Genetic determination of the queen caste in an ant hybrid zone

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The question of how reproductives and sterile workers differentiate within eusocial groups has long been a core issue in sociobiology because it requires the loss of individual direct fitness in favor of indirect or group-level fitness gains. The evolution of social behavior requires that differentiation between workers and female reproductives be environmentally determined, because genetically determined sterility would be quickly eliminated. Nevertheless, we report clear evidence of genetic caste determination in populations of two seed harvester ant species common to the southwestern USA, *Pogonomyrmex rugosus* and *Pogonomyrmex barbatus*. The genetic differentiation between workers and queens is found only in areas of sympatry of the two species, and thus appears to arisen from hybridization. Our data suggest that this hybridization has had a profound historical effect on the caste determination systems and mating patterns of each of these species.

An overriding principle of social insect biology is that the determination of reproductive (queens) versus sterile (workers) individuals within a social group is primarily environmentally governed (1–3). A sterile caste can evolve under kin selection only if the genes for sterility are expressed conditionally, because any allele that invariably caused sterility could not be passed on and would be quickly eliminated from the population (4, 5).

Numerous studies have accumulated evidence that differences in larval environment, particularly larval nutrition, determine whether a female egg will develop into a fully capable reproductive or a small worker female (6, 7). When a nutritional or other physiological threshold is reached during larval development, neurosecretory changes result in elevated juvenile hormone levels, triggering development of reproductive potential (8). Recent research has focused on identifying genes involved in the developmental cascade underlying this polyphenism (9). However, the hypothesis that the underlying mechanism for queen-worker caste differentiation could itself become genetically based lacks support.

There have been rare reported exceptions of genetic influence on caste determination; however, these have involved the generation of different queen types in ants with polymorphic queens, rather than the fundamental differentiation of queens and workers. Allelic differences between regular, winged queens and an intermediate, wingless queen have been described in the slave maker ant, *Harpegoxenus sublaevis* (10), and a genetically based queen polymorphism also has been demonstrated in a species of an Australian ant, *Monomorium* (11). A third case, using allozymes as genetic markers, has demonstrated that a second queen morph in *Acanthomyops* is actually a genotype resulting from hybridization (12). In *Melipona* bees, it has been suggested that queen determination is in part genetically controlled by double heterozygosity at two independent loci (13). However, this example is highly controversial (14), and the environmental influence on caste remains strong because all genotypes become workers if given insufficient food.

This article presents clear evidence for genetic queen-worker caste determination in populations of two species of harvester ants, *Pogonomyrmex rugosus* and *Pogonomyrmex barbatus,* which seems to be associated with hybridization. These two sister species are widespread in deserts and grasslands of the south-central and southwestern United States and northern Mexico (15). Of the two species, *P. barbatus* has a more eastern distribution, occurring from western Louisiana to central Arizona, whereas *P. rugosus* occurs from central Texas to California. The two species have broadly overlapping geographic ranges from central and southeastern Arizona to western Texas, but both species also inhabit large areas of allopatry (15).

We used randomly amplified polymorphic DNA (RAPD) genetic markers to examine genotypic patterns among sibling workers, alate queens (alates), and males from colonies of *P. rugosus* and *P. barbatus* in areas of sympatry and allopatry. Morphological data suggest that these two ant species hybridize in areas of overlap (16), and both species often possess mtDNA of the sister species in these areas (17). Mating aggregations of both species within contact zones have been observed to contain low numbers of congeneric reproductives, supporting the possibility of hybridization (18).

Methods

We collected workers, alates, and males from colonies of *P. rugosus* and *P. barbatus* during the mating flight season (July through September) in areas of sympatry and allopatry. Ants were immediately frozen at −80°C or placed in 100% ethanol. Both *P. barbatus* and *P. rugosus* have one reproductive queen per colony, and queens of both species mate with multiple males (18). Thus, all individuals collected from a given colony were offspring of the same queen.

