SIMPLE GENETIC BASIS FOR IMPORTANT SOCIAL TRAITS IN THE FIRE ANT *SOLENOPSIS INVICTA*

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Abstract.—Variation in queen phenotype and reproductive role in the fire ant *Solenopsis invicta* has been shown to have a simple genetic basis in a single introduced population in the United States. The evidence consists of an association between this variation and queen genotype at *Pgm-3*, a phosphoglucomutase-encoding gene. In the present study, we surveyed *Pgm-3* allele and genotype frequencies in diverse populations from the native and introduced ranges of this ant to learn whether this simple genetic basis for reproductive traits is a general feature of the species or a genetic anomaly in introduced ants stemming from a recent bottleneck or the invasion of novel habitats. No egg-laying queens living in polygyne (multiple-queen) nests possessed the homozygous genotype *Pgm-3ᵃ/ᵃ* in any of the study populations, yet nonreproductive females from such nests (workers as well as queens that had not yet initiated oogenesis) possessed this genotype at moderate frequencies. Remarkably, *Pgm-3ᵃ/ᵃ* was the most common genotype among all classes of females, including egg-laying queens, in monogyne (single-queen) nests from all populations studied. Genotype proportions at *Pgm-3* in polygyne populations typically departed strongly from the proportions expected under Hardy-Weinberg equilibrium, whereas those in monogyne populations did not. These patterns establish that a single mendelian gene influences queen reproductive role in *S. invicta* and that this gene uniformly is under strong directional selection in the polygyne social form only. Moreover, the perfect association of *Pgm-3* genotype and reproductive role in all populations, combined with the known function of phosphoglucomutase in insect metabolism, suggest that this gene may directly influence queen phenotypes rather than merely serving as a marker for a linked gene that causes the effects.

Key words.—Enzyme polymorphism, fire ants, phosphoglucomutase, physiological genetics, selection, social insects, *Solenopsis invicta*.

Evolutionary biologists have a long-standing interest in the genetic basis of traits that are under selection in natural populations. Predicting such features of long-term evolution as rates of phenotypic change and the limits to selective responses requires knowledge of several key genetic parameters, most importantly, the number of loci underlying mean trait values and reaction norms, and the distribution of effects among these loci (e.g., Barton and Turelli 1989; Charlesworth 1990; Mitchell-Olds 1995; Via et al. 1995). Moreover, identification of genes with substantial phenotypic effects can be important for understanding how genotypic variation causes differences in individual performance and, ultimately, how this translates into fitness variation in the wild (Pough 1989; Clark and Koehn 1991; Feder and Watt 1991). The classical neo-Darwinian view concerning the genetic architecture underlying selectively important traits has been that most adaptive microevolution occurs via the gradual substitution of many mutations of slight individual effect, that is, that traits under selection normally are polygenic and that no single gene contributes a major portion of an adaptive response (Charlesworth 1990; Grafen 1991; Orr and Coyne 1992). Nonetheless, recent theory suggests that adaptively significant variation encoded by one or a few loci with major effects could be widespread (Watt 1985; Barton and Turelli 1989; Clark and Koehn 1991; Orr and Coyne 1992), and examples from wild populations apparently are not uncommon (e.g., Gottlieb 1984; Watt 1985, 1992; de Belle et al. 1989; Clark

and Koehn 1991; Orr and Coyne 1992; Hoffmann 1994; McKenzie and Batterham 1994; Van Oortmerssen and Sluyter 1994; Clark et al. 1995; Hunt et al. 1995; Mitchell-Olds 1995; Paterson et al. 1995).

Studies of the genetic bases of selectively important traits involved in behavioral evolution have not been conducted frequently in natural populations (Ferguson and Danzmann 1985; Boake 1994; Hoffmann 1994; Lank et al. 1995; Pontier et al. 1995; Shaw 1996). Nowhere is this lack of attention more apparent than in the case of social organisms, where genetic analysis of behaviors integral to the evolution of complex forms of sociality is in its infancy (Moritz and Hillesheim 1985; Page and Robinson 1991, 1994; Keller and Ross 1993a; Rinderer and Beaman 1995). This last fact is surprising given that the evolution of social behavior has been studied extensively by use of both explicit genetic models and phenotypic models with implicit genetic assumptions (e.g., Crozier 1979; Michod 1982; Mueller and Feldman 1985; Grafen 1991; Queller 1992; Reeve 1993).

One example in which the genetic basis of a complex social trait under selection in the wild has been elucidated comes from populations of the fire ant *Solenopsis invicta* that have been introduced to the United States (Ross 1992; Keller and Ross 1993a; Ross and Keller 1995a). Egg-laying queens in multiple-queen (polygyne) societies of this ant never possess a particular homozygous genotype at the enzyme-encoding gene *Pgm-3*, despite the fact that this genotype, designated *Pgm-3ᵃ/ᵃ*, occurs commonly in nonreproductive females (young winged queens and the sterile workers). Laboratory

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studies have revealed that $Pgm-3^{a/a}$ queens from polygyne nests do not make the transition from nonreproductive to reproductive status because workers selectively destroy queens with this genotype as the queens initiate oogenesis (Keller and Ross 1993a). Such queens undergo accelerated sexual maturation and oogenesis compared to queens with the other two genotypes, $Pgm-3^{a/b}$ and $Pgm-3^{b/b}$, suggesting that the product of $Pgm-3$ (or a closely linked gene) strongly affects the process of reproductive development and that workers use cues associated with such development to discriminate among queens with different genotypes (Keller and Ross 1993a, 1995). Remarkably, although $Pgm-3^{a/a}$ is completely absent among egg-laying queens of the polygyne form, it is the most common genotype among egg-laying queens of an alternate social form in which nests contain only one such queen (monogyne form). Furthermore, there are no apparent phenotypic differences among queens with different $Pgm-3$ genotypes in this alternate form. Taken together, these findings suggest that a single gene underlies major phenotypic variation of selective importance in introduced fire ants and that the phenotypic effects of the gene are strongly influenced by the social environment.

This unusual example of a simple genetic basis for a trait under consequential social selection has been described in ants from a single locality in the introduced range, that is, in a restricted set of individuals recently descended from a founder population that invaded a novel environment and experienced a pronounced bottleneck (e.g., Lofgren 1986; Porter et al. 1992; Ross et al. 1993). Such recent events in the history of a population may alter the relationship between genotype and phenotype by several means. For example, a novel environment may impose strong directional selection that generates significant phenotypic change by mobilizing mutations of large phenotypic effect, mutations that ordinarily would be deleterious in equilibrium populations experiencing stabilizing selection (Lande 1983; Barton and Turelli 1989; Orr and Coyne 1992; López and López-Fanjul 1993; McKenzie and Batterham 1994). Low population size in early founding populations may impose other substantial changes in selective regimes due simply to the low density of conspecifics, such as through a reduction in intraspecific competition or disruption of the mating system. Most importantly, perhaps, a severe bottleneck may cause loss of allelic variation at structural or modifier loci, converting a potentially complex genetic architecture into a simpler one. Such a reduction in variation could be accelerated in a small colonizing population due to drift or in an expanding post-bottleneck population due to altered environmentally and genetically induced selective regimes (e.g., Templeton 1980; Carson 1990). Thus, the simple genetic basis for selectively important variation in social traits discovered in a single population of United States fire ants conceivably could be a genetic anomaly attributable to their recent introduction.

