Strong selection on a gene that influences reproductive competition in a social insect

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COMPETITION among nestmate females for reproductive opportunities is a fundamental property of insect societies, the outcome of which defines the form of colony social organization and the nature of subsequent social evolution[1–3]. Several factors influencing the outcome of competition among potential reproductives have been identified in eusocial Hymenoptera, including age[4], size[5–7] and degree of ovary or exocrine gland development[7–10]. In no case is the genetic basis of traits associated with success in these social contests understood, although there is a heritable component to their expression in laying worker honey bees[11]. I report here the existence of a single mendelian factor that strongly influences success in reproductive competition in multiple-queen societies of an ant, and I also propose a mechanism by which variation is maintained at this gene despite the presence of strong directional selection.

The fire ant *Solenopsis invicta* is an advanced eusocial insect in which two distinct forms of social organization exist. Colonies in monogyne populations are simple families headed by a single reproductive queen, whereas colonies in polygyne populations contain up to several hundred reproductive queens[12,13]. Initiation of reproduction by queens occurs through different routes in the two social forms. Monogyne queens found new nests independently, either alone or in groups, but never with workers in attendance[14]. By contrast, polygyne queens initiate reproduction in their natal or other polygyne nest in the company of workers and older queens[15,16].

An electrophoretic survey of wingless reproductive queens in a polygyne population from northern Georgia, United States, revealed consistent large-scale departures of genotype proportions from those expected under Hardy–Weinberg equilibrium (HWE) at the enzyme locus *Pgm-3*. Most striking was the complete absence of one genotypic class among these queens over both years of the survey (Table 1). These results are not artefacts of interpretation because extensive family studies show that this marker is inherited as the product of a single mendelian locus

TABLE 1 Observed and expected population genotype and allele frequencies and fit to Hardy–Weinberg genotype proportions at the locus *Pgm-3* in *S. invicta*

	Genotype frequencies observed (expected)			Allele frequencies		Deviations from Hardy–Weinberg genotype proportions
	aa	*ab*	*bb*	*a*	*b*	
Polygyne population						
Queens						
Wingless reproductives						
1990						
mated ($N=28$, $n=760$)	0 (0.139)	0.746 (0.468)	0.254 (0.393)	0.373	0.627	0.98
virgin ($N=21$, $n=113$)	0 (0.170)	0.827 (0.485)	0.173 (0.345)	0.413	0.587	0.98
all ($N=28$, $n=873$)	0 (0.143)	0.756 (0.470)	0.244 (0.387)	0.378	0.622	0.94
1991						
mated ($N=38$, $n=307$)	0 (0.138)	0.744 (0.468)	0.256 (0.394)	0.372	0.628	1.0
virgin ($N=24$, $n=57$)	0 (0.112)	0.670 (0.446)	0.330 (0.442)	0.335	0.665	0.83
all ($N=52$, $n=467$)	0 (0.138)	0.744 (0.468)	0.256 (0.394)	0.372	0.628	1.0
Winged nonreproductives						
1990 ($N=28$, $n=886$)	0.212 (0.288)	0.649 (0.497)	0.139 (0.215)	0.537	0.463	0.20
1991 ($N=27$, $n=446$)	0.099 (0.193)	0.680 (0.493)	0.221 (0.314)	0.439	0.561	0.30
Workers						
Pupae ($N=28$, $n=922$)	0.194 (0.272)	0.655 (0.499)	0.151 (0.229)	0.521	0.479	0.24
Adults ($N=28$, $n=724$)	0.263 (0.320)	0.606 (0.491)	0.131 (0.189)	0.566	0.434	0.20
Males ($N=22$, $n=112$)	—	—	—	0.251	0.749	—
Monogyne population						
Queens						
Wingless reproductives						
mature mother queens ($n=59$)	0.542 (0.582)	0.441 (0.362)	0.017 (0.056)	0.763	0.237	n.s. ($P>0.10$)
newly-mated queens ($n=271$)	0.542 (0.555)	0.406 (0.380)	0.052 (0.065)	0.745	0.255	n.s. ($P>0.10$)
Winged nonreproductives ($N=65$, $n=601$)	0.476 (0.498)	0.459 (0.416)	0.065 (0.086)	0.706	0.294	0.04
Workers						
Pupae ($N=52$, $n=1316$)	0.575 (0.573)	0.362 (0.368)	0.063 (0.059)	0.757	0.243	0
Adults ($N=39$, $n=160$)	0.489 (0.466)	0.388 (0.433)	0.123 (0.101)	0.683	0.317	0
Males ($n=108$)	—	—	—	0.815	0.185	—