Our sympatic site was in southeastern Arizona (Cochise County) and southwestern New Mexico (Hidalgo County), where we collected five colonies of *P. rugosus* and nine colonies of *P. barbatus*. Our sites for allopatric samples were in Texas and Arizona, where we collected 17 colonies of *P. rugosus* and seven colonies of *P. barbatus*. Colonies of *P. rugosus* were collected in Maricopa County (six colonies), Pinal County (five colonies), and Navajo County (six colonies), Arizona, whereas samples of *P. barbatus* were collected in Yavapai County (three colonies), Arizona, and Tarrant County (three colonies) and Wichita County (one colony), Texas.

DNA was extracted from each individual according to Landry *et al.* (19) or Gadau (20); abdomens of workers and alates were removed before extraction. The RAPD PCR was performed according to Williams *et al.* (21). We screened an initial colony of workers, alates, and males of each species with 45 different 10-base random primers. We then chose a 10-bp primer OPC9 (Operon Technologies, Alameda, CA) showing a fragment-length polymorphism that was useful for identifying heterozygotes (22, 23). All colonies were then screened with the C9 primer. Heterozygous individuals displayed three different bands, one at 510 kb, one at 550 kb, and a heteroduplex band at 590 kb. We verified that the marker was a codominant fragment-

Abbreviation: RAPD, randomly amplified polymorphic DNA.

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EVOLUTION

Table 1. Results of presence/absence RAPD markers among sibling alate queens, workers, and males from a single colony of *P. barbatus* and *P. rugosus*

	Alate queens					Workers										Males				
P. barbatus (Colony 5)																				
Primer (size)	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5
C9 (600)	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
R11 (300)	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
R11 (400)	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
D18 (490)	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
S19 (1000)	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
G4 (650)	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
Q9 (850)	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
R11 (600)	0	0	0	1	0	0	1	1	0	0	0	0	1	1	0	1	0	0	0	1
Q9 (450)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
P. rugosus (Colony D)																				
Primer (size)	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5
C9 (600)	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
P13 (630)	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
S8 (1300)	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
T20 (750)	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
N11 (650)	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
K4 (300)	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
D18 (495)	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1
A1 (800)	—	0	0	1	0	0	0	1	0	—	1	0	1	0	0	1	0	0	1	1

1 = band present, 0 = band absent.

length polymorphism by mixing PCR-amplified DNA products from males of each alternative allele, heating the samples, and running them on a gel, which allows reannealing of the heteroduplex. All three bands appeared, indistinguishable from a heterozygous individual.

Results

We found distinct intra-colonial differences between the genotypes of workers and their reproductive siblings in colonies within the contact zone. In a sample colony of each species, 13 primers revealed 16 variable markers; 12 displayed complete linkage with the worker caste (Table 1). Specifically, workers displayed bands that were absent in their alate queen sisters or in the males. The most parsimonious explanation for these genotype differences is that the workers and queens come from different patrilines. Because Hymenoptera are haplo-diploid, workers from the same father share all markers specific to that patriline. Males are produced parthenogenetically, and their genotypes reveal the queen's genotype. Therefore, markers absent in males but present in workers are inherited patrilinially. Not all markers showed this distinct caste difference; four markers showed variation independent of caste and sex (Table 1). Most likely the queens are heterozygous (present/absent) for these markers and the genotypes of their mates are absent, because males—representing the queens genotype—also segregated for all of these markers.

In addition, we found distinct differences between alates and workers by using a fragment-length polymorphism (C9–550/510) for colonies within the contact zone. All workers in the sympatric populations of both *P. rugosus* and *P. barbatus* were heterozygous for the C9 marker (Table 2, Fig. 1). In contrast, their alate sisters were significantly more likely to be homozygous for one of the two alleles. All *P. barbatus* alates ($n = 67$) and 80% of *P. rugosus* alates ($n = 21$) were homozygous. All males within a given colony shared the same allele, indicating that the queens of colonies in the sympatric zone are all homozygous. This allele matched the one present in their sibling alates.

