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JOINT INFLUENCE OF GENE FLOW AND SELECTION ON A REPRODUCTIVELY IMPORTANT GENETIC POLYMORPHISM IN THE FIRE ANT *SOLENOPSIS INVICTA*

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Abstract.—We present evidence that gene flow counteracts directional selection to maintain a high level of polymorphism at *Pgm-3,* a gene known to have a major effect on reproduction in the population of *Solenopsis invicta* that we studied. Reproductive queens in this population never possess the homozygous genotype *Pgm-3*$^{a/a}$, whereas prereproductive and nonreproductive females possess it at substantial frequencies. The loss of *Pgm-3*$^{a/a}$ queens, which occurs because workers selectively destroy all such queens as they initiate reproductive development, constitutes a process of strong negative selection on the allele *Pgm-3*^{a} that now has been observed over a 4-yr period. This allele is maintained at high frequency in the study population in spite of such directional selection by means of gene flow from a population of a different social form, in which equivalent selection is not found and the allele *Pgm-3*^{a} is common. Evidence for such gene flow comes from two sources in this study. First, *Pgm-3* genotype and allele frequencies for parental and offspring generations suggest that a large majority of matings at our six study sites are between resident females and immigrant males of the alternate social form. The inferred proportions of matings attributable to immigrant males at each site vary predictably according to the distance from an upwind source of such males. Second, the proportions of queens that are mated within the study population depend to a large extent on the proximity of the queens to an upwind source of immigrant males. This system offers a unique view of how gene flow occurring in the face of strong, locally restricted selection can affect the extent and distribution of genetic variation.

Determination of the relative importance of the forces that influence levels of population genetic diversity is a principal goal of evolutionary biology. Natural selection and gene flow frequently are cited as playing prominent roles in generating, preserving, or diminishing genetic diversity, but the difficulties of studying these forces directly or indirectly in wild populations often hinder their detailed evaluation (Slatkin 1985, 1987; Endler 1986). Natural selection may be an important force either in maintaining existing diversity in a population, such as through heterosis, or in diminishing it, such as through directional selection (see, e.g., Lewontin 1974; Lewontin et al. 1978; Endler 1986; Hedrick 1986; Nei 1987; Spiess 1989). In contrast, gene flow into populations from genetically differentiated pop-

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ulations is expected to be important in generating at least transient diversity, through the introduction of novel variants, or in maintaining existing diversity at levels higher than would occur in its absence (Wright 1969, 1978; Karlin 1982; Slatkin 1987). Thus gene flow may act in opposition to directional selection, with the gene frequencies and levels of genetic polymorphism that exist in a population reflecting a balance between these two evolutionary forces (Haldane 1930; Jain and Bradshaw 1966; Wright 1969; Nagylaki 1975; Felsenstein 1976; Karlin 1976; Slatkin 1985; Barton and Clark 1990).

One recently proposed example of gene flow acting in opposition to directional selection to determine levels of genetic diversity involves the locus *Pgm-3* in the fire ant *Solenopsis invicta*. Two distinctive population types or social forms exist in this species: the "monogyne" form, in which colonies are headed by a single reproductive queen, and the "polygyne" form, in which colonies are headed by multiple reproductive queens. The locus *Pgm-3* (or a tightly linked gene) is under strong directional selection in polygyne populations but not in monogyne populations of this ant (Ross 1992). Young polygyne queens with the homozygous genotype *Pgm-3*$^{a/a}$ are prevented from becoming active egg layers by workers, which invariably destroy such queens before they can complete their reproductive development (Keller and Ross 1993*a*). These homozygous queens undergo accelerated sexual maturation and oogenesis compared with queens with other genotypes, which suggests that the product of *Pgm-3* is involved in the process of reproductive development and that workers use cues associated with differential development to discriminate among queens of different genotypes (Keller and Ross 1993*a*). Because no queens homozygous for the allele *Pgm-3*^{a} survive to become egg layers in polygyne nests, there is a consistent drop in the frequency of this allele from initially high levels in prereproductive females to lower levels in reproductive females. The elimination of all *Pgm-3*$^{a/a}$ queens before they reproduce thus constitutes a bout of strong directional selection that occurs in every sexual generation in the polygyne social form of this ant.

The anticipated result of such strong directional selection, if it acts in the absence of other evolutionary forces, is a rapid decrease toward a low equilibrium frequency of *Pgm-3*^{a} in the polygyne form, with a concomitant reduction in diversity at this locus. However, such a reduction in *Pgm-3*^{a} frequency and in diversity does not seem to occur, and available evidence implicates gene flow from the monogyne form as the force responsible for preventing it (Ross 1992; Ross and Shoemaker 1993). The allele *Pgm-3*^{a} is present at high frequencies in monogyne populations, apparently because there is no selection against the allele in this social form (Ross 1992; Keller and Ross 1993*a*). Thus, monogyne populations can serve as effective reservoirs for the continuing exportation of this allele into polygyne populations. This gene flow from monogyne to polygyne populations, as marked by the recurring introduction of *Pgm-3*^{a} into the polygyne form, seems to be mediated largely or solely by monogyne males, which are thought to disperse into polygyne populations and outcompete resident polygyne males for matings with polygyne queens (Ross and Shoemaker 1993; see also Keller and Ross 1993*b*). The best evidence for this mode of gene flow comes from progeny studies in which *Pgm-3* mating types were reconstructed in a polygyne population in northeastern Georgia (Ross and Shoemaker 1993); over 90% of the inseminated polygyne queens sampled were inferred to have mated with immigrant monogyne males.

