

Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants

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The process of reproductive caste determination in eusocial insect colonies is generally understood to be mediated by environmental, rather than genetic factors. We present data demonstrating unexpected genetic differences between reproductive castes in a variant of the rough harvester ant, *Pogonomyrmex rugosus* var. *fuscatus*. Across multiple loci, queens were consistently more homozygous than expected, while workers were more heterozygous. Adult colony queens were divided into two highly divergent genetic groups, indicating the presence of two cryptic species, rather than a single population. The observed genetic differences between castes reflect differential representation of heterospecific and conspecific patrines in these offspring groups. All workers were hybrids; by contrast, winged queens were nearly all pure-species. The complete lack of pure-species workers indicates a loss of worker potential in pure-species female offspring. Hybrids appear to be bipotential, but do not normally develop into reproductives because they are displaced by pure-species females in the reproductive pool. Genetic differences between reproductive castes are expected to be rare in non-hybridizing populations, but within hybrid zones they may be evolutionarily stable and thus much more likely to occur.

Keywords: allozymes; caste determination; cryptic species; hybridization; kin selection

1. INTRODUCTION

A defining feature of eusocial insect colonies is reproductive division of labour (Michener 1974). Reproduction is generally limited to morphologically distinct queens, while non-reproductive tasks are performed by workers. The inclusive fitness benefits of belonging to each caste vary as a function of colony size, relatedness structure and individual condition (Hamilton 1964; Bourke 1999; Bourke & Ratnieks 1999). The process by which an individual caste is determined is of fundamental importance to the fitness interests of developing offspring. Traits that increase the likelihood of an individual being in the reproductive caste should be favoured, and there is evidence of conflict over representation in reproductive broods (Bourke 1999; Bourke & Ratnieks 1999; Sundstrom & Boomsma 2000). However, such competition should quickly select against genotypes less represented in the reproductive line, making heritable differences in reproductive propensity a transient phenomenon. Caste determination is virtually always environmentally, rather than genetically, based and genetic differences between the

sterile and reproductive castes are extremely rare (Winter & Buschinger 1986; Hölldobler & Wilson 1990).

The few cases in which genetic caste differences appear stable tend to show an association between heterozygosity and reproductive caste, rather than specific 'reproduction' alleles. In *Melipona* bees, females heterozygous at two caste-determining loci are bipotential, while individuals homozygous at one or both loci are restricted to worker development (Kerr 1950*a,b*). Heterozygote advantage in queens assures that although workers and reproductives differ genotypically, the relative representation of each allele in the reproductive line is equal, allowing the system to be repeated in each generation. Heterozygosity also underlies genetic caste differences in polygynous populations of the red imported fire ant, *Solenopsis invicta*, where queens are invariably heterozygous (*Bb*) at the locus *Gp-9*, while workers can be either *Bb* or *BB* (Keller & Ross 1999). In this case, the relative fitness disadvantage of *BB* workers is counteracted by gene flow from single-queen populations fixed for the *B* allele (Ross & Keller 1995; Parker & Hedrick 2000). Other instances of stable genetic differences between reproductive castes remain elusive.

Here, we report extreme genetic differences between worker and reproductive female offspring associated with hybridization in the genus *Pogonomyrmex*. We conducted a population genetic study of a variant of the rough harvester ant, *Pogonomyrmex rugosus* var. *fuscatus*, whose taxonomic status is considered unclear. We present evidence that this 'population' is composed of two cryptic sympatric

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species that appear to hybridize frequently. However, the reproductive fates of pure-species and hybrid female offspring differ dramatically: all workers are hybrids, while reproductive offspring are overwhelmingly of non-hybrid ancestry. To determine whether genetic differences between castes were restricted to this zone of hybridization, we compared colony genetic structure at this site with a population of the typical form of *P. rugosus* that occurs in central Arizona, where we found no genetic differences across castes. We propose that hybridization is one of the few contexts in which genetic determination of reproductive caste could evolve and be maintained evolutionarily.