The allopatric populations of both species showed a more typical pattern of within-colony genetic variation for the C9 locus, in which genotypic frequencies were similar for alates and workers (Table 2). As a result, the proportion of heterozygotes differed significantly between the sympatric and allopatric groups (Fig. 1). In *P. barbatus* within the contact zone, heterozygosity was completely associated with caste, whereas in the allopatric population heterozygosity was not different between alates and workers (Fig. 1). Heterozygosity levels in allopatric *P. rugosus* were low for both alates and workers, because the 550 allele was rare. However, heterozygosity was not associated with caste (Table 2), and some colonies contained both a heterozygous queen and heterozygous daughter alates.

Table 2. Genotypes revealed by RAPD genetic marker C9 for alate queens, workers, and males from sympatric and allopatric populations of *P. rugosus* and *P. barbatus*

Caste	Homozygotes 510/510	Heterozygotes 510/550	Homozygotes 550/550	Total
P. rugosus (sympatric) ($n = 5$ colonies)				
Queens	15	4	5	21
Workers	0	33	0	33
Males	17	—	12	29
P. rugosus (allopatric) ($n = 17$ colonies)				
Queens	41	2	0	43
Workers	56	5	0	61
Males	46	—	1	46
P. barbatus (sympatric) ($n = 9$ colonies)				
Queens	29	0	38	67
Workers	0	60	0	60
Males	37	—	30	67
P. barbatus (allopatric) ($n = 7$ colonies)				
Queens	4	6	5	15
Workers	12	11	13	36
Males	4	—	5	9

Genotypes were assigned based on the fragment-length polymorphic marker that revealed two alleles of different lengths, one at 510 bp, the other at 550 bp. Males are hemizygous and therefore possess only one allele.

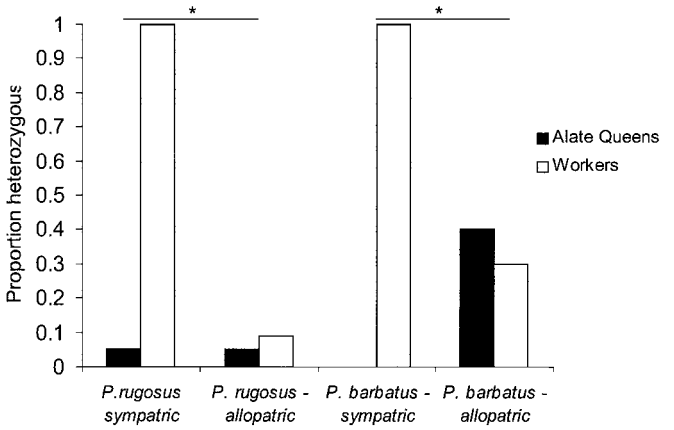

Fig. 1. Proportion heterozygous individuals comparing alates and workers of *P. barbatus* and *P. rugosus* (identified morphologically) in areas of sympatry versus allopatry. There is a significant difference between sympatric and allopatric populations in the frequency of heterozygotes in queens and workers. (*P. rugosus*, $\chi^2 = 26.1$, $P < 0.000$; *P. barbatus*, $\chi^2 = 40$, $P < 0.000$.) The low amount of heterozygosity in allopatric *P. rugosus* is a result of one allele, 550, being rare in that population. However, allopatric populations show no difference in the amount of heterozygosity between castes. The asymmetry in heterozygosity between alates and workers in sympatric populations reveals a genetic caste determination.

Discussion

The evolution of caste determination is central to the evolution of complex sociality. Kin selection as an explanation for eusociality is based on the assumption that sterile workers forgo direct fitness to help their closely related siblings reproduce (1). This argument for social evolution also depends on conditional expression by any genes underlying caste determination (4, 5). In contrast to this expectation, our data show clear evidence of genetically based caste determination between reproductive and sterile females. Within areas of sympatry, genetic differentiation between alates and workers was essentially absolute. All workers were heterozygous for the C9 locus, whereas almost all alates were homozygous. An additional 14 RAPD primers also indicated genotypic differentiation between alates and workers (Table 1). These data collectively suggest that the C-9–550/510 locus is a marker for a large genomic linkage group affecting caste determination.