The proposed occurrence in these ants of directional selection and gene flow acting in opposition to one another to influence levels of polymorphism at *Pgm-3* generates several hypotheses, the testing of which may reveal useful insights into the nature of these and other evolutionary forces. The major hypothesis yet to receive critical testing is that gene flow from monogyne to polygyne populations actually is sufficiently strong to prevent the gradual erosion and eventual loss of diversity at *Pgm-3* due to negative selection on the allele *Pgm-3*^{a} in the polygyne form. Additional hypotheses concern the genetic and mating structures expected in polygyne populations when such gene flow and selection act in combination. One prediction of the scenario outlined above is that an allele frequency cline may be produced in which *Pgm-3*^{a} decreases in frequency from the periphery toward the center of a polygyne population. Such a cline is expected if the distance over which selection on *Pgm-3* occurs is large compared with the effective gene flow distance associated with dispersal of monogyne males (see, e.g., Slatkin 1973; May et al. 1975). Another prediction is that the proportionate success of males of the two forms in mating with polygyne queens will depend on the local abundance of each type of male, which should vary predictably within a polygyne population according to such factors as the spatial distributions of the forms and the prevailing wind directions during mating flights. Finally, mating opportunities for polygyne queens are predicted to be limited to at least some extent by the availability of immigrant monogyne males. This last prediction stems from the observation that fertile males are produced relatively infrequently in polygyne nests, which leads to a strong female bias in the operational sex ratios for resident sexuals in these populations (Ross and Fletcher 1985*a;* Vargo and Fletcher 1987; Ross and Shoemaker 1993; E. L. Vargo, unpublished results). The presence of many permanently unmated reproductive queens in polygyne nests (Fletcher et al. 1980; Vargo and Fletcher 1987, 1989; Ross 1992) is the apparent result of this imbalanced sex ratio in the polygyne form.

The results presented here confirm most of the above predictions and document in some detail how gene flow and selection jointly influence a simple genetic polymorphism in a wild population of *S. invicta.* The data are of further interest in that they provide a detailed view of natural dispersal distances and male-mediated gene flow patterns in these ants. Finally, the analyses conducted raise the possibility that the selection/gene flow dynamics in the study population are more complex than previously suspected by virtue of more than one form of selection acting on *Pgm-3.*

METHODS

Wind Direction during Mating Flights

Raw meteorological data were obtained from the National Climatic Data Center (Asheville, N.C.) for Athens, Georgia, site of the National Weather Service

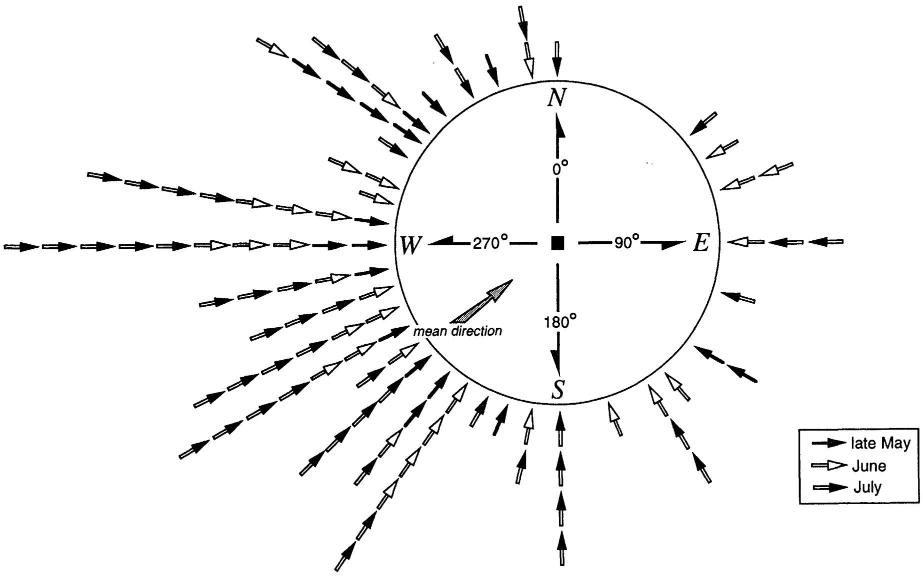

FIG. 1.—Prevailing wind directions during the anticipated times of major mating flights of *Solenopsis invicta* in the vicinity of Athens, Ga. Each small arrow represents the prevailing wind direction at 1300 or 1600 hours on days when mating flights are likely to have occurred. These data are compiled for the 4-yr period 1989–1992. The overall mean wind direction estimated from these data is shown by the large arrow.