2. METHODS

(a) *Study species and collection localities*

Pogonomyrmex rugosus is a large-bodied seed-harvester ant species that is common in south-central and southwestern United States, from west Texas to California, and south into central Mexico (Johnson 2000). Colonies are founded independently by a single multiply mated queen and can live for 15–20 years (Gordon & Kulig 1996). Mating occurs in mass mating aggregations once or twice a year, triggered by summer monsoon rains in July and August (Hölldobler 1976). Across its range, *P. rugosus* displays high levels of phenotypic variation and several variants have been described (Creighton 1950; Cole 1968). The variant *fuscatus*, reported from sites in southeast Arizona, New Mexico and west Texas, displays body sculpturing intermediate between typical *P. rugosus* and its sister species *P. barbatus*, and has been proposed alternatively as a *P. rugosus* variant, a subspecies of *P. barbatus*, a *P. rugosus* × *barbatus* hybrid and a separate species, *P. fuscatus* (Creighton 1950; Cole 1954, 1968; Gregg 1963). Colonies were sampled at two low elevation sites separated by ca. 500 km. The Hidalgo site was within the described distribution of *P. rugosus* var. *fuscatus* at the junction of NM80 and NM9, Hidalgo County, New Mexico. The Queen Creek site, along Queen Creek in Pinal County, Arizona, was located within the range of the typical form of *P. rugosus*.

(b) *Collection and colour scoring*

Colonies were sampled at the beginning of the reproductive season in late June and early July 1996. An additional four colonies were sampled in June 2001 at Hidalgo. At this time of year, most adult colonies contain three easily distinguishable morphs or castes: sterile workers, winged males and winged (virgin) queens. The mated queen is normally deep within the nest and difficult to collect from adult colonies (Tschinkel 1999; S. W. Rissing, unpublished data); to avoid possible confusion of mother and offspring, however, morphological queens lacking wings were not collected. Colony entrances were excavated to a depth of ca. 4 cm and scanned for males. If six or more males were seen, colonies were excavated to ca. 10 cm and six or more individuals of all available castes were collected. Colonies containing fewer than six males were not sampled. Males were inspected visually in the field and colour type assigned by two observers. Colour types were identically assigned in 42 out of 43 colonies at Hidalgo; later assessment of the last colony confirmed that the conflicting assignment was due to observer error rather than ambiguous male colour. Workers were also collected from 15 colonies at Hidalgo on 24 November 1996. These constituted a subset of the colonies sampled in June 1996; because colonies were not marked for long-term identification, however,

male colour in these colonies was not known. Samples were stored at -70°C .

(c) *Protein electrophoresis*

Individuals of all castes (males, winged queens and workers) were screened for six variable protein markers. Heads of males and winged queens and heads and thoraxes of workers were homogenized in 70 μl extraction buffer containing 100 μl Triton 100X, 10 mg NADP and 0.1 g DTT 100 ml^{-1} H_2O . Samples were identified by number to eliminate scoring bias. Samples were run on cellulose acetate gels in discontinuous buffer systems; soaking and running buffers are listed as soak/run. Buffers g and I are described in Richardson *et al.* (1986) and buffer CAEA in Hebert & Beaton (1989). Running conditions were as follows: phosphoglucosomerase (PGI): I/G 23 min at 200 V; phosphoglucosomutase 1 and 2 (PGM-1 and PGM-2): I/G 25 min at 200 V; hexokinase (HEX): I/CAEA 18 min at 200 V; and esterase 1 and 2 (EST-1 and EST-2): I/G 23 min at 200 V. All gels were scored by one observer. For Hidalgo, six individuals from each available caste were run in 11 colonies, a single gyne and worker and six males were run in seven colonies, and a single representative of each available caste was run in nine colonies. For Queen Creek, six individuals of each caste were run for 12 colonies. Two loci, *PGM-2* and *EST-2*, were not identified initially and were scored from preserved gels after electrophoresis was completed. Because gels varied in their level of preservation, significantly fewer individuals were scored for these loci. Sample sizes are listed in table 1 (males) and figure 2 (winged queens and workers). Too few individuals from Queen Creek were scorable at either *PGM-2* or *EST-2* to allow statistical comparisons; thus, these loci were not considered for the Queen Creek population.