How can a system of genetic caste determination arise? We suggest that caste determination in *P. rugosus* and *P. barbatus* is related (at least historically) to hybridization. Our results show an extreme genetic effect on caste within areas of sympatry, but we found no similar pattern in allopatric populations of either species. We hypothesize that ants in the sympatric zone have responded to introgression with a unique form of colony-level or social hybridogenesis (24). This hybridogenesis is similar on a colony level to that found on an organismal level in certain species of guppies (*Poeciliopsis*) (25). These *Poeciliopsis* females hybridize with males of another species, but during meiosis of the offspring only the maternal genes remain in viable gametes (26). Thus the male's genome is not transferred beyond the F_1 generation, which is the same in our system.

How could hybridogenesis in *Pogonomyrmex* work? Our data indicate two separate multilocus genotypes present within both species, which are revealed by the 510 and 550 markers and the additional RAPD markers (Tables 1 and 2). To survive and reproduce, homozygous queens must mate with a male of matching type to produce reproductively capable daughters. To produce sterile workers they must also mate with a male of the opposite type. However, because workers do not reproduce, the genetic information of nonmatching males is not transferred across successive generations. Because any successful colony must produce both workers and alate queens, this system has become a case of obligate polyandry. Note that queens who mated only with males of the opposite type can still successfully found colonies (produce workers) but should have a reduced fitness because they can produce only male sexuals.

We hypothesize that the two multilocus genotypes, which are currently within each species, arose from a past hybridization event. This hybridization between the two species generated genetic incompatibilities in diploid females, which were then apparently only able to develop into sterile workers. However, because queens of these species mate with multiple males, they could still raise female sexuals, too, as long as they had also mated with at least one compatible male. Thus, because of the already present polyandry, the hybridization was not selected against. Once hybridization occurred, a second evolutionary step had to occur, the elimination of homozygotic (or nonhybrid) workers. Although hybridization would theoretically provide an excess of heterozygote workers, there is no *a priori* expectation that it would also prevent homozygotes from becoming workers. This second step was presumably under strong selection because of kin conflict over caste determination. In a polyandrous system (with associated reduction in within-colony relatedness) and with a hybrid worker caste in place, it becomes more advantageous for a nonhybrid female to become a queen. Selection on these females also may have been enhanced by selection on males to father reproductives rather than workers. An alternative, more mechanistic explanation for the genotype-phenotype correlation in hybrid colonies would be that in colonies where most individuals (all heterozygotes) cannot develop into queens because of hybridogenesis, the remaining homozygous larvae may monopolize the attention of workers seeking to turn female larvae into queens. This mechanism could also explain the otherwise puzzling fact that same-species gamete combinations do not produce viable workers in mixed colonies but do in allopatric populations. However, under this scenario we would expect that in founding colonies of mixed colonies we should find homozygous workers, but this remains to be tested.

This pattern of worker heterozygosity and queen homozygosity may not be unique to *Pogonomyrmex*. Hung and Vinson (27) presented allozyme evidence consistent with genetic caste determination in fire ants, *Solenopsis geminata* and *Solenopsis xyloni*. Because they did not have DNA-based markers, they interpreted their results to suggest differential enzyme expression between reproductive queens and workers.

The ability to shunt nonconspecific offspring into sterile castes offers eusocial species a unique mechanism for countering the negative fitness consequences of hybridization (24). Because workers are valuable to colony success, a differential caste trajectory for conspecific versus nonconspecific sperm would offset negative fitness consequences of interspecies mating. Shunting heterospecific sperm into workers would also limit the movement of hybrid genomes into F_2 generations, because reproductives are almost universally the product of conspecific matings. If so, eusociality may paradoxically contribute to hybrid colony success, but in turn limit the extent of hybridization beyond the F_1 generation.

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