monitoring station closest to our study area in northeastern Georgia. The major mating flights of *Solenopsis invicta* occur in our study area during late May, June, and July on calm, sunny, warm afternoons of the first few days following significant rainfall (Markin et al. 1971; Morrill 1974; Ross and Fletcher 1985*a*). The meteorological data were used to infer the days on which mating flights are likely to have occurred during this peak flight season over the 4-yr period 1989–1992, and the prevailing wind directions at 1300 and 1600 hours (Eastern Standard Time) on these days were recorded. Data on wind direction were obtained for 52 d (9 in late May, 18 in June, 25 in July), which yielded a total of 104 observations.

Winds blew predominantly from the southwest during times when the major mating flights in this area are expected to have occurred (fig. 1), with almost 60% of the wind readings indicating winds blowing from the southwest quadrant. The mean wind direction was 256° from true north in May, 222° in June, and 236° in July, with the overall mean wind direction for the peak flight season 234° from true north.

Collection of Samples

The polygyne population of *Solenopsis invicta* that is the focus of our study is centered around Monroe, Georgia (Walton County). This population, which has been the subject of considerable study since its discovery over a decade ago (see,

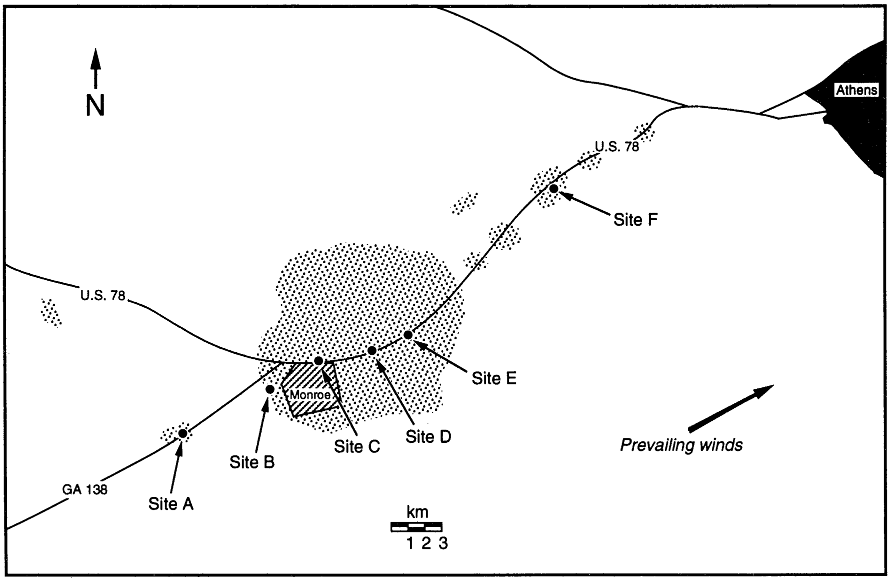

FIG. 2.—Distribution of monogyny and polygyny in *Solenopsis invicta* in the study area in northeastern Georgia. Areas in which only polygyne nests were identified are indicated by stippling; other areas are known or presumed to contain only monogyne nests. The six sampling sites for this study are distributed along a transect that lies approximately parallel to the mean direction of the prevailing winds during major mating flights.

e.g., Fletcher 1983; Vargo and Fletcher 1987, 1989; Ross 1992, 1993), is a relatively discrete population of modest size consisting almost exclusively of polygyne nests. It is surrounded by populations composed almost exclusively of nests of the monogyne form. We generated a map of the distribution of polygyne and monogyne nests in and around this main polygyne population in spring 1993 in order to identify appropriate sampling sites for our study. Over 100 locations were visited in Walton, Oconee, and Clarke Counties, and the social organization of 6–10 nests at each location was inferred using well-established criteria to distinguish nests of the two social forms (Greenberg et al. 1985; Vargo and Fletcher 1987). Particular attention was paid to characterizing nests at the boundaries of the main polygyne population. The resulting distributional map of the two forms is presented as figure 2.

Six sampling sites for polygyne *S. invicta* were identified on a transect that lies approximately parallel to the mean prevailing wind direction during the major mating flights (fig. 2). Three of the sites (C, D, E) lie well within the main polygyne population, at varying distances from the windward (southwestern) boundary with the monogyne form. The two sites at either end of the transect (A, F) are in restricted pockets of polygyny that occur outside of the main polygyne population. On the windward (southwestern) end of the transect, site A is situated in what seems to be an isolated enclave of polygyny several kilometers distant from the main population of this form. On the leeward (northeastern) end, site F occurs in one of many pockets of polygyny that characterize the gradual transition from pure polygyny around Monroe to pure monogyny around Athens. Finally, site B is located on the comparatively abrupt windward boundary between the main polygyne population and the surrounding monogyne population.