The objective of the present study was to determine whether the association of $Pgm-3$ genotype with queen reproductive role is a general phenomenon in the polygyne form of *S. invicta*. This was accomplished by examining $Pgm-3$ genotype distributions in a large number of ants in many samples collected from the introduced range in the United States and, most importantly, from the native range in South America.

Our data reveal that egg-laying queens with the genotype $Pgm-3^{a/a}$ are not found in polygyne nests from any of these diverse and remotely related populations but that nonreproductive females with this genotype do occur in such nests. Thus, we conclude that elimination of $Pgm-3^{a/a}$ queens by workers is a social behavior common to all populations of the polygyne social form and that a simple genetic basis for queen phenotype and reproductive role is a ground plan trait of this species. Furthermore, the complete association between $Pgm-3$ genotype and queen fate, coupled with the key role of the product of this gene (phosphoglucomutase) in insect growth and metabolism, suggest that $Pgm-3$ may be the actual gene causing the observed effects on queen reproductive phenotype rather than simply a marker of a closely linked gene that produces these effects.

MATERIALS AND METHODS

Sample Collection

Most samples from the United States were collected from nests in northern Georgia (Walton, Oconee, Clarke, and Putnam Counties), an area where the distributions of the monogyne and polygyne social forms of *S. invicta* are well known (Ross and Keller 1995a). Several of these samples have been described elsewhere (Ross 1992; Keller and Ross 1995; Ross and Keller 1995a). One sample of "reproductive" queens (queens that have shed their wings, a behavior associated with the onset of oogenesis) was collected from polygyne nests in Travis County, Texas, 1400 km from the sites of the Georgia collections. Another sample of reproductive queens (in this case newly mated queens that had not yet started nests) was collected from a population of the monogyne form in Leon County, Florida, 400 km from the Georgia sites.

Initial determination of the social form of each nest in the United States was made in the field using well-established criteria that distinguish the forms (Greenberg et al. 1985). Confirmation was obtained in most instances by examining genotype distributions for eight or more nestmates at 4–10 polymorphic allozyme loci (see Shoemaker et al. 1992) or, in the case of polygyny, by recovering several mated reproductive queens from a nest. Nestmate genotype distributions are diagnostic for the two social forms because fire ant queens mate only once (Ross and Fletcher 1985; Ross et al. 1993); thus, monogyne nestmates exhibit the simple genotype distributions expected for the offspring of a single diploid mother and single haploid father (e.g., Ross and Fletcher 1985; Shoemaker et al. 1992; Ross 1993). Monogyne reproductive queens ("mother" queens in mature nests or newly mated queens) were collected from areas known to be inhabited only by the monogyne form. Mother queens were collected from large nests identified as being monogyne on the basis of field criteria. Newly mated queens, collected from the ground immediately after their mating flights, were weighed within 24 h of collection to confirm that they originated from monogyne nests; mature winged queens of this form are substantially heavier than such queens of the polygyne form (Porter et al. 1988; Keller and Ross 1993b, 1995).

Samples of both social forms from the native range were collected from two geographic populations in northern Argentina. The two populations, centered around the cities of
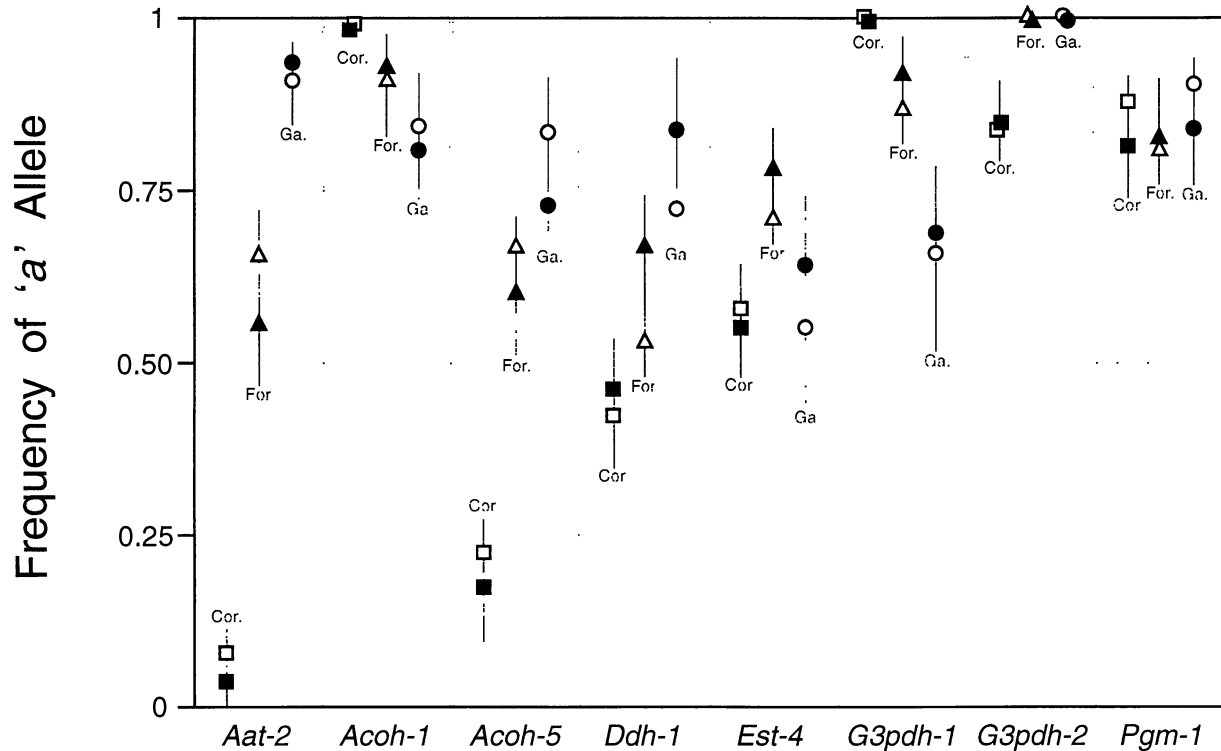
FIG. 1. Frequency of the "*a*" allele at eight polymorphic electrophoretic loci in three geographic populations of *Solenopsis invicta* (Corrientes, Argentina—*Cor.*; Formosa, Argentina—*For.*; Georgia, U.S.—*Ga.*). The "*a*" allele is defined as the most common allele at each locus in Georgia. Only loci for which this most common allele was present at a frequency ≤ 0.90 in at least one of the populations are depicted. Open symbols represent values for monogyne nests, whereas filled symbols represent values for polygyne nests. Vertical bars indicate the 95% confidence intervals around the estimates for both social forms at a site generated using a resampling procedure (see *Genetic Analyses* section of text). Data for *Est-4* and *G3pdh-1* are from nonreproductive queens, whereas data for the other loci are from workers. Sample sizes for Corrientes and Formosa are as in the Appendix; those for Georgia are reported in Ross and Shoemaker (1993).