Pgm-3 encodes the enzyme phosphoglucomutase (EC 5.4.2.2). *N*, Number of nests; *n*, number of individuals studied. In cases where only *n* is shown, only one individual was sampled per nest (monogyne males, monogyne mother queens) or individuals were not collected in association with nests and are assumed to each originate from a different nest (newly mated monogyne queens). All individuals of a single class were collected during a single season and, unless otherwise indicated, a single year. Observed genotype and allele frequencies represent the means of 50 random draws (with replacement) of single genotypes from each nest in cases where more than one individual was sampled per nest. This procedure ensures the independence of sampled genotypes in strongly family-structured social insect populations (see for example ref. 26). Genotype proportions expected under Hardy–Weinberg equilibrium are shown in parentheses. Deviations from Hardy–Weinberg genotype proportions are either the proportion of the 50 random draws in which the drawn genotypes departed significantly from ratios expected under Hardy–Weinberg equilibrium (cases where >1 individual sampled per nest) or are the results of a single test for departure of the entire sample from Hardy–Weinberg equilibrium (monogyne wingless reproductives) (χ^2 test with Yates' continuity correction[27], 5% significance level). Haploid polygyne males were distinguished from their more common diploid (infertile) male nestmates on the basis of banding patterns at four polymorphic enzyme loci (*Est-4, G3pdh-1, Pgm-1, Pgm-3*) and by their smaller size[24]. Haploid male genotype frequencies are the same as allele frequencies. Genotypes at *Pgm-3* were scored using the same electrophoretic methods that were used for *Pgm-1* (see Table 3).

TABLE 2 Observed and expected genotype classes at the locus *Pgm-3* in simple families of *S. invicta*

	Genotype classes observed (expected)					Deviations from 50:50 ratio for split genotype classes
	aa	*ab*	*bb*	*aa* + *ab*	*bb* + *ab*	
Monogyne population						
winged nonreproductive queens (N=33, n=574)	11 (11.6)	7 (6.9)	0 (0.7)	9 (9.7)	6 (4.1)	0
worker pupae (N=52, n=1316)	23 (22.6)	7 (9.6)	0 (0.7)	16 (14.5)	6 (4.6)	0
Polygyne population						
worker pupae (N=69, n=766)	0 (0)	14 (12.1)	2 (5.1)	39 (36.4)	14 (15.4)	0.06

N, Number of families; n, number of individuals studied (from eight to 54 offspring were genotyped for each family). Data for the monogyne population come from colonies collected in the field (such colonies are simple families[12]). Data for the polygyne population were obtained by collecting 2–4 wingless reproductive queens from each of 25 randomly selected polygyne field nests and isolating these queens in laboratory rearing units for at least 6 weeks, at which time pupae collected from the units were known to be the resident queens' offspring. The genotypes of the mother queens were obtained in addition to offspring genotypes for all 69 of these polygyne families, as well as for 31 of the 85 monogyne families. Values shown are the observed numbers of families with particular classes of genotypes; the expected numbers of such families (in parentheses) were calculated from the population genotype frequencies for the two sexes and consequent expected frequencies of *Pgm-3* mating types. All matings in the monogyne population and 80% of matings in the polygyne population were assumed to be by monogyne males (see text and Fig. 1). Evidence for mendelian inheritance of the product of *Pgm-3*: Only one or two genotype classes were observed among female offspring in single monogyne or polygyne families. When two genotype classes were present, one was always the heterozygote class and the two classes typically segregated in a 50:50 ratio (the proportion of significant deviations from this ratio, estimated from the binomial distribution at the 5% probability level, is given in the final column). The mother's genotype, when available, was always consistent with the offspring genotypes being the result of a single mating[12]. The close correspondence between the observed and expected numbers of families with particular genotype classes, the identities of classes within families, the segregation ratios, and the concordance of mother/offspring genotypes demonstrate that *Pgm-3* behaves as a single mendelian locus.

