

Patriline shifting leads to apparent genetic caste determination in harvester ants

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The harvester ant, *Pogonomyrmex occidentalis*, is characterized by high levels of intracolony genetic diversity resulting from multiple mating by the queen. Within reproductively mature colonies, the relative frequency of different male genotypes (patrilines) is not stable. The difference between samples increases with time, nearing an asymptote after a year. Patriline distributions in gynes and workers display similar patterns of change. A consequence of changing patriline distributions is that workers and gynes appear to have different fathers. However, apparent genetic differences between castes are caused by changing paternity among all females. Temporal variation in the relative frequency of patrilines may be a consequence of processes that reflect sexual conflict, such as sperm clumping. Recent work documenting genotype differences between physical castes (workers and gynes; major and minor workers) in several species of ants has been interpreted as evidence of genetic caste determination. Reanalysis of these studies found little support for this hypothesis. Apparent caste determination may result from temporal variation in sperm use, rather than from fertilization bias among male ejaculates.

genetic caste determination | multiple mating | sperm use | social insects | sexual conflict

Morphological traits frequently display phenotypic plasticity (reviewed in refs. 1, 2); such variation is especially common in insects (3). Phenotypic plasticity generally is assumed to be responsible for physical castes in the social insects, including differences between gynes (virgin queens) and workers (nonreproductive caste) and differences between workers (major and minor worker castes). The proximal cause of the plasticity is thought to be largely nutritional (4, 5), and in some species, such as the honey bee, the underlying nutritional mechanisms are well understood (6, 7). As with any phenotype, physical caste is unlikely to be completely environmentally determined, and genotypes may differ in their propensity to develop into gynes or workers under a given set of environmental conditions (4, 8). Historically, genetic variation among individuals has not been thought to contribute to caste determination, although a number of scattered publications have suggested a role for genetic differences, especially in the ants (9–13).

However, several recent studies have reported genetic determination of caste in ants. Reproductive females in some species of ants are produced clonally, whereas workers are produced sexually [e.g., *Cataglyphis cursor* (14); *Wasmannia auropunctata* (15)]. Some populations of harvester ants (*Pogonomyrmex*) comprise two independent but interacting genetic lineages. The genome of reproductive females in such populations is composed of a single lineage, whereas females that contain genes from both lineages become workers (16–18). A similar phenomenon occurs in hybridizing populations of fire ant species (19). In these species, there is a clear genetic effect.

A second set of studies involves species with polyandrous queens that find differences in the distribution of male genotypes between gynes and workers or between workers belonging to different castes (20–24). The general pattern is to find that in some, but not all, colonies the distribution of male genotypes (patrilines) differs significantly between castes, leading to a significant overall difference when results are pooled across colonies. Such results are the basis for the inference that physical

caste in these species has a genetic basis. These studies form part of the basis for an emerging consensus that genetic differences are responsible for many differences in behavior, morphology, and physical caste in social insects (25).

Two general mechanisms could favor the evolution of genetically biased caste determination for species with multiply mated queens. Just as task specialization leads to division of labor and increased efficiency in colonies (e.g., ref. 26), so too could a genetic predisposition to become a major or minor worker enhance colony-level performance (23). Similarly, utilization of high-quality genotypes as gynes could increase colony fitness (27). Genetically biased determination of reproductive caste also could arise from conflict among males and between males and queens over how sperm is allocated to workers versus gynes (28, 29).

Studies that infer genetic caste determination from differences in the patriline distribution between castes rest on the assumption that the frequency distribution of patrilines is stable over time. Ross (30, 31) has discussed how the pattern of colony genetic variation and/or relatedness could vary over time as a consequence of either queen turnover or temporal variation in sperm use. Matrilineal turnover has been documented in colonies of polygynous species (32, 33), and in some cases there is evidence of reproductive skew—unequal contribution of queens to production of gynes by the colony (30, 34–37). More relevant to the question of genetic caste bias is whether apparent unequal contributions by different patrilines could be explained by temporal variation in sperm use.