A single worker pupa was collected from each of 81–87 polygyne nests at each sampling site during spring 1993 (total of 504 nests sampled). The greatest distance between any two nests sampled within a site (200–300 m) was about 10% of the minimum distance between any two nests from different sites. From one to four adult reproductive (wingless) queens also were collected from each of these nests when they could be found. Nests from which queens were collected represented 89% (site A), 85% (site B), 92% (site C), 93% (site D), 77% (site E), and 99% (site F) of the nests from which worker pupae were collected. The collection of reproductive queens from almost all of the sampled nests was important, especially at the outlying sites, in that it allowed us to confirm polygyny in these nests. A total of 504 worker pupae and 1,171 reproductive queens was collected for this study.

A substantial number of the reproductive queens present in polygyne colonies of *S. invicta* remain permanently unmated (Fletcher et al. 1980; Vargo and Fletcher 1987, 1989; Ross 1992). Therefore, all reproductive queens were dissected prior to electrophoresis to determine their mating status. The mean proportions of queens that were unmated and the 95% confidence intervals (CIs) about these means were estimated using a resampling procedure, as described below for estimation of *Pgm-3* genotype proportions in this caste.

Genetic Data Analyses

The locus *Pgm-3* is biallelic in *Solenopsis invicta* in northeastern Georgia, so three genotypes segregate among females from populations in this area (Ross 1992, 1993). Genotypes at *Pgm-3* were determined for all individuals collected for our study by means of gel electrophoresis (Ross 1992; Shoemaker et al. 1992). Distributions of *Pgm-3* genotypes in worker pupae were tested for correspondence to Hardy-Weinberg equilibrium (HWE) for each site and for the total sample using χ^2 tests with Emigh's (1980) continuity correction and Levene's (1949) small-sample correction to the expected frequencies (see Lessios 1992). The 95% CIs about the *Pgm-3* allele frequency estimates for workers were obtained by drawing 1,000 bootstrap samples from the original data sets and dropping the 25 highest and 25 lowest allele frequency estimates (see, e.g., Efron and Tibshirani 1986; Weir 1990; Crowley 1992).

Estimates of genotype and allele frequencies at *Pgm-3,* as well as tests for correspondence to HWE, are more complicated for queens than worker pupae because often more than one queen was collected per nest, and nest mate genotypes may not always be independent. A resampling procedure was used whereby a single genotype was drawn at random from each nest at a collection site, which yielded a distribution of independent genotypes for that site. Genotype and allele frequencies then were estimated as the arithmetic mean frequencies for 200 such resampled genotype distributions. The 95% CIs about the allele frequency estimates were obtained by dropping the five highest and five lowest allele frequency

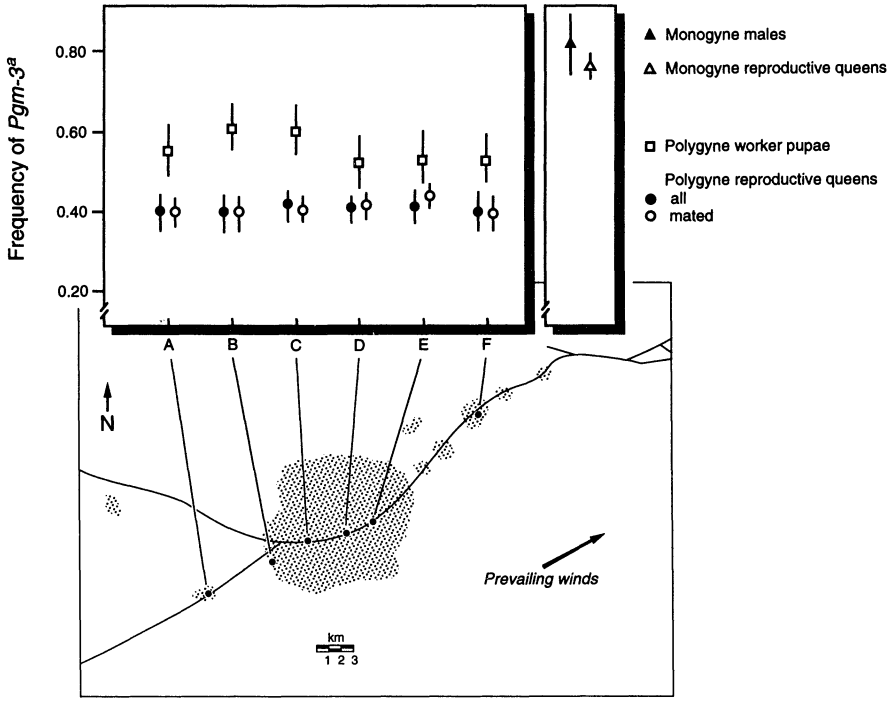

FIG. 3.—Frequencies of the allele *Pgm-3*^{a} in worker pupae and reproductive queens of polygyne *Solenopsis invicta* sampled from six sites. Shown for comparison in the smaller panel are frequencies of this allele in males and reproductive queens from an adjacent monogyne population (data from Ross and Shoemaker 1993). Lines around the point estimates indicate the 95% confidence intervals.

values derived from the 200 resampled distributions. The probabilities of significant departure of the observed genotypes from HWE were calculated as the proportions of the 200 resampled genotype distributions that deviated significantly from genotype distributions expected under HWE, using the χ^2 tests described above and $\alpha = 0.05$. Genotype and allele frequencies, as well as the extent of departures from HWE, were estimated separately for all reproductive queens collected and for only those reproductive queens that were mated.