Corrientes (Corrientes Province) and Formosa (Formosa Province), are separated by a potentially significant barrier to gene flow (the Río Paraná drainage basin) and are quite distinct genetically. For instance, the value of Nei's genetic distance ($D \pm$ SE) based on 70 protein-encoding electrophoretic loci is 0.019 ± 0.010 between Corrientes and Formosa ants, in contrast to the value of only 0.004 ± 0.003 between Formosa and Georgia ants estimated using these same loci (unpubl. data). Significant and often pronounced allele frequency differences at several well studied, presumably neutral loci contribute to this differentiation between ants from the two Argentine localities (Fig. 1). These observations suggest that the native populations from these two areas, which provide the most relevant comparative data base for *Pgm-3* in this study, may be united by only rather weak and/or recent gene flow. All of the Argentine samples were confirmed to be *S. invicta* on the basis of diagnostic morphological characters (Trager 1991).

Initial determination of the social form of nests sampled in Argentina was made using field criteria. Verification of field determinations was accomplished for each nest by inspecting genotype distributions for 12 or more nestmates at ten polymorphic allozyme loci (see Ross et al. 1993 for information on these loci). Verification of the social form of each nest by this means was crucial in Argentina, where nests

of the two forms commonly are intermixed and the field criteria for distinguishing the forms are not as reliable as in the United States. Multiple reproductive queens were captured from two-thirds of the Argentine nests identified on the basis of the genotype distributions as being polygyne nests. Reproductive queens of the monogyne form were not collected in Argentina because of logistical constraints.

Determination of Pgm-3 Genotypes

Genotypes at *Pgm-3* were determined for all collected individuals ($n = 15,359$) by means of starch-gel electrophoresis (Ross 1992; Shoemaker et al. 1992). This locus is effectively biallelic in all U.S. samples studied to date, with the two alleles designated *Pgm-3^a* and *Pgm-3^b* (progeny studies have confirmed that the electromorphs behave as allelic variants of a single mendelian gene: Ross 1992; Shoemaker et al. 1992). Several other alleles are found at low frequency in the two Argentine study populations, a finding not unexpected given that many rare alleles present in native populations at other electrophoretic loci are absent in introduced *S. invicta* (Ross et al. 1993). The distributions of genotypes carrying these rare *Pgm-3* alleles within native monogyne nests were consistent with these alleles also being inherited in mendelian fashion. The frequencies of the *Pgm-3* alleles

TABLE 1. Deviations of observed genotype proportions from those expected under Hardy-Weinberg equilibrium (HWE) at presumably neutral polymorphic allozyme loci in polygyne populations of *S. invicta* from Texas and Argentina. The numerical values presented are the proportions of 1000 resampled genotype distributions (one genotype per nest) in which the genotype proportions deviated significantly from the proportions expected under HWE (using χ^2 tests with $\alpha = 0.05$); values greater than 0.05 (in bold with an asterisk) indicate significant departures from HWE (see text). The term "n.s." indicates that the sample genotype proportions did not differ significantly from HWE proportions according to an exact test; this method was used when one allele was sufficiently common at a locus so as to preclude using χ^2 tests on the resampled distributions. Dashes indicate that a particular locus is not variable in a particular population or is not expressed in a particular female caste. Sample sizes are for numbers of nests (N) and numbers of individuals (n) studied. Procedures for electrophoresis are given in Shoemaker et al. (1992).

Sample	Locus									
	Aat-2	Acoh-1	Acoh-5	Ddh-1	Est-4	Fbp-2	G3pdh-1	G3pdh-2	Pgdh-3	Pgm-1
Texas, U.S.										
Reproductive queens ($N = 30$, $n = 461$)	n.s.	n.s.	0.01	—	**0.07***	—	0.04	—	—	0.03
Corrientes, Arg.										
Workers ($N = 44$, $n = 528$)	n.s.	n.s.	0.02	0.05	0.03	—	—	0.02	n.s.	0.01
Reproductive queens ($N = 28$), $n = 187$)	n.s.	—	0.01	—	0.03	—	—	—	—	0.05
Formosa, Arg.										
Workers ($N = 35$, $n = 411$)	**0.10***	n.s.	0.02	0.02	0.02	—	0.01	—	—	0.03
Reproductive queens ($N = 26$, $n = 123$)	0.02	n.s.	0	—	0	n.s.	0.01	—	—	0.05

found in each sample in this study and the relative electrophoretic mobility of each allelic electromorph are reported in the Appendix.

Genetic Analyses

Nestmate genotypes are not independent in monogyne nests, nor in polygyne nests from the native range (where nestmate relatedness is substantial: Ross et al. 1996), because in these cases nestmates are members of more or less extended families. Even in polygyne nests in the United States, where nestmate relatedness typically is low (Ross and Fletcher 1985; Ross 1993), some sampled genotypes may not be independent, depending on the particular relatedness structure of a nest and the number of individuals sampled from it. Therefore, to obtain unbiased estimates of *Pgm-3* genotype and allele frequencies, a resampling procedure was instituted whereby a single genotype was drawn at random from each nest in a sample, yielding a distribution of independent genotypes for that sample. Genotype and allele frequencies then were estimated as the arithmetic mean frequencies for 1000 such resampled genotype distributions from each sample. The 95% confidence intervals (CIs) about the allele frequency estimates were obtained by dropping the 25 highest and 25 lowest allele frequency values derived from the 1000 resampled distributions (e.g., Weir 1990). In those Georgia samples for which only one individual was collected per nest (polygyne worker pupae in 1993, monogyne mother queens in 1991, monogyne worker adults in 1994, and monogyne nonreproductive queens in 1996), or samples for which individuals were not collected in association with nests (both samples of monogyne newly mated queens), 95% CIs about the allele frequency estimates were obtained by drawing 1000 bootstrap samples from the original data sets and dropping the 25 highest and 25 lowest allele frequency values.