(d) *Analyses*

Pairwise gametic disequilibrium and comparisons of allele and genotype frequencies were tested with *G*-tests of independence using sequential Bonferroni-corrected alpha values. Disequilibria were tested directly from gametic data from haploid male offspring. When multiple individuals of the same caste were sampled from the same colony, their genotypes were averaged to form a composite genotype to equalize the contribution of each colony to the population average. Conformation to Hardy–Weinberg equilibrium expectations were tested with χ^2 -tests comparing observed and expected heterozygosity to avoid low expected numbers that could bias the χ^2 -statistic (Richardson *et al.* 1986). Within-colony relatedness was calculated from genotypic data using the program RELATEDNESS 5.0.8. Errors around average relatedness estimates were calculated by jackknifing over colonies.

3. RESULTS

(a) *Male colour patterns*

Two distinct male colour morphs were found at Hidalgo. ‘Red’ males were a bright red colour on all body segments and occurred in 62% of colonies (27 out of 44). ‘Black’ males, black on the head and thorax with a red gaster, occurred in 41% of colonies (18 out of 44). In all but one colony, only a single male colour morph was found within each nest. Colour patterns of all workers and winged queens were similar to black males and did not differ between red- and black-male colonies. Colonies of

Table 1. Allele frequencies of 'red' and 'black' males of *Pogonomyrmex rugosus* var. *fuscatus* in Hidalgo County. (Differences in allele frequencies were tested with a G -test of heterogeneity (G_H) with Bonferroni correction for multiple comparisons. All comparisons remained significant after corrections. n indicates the number of colonies sampled; when more than one male was sampled from a colony, genotypes were averaged to create a single composite genotype for the colony.)

enzyme	allele	male colour		G_H	d.f.	p
		red	black			
PGI	1	1.000	—	48.2	1	< 0.0001
	2	—	1.000			
	n	27	15			
EST-2	1	—	1.000	36.8	1	< 0.0001
	2	1.000	—			
	n	23	11			
PGM-2	1	0.105	—	21.7	2	< 0.001
	2	0.895	—			
	3	—	1.000			
	n	19	6			
HEX	1	0.853	—	31.3	2	< 0.0001
	2	0.147	0.978			
	3	—	0.022			
	n	27	15			
EST-1	1	—	0.012	20.0	3	< 0.001
	2	0.227	0.988			
	3	0.658	—			
	4	0.115	—			
	n	26	12			
PGM-1	1	0.071	—	8.0	2	< 0.02
	2	0.929	0.718			
	3	—	0.282			
	n	27	16			

the two morphs appeared intermixed in the field at Hidalgo. Only the red male morph occurred at Queen Creek.

(b) Population genetic structure

At Hidalgo, all enzyme loci showed strong and significant gametic disequilibrium with male colour (G -tests of independence, colour versus PGI: $G_1 = 41.2$, $p < 0.0001$; versus PGM-1: $G_2 = 9.1$, $p = 0.01$; versus PGM-2: $G_2 = 19.1$, $p < 0.0001$; versus EST-1: $G_3 = 20.2$, $p < 0.001$; versus EST-2: $G_1 = 36.8$, $p < 0.0001$; and versus HEX: $G_2 = 26.0$, $p < 0.0001$). Indeed, when considered as two separate groups, red and black male allele frequencies were diagnostically or significantly different at all six loci (table 1).

Although there was variability at three of the four loci scored in the Queen Creek population (PGI, EST-1, HEX), there was no evidence of gametic disequilibria between pairs of loci that would suggest either physical linkage among these genetic markers or genetic subdivision of the population (PGI versus EST-1: $G_1 = 0.54$, $p = 0.76$; PGI versus HEX: $G_1 = 0.17$, $p = 0.68$; EST-1 versus HEX: $G_1 = 0.34$, $p = 0.84$).