The best empirical support for the assumption that sperm from multiple ejaculates is distributed homogeneously in the spermatheca comes from a single species—the honey bee, *Apis mellifera*. Genetic analysis has shown that different ejaculates become progressively more mixed over the course of several months (38, 39), leading to a homogeneous distribution in mature colonies (40, 41). If different ejaculates remain clumped in the spermatheca, the colony phenotype may vary over time, both in which patrilines are present and their frequency. Such temporal variation has been observed throughout the life cycle of annual yellowjackets (*Vespa maculifrons*) (42). A shifting patriline distribution characterized a multiyear study of *Formica exsecta* (28), suggesting that sperm have the potential to remain clumped in the spermatheca for years. Snapshot studies, which compare the patriline distribution between castes at a single time point, potentially confound temporal variation in sperm use with paternity bias with respect to caste.

We assayed repeated samples of workers and gynes from colonies of *Pogonomyrmex occidentalis* and show here that a mechanism other than genetic differences in caste determination can lead to the appearance of genetic differences between castes. Species of *Pogonomyrmex* are characterized by colonies headed by a single, polyandrous queen (17, 43–45). In our study population, the queens typically mate with six or seven males (46), may live for as many as

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Table 2. Regression of standardized distance on interval of measurement with the type of comparison (worker–worker vs. worker–queen) as a classification variable

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Time interval	8.01	1	8.01	6.885	0.0097
Worker–worker	3.53	1	3.53	3.035	0.084
Queen–worker	0.005	1	0.005	0.004	0.95
Error	153.574	132	1.163		

The regression model is linear regression, although it is clear that the time effect is nonlinear (i.e., Fig. 1).

Discussion

The genetic composition of females in the colonies of *P. occidentalis* displays considerable temporal variation. The distribution of patriline between gynes and workers collected during the same year differs markedly, but this difference is not a consequence of differential allocation of male genotypes between castes. Instead, which ejaculates are used to fertilize female-destined eggs changes with time.

Substantial patriline shifting also was found in *Formica truncorum* (28), the only other species for which data from a multi-year study have been published. Sündstrom and Boomsma (28) suggested that patriline shifting in *F. truncorum* originated from male–queen conflict over the sex ratio. In their study, the extent of patriline shifting was correlated positively with increased investment in gynes, which increased male fitness. We failed to detect any evidence of a similar pattern in *P. occidentalis* in 8 y of sex-ratio data (Table 3).

In species with polyandrous queens, sexual selection will favor alleles that bias the development of females into gynes rather than workers (8) or that increase the probability that sperm fertilize eggs at the time that gynes are being produced. Resistance to fertilization during worker production could manifest as temporal variation in paternity skew. The genus *Pogonomyrmex* is distinguished by the presence of four genetically distinct pairs of interacting lineages in which caste is determined largely by genotype [“strong genetic caste determination” *sensu* Anderson et al. (8)]. Harvester ant species such as *P. occidentalis*, which lack strong genetic caste determination, may provide opportunities to study the early stages of the evolution of sexual conflict in social insects.

Sündstrom and Boomsma (28) hypothesized that the annual variation in paternity skew in *F. truncorum* was a consequence of sperm clumping and that colonies differed in the degree of clumping. In *Eciton burchellii*, clumped sperm have been observed in the spermathecae of newly mated queens (50). Patriline shifting in *P. occidentalis* could result from similar sperm clumping. Variation among colonies suggests either that some male genotypes

Table 3. Correlation between standardized distance between workers and the sex ratio in *P. occidentalis*

Year	Correlation	Probability	N
1994	0.072	0.825	12
1995	0.285	0.188	23
1996	0.272	0.210	23
1997	0.256	0.217	25
1998	0.129	0.621	17
2001	0.187	0.297	33
2003	0.196	0.299	30
2004	0.007	0.978	17

Sex ratio is calculated as the ratio of female biomass to total biomass. Sample size varies because not every colony reproduces every year.

are more successful at preventing sperm mixing or that some queens are better able to effect sperm mixing independent of the males with whom they have mated. The degree of mixing may depend on the genotypes of all males present (i.e., a male × male × queen interaction). Support for male × female genotype interactions comes from directed mating studies in *Pogonomyrmex rugosus* (29), although, because each female was mated to one male, the effect of other males could not be assessed. Alternatively, this variation could result from some nonadaptive mechanism that leads to incomplete sperm mixing in some queens. Further work is needed to determine whether the pattern we observe is the result of sexual selection or other processes.