Tests for departures of *Pgm-3* genotype proportions from Hardy-Weinberg equilibrium (HWE) were in most cases conducted by applying χ^2-tests with Emigh's continuity correction and Levene's small-sample correction (Lessios 1992) to each of the 1000 resampled data sets (one genotype sampled per nest), and then determining the proportion of the 1000 tests that yielded values of χ^2 greater than the critical value for $\alpha = 0.05$. Samples for which greater than 5% of these tests yielded χ^2-values above the critical value were judged to depart significantly from HWE. In those samples for which only one individual was collected per nest or individuals were not collected in association with nests, only a single χ^2-test for departure from HWE was conducted, with the above-listed corrections employed. Rare alleles (which generally occurred at frequencies < 0.05, see the Appendix) were pooled with the allele *Pgm-3^b* and the observed and expected genotype proportions recalculated for these analyses (Lessios 1992).

Previous studies of polygyne *S. invicta* in Georgia have shown that genotype proportions at polymorphic allozyme loci other than *Pgm-3* match the proportions expected under HWE (Ross 1992, 1993). Thus, it was desirable to test for departures from HWE genotype proportions at such loci in the polygyne samples collected for the present study from Texas and Argentina. The polymorphic loci tested in each of these samples are listed in Table 1; procedures for electrophoresis and scoring of these markers are found in Shoemaker et al. (1992). Genotype and allele frequencies at these loci were estimated from 1000 resampled genotype distributions from each sample (one genotype sampled per nest). Tests for departures of the observed genotype proportions from HWE generally were conducted by applying χ^2-tests to each of the resampled data sets and determining the proportion of the 1000 tests that yielded values of χ^2 greater than the critical

value for $\alpha = 0.05$. In those cases in which one allele at a locus was sufficiently common to invalidate the use of χ^2-tests because of low expected genotype counts, an exact test was performed on the unbiased estimates of the sample genotype frequencies using the tables of Vithayasai (1973). When present, multiple rare alleles were pooled for the tests of HWE (Lessios 1992).

RESULTS

Genotype proportions at *Pgm-3* in samples of polygyne and monogyne *S. invicta* from the introduced and native ranges are presented in Figures 2 and 3. Considering first the polygyne form (Fig. 2), the genotype *Pgm-3$^{a/a}$* is present in nonreproductive females (workers and nonreproductive [winged] queens) in all samples, typically at moderate frequency (0.2–0.4). In contrast, this genotype is completely lacking among reproductive queens of this social form. As expected given this genotypic pattern, the frequency of allele *Pgm-3^a* is consistently lower in reproductive females than in nonreproductive females from the same site (see the Appendix for allele frequencies). The total absence of reproductive queens with genotype *Pgm-3$^{a/a}$* in polygyne nests now is based on samples of 3819 queens from Georgia, 449 from Texas, and 310 from Argentina. Thus, the earlier conclusion that queens with this genotype are systematically destroyed by nestmate workers and thus precluded from assuming a reproductive role, which was based on population genetic data and laboratory studies of queen execution in Georgia, would appear to be applicable to all polygyne populations of this species.

The consistent exclusion of *Pgm-3$^{a/a}$* females from the pool of reproductive individuals constitutes strong directional selection against the allele *Pgm-3^a* in the polygyne social form. Such strong selection is expected to draw *Pgm-3* genotype proportions away from those expected under HWE, and indeed, large and significant departures of the observed proportions from HWE expectations are a general feature of the polygyne populations studied (Fig. 2) (gene flow from monogyne colonies apparently also contributes to this disequilibrium: see Ross 1992; Ross and Shoemaker 1993; Ross and Keller 1995a). Disequilibrium at *Pgm-3* has been shown to occur against a background of general conformity to HWE at other, presumably neutral, polymorphic loci in polygyne *S. invicta* from Georgia (Ross 1992, 1993). This pattern is further documented here for the polygyne samples from Texas and Argentina (Table 1). The general occurrence of Hardy-Weinberg genotype proportions at these other markers indicates that such features as inbreeding or restricted dispersal are unlikely to influence genotype frequencies at *Pgm-3* in polygyne *S. invicta*.

Genotype proportions at *Pgm-3* in the monogyne form of *S. invicta* stand in dramatic contrast to the genotype proportions in the polygyne form (Fig. 3). For both the introduced and native ants, the genotype *Pgm-3$^{a/a}$* invariably is the most common genotype in all classes of females surveyed (reproductives as well as nonreproductives), and it occurs in similar proportions in all of these classes within a given population. As expected, the frequency of the allele *Pgm-3^a* also is very similar across all classes of monogyne females

within each population (see the Appendix). Observed genotype proportions at *Pgm-3* conform to the proportions expected under HWE in every monogyne sample, in both the introduced and native ranges.

The results from these diverse monogyne samples strongly support the earlier conclusion that the locus *Pgm-3* in this social form is not under selection of the type and magnitude evident in the polygyne social form (Ross 1992; Keller and Ross 1993a). Perhaps the most compelling feature of the data with respect to this conclusion is the fact that *Pgm-3$^{a/a}$* is the most common genotype among monogyne reproductive queens in the United States, in contrast to its complete absence among polygyne reproductive queens. Although genotypic data are not directly available for monogyne reproductive queens in Argentina, the prevalence of genotype *Pgm-3$^{a/a}$* among such queens can be inferred from the genotypes of their daughters (at least 12 daughters from each nest were scored for *Pgm-3*). Colonies containing only *Pgm-3$^{a/a}$* daughters must be headed by a *Pgm-3$^{a/a}$* queen mated to a *Pgm-3^a* male. Thus, the proportion of such colonies provides a minimum estimate of the proportion of *Pgm-3$^{a/a}$* mother queens in a monogyne population (some colonies with only *Pgm-3$^{a/b}$* daughters also are headed by *Pgm-3$^{a/a}$* queens). In Formosa, 21 of 35 monogyne colonies (60%) had only *Pgm-3$^{a/a}$* daughters, whereas in Corrientes the figure is 21 of 37 colonies (57%). Thus, *Pgm-3$^{a/a}$* is inferred to be the most common genotype among reproductive queens of the monogyne form in the native range as well as the introduced range.