(c) Within-colony genetic structure

At Hidalgo, 42 out of 44 colonies contained both female offspring fathered by red males and those fathered by black males. However, workers and winged queens showed strong genetic differences within colonies consistent with differential representation of red- and black-male patriline across castes (figure 1). All workers in both red-male and black-male colonies were hybrids. These were

predominantly F_1 hybrids; such individuals were heterozygous at all diagnostic loci (PGI, EST-2, PGM-2). Heterozygosity at the two other highly divergent loci was significantly greater than expected under Hardy-Weinberg equilibrium (red-male colonies: HEX, $\chi^2_1 = 10.8$, $p = 0.001$; EST-1, $\chi^2_1 = 10.5$, $p = 0.001$; black-male colonies: HEX, $\chi^2_1 = 4.6$, $p < 0.04$; EST-1, $\chi^2_1 = 5.7$, $p < 0.02$) but the level of heterozygosity was consistent with that expected for F_1 hybrids (red-male colonies: HEX, $\chi^2_1 = 0.09$, $p = 0.8$; EST-1, $\chi^2_1 = 1.4$, $p = 0.2$; black-male colonies: HEX, $\chi^2_1 = 1.01$, $p = 0.3$; EST-1, $\chi^2_1 = 1.76$, $p = 0.2$). Only three workers were homozygous at one of the three diagnostic loci, indicating backcrossing. Worker allele frequencies were intermediate between those of red and black males at all loci and significantly different from their male siblings at most loci in both red-male and black-male colonies (figure 2). Workers sampled in late November, when no reproductive offspring were present, were also uniformly F_1 hybrids ($n = 15$).

By contrast, hybrids were virtually absent from the reproductive caste (figure 1). In colonies containing both pure-species and hybrid female offspring, winged queens were either completely ($n = 2$ colonies) or predominantly (83%; $n = 2$ colonies) non-hybrid females. Hybrid winged queens were produced in large numbers only in two black-male colonies in which no non-hybrid offspring were detected in either caste. Overall, allele frequencies of winged queens of the two colony types were nearly identical to male allele frequencies, and significantly different from their worker siblings at four of the six enzyme loci in red-male colonies (figure 2). The same pattern occurred

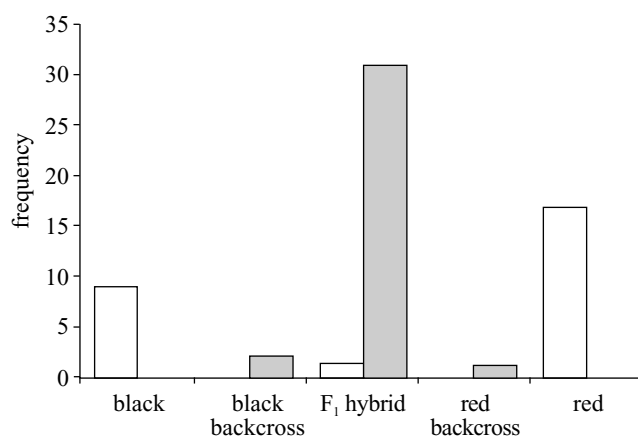


Figure 1. Frequencies of pure-species and hybrid genotypes within the pre-reproductive winged queen (white bars) and sterile worker (grey bars) castes of red-male and black-male colonies of *Pogonomyrmex rugosus* var. *fuscatus*. A single individual from each caste (when available) was included per colony. Considering only colonies containing both pure-species and hybrid females, pure-species females were significantly underrepresented in the worker caste (G -test, red-male colonies: $G_1 = 30.9$, $p < 0.0001$; black-male colonies: $G_1 = 7.4$, $p < 0.01$) but significantly overrepresented among winged queens (red-male colonies: $G_1 = 10.7$, $p < 0.005$; black-male colonies: $G_1 = 9.0$, $p < 0.005$) when compared with the proportions expected from random mating.

in black-male colonies, although statistically significant at only one locus (PGI; figure 2).