Frequencies of the allele *Pgm-3*^{a} differ substantially between monogyne and polygyne sexuals of *S. invicta* because of the different selection pressures acting on *Pgm-3*$^{a/a}$ queens in the two forms (Ross 1992; Ross and Shoemaker 1993; see fig. 3). These frequency differences allow us to predict the proportionate success of males of the two forms in mating with polygyne queens. This is possible because allele frequency differences between the sexes in the parental generation, such as those that occur at *Pgm-3* between polygyne queens and monogyne males, lead to offspring genotype proportions that deviate from those predicted under the Hardy-Weinberg law (which assumes that allele frequencies are identical between the sexes). Specifically, there should be an excess of heterozygotes and deficiencies of homozygotes relative to Hardy-Weinberg expectations (Robertson 1965; Purser 1966), with the magnitudes of these deviations dependent on the differences in allele frequencies between the male and female parents, as shown by the following equations:

$$aa_{\mathrm{obs}} = \bar{p}^2 - {}^1\!/\!_4(p_{\mathrm{f}} - p_{\mathrm{m}})^2 , \tag{1}$$

$$h_{\mathrm{obs}} = 2\bar{p}\bar{q} + {}^1\!/\!_2(p_{\mathrm{f}} - p_{\mathrm{m}})^2 , \tag{2}$$

and

$$bb_{\mathrm{obs}} = \bar{q}^2 - {}^1\!/\!_4(q_{\mathrm{f}} - q_{\mathrm{m}})^2 , \tag{3}$$

where, in our case, aa_{obs}, h_{obs}, and bb_{obs} are the observed proportions of the genotypes $Pgm\text{-}3^{a/a}$, $Pgm\text{-}3^{a/b}$, and $Pgm\text{-}3^{b/b}$, respectively, in the female offspring of polygyne queens; p_{f} and p_{m} are the frequencies of $Pgm\text{-}3^{a}$ in polygyne queens and their male mates, respectively (q_{f} and q_{m} are the frequencies of $Pgm\text{-}3^{b}$); and $\bar{p}$ and $\bar{q}$ represent the overall frequencies of the two alleles in both sexes of the parental generation (Hedrick 1985, pp. 48–49). The overall frequencies are $\bar{p} = {}^1\!/\!_2(p_{\mathrm{f}} + p_{\mathrm{m}})$ and $\bar{q} = {}^1\!/\!_2(q_{\mathrm{f}} + q_{\mathrm{m}})$ because each sex contributes half of the gametes to offspring generation females. For our situation, the allele frequencies of the male mates of polygyne queens must be decomposed to reflect the proportionate mating contributions of males of the two social forms, so that $p_{\mathrm{m}} = (y_{\mathrm{mm}} \cdot p_{\mathrm{mm}} + y_{\mathrm{pm}} \cdot p_{\mathrm{pm}})$ and $q_{\mathrm{m}} = (y_{\mathrm{mm}} \cdot q_{\mathrm{mm}} + y_{\mathrm{pm}} \cdot q_{\mathrm{pm}})$, where y_{mm} is the proportion of polygyne offspring fathered by monogyne males, y_{pm} is the proportion of polygyne offspring fathered by polygyne males, p_{mm} and p_{pm} are the frequencies of $Pgm\text{-}3^{a}$ in monogyne and in polygyne males, respectively, and q_{mm} and q_{pm} are the frequencies of $Pgm\text{-}3^{b}$ in these males. As monogyne males account for an increasing proportion of matings of polygyne queens, p_{m} and q_{m} diverge increasingly from p_{f} and q_{f} (i.e., the allele frequency differences between the sexes increase), which leads to ever-greater excesses of heterozygotes and deficiencies of homozygotes in the offspring of polygyne queens. Substituting the equations above into equations (1)–(3), solving for y_{mm}, and simplifying, we obtain

$$y_{\mathrm{mm}} = (aa_{\mathrm{obs}}/p_{\mathrm{f}} - p_{\mathrm{pm}})/(p_{\mathrm{mm}} - p_{\mathrm{pm}}) , \tag{4}$$

$$y_{\mathrm{mm}} = [(h_{\mathrm{obs}} - p_{\mathrm{f}})/(1 - 2p_{\mathrm{f}}) - p_{\mathrm{pm}}]/(p_{\mathrm{mm}} - p_{\mathrm{pm}}) , \tag{5}$$

and

$$y_{\mathrm{mm}} = [bb_{\mathrm{obs}}/(1 - p_{\mathrm{f}}) + p_{\mathrm{pm}} - 1]/(p_{\mathrm{pm}} - p_{\mathrm{mm}}) . \tag{6}$$

We focus on estimating y_{mm} to quantify interform gene flow because such gene flow is likely to occur predominantly or solely by means of monogyne males mating with polygyne queens (Ross and Shoemaker 1993). The variable y_{mm} not only represents the proportion of polygyne offspring fathered by monogyne males but is also a measure of the extent of mating between polygyne queens and monogyne males, because *S. invicta* queens mate with only a single male (Ross and Fletcher 1985*b*).