DISCUSSION

The results of this study indicate that an identical, simple genetic basis for variation in queen reproductive role occurs throughout various native and introduced populations of the polygyne social form of *S. invicta*. This conclusion is based on the invariant patterns in the distribution of *Pgm-3* genotypes among females of this social form; nonreproductive females possess the genotype *Pgm-3$^{a/a}$* at moderate frequencies, yet this genotype is not found among reproductive queens. These findings are important because they demonstrate that the genetic basis for queen reproductive role inferred from studies at a single U.S. locality is not an artifact of the recent introduction, such as might result from a massive loss of genetic variation. Of special significance is the fact that the identical genetic basis occurs in two genetically differentiated native populations separated from one another by a potentially important biogeographic barrier, as well as in introduced populations that have diverged strongly from the native ants in such key features of their social organization as colony queen number and relatedness (Ross et al. 1996). Thus, the genetic component of reproductive role associated with variation at *Pgm-3* appears to be a fundamental and ancient feature in the regulation of social behavior in the polygyne form of *S. invicta*.

The genetic data obtained from the monogyne form are crucial to developing a complete picture of the role of selection acting on *Pgm-3* in fire ants. Monogyne populations do not exhibit the unusual patterns in *Pgm-3* genotype proportions seen in the polygyne form. Specifically, genotype proportions in the monogyne form are similar between all
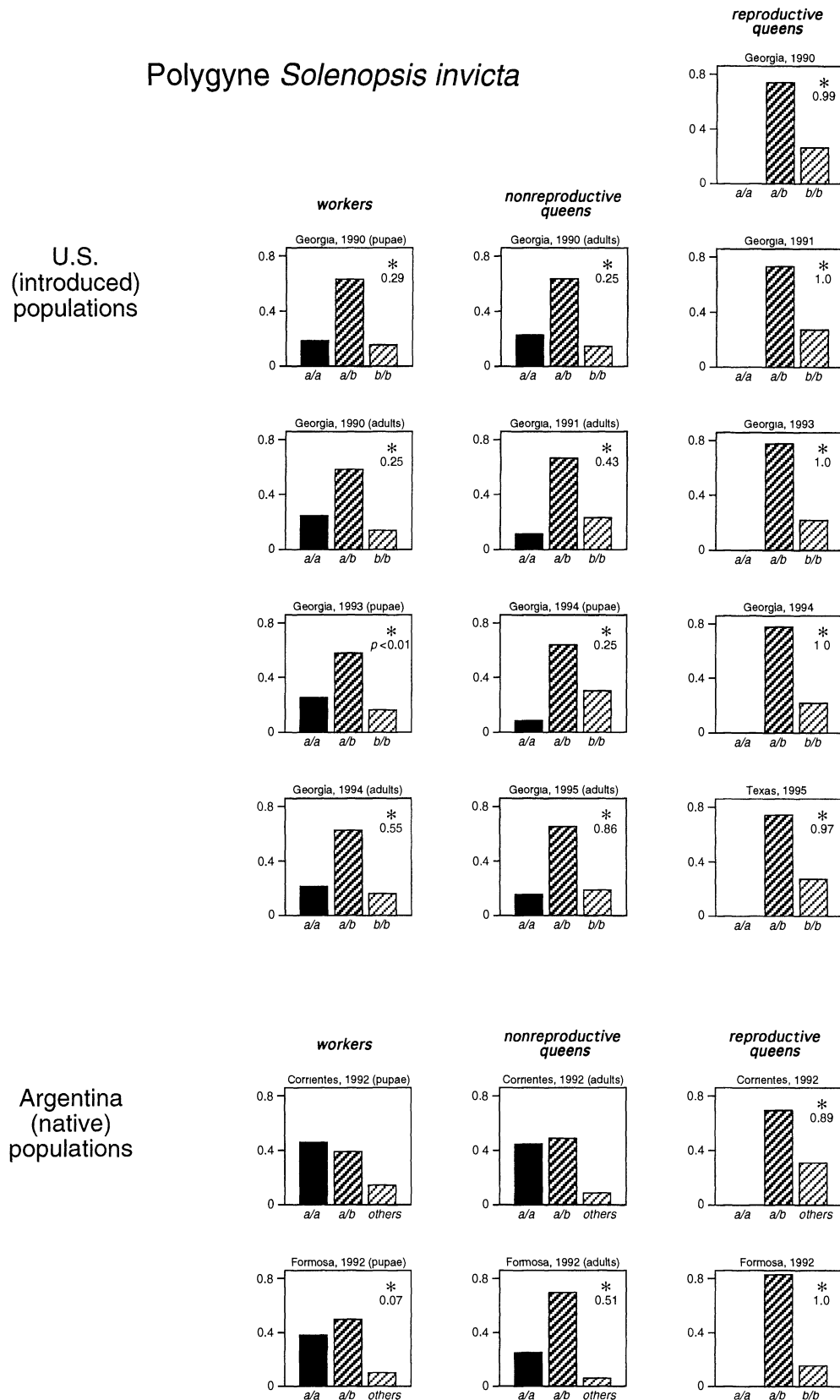
Polygyne *Solenopsis invicta*