The Queen Creek population did not deviate significantly from Hardy–Weinberg equilibrium for either winged females (PGI: $\chi^2_1 = 0.01$, $p = 0.91$; PGM-1: $\chi^2_1 = 0.02$, $p = 0.89$; EST-1: $\chi^2_1 = 0.03$, $p = 0.85$; HEX: $\chi^2_1 = 0.07$, $p = 0.80$) or workers (PGI: $\chi^2_1 = 0.04$, $p = 0.84$; PGM-1: $\chi^2_1 = 0.27$, $p = 0.60$; EST-1: $\chi^2_1 = 0.17$, $p = 0.68$; HEX: $\chi^2_1 = 0.04$, $p = 0.84$). Colonies showed no allele frequency differences between winged female and worker offspring (PGI: $\chi^2_2 = 0.2$, $p = 0.9$; PGM-1: $\chi^2_2 = 0.4$, $p = 0.82$; EST-1: $\chi^2_2 = 1.6$, $p = 0.44$; HEX: $\chi^2_1 = 0.01$, $p = 0.91$). Pooling both winged queens and workers, average female–female within-colony relatedness was 0.41 ± 0.11 . Within-colony relatedness and genetic effective mate number are related by the formula (Starr 1984)

$$n_e = 1/(2r_f - 0.5),$$

where n_e is effective mate number and r_f is female–female relatedness. The observed relatedness value gives an effective mate number estimate of 3.13.

4. DISCUSSION

This study demonstrates strong genetic differences between the reproductive and sterile female castes in both black- and red-male colonies of *P. rugosus* var. *fuscatus*. While males and winged queens of the two colony types showed diagnostic genetic differences indicative of pure-species offspring, the workers from both colony types were invariably hybrids. This differs markedly from previously described cases of genetic differences between castes, in which heterozygous offspring are more likely to become

reproductive. In such cases, genotypic differences can be maintained over generations because all alleles found in workers are represented equally in queens. In this system, however, queens and males contain only conspecific alleles, so half of the workers' genome is not represented in the reproductive line and must be replenished each generation via hybridization.

The fixed genetic differences between red and black males, despite occurring in sympatry and evidently hybridizing frequently, indicate that these are reproductively isolated and should be considered as members of separate species (Mallet 1995; table 1). Because haploid male ants develop from unfertilized eggs, the genotype of the queen can be determined from multiple male offspring (Umphrey & Danzmann 1998). In all 18 colonies in which multiple males were screened genetically, alleles of only a single colour type were present, indicating that adult colony queens also belong to two distinct genetic groups. Using male colour as a marker for the remaining colonies, we conclude that the vast majority of colonies (*ca.* 98%) are headed by a pure-species queen, with no evidence of genetic introgression.

Like their mothers, reproductive female offspring nearly always possessed alleles of only a single species, indicating that they were produced from conspecific matings. Other potential mechanisms to produce such genotypes are not supported by the patterns described here. Genetic caste differences in hybridizing *Solenopsis* species were attributed to elimination or imprinting of the paternal genome in winged queens (Hung & Vinson 1977); this is not supported in *Pogonomyrmex* because winged queens were often heterozygous at non-diagnostic loci (e.g. PGM-1; figure 2). The deficit in hybrid queens also does not appear to result from parthenogenesis of reproductive offspring (Stouthamer & Kazmer 1994), because winged queens often possessed alleles not present in their mother, and thus must have acquired such alleles from a paternal source.

When workers are considered, however, *P. rugosus* var. *fuscatus* at the Hidalgo site appears to be a 'hybrid population', not between *P. rugosus* and *P. barbatus* as originally proposed (Cole 1968), but between *P. rugosus* and the black-male species (figure 1). Because this is the first description of the black-male species, it is unclear what its distribution is, and whether it exists independently outside of the hybrid zone. The *fuscatus* variant has been described across a wide area ranging from southeastern Arizona through southern New Mexico and west Texas, but it is not known whether these represent hybridizing or pure-species populations (Cole 1968). Black males have also been found in northern Arizona (Navajo County; R. A. Johnson, unpublished data) and could indicate independent populations of this species, but they have not yet been assessed genetically.