Estimates of y_{mm} in this study are derived from equations (4)–(6) using values of aa_{obs}, h_{obs}, bb_{obs}, and p_{f} obtained directly from the empirical data reported here. Values of p_{pm} are assumed to equal p_{f}. That is, the frequency of $Pgm\text{-}3^{a}$ in polygyne males is assumed to be identical to that in reproductive queens from the same site, which is reasonable because males are impaternate and thus should exhibit the same allele frequencies as their mothers. The value of p_{mm} used in the estimates ($p_{mm} = 0.815$, 95% CI = 0.741–0.888) is obtained from previously published genotypic data for males collected from monogyne nests located in several counties immediately south of the polygyne study population (see Ross 1992; Ross and Shoemaker 1993). Additional estimates of y_{mm} are calculated for this same polygyne population using genotypic data obtained in 1990 and 1991 for workers, prereproductive (winged) queens, and reproductive (wingless) queens (Ross 1992), and using $p_{mm} = 0.815$.

Estimation of the extent of interform mating using the above method assumes that queens mate randomly with respect to their mate's *Pgm-3* genotype. This assumption is reasonable in light of the behavior of queens during mating flights. Queens ascend to altitudes of up to several hundred meters, mate on the wing, and descend to the ground within a period of about 30 min (Markin et al. 1971). Such rapid mating by queens in the presence of what must be low densities of males suggests that males respond to pheromonal signals from queens and that there is scramble competition among males for access to receptive queens. Female choice is not likely to be an important component of the mating system.

RESULTS

The proportions of different *Pgm-3* genotypes observed in worker pupae and reproductive queens of polygyne *Solenopsis invicta* collected from the six sampling sites are presented in table 1. Proportions of heterozygotes observed in worker pupae consistently exceeded the proportions expected under HWE, although this excess heterozygosity is not judged to be statistically significant at any individual site on the basis of the sequential Bonferroni procedure for evaluating the multiple χ^2 tests (see Hochberg 1988). A large and highly significant excess of heterozygotes exists when the entire collection of worker pupae is considered together. Such consistent excess heterozygosity at *Pgm-3*, which has been observed earlier in this polygyne population (Ross 1992), is expected if interform gene flow is substantial and occurs via polygyne queens mating chiefly with monogyne males, because allele frequencies differ strongly between these two classes of sexuals at *Pgm-3* (see above text, as well as Ross and Shoemaker 1993).

Among reproductive queens the homozygous genotype *Pgm-3*$^{a/a}$ is entirely absent (table 1). The invariable absence of queens of this genotypic class, combined with consistently high proportions of heterozygous queens, leads to strong departures of the observed genotype proportions from those expected under HWE at all sites. These genotypic patterns, which exist regardless of whether all reproductive queens or just mated ones are considered, also have been observed earlier in this population (Ross 1992).

The relative frequencies of the genotypes *Pgm-3*$^{a/b}$ and *Pgm-3*$^{b/b}$, shown in table 1 as simple ratios of the two genotypes, are statistically indistinguishable between worker pupae and reproductive queens collected from the same sites ($\chi^2_1 = 0.005$–2.83, all $P > .05$). That is, heterozygotes tend to outnumber

TABLE 1

Observed Genotype Proportions at the Locus *Pgm-3* in Worker Pupae and Reproductive Queens of Polygyne *Solenopsis invicta* Collected from Six Sites