FIG. 2. Genotype proportions at *Pgm-3* in samples of polygyne *Solenopsis invicta* from introduced and native populations. The category "others" in the Argentine populations comprises genotype *Pgm-3*$^{b/b}$ pooled with all genotypes containing rare alleles (see the Appendix for allele frequencies). An asterisk in a panel signifies a significant departure of the genotype porportions observed in that sample from
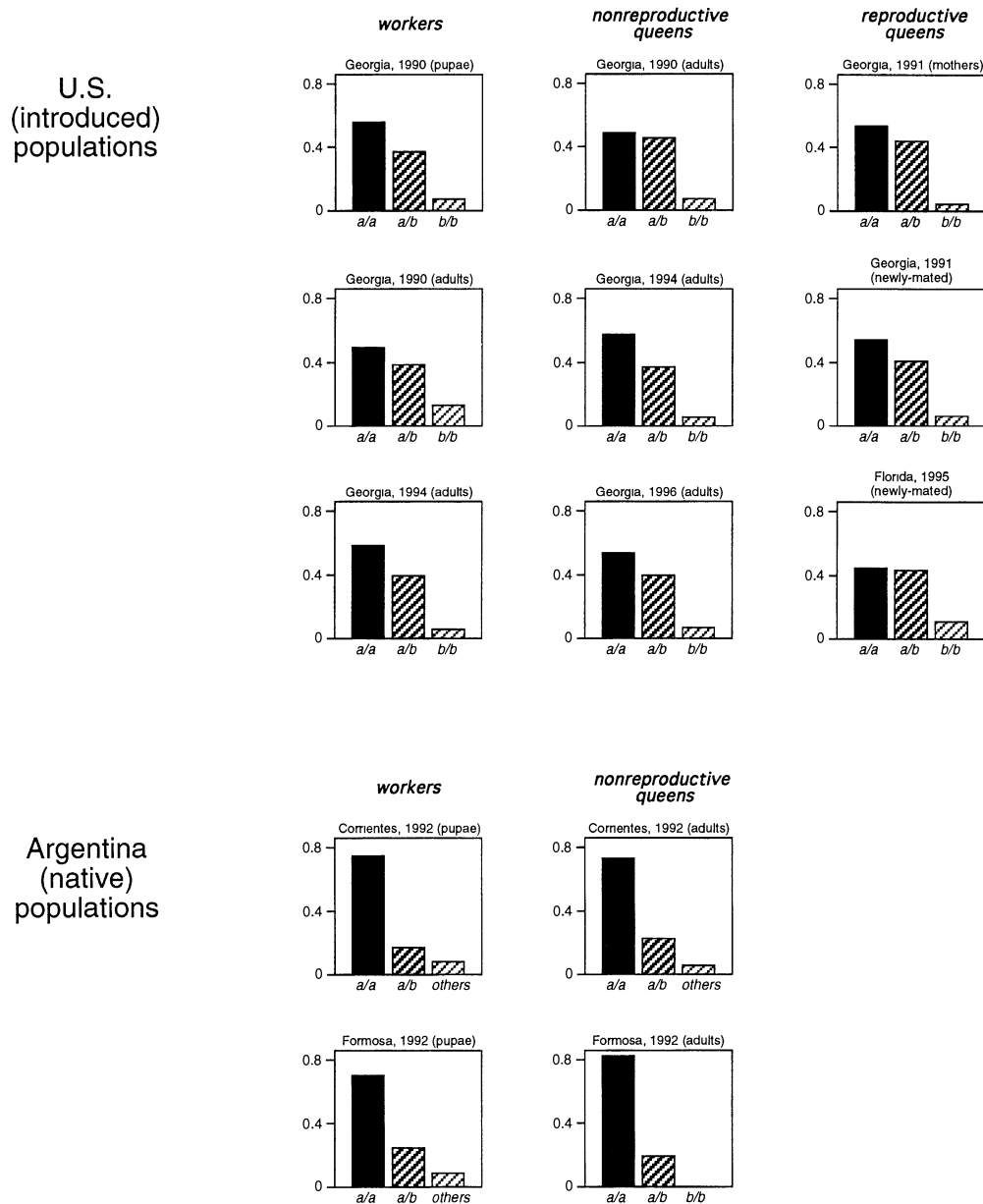
Monogyne *Solenopsis invicta*

FIG. 3. Genotype proportions at *Pgm-3* in samples of monogyne *Solenopsis invicta* from introduced and native populations. The category "others" in the Argentine populations comprises genotype *Pgm-3$^{b/b}$* pooled with all genotypes containing rare alleles. None of the genotype porportions observed in these samples departed significantly from the proportions expected under Hardy-Weinberg equilibrium (HWE). Sample sizes are listed in the Appendix.

←

the proportions expected under Hardy-Weinberg equilibrium (HWE). The value under each asterisk indicates the magnitude of the departure, expressed as the proportion of 1000 resampled genotype distributions that exhibited significant deviations from HWE (see text). For the Georgia 1993 worker sample, the value under the asterisk represents the significance level for the deviation from HWE based on a single χ^2 test (only one genotype per nest was scored for this sample). Sample sizes are listed in the Appendix.

classes of females within a population and always match Hardy-Weinberg expectations. These results indicate that the monogyne form of *S. invicta* does not experience selection on *Pgm-3* of the type and magnitude experienced by the polygyne form, validating the important concept that the form of selection acting on this gene is critically dependent on the type of social environment in which the ants exist (Keller and Ross 1993a).

Although the effects of *Pgm-3* genotype on queen phenotype and on worker responses toward queens of various genotypes have not been investigated outside of Georgia, the genotypic data reported here are entirely consistent with phenotypic effects and worker discrimination tendencies that are uniform among all polygyne populations of *S. invicta*. That is, it is likely that polygyne queens with genotype *Pgm-3*$^{a/a}$ are physiologically distinct from other queens and that workers use cues associated with the phenotypic differences to selectively destroy these queens before they become egg layers (Keller and Ross 1993a, 1995). Again, it is remarkable that the proximate mechanism of selection on *Pgm-3* seems to be the same in the native populations, in which colony queen number is low and new queens are recruited from their parent nest (Ross et al. 1996), as it is in the introduced populations, in which queen number is high and new queens often are recruited from foreign nests (Glancey and Lofgren 1988; Porter 1991; Ross and Keller 1995b). The consistency of this proximate mechanism in the face of conspicuous variation in colony social organization suggests that it involves a strongly conserved property of worker social behavior that is fundamental to the workers' role in recruiting new reproductive queens. Invariant worker discrimination against young *Pgm-3*$^{a/a}$ queens in polygyne nests containing any number of reproductive queens further suggests that monogyny and polygyny in *S. invicta* are not simply artificial designations along a continuum of colony queen number, but rather, that these are discrete social systems.

The results of this study appear to establish another case in which evolutionarily meaningful selection acts on a single gene of major effect, a proposition considered unlikely under a strict neo-Darwinian view of adaptive evolution (Orr and Coyne 1992). It might be argued that the neo-Darwinian view properly is meant to be restricted to major, "complex" morphological adaptations, such as the substantial modifications required for organs or limbs to acquire new functions (cf. Charlesworth 1990). However, single major genes have been shown to have effects on important behavioral (Watt et al. 1986; Carter and Watt 1988; de Belle et al. 1989), physiological (Roush and McKenzie 1987; Pough 1989; Watt 1985, 1991; Rountree and Nijhout 1995), and morphological (Allendorf et al. 1983, Orr and Coyne 1992) traits, with significant consequences for the growth, survival, and reproduction (and thus fitness) of individuals with different genotypes. Restricting consideration of the genetic underpinnings of adaptation to "complex" features would seem to arbitrarily exclude a large portion of the variation involved in anagenetic evolution.

The accumulating information on the phenotypic effects of and mode of selection acting on *Pgm-3* in *S. invicta* allows us to view this system in the broad context of the functional biology of adaptation (Feder and Watt 1991). A growing number of studies have integrated information on the physiology, population genetics, and natural history of particular model organisms in attempts to discern the detailed workings of adaptive evolution at multiple levels of biological organization. The approach involves observing genetic variation in wild populations, determining the biochemical or physiological ("design") consequences, if any, of this variation, linking such differences in design to differences in performance, and finally, tracking the effects of variation in performance on fitness (Clarke 1975; Pough 1989; Clark and Koehn 1991; Feder and Watt 1991). Because fitness differences influence the distribution of genetic variation in subsequent generations, an additional useful element of this approach is to follow the fate of the genetic variants through time.