The strong segregation of reproductive castes by paternal genotype observed in both red-male and black-male colonies represents a fundamental shift from the typical colony genetic structure in *P. rugosus* outside of the *fuscatus* zone, where, as in virtually all other ants, reproductive and sterile castes do not differ genetically. The simplest explanation for this difference is that colonies inside and outside of the hybrid zone do not differ intrinsically in their caste determination mechanisms, but

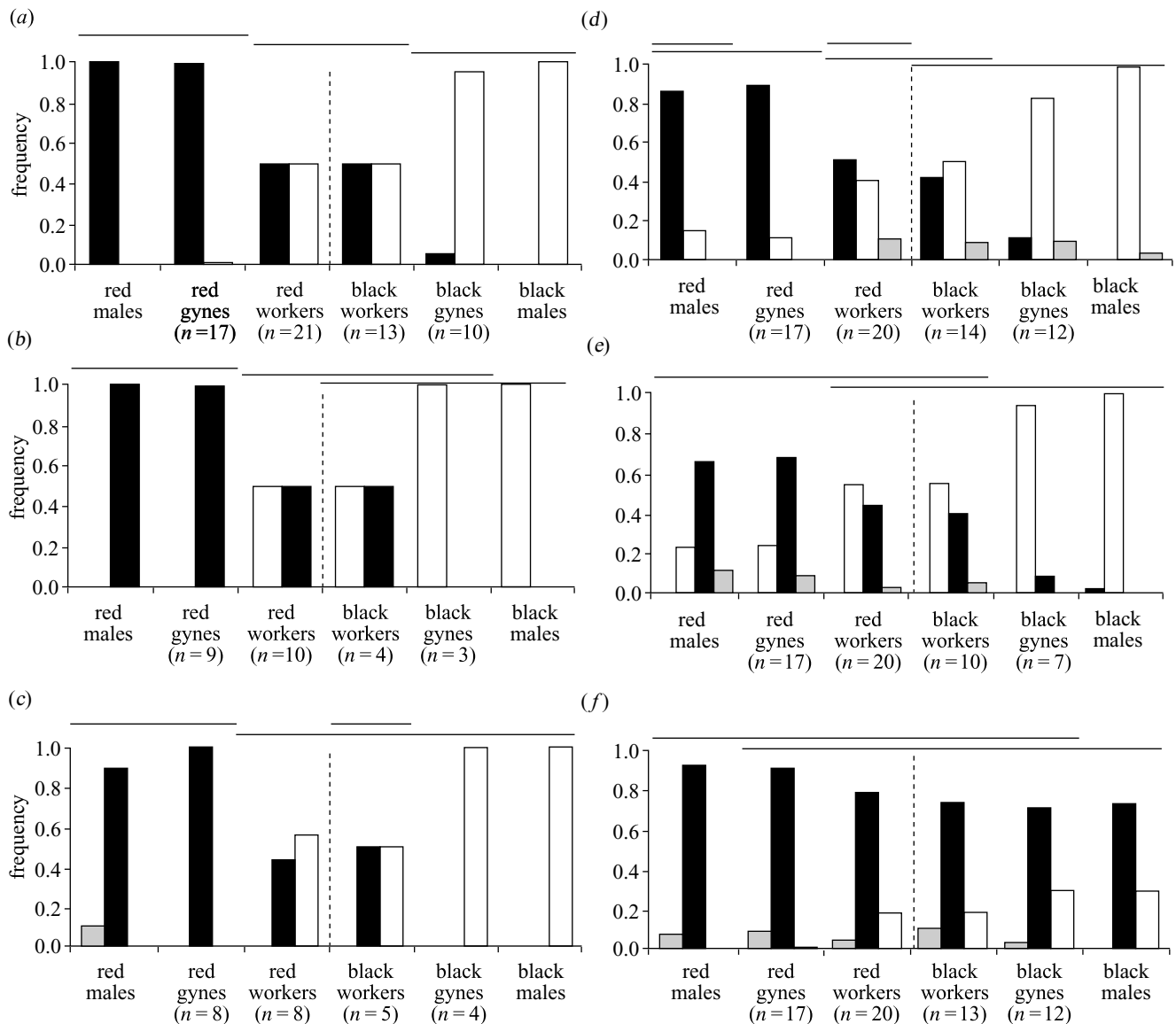


Figure 2. Allele frequencies across castes of red-male and black-male colonies of *P. rugosus* var. *fuscatus* from Hidalgo County. (a) PGI, (b) EST-2, (c) PGM-2, (d) HEX, (e) EST-1 and (f) PGM-1. Alleles are ordered by migration rate; different alleles are represented by different bar shadings. *n* indicates the number of colonies sampled. Horizontal lines indicate distributions not significantly different from one another as determined by an $R \times C$ test of independence with pairwise post-hoc comparisons. Alpha values for all subset comparisons were adjusted for the total number of pairwise comparisons per locus to avoid type I errors.

hybridization automatically results in genetic caste bias because hybrids are intrinsically poorer competitors for reproductive resources during development. This would reduce the proportion of hybrids among winged queens but increase their representation in the worker caste. Such a disadvantage is supported by the occurrence of numerous hybrid winged queens in the two colonies lacking pure-species progeny. Thus, hybrid females can potentially develop into both castes, but appear to be prevented from becoming queens by the presence of pure-species females.

However, poor hybrid reproductive development does not account for the complete absence of pure-species workers. This hypothesis predicts that hybrids should be over-represented in the worker brood only when winged queens are produced in late spring, and not when only workers are raised in summer and autumn (Tschinkel

1999), leading to an annual cycle of hybrid frequency in the worker caste. However, when we sampled 15 colonies in late November, well past the reproductive season, pure-species workers were still absent, indicating that a purely hybrid workforce is a year-round phenomenon.