Our reanalysis of previous work in ants failed to support the interpretation that caste is genetically biased in these species. Taken individually, the evidence for genetic differences between physical castes is not convincing. Although effect sizes were consistently positive, this result could be explained as a publication bias (51): An observation that gynes and workers did not differ would not be deemed suitable for publication. For the data as a whole, there is a negative relationship between the mean effect size and the number of colonies in the study (correlation = -0.3 , although this relationship is not significant among the nine studies). In *P. occidentalis*, the species for which there is the largest amount of data, significant differences between workers and gynes are the result of the time interval between comparisons rather than a consequence of caste. Patriline shifting means that snapshot studies must account for differences in individual age (and thus time of oviposition). Whether this phenomenon occurs in other species of ants is unknown, although it seems probable in the congeneric *Pogonomyrmex badius*. The magnitude of temporal variation in other highly multiply mated species may be even larger than in *P. occidentalis*. When patriline distributions between physical castes are compared, the null hypothesis should not be that there are no differences between castes (mean effect = 0) but rather that the castes will differ because of the degree of patriline shifting expected from samples taken at different times (mean effect related to sampling interval).

Differences in the seasonal timing of the production of different castes, coupled with temporal variation in sperm use, could generate apparent paternity bias with respect to caste. Gyne–worker comparisons, even if made when the adults are collected simultaneously, may represent eggs laid over a significant time interval. This explanation is likely in *P. badius* (24), because gynes are produced during the spring, whereas the workers in the colony may have been produced the previous summer (52). The degree to which paternity skew is consistent over time in *A. echinator* (53) is uncertain, because patrilines were identified on the basis of a single microsatellite locus with low genetic power (four alleles). Comparisons between major and minor workers also may be problematic. Among ants, the longevity of major workers is usually greater than that of minor workers (54–57; but see ref. 58 for an instance of greater longevity of minor workers). Based on differences in lon-

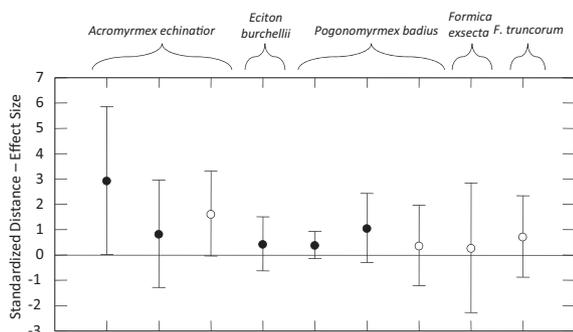


Fig. 2. Average effect size for the differences between samples of queens and workers (hollow circles) or between worker physical castes (solid circles) (see Table 1). The bars indicate the 95% confidence limits of the mean. All but one of the 95% confidence limits include zero.

geivity, simultaneous samples of major and minor workers are likely to collect individuals produced from eggs that were fertilized at different times. Inferences of a genetic bias to caste cannot be based on a single comparison but must eliminate the possibility of changing patriline distributions over time.

Materials and Methods

The colonies of *P. occidentalis* analyzed in this study were a subset of those that comprise our long-term study of reproductive allocation. The minimum colony age is 17 y, and most colonies are considerably older (59). We selected colonies whose patriline (male genotype) diversity, based on a sample of 20 workers, ranged from five to eight males, and we collected several additional samples ($n = 20$) to compare patriline distributions between castes and within castes over time. We compared worker samples that were 1 y apart, worker samples from the beginning (early June) and end of the summer (mid-August) of a single year, workers and gynes that were present simultaneously in the colony, and gyne samples separated by at least 2 y. Although the gynes and workers sampled were present in the colony at the same time, the gynes were produced from eggs laid 1–2 mo earlier (approximately mid-April), whereas the large majority of workers probably had been produced the previous summer (approximately mid-August). As control for the randomization procedure (described below), we collected larger ($n = 40$) samples of workers during June, split the sample into two “simultaneous” samples, and genotyped each separately. As a consequence, samples were separated in time by intervals of 0 for the simultaneous samples, 0.25 y for June and August in the same year (we also used a time interval of 0.5 in analyses; this interval did not change the results), 0.75 y for gynes and workers present during the same summer, and 1 or more years for individuals collected in different years.