(Table 2). The genotypic disequilibrium observed at *Pgm-3* in polygyne queens disagrees with results from all other polymorphic enzyme loci surveyed in the same polygyne population; genotype distributions for these 10 additional markers show close correspondence to HWE (Table 3). Disequilibrium at *Pgm-3* therefore arises from forces acting uniquely on this or a closely linked gene rather than from processes related to the breeding or dispersal systems, which are expected to affect all genomic markers similarly.

Strong selection on *Pgm-3* in the context of reproductive competition provides the most plausible explanation for the observed genotypic disequilibrium. Although all classes of females in the polygyne population show disequilibrium at *Pgm-3*, specific genotype proportions and the consequent levels of disequilibrium differ considerably between reproductive and nonreproductive females (Table 1). Reproductive queens lack the homozygous $Pgm\text{-}3^a/\text{-}3^a$ genotype, and the unusual ratios of the other two genotypes create extreme genotypic disequilibrium in these reproductives. By contrast, all three genotypes are found among nonreproductive polygyne females (winged queens and workers), although consistent excesses of heterozygotes create moderate levels of disequilibrium. It is important, that genotype and allele frequencies in the monogyne form are similar among all classes of females and differ strongly from frequencies in the polygyne form, and monogyne genotype proportions are in HWE.

The absence of $Pgm\text{-}3^a/\text{-}3^a$ homozygotes among reproductive polygyne queens and their presence among nonreproductive queens suggest that selection acts by eliminating queens with this genotype before they are recruited into a colony's pool of reproductives. This view is consistent with the similar ratios of *ab* to *bb* genotypes in reproductive queens (3.0:1) and the winged nonreproductive queens from which they originate (3.9:1). Also, preliminary data indicate a greatly diminished survival probability for $Pgm\text{-}3^a/\text{-}3^a$ queens relative to the other genotypes when these queens initiate reproduction in isolation and are reintroduced into their natal polygyne nests (L. Keller and K.G.R., manuscript in preparation). That selection acting on polygyne queens does not result from wholesale breakdown of adult viability is indicated by the fact that $Pgm\text{-}3^a/\text{-}3^a$ is the most common genotype among reproductive queens in mature monogyne colonies (Table 1).

Selection on *Pgm-3* seems to be related to some unique feature of the process by which polygyne queens of *S. invicta* become functionally reproductive. Genotype proportions in monogyne queens remain relatively unchanged from the time before their mating flights, to just after mating, to when they are reproductives in large colonies (Table 1), indicating no comparable type of selection in this social form. The most conspicuous difference between the two forms in the process of reproductive development is that it is initiated in the presence of fully functional reproductive queens in the polygyne but not the monogyne form, a difference likely to have important consequences for reproductive competition. Reproductive competition among polygyne fire ant queens is mediated largely by workers, who seem actively to select the queens that become reproductives while destroying the remainder, most probably in response to each queen's pheromonal output[8,17–19]. This study demonstrates that a queen's genotype at a single mendelian locus is a determinant of the outcome of this competitive process, suggesting that the gene product may be involved in regulating production of a queen control pheromone.

Complete loss of reproductive opportunities for polygyne

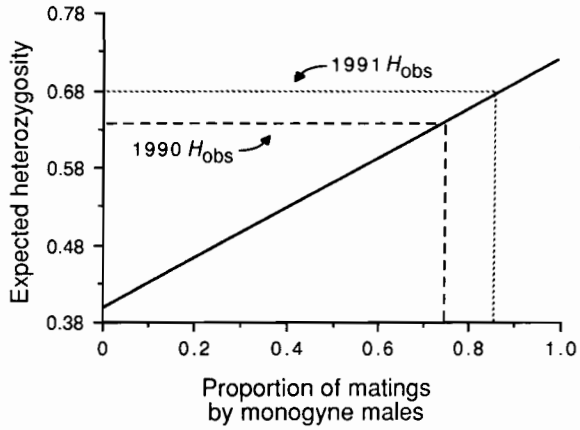

FIG. 1 Relationship between proportion of matings of polygyne queens attributed to monogyne males and expected heterozygosity of female offspring at *Pgm-3*. The solid line was generated from equation 2.15 of ref. 20, which estimates values of heterozygosity expected under male haploidy when allele frequencies differ between the sexes. Values are based on the average allele frequencies for polygyne reproductive queens over both years of the study and on the allele frequencies expected for their male mates when monogyne males are responsible for varying proportions of successful matings (haploid polygyne males being responsible for the remainder). The average heterozygosities observed for nonreproductive females for each year of the study (H_{obs}) correspond to heterozygosities expected when 74 or 85% of matings by polygyne queens are attributed to monogyne males.