The complete lack of pure-species workers raises the possibility that queens from the hybridizing populations have lost the ability to produce workers from pure-species offspring. At present, the mechanism that might prevent such development is unclear. It is possible that a genetic determinant of caste intrinsically biases non-hybrids towards a reproductive trajectory. Alternatively, developing brood may be fully bipotential but receive differential treatment from nest-mates based on paternity (Page & Erickson 1986; Tilley & Oldroyd 1997). In addition to creating a completely hybrid workforce, both of these mechanisms would tend to increase the likelihood

that pure-species brood would displace hybrids during reproductive development. A third alternative is that queens clump production of hybrid and non-hybrid diploid offspring to correspond with production of worker and reproductive offspring; however, there is no evidence that ants can selectively store or utilize sperm of different males, especially in the cyclic manner that would be required to generate the observed patterns (Keller *et al.* 1997).

For colonies to contain both pure-species and hybrid female offspring, queens must mate with conspecific and heterospecific males. Both the results of this study and previous observations of queens at mating swarms near the Hidalgo site confirm that queens are polyandrous, with effective mate number in this and another species of *Pogonomyrmex* estimated between 3.13 (this study) and six (Hölldobler 1976; Cole & Weirnsz 1999b). We calculated the likelihood that a given queen will mate with both male types over n matings with the equation

$$P(\text{red, black}) = 1 - p^n - q^n,$$

where p and q are the frequencies of red-male and black-male colonies, respectively. Given the two estimates as minimum mate numbers, 76–92% of queens mating randomly with respect to species identity should mate with at least one male of each species, slightly lower than the observed proportion of colonies (93%) containing both non-hybrid and hybrid female offspring. It is important to note, however, that over-representation of such colonies might be expected if queens failing to hybridize are unable to produce workers and thus die during colony founding. Consistent with this expectation, all colonies with only a single offspring type contained hybrids.