Worker samples were collected by agitating the colony (blowing into the entrance) until several hundred workers were on the surface of the nest and then randomly picking up the desired number. Gynes were collected from colonies using the technique of artificial watering (59). First, males and gynes were separated from workers; then, from the colonies that had produced at least 50 gynes, 20–40 gynes were removed at random. Workers and gynes were stored in labeled 1.5-mL microcentrifuge tubes at 5 °C for 2–3 d until being shipped to the University of Houston, where they were stored at –80 °C until DNA extraction.

Each individual was genotyped at four highly polymorphic microsatellite loci (with 12, 15, 19, and 28 alleles; range of heterozygosities: 0.81–0.97; for details of DNA extraction and PCR, see ref. 46). We determined the maternal and paternal genotype and the number of unique patrilines present in the sample. Given the high degree of polymorphism at these loci, the likelihood of two males having an identical four-locus genotype is extremely low ($< 10^{-4}$). Typically, patrilines within a colony differed in alleles at least three loci. We used the Genographer (ver 1.6.0) software program (60) to determine each worker’s multilocus genotype.

We used a randomization procedure to establish the difference between each pair of samples (e.g., workers from different years or worker and gyne samples collected in the same year) and to measure the significance of the difference in patriline distribution. We first calculated the distance between a pair of samples based on the proportion of the sample represented by each patriline (where a , b , c , ... represent the differences in the proportion of a given patriline in the two samples): $\sqrt{a^2 + b^2 + c^2 + \dots}$. This measure is essentially Rogers’ (61) genetic distance using patriline frequency instead of

allele frequency. We then compared the observed distance with a distribution of 10,000 randomly generated distances. A random distance was generated by constructing two sets of 20 individuals by sampling with replacement from the complete sample of a given colony and calculating the distance between the pair. The distribution of distances was square root transformed to produce a normally distributed null distribution. We compared the transformed distance from our samples with the underlying null distribution and expressed the difference in standard normal variate units (difference between the observed statistic and the mean null distance divided by the SD of the null distribution). This standardized distance measure is equivalent to the effect size used in meta-analyses. Simulations confirmed that this measure was independent of the size of the samples taken, the number of patrilines, or the skew among patrilines. It thus serves as a convenient way to compare results among colonies, data sets, and species. Two random samples from a distribution will have a positive absolute distance (unless they are identical); however, they are equally likely to have standardized distances that are positive (more dissimilar than expected) or negative (more similar than expected), with a mean of zero.

Data were available, either in tabular or graphical form, from previous studies that allowed us to calculate the effect size equivalent to the standardized distance metric calculated for *P. occidentalis* in this study. In most of the studies, the presentation of the data made it possible to calculate a heterogeneity G-statistic, which was converted into the effect size by the following method: For data from each colony we calculated the probability of the G-statistic and the z-statistic that would produce the same probability. This z-statistic is the effect size for a particular colony and has the desirable property that the null expectation is a standard normal distribution. From earlier studies of *A. echinator* (20, 23), *E. burchellii* (22), *P. badius* (21, 24), *F. exsecta*, and *F. truncorum* (62), we calculated the heterogeneity G-statistic and the exact probability, the effect size for each colony in the study, and the 95% confidence interval of the mean effect size (across colonies) for each species. Data for a recent similar study in *P. rugosus* (29) were not presented in a form that allowed calculation of appropriate statistics. To be comprehensive, we included the data from *F. truncorum* and *F. exsecta* because worker and gyne genotype distributions were compared, although this study did not argue for a genetic component to physical caste (62). In calculating the effect sizes, we weighted all colonies from a particular study equally, although there were occasional differences in sample size among colonies.

To evaluate the evidence for genetic differences between physical castes in all studies, we combined the data from separate studies into a measure of the cumulated mean effect size across studies (51). The overall effect size was weighted by the sample sizes for each study. We considered separately studies that compare major and minor worker castes and those that compare the gyne and worker castes. For the comparison of worker castes, the data are from five data sets, but only three species, thus limiting the independence of this measure and therefore the validity of the effect size for the comparison. However, for the four studies comparing gynes and workers, each data set comes from a different species (Table 1).

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