TABLE 3 Deviations from Hardy-Weinberg genotype proportions at 10 polymorphic loci in polygyne *S. invicta*

	Aat-2	*Acoh-1*	*Acoh-5*	*Acy1*	*Ddh-1*	*Est-4*	*Est-6*	*G3pdh-1*	*Pgm-1*	*Pro-5*
Workers										
larvae ($N=31$, $n=1012$)	0	—	—	—	0.02	0	—	—	0	—
pupae ($N=31$, $n=1015$)	—	0	0	—	—	—	0	—	0.04	—
adults ($N=31$, $n=976$)	0	—	—	—	—	0.04	—	0	0	—
Queens										
wingless reproductives ($N=31$, $n=616$)	0	0	0	—	—	0.02	—	0	0.02	—
winged nonreproductives ($N=31$, $n=954$)	0.02	0	—	0	—	0.04	—	0.02	0.04	0.02

N, Number of nests; *n*, mean number of individuals studied per locus for each class of female. Values represent the proportion of 50 random draws of single genotypes per nest in which the ratios of the drawn genotypes departed significantly from those expected under Hardy-Weinberg equilibrium (χ^2 test with Yates' continuity correction, 5% significance level; see Table 1 legend). Dashes indicate that the markers were not scored in a particular material. Genotypes were scored following horizontal electrophoresis in 14% starch gels using a pH 6.0 amine-citrate (morpholine) continuous buffer system (*Ddh-1, Est-4, Est-6, G3pdh-1, Pgm-1, Pro-5*), a pH 8.6 Tris-borate-EDTA continuous buffer system (*Aat-2, Acy1*), or a pH 6.5 Tris-citrate continuous buffer system (*Acoh-1, Acoh-5*), with the protein bands visualized by specific histochemical staining[12,28,29]. Mendelian inheritance of all 10 markers has been confirmed by family studies (ref. 12; D. Shoemaker, J. Costa and K.G.R., manuscript in preparation).

queens homozygous for *Pgm-3*^{a} suggests that the dynamics of this allele should mimic those of a recessive lethal, resulting in virtual monomorphism for the alternate allele in relatively few generations in the absence of other evolutionary forces[20]. Yet *Pgm-3*^{a} was common in the polygyne population over both years of this study. Because a high frequency of this allele in polygyne males can be ruled out as an explanation for the maintenance of this polymorphism (Table 1), the most likely cause is that *Pgm-3*^{a} is continually reintroduced into the polygyne population through dispersing monogyne males. *Pgm-3*^{a} occurs at high frequency in these males (Table 1), and their ability to disperse widely[21] and the modest size of the polygyne population studied[22] allow ample opportunity for males from surrounding monogyne populations to reach polygyne females. Moreover, the *Pgm-3*^{a} allele is sufficiently more common in monogyne males than in polygyne reproductive queens that substantial excess heterozygosity should be generated at *Pgm-3* if they interbreed[20], as is observed. Indeed, the excess heterozygosity found in nonreproductive polygyne females can be fully accounted for if some 80% of matings in the polygyne population involve monogyne males (Fig. 1). Additionally, the distribution of genotype classes observed in 69 families derived from single polygyne queens closely matches the distribution expected if monogyne males are responsible for 80% of the matings (Table 2). Finally, because of reduced allelic variation at the putative sex-determining locus, 80–95% of males produced in polygyne populations of *S. invicta* are infertile diploids, whereas males in monogyne populations are invariably haploid[23–25]. Thus there may be too few fertile haploid males produced in polygyne populations to compete effectively with immigrant monogyne males for fertilizations.

This study provides evidence that a single gene can strongly influence the outcome of reproductive competition in a social insect, that this effect is dependent on social context, and that both selection and gene flow are important in determining genotype and allele frequencies at this gene in a specific social environment. Identification of the gene and physiological role of its product may elucidate the manner in which its phenotypic social effects are achieved and so provide a model for the genetic basis of queen competition and control in insect societies. □

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