Why would genetic differences between reproductive castes evolve, given that genetic similarity between queens and workers is so central to the kin-selected benefits of sociality? Paradoxically, it is kin selection in the hybridization context that may be the best candidate to explain genetic caste differences in these ants. If hybrid queens are substantially less fit than their pure-species counterparts, alleles reducing investment into hybrid offspring would be favoured by selection when resources that would have gone into low-fitness hybrids can be reliably redirected towards high-fitness pure-species relatives (Leibowitz 1994). Although such kin-selected models were not originally developed for social organisms, hybrid social insects can both redirect reproductive resources and contribute additional labour towards pure-species half-siblings by becoming workers, making evolution of reproductive bias toward non-hybrids even more probable. At present, little is known about the relative fitness of hybrid queens, but the fact that virtually no gene flow between species is evident despite hybrid queen production in 7% of colonies indicates that, as in many hybrid zones (Barton & Hewitt 1985), hybrid queens may be less fit than their pure-species counterparts. In eusocial insects with a simple family structure (i.e. a single, once-mated queen), where all diploid offspring are either hybrid or non-hybrid, hybrid colonies could specialize on haploid, non-hybrid males (Umphrey & Danzmann 1998; Seifert 1999). In polyandrous or polygynous species, however, manipulation of the reproductive potential of female offspring based on ances-

try is also possible as a strategy to reduce the costs of hybridization.

Although biasing reproduction towards pure-species offspring may be adaptive from the female perspective, it has negative fitness consequences for those males who mate with a heterospecific queen. This system has striking parallels to hybridogenesis in unifemale lineages of several vertebrates (Schultz 1969; Uzzell & Berger 1975) and invertebrates (Mantovani & Scali 1992), which utilize sperm from host species for somatic expression but discard it during reproduction. In eusocial insects, where the colony itself goes through distinct phases of growth and reproduction, the process of selective genome usage can occur both at the organism and colony level (Seifert 1999). In *Pogonomyrmex*, red- and black-male colony queens parasitize heterospecific males for the workers responsible for colony growth, while only conspecific sperm is represented in reproductive daughters. This indicates that it would pay for males to be able to discriminate between conspecific and heterospecific queens, but the intensely competitive lek mating system may put discriminating males at a significant disadvantage in mate acquisition that outweighs the benefits of avoiding a heterospecific partner (Hölldobler 1976).

It is currently difficult to assess how common such 'social hybridogenesis' may be, as genotypes of winged queens and workers are generally assumed to be similar and thus not both sampled in most studies (Douwes & Stille 1991; Shoemaker *et al.* 1996). However, interspecific hybridization in ants is apparently not overly rare (Seifert 1999), and selection against hybrid queens has been demonstrated in several cases (Plateaux 1984; Buschinger & Fisher 1991; Shoemaker *et al.* 1996; Umphrey & Danzmann 1998). At least one other case of genetic mediation of caste occurs in a hybrid zone between *Solenopsis xyloni* and *S. geminata* (Hung & Vinson 1977); although originally described as differences in gene expression, later work has confirmed that, as in this study, winged queens are pure-species offspring, while workers are F_1 hybrids (S. Helms Cahan and S. B. Vinson, unpublished data). Similarly, museum specimens of colonies of *Lasius jensi* × *L. umbratus* hybrids included numerous hybrid workers but no hybrid winged queens, suggesting that these are not produced (Seifert 1999). It seems possible that further work on ant hybrid systems will reveal more cases of genetically mediated reproductive division of labour. It is worth noting that the genus *Pogonomyrmex* is one of the most conspicuous and well-studied ant taxa in western North America (Cole 1968; Whitford *et al.* 1976; Davidson 1977; Cole & Weirnsz 1999a; Gordon 1999), and while speculations about hybridization within the genus were made as early as 35 years ago (Cole 1968), the unusual patterns and consequences of hybridization even in such an obvious species have only now come to light.

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