	Pgm-3 Genotypes			Probability of Deviation from HWE	Ratio of Genotypes *a*/*b*:*b*/*b*
	a/*a*	*a*/*b*	*b*/*b*		
Site A:					
Worker pupae (N = 85, n = 85)	.247 (.299)	.600 (.496)	.153 (.205)	.93	3.9:1
Reproductive queens (all) (N = 76, n = 188)	0 (.156)	.791 (.478)	.209 (.366)	1	3.8:1
Reproductive queens (mated) (N = 71, n = 154)	0 (.157)	.792 (.478)	.208 (.365)	1	3.8:1
Site B:					
Worker pupae (N = 81, n = 81)	.296 (.366)	.617 (.478)	.087 (.156)	.99	7.1:1
Reproductive queens (all) (N = 69, n = 179)	0 (.151)	.776 (.475)	.224 (.374)	1	3.5:1
Reproductive queens (mated) (N = 68, n = 170)	0 (.151)	.776 (.475)	.224 (.374)	1	3.5:1
Site C:					
Worker pupae (N = 87, n = 87)	.310 (.357)	.575 (.481)	.115 (.162)	.91	5.0:1
Reproductive queens (all) (N = 80, n = 217)	0 (.172)	.828 (.485)	.172 (.343)	1	4.8:1
Reproductive queens (mated) (N = 74, n = 149)	0 (.162)	.806 (.481)	.194 (.357)	1	4.2:1
Site D:					
Worker pupae (N = 85, n = 85)	.200 (.262)	.624 (.500)	.176 (.238)	.97	3.5:1
Reproductive queens (all) (N = 79, n = 208)	0 (.157)	.793 (.479)	.207 (.364)	1	3.8:1
Reproductive queens (mated) (N = 66, n= 109)	0 (.164)	.811 (.482)	.189 (.354)	1	4.3:1
Site E:					
Worker pupae (N = 82, n = 82)	.268 (.281)	.525 (.498)	.207 (.221)	.27	2.5:1
Reproductive queens (all) (N = 63, n = 147)	0 (.159)	.797 (.479)	.203 (.362)	1	3.9:1
Reproductive queens (mated) (N = 55, n = 97)	0 (.179)	.847 (.488)	.153 (.333)	1	5.5:1

TABLE 1 (*Continued*)

	Pgm-3 Genotypes			Probability of Deviation from HWE	Ratio of Genotypes *a*/*b*:*b*/*b*
	a/*a*	*a*/*b*	*b*/*b*		
Site F:					
Worker pupae (N = 84, n = 84)	.238 (.274)	.571 (.499)	.191 (.227)	.77	3.0:1
Reproductive queens (all) (N = 83, n = 221)	0 (.150)	.775 (.475)	.225 (.375)	1	3.4:1
Reproductive queens (mated) (N = 81, n = 177)	0 (.148)	.768 (.473)	.232 (.379)	1	3.3:1
All sites:					
Worker pupae (N = 504, n = 504)	.260 (.305)	.585 (.495)	.155 (.200)	>.999	3.8:1
Reproductive queens (all) (N = 450, n = 1,160)	0 (.159)	.797 (.479)	.203 (.362)	1	4.0:1
Reproductive queens (mated) (N = 415, n = 856)	0 (.152)	.780 (.476)	.220 (.372)	1	3.5:1

NOTE.—The proportions of genotypes expected under HWE are shown in parentheses under the observed values. Values for the probability of deviation from HWE indicate the probability that the genotype proportions observed differ significantly from those expected based either on single χ^2 tests (worker pupae) or on 200 χ^2 tests conducted on resampled data (reproductive queens) (see text). N indicates the number of nests from which samples were collected, whereas n indicates the number of sampled individuals.

Pgm-3$^{b/b}$ homozygotes to a similar extent in the two castes, despite the fact that the alternate homozygote is common in workers but absent in queens. This is the pattern expected if genotype distributions in young queens initially resemble those in workers and if the major component of selection acting on *Pgm-3* involves the subsequent elimination of all queens with the genotype *Pgm-3*$^{a/a}$ as they mature sexually (see, e.g., Ross 1992; Keller and Ross 1993*a*).

The observed frequencies of allele *Pgm-3*^{a} in worker pupae and reproductive queens sampled from the six polygyne sites are shown in figure 3, as are the frequencies in males and reproductive queens from an adjacent monogyne population. As expected given the strong directional selection against *Pgm-3*^{a} in the polygyne form but not the monogyne form, the frequencies differ sharply between sexuals of the two social forms. Within the polygyne form, *Pgm-3*^{a} is significantly more common in workers than in queens at every site (i.e., the 95% CIs never overlap), the predicted outcome of selective elimination of the genotype *Pgm-3*$^{a/a}$ in the queen caste only. Within each caste of the polygyne form, the allele frequencies are statistically indistinguishable across the sampling transect (i.e., all of the 95% CIs overlap), with these frequencies especially uniform for queens. Thus, there is no statistically significant clinal pattern in the distribution of *Pgm-3*^{a} from the upwind periphery to the downwind regions of the main polygyne population.

Genotypic data for *Pgm-3* obtained from mated queens and their worker offspring can be used to estimate the proportions of matings of polygyne queens for which monogyne males are responsible (y_{mm}) at each site using equations (4)–(6). Estimates of y_{mm} are expected to be similar for a given data set regardless of which of these three equations (offspring genotypic classes) is used and should vary between 0 and 1.0, if interform gene flow is the only force acting in conjunction with selection against $Pgm\text{-}3^{a/a}$ queens to determine offspring genotypes. These two expectations are not met in a surprising number of instances. First, estimates of y_{mm} derived from the observed proportions of heterozygous offspring (using eq. [5]) invariably exceed those derived from the proportions of homozygous offspring at the same site (using eqq. [4] and [6]), a pattern seen also in four other data sets generated from this population in 1990 and 1991 (fig. 4). The probability of this specific pattern recurring in all 10 data sets is 0.000017 (i.e., $1/3^{10}$), if all three equations are providing estimates of the same value of y_{mm} for each data set. The improbability that all three equations are in fact yielding estimates of the same values is further shown by a bootstrap analysis that employs repeated resampling of the 10 original data sets. The proportion of 1,000 bootstrap replicates of each data set in which y_{mm} derived from equation (5) exceeded the estimates from both equations (4) and (6) ranges from 0.54 to 1.0 (fig. 4), in contrast to the null expectation of 0.33 for each data set. These analyses show that offspring heterozygosity gives inflated estimates of y_{mm} relative to the estimates obtained using offspring homozygosity.