The distribution of variation at *Pgm-3* among different individuals in the two social forms of *S. invicta* provided initial clues that this gene may have significant design and performance effects. In particular, the absence of genotype *Pgm-3*$^{a/a}$ among egg-laying polygyne queens raised the possibility that such queens might differ from others with respect to measurable phenotypic attributes. A search for phenotypic differences in the laboratory and field revealed that *Pgm-3*$^{a/a}$ queens differ in a number of traits linked to reproduction, including the pace of sexual maturation and ovarian development (Keller and Ross 1993a, 1995). These genetically based physiological differences apparently correlate with quality or quantity of pheromones produced by a queen, which workers seem to use as the actual proximate cues to discriminate among queens with different genotypes (Keller and Ross 1993a). Thus, queen pheromonal "signature" probably constitutes the relevant genetically influenced performance variable in this system.

The link between performance and fitness is unusual in the case of *Pgm-3* in fire ants because queen performance is evaluated by members of the social group (nestmate workers) rather than by biotic or abiotic components of the environment external to the group. Queens with a less-preferred pheromonal signature (all those with genotype *Pgm-3*$^{a/a}$) have zero direct fitness because they are destroyed by their nestmates before they can reproduce. This occurs despite such queens exhibiting relatively high energy reserves and rapid ovarian development, traits that presumably would be advantageous in attempting to found new colonies independently, outside of the existing social group. The case of *Pgm-3* in polygyne fire ants thus provides an unusually clear example of the distinction between social selection and natural selection (e.g., West-Eberhard 1983) and demonstrates the potency of the former as an evolutionary force in social organisms.

The final element of the functional approach is concerned with the fate of the genetic variants underlying fitness differences, in the present case, the *Pgm-3* alleles in the polygyne social form. Destruction of *Pgm-3*$^{a/a}$ queens as they mature in polygyne nests constitutes a process of strong directional selection against allele *Pgm-3*^{a}, which is observed as the reduced frequency of this allele in older, reproductive queens compared with young, nonreproductive queens. However, this within-generation change appears not to translate into a sustained decrease in the frequency of *Pgm-3*^{a} across

generations (Ross and Keller 1995a; also see the Appendix). Studies of the two forms in Georgia suggest that this may be because selection against *Pgm-3ᵃ* in the polygyne form is opposed by gene flow from the monogyne form, in which the allele is common (Ross 1992; Ross and Shoemaker 1993; Ross and Keller 1995a). The presence of *Pgm-3ᵃ* at high frequencies in native polygyne populations despite the presence of the same selection pressures against it implicates gene flow from the alternate form as an important evolutionary force in these populations as well.

The most poorly understood aspect of the functional biology of *Pgm-3* in *S. invicta* is the manner in which variation at this gene affects biochemical parameters to cause the observed physiological differences among queens. Indeed, the link between natural genetic variation and "design" differences that affect fitness has been clarified in relatively few model organisms because it requires in vitro determination of the biochemical properties of the variant gene products (see Watt 1985, 1991; Feder and Watt 1991). A concern in the absence of such biochemical studies is whether the surveyed genetic variants actually are responsible for the observed phenotypic differences with which they are associated (and thus are the true targets of selection) or whether they merely are linked to and in strong linkage disequilibrium with unknown genes causing the effects. Several lines of evidence suggest that *Pgm-3* may be directly responsible for the phenotypic effects observed in polygyne *S. invicta* queens.

Not a single reproductive polygyne queen among the 4600 studied from a variety of native and introduced populations has been found to possess the genotype *Pgm-3ᵃ/ᵃ*. That is, there is no evidence of any breakdown in the strict association of *Pgm-3* genotype and reproductive role. This result is particularly significant with respect to the native ants, because the two native populations that we studied appear to have been more or less genetically isolated for a significant period. The implied number of meiotic events since separation of the Corrientes, Formosa, Georgia, and Texas populations suggests an enormous number of opportunities for recombination between *Pgm-3* and a putative second gene causing the phenotypic effects (e.g., Carter and Watt 1988), which leads to the expectation that some reproductive queens with genotype *Pgm-3ᵃ/ᵃ* would be found in a large sample if such a second gene existed. Of course, mechanisms that effectively eliminate the products of crossing-over, such as the presence of an inversion containing both *Pgm-3* and the putative second gene, could block recombination even if the two genes were not particularly closely linked. Inversions (pericentric) do appear to be widespread in ants such as *S. invicta* that possess intermediate chromosome numbers (Imai et al. 1988), although none has been reported in fire ants.

Allozymes most likely to influence physiological phenotypes and performance are those that function in central metabolic pathways involving a large fraction of the organism's energy budget (Watt 1985). Phosphoglucomutase (PGM) catalyzes the interconversion of glucose 6-phosphate and glucose 1-phosphate, a reaction that stands at the intersection of three central metabolic pathways (glycolysis, glycogen metabolism, and the pentose phosphate shunt). Thus, variation in PGM activity or stability potentially may alter the flux capacity of any of these pathways and directly influence the phenotypes that they support (Pough 1989; Clark and Koehn 1991; Pogson 1991). Given the crucial role of this enzyme in metabolism, it is not surprising that variation at genes encoding PGM (or their regulatory elements) is associated with variation in growth rates (Snyder and Ayala 1979; Allendorf et al. 1983; Pogson 1991; Whitehurst and Pierce 1991), fecundity (Riddle et al. 1983), and survival (Leigh Brown 1977) in a diversity of animals, and with variation in flight activity (Carter et al. 1989; Schutz et al. 1990; Goulson 1993) and male mating success (Carter and Watt 1988) in insects. Of probable great significance for queens of *S. invicta* is the role of PGM in moving carbohydrate reserves to and from glycogen synthesis and breakdown. Accumulation of large glycogen reserves is an important part of the process of sexual maturation of young adult queens of fire ants (Toom et al. 1976a,b) and other ants (Passera et al. 1990), and free glucose liberated from the breakdown of glycogen appears to be used to support ovarian maturation in newly mated *S. invicta* queens (Toom et al. 1976a). Differences in weight gained during sexual maturation and in rate of initial ovarian development are the major phenotypic correlates of genotypic variation at *Pgm-3* that we have detected in fire ant queens (Keller and Ross 1993a, 1995). Thus, the role of PGM in metabolism and the nature of physiological differences among queens with different *Pgm-3* genotypes are consistent with variation at this gene actually causing the physiological effects and with *Pgm-3* being the target of selection in polygyne *S. invicta*.

