of gyne-destined genotypes carried by the common lineage represents a severe genetic and nutritional load during colony founding and that as a result the fitness of the rare lineage increases via negative frequency-dependent selection. These findings suggest that the colony-level phenotype of GCD is regulated in part by population-level processes. Because mating frequency and sex ratio are intimately tied to both colony founding success and gyne fitness, quantifying these parameters relative to lineage skew across DL populations would be informative in determining the stability of DL systems.

Materials and Methods

We compared brood production of founding queens from two populations that differ in their mode of caste determination: (i) a DL population with the morphology of *P. barbatus*, consisting of two lineages that interbreed to produce GCD colonies, and (ii) a population of *P. rugosus* in which caste is determined environmentally (ECD). DLs are labeled J1/J2 based on the location of initial description (18). In the summer of 2005, lineages J1/J2 were collected from a DL population near Scottsdale Community College, Maricopa County, AZ (33° 31' N, 111° 53' W; 386 m). The ECD colonies were collected from *P. rugosus* population no. 28 (17) near the junction of Interstate 10 and McCartney Road, Pinal County, AZ (32° 56' N, 111° 42' W; 439 m). We selected these populations because they are closely related based on shared nuclear alleles and the results of an inclusive phylogenetic analysis of mtDNA (17, 18). The ECD *P. rugosus* haplotypes root the J1 mtDNA lineage (17), and the nuclear genome of J1 is the result of introgression with ECD *P. rugosus* (17, 18).

We determined the effect of caste determination mode on brood production by rearing newly mated queens collected within several hours of their mating flights ($n = 91$ GCD queens and 101 ECD queens). We collected queens that had removed their wings (were mated) and initiated colony excavations. Queens were maintained for several hours in Eppendorf tubes containing a small piece of moist paper towel until they were placed into nests. We constructed glass “ant farms” (16 × 10 × 1.0 cm), which were filled with native soil passed through a 2-mm sieve and mixed into a composite sample. Queens were placed into farms and maintained in darkness at 30°C; water was added as necessary to keep the soil moist. Throughout colony observations, all queens were provided an ad libitum diet of Kentucky bluegrass seeds.

We monitored brood development every 2–3 days and recorded the total number of larvae, pupae, and workers after 8 weeks. Workers and pupae of colonies from both populations were inspected for expression of gyne phenotype, including wing buds, thorax development, and ocelli. We estimated the genetic load associated with mode of caste determination by comparing the total number of larvae, pupae, and workers produced by GCD and ECD

colonies. Data were analyzed by using a χ^2 contingency table with the number of individuals in the three brood classes as dependent variables and the mode of caste determination as the independent variable. We examined the effect of lineage frequency by comparing brood production (dependent variable) among the common lineage, rare lineage, and the ECD population (independent variables) using a Kruskal–Wallis test with a significance level based on the χ^2 approximation, followed by a post hoc multiple-comparison test for unequal sample sizes (Q statistic) (42, 43).

We tested the hypothesis that initial lineage frequencies in the GCD population predict lineage-specific brood production. We first determined initial lineage frequencies of the GCD population by genotyping one male from each of 47 reproductively mature field colonies. Colonies contain one reproductive queen (20, 44), so the mitochondrial haplotype of one male represents that of the queen and the colony. Each male was sequenced for a 630-bp portion of *cox1* mitochondrial gene, which is diagnostic of the two lineages (17, 18). We then tallied the lineage-specific haplotypes of these males to estimate the lineage frequencies in the population.

To generate predicted values of brood production it was necessary to quantify the number and type (lineage) of males a particular gyne may encounter randomly in the mating aggregation. We first determined existing lineage frequencies as described above. At the beginning of the mating flight we surveyed the 47 colonies used to determine lineage frequencies to assess the number of each lineage contributing reproductives. We generated predictions of brood production using the binomial expansion as $[f(a) + f(b)]^n$ where f is lineage frequency, a and b represent each lineage, and n is the queen mating frequency (Table 1).

Assuming a mating frequency of 1, two discrete brood production categories are expected for each lineage: queens that have mated with the alternate lineage and produce only worker-destined genotypes, and queens that mated with the same lineage and cannot produce workers. At a mating frequency of 1, the expected success of a rare lineage gyne is the chance of mating once with a common lineage male, and this chance equals the frequency of the common lineage. The expected success of a queen from the common lineage is the chance of mating once with a rare lineage male, and this chance equals the frequency of the rare lineage.

Assuming a mating frequency of 2, three discrete brood production categories are expected for each lineage: (i) queens that mated

twice within lineage and fail to produce workers, (ii) queens that mated once with the same lineage and once with the alternate lineage and suffer reduced worker production, and (iii) queens that have mated twice with the alternate lineage and produce workers similar in number to an ECD colony. A mating frequency of 3 results in four different sperm ratios predicted to influence brood production (see Table 1).

In summary, we used the relative frequency of each lineage in the DL population and three different mating frequencies to predict brood production by each lineage. If mating is random, the relative contribution of each lineage to the mating swarm should predict lineage-specific brood production in the next generation. At all three mating frequencies, expected vs. observed brood production was compared by using χ^2 tests.

Gynes, workers, and larvae from each colony were assigned to lineage (J1/J2) by using the *cox1* mtDNA sequence of one individual per colony (17). DNA was extracted from ant heads following the Chelex extraction protocol outlined by Volny and Gordon (13) and amplified according to methods of Anderson *et al.* (17) by using the primer pairs LCO and HCO (45). The PGI locus is diagnostic of caste and was used to distinguish worker-destined from gyne-destined larvae in GCD laboratory colonies (16–18, 30). At PGI, gyne-destined larvae are homozygous and worker-destined larvae are heterozygous. After observations, we determined a PGI genotype of one to two larvae from each surviving laboratory colony using standard extraction and staining protocols (46). We determined the effect of larval genotype (independent variable) on brood production (dependent variable) using a Kruskal–Wallis test with a significance level based on the χ^2 approximation, followed by a post hoc multiple-comparison test for unequal sample sizes (Q statistic) (42, 43).

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