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APPENDIX

Frequencies of alleles at *Pgm-3* in samples of *S. invicta* from polygyne and monogyne nests in introduced and native populations.*

Sample	\multicolumn{9}{c}{*Pgm-3* alleles}	Sample size
	a	*b*	*c*	*d*	*e*	*f*	*g*	*j*	*k*	
Polygyne form (*introduced populations*)										
Workers										
Georgia, 1990 (pupae)	0.53 (0.43–0.64)	0.47 (0.36–0.57)	—	—	—	—	—	—	—	$N = 28$, $n = 922$
Georgia, 1990 (adults)	0.56 (0.45–0.66)	0.44 (0.34–0.55)	—	—	—	—	—	—	—	$N = 28$, $n = 724$
Georgia, 1993 (pupae)	0.55 (0.52–0.58)	0.45 (0.42–0.48)	—	—	—	—	—	—	—	$N = 504$, $n = 504$
Georgia, 1994 (adults)	0.52 (0.46–0.58)	0.48 (0.42–0.54)	—	—	—	—	—	—	—	$N = 60$, $n = 257$
Nonreproductive queens										
Georgia, 1990 (adults)	0.54 (0.45–0.64)	0.46 (0.36–0.55)	—	—	—	—	—	—	—	$N = 28$, $n = 886$
Georgia, 1991 (adults)	0.44 (0.35–0.54)	0.56 (0.46–0.65)	—	—	—	—	—	—	—	$N = 27$, $n = 446$
Georgia, 1994 (pupae)	0.37 (0.25–0.47)	0.63 (0.53–0.75)	—	—	—	—	—	—	—	$N = 18$, $n = 427$
Georgia, 1995 (adults)	0.48 (0.44–0.53)	0.52 (0.47–0.56)	—	—	—	—	—	—	—	$N = 71$, $n = 395$
Reproductive queens										
Georgia, 1990	0.38 (0.30–0.45)	0.62 (0.55–0.68)	—	—	—	—	—	—	—	$N = 28$, $n = 873$
Georgia, 1991	0.37 (0.32–0.42)	0.63 (0.57–0.68)	—	—	—	—	—	—	—	$N = 52$, $n = 467$
Georgia, 1993	0.39 (0.37–0.41)	0.61 (0.59–0.63)	—	—	—	—	—	—	—	$N = 450$, $n = 1160$
Georgia, 1994	0.39 (0.33–0.45)	0.61 (0.55–0.67)	—	—	—	—	—	—	—	$N = 94$, $n = 1319$
Texas, 1995	0.37 (0.28–0.43)	0.63 (0.57–0.72)	—	—	—	—	—	—	—	$N = 30$, $n = 449$
Polygyne form (*native populations*)										
Workers										
Corrientes, 1992 (pupae)	0.69 (0.61–0.77)	0.25 (0.18–0.32)	—	0.01 (0–0.02)	0.01 (0–0.03)	—	0.01 (0–0.03)	—	0.03 (0–0.06)	$N = 44$, $n = 527$
Formosa, 1992 (pupae)	0.65 (0.57–0.74)	0.34 (0.26–0.43)	0.01 (0–0.01)	—	—	—	—	—	—	$N = 35$, $n = 406$
Nonreproductive queens										
Corrientes, 1992 (adults)	0.71 (0.62–0.79)	0.26 (0.19–0.33)	—	—	0.01 (0–0.02)	—	—	—	0.02 (0–0.02)	$N = 21$, $n = 180$
Formosa, 1992 (adults)	0.60 (0.52–0.67)	0.40 (0.33–0.48)	—	—	—	—	—	—	—	$N = 23$, $n = 172$
Reproductive queens										
Corrientes, 1992	0.45 (0.41–0.46)	0.45 (0.43–0.48)	—	—	0.01 (0–0.04)	—	—	—	0.09 (0.09–0.11)	$N = 28$, $n = 187$
Formosa, 1992	0.44 (0.40–0.46)	0.56 (0.54–0.60)	—	—	—	—	—	—	—	$N = 26$, $n = 123$

APPENDIX. Continued.

Sample	Pgm-3 alleles									Sample size
	a	b	c	d	e	f	g	j	k	
Monogyne form (introduced populations)										
Workers										
Georgia, 1990 (pupae)	0.76 (0.72–0.81)	0.24 (0.19–0.28)	—	—	—	—	—	—	—	N = 52, n = 1316
Georgia, 1990 (adults)	0.69 (0.64–0.74)	0.31 (0.26–0.36)	—	—	—	—	—	—	—	N = 39, n = 160
Georgia, 1994 (adults)	0.78 (0.72–0.84)	0.22 (0.16–0.28)	—	—	—	—	—	—	—	N = 81, n = 81
Nonreproductive queens										
Georgia, 1990 (adults)	0.71 (0.68–0.74)	0.29 (0.26–0.32)	—	—	—	—	—	—	—	N = 65, n = 601
Georgia, 1994 (adults)	0.75 (0.73–0.78)	0.25 (0.22–0.27)	—	—	—	—	—	—	—	N = 147, n = 957
Georgia, 1996 (adults)	0.73 (0.69–0.76)	0.27 (0.24–0.31)	—	—	—	—	—	—	—	N = 343, n = 343
Reproductive queens										
Georgia, 1991 (mothers)	0.76 (0.69–0.83)	0.24 (0.17–0.31)	—	—	—	—	—	—	—	N = 59, n = 59
Georgia, 1991 (newly mated)	0.75 (0.71–0.78)	0.25 (0.22–0.29)	—	—	—	—	—	—	—	n = 271
Florida, 1995 (newly mated)	0.66 (0.59–0.72)	0.34 (0.28–0.41)	—	—	—	—	—	—	—	n = 118
Monogyne form (native populations)										
Workers										
Corrientes, 1992 (pupae)	0.86 (0.81–0.91)	0.12 (0.07–0.16)	0.01 (0–0.03)	0.01 (0–0.01)	—	—	—	—	—	N = 37, n = 443
Formosa, 1992 (pupae)	0.85 (0.81–0.90)	0.12 (0.09–0.16)	—	—	—	0.02 (0.02–0.02)	—	0.01 (0.01–0.01)	—	N = 35, n = 417
Nonreproductive queens										
Corrientes, 1992 (adults)	0.86 (0.81–0.90)	0.13 (0.08–0.17)	—	0.01 (0–0.02)	—	—	—	—	—	N = 24, n = 135
Formosa, 1992 (adults)	0.90 (0.87–0.93)	0.10 (0.07–0.13)	—	—	—	—	—	—	—	N = 28, n = 152

* The 95% confidence intervals about the allele frequency estimates are shown in parentheses. Relative anodal mobilities of the allelic electromorphs are as follows: $a = 1.000$; $b = 0.890$; $c = 0.875$; $d = 0.780$; $e = 0.715$; $f = 0.840$; $g = 0.980$; $j = 0.950$; $k = 0.745$. Sample sizes are for numbers of nests (N) and numbers of individuals (n) studied (newly mated queens from monogyne introduced populations were not collected from nests and so no values are given for N). The genotype proportions for these samples are illustrated in Figures 2 and 3.