A second indication that estimates of y_{mm} derived from offspring heterozygosity are artificially high is that they are greater than 1.0 for four of the six data sets from 1993, for the data from this year pooled over all sites, and for all four of the earlier data sets from this population (fig. 4). Under the conservative assumption that polygyne queens invariably mate with monogyne males (i.e., all $y_{\mathrm{mm}} = 1.0$), the binomial probability of obtaining estimates of y_{mm} greater than 1.0 in eight or more of the 10 data sets is 0.055.

The above results suggest that residual excess heterozygosity exists in the offspring of polygyne queens above that which can be accounted for by interform gene flow alone. This conclusion leads us to consider additional factors that may inflate offspring heterozygosity, with the objective of accounting for these factors to obtain more reliable measures of gene flow. The most obvious such factors are a fecundity advantage of $Pgm\text{-}3^{b/b}$ over $Pgm\text{-}3^{a/b}$ queens and a survival advantage of $Pgm\text{-}3^{a/b}$ offspring over the other two genotypes. To investigate the influence of these factors on the gene flow estimates, values of y_{mm} were calculated from the proportions of the three offspring genotypes in the pooled data for 1993 by using equations (4)–(6), with the relative fecundity or survival of the genotypes adjusted so that the estimates from the three equations converged to a common value. A 1.67-fold fecundity advantage of $Pgm\text{-}3^{b/b}$ queens or a 1.17-fold survival advantage of $Pgm\text{-}3^{a/b}$ offspring was found to give identical estimates of y_{mm} for the pooled data no matter which genotypic class of offspring was used for the estimations. Therefore, it appeared desirable to incorporate these fecundity and survival differentials in a subset of the estimations of y_{mm} for the individual sites from this study. This approach resulted in our use of three procedures to estimate

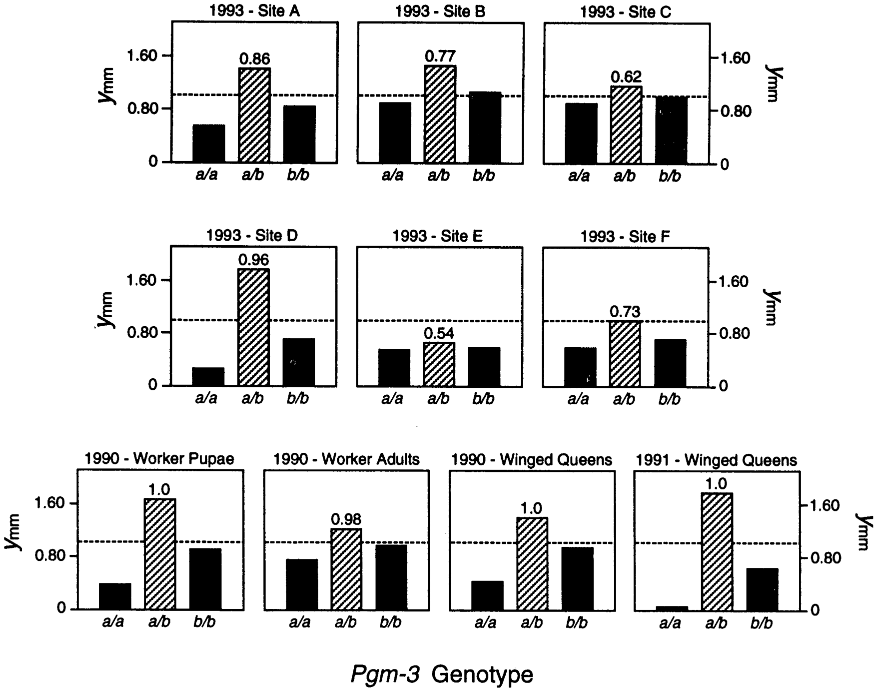

FIG. 4.—Comparisons of estimates of y_{mm}, the proportion of matings of polygyne *Solenopsis invicta* queens attributable to monogyne males, derived from the proportions of the three different genotypic classes at *Pgm-3* in polygyne offspring females. Estimates based on the frequencies of *Pgm-3*$^{a/b}$ heterozygotes (*hatched columns*) are derived from eq. (5), whereas those based on the frequencies of *Pgm-3*$^{a/a}$ and *Pgm-3*$^{b/b}$ homozygotes (*black columns*) are derived from eqq. (4) and (6), respectively (see text). Values above the hatched columns represent the proportion of 1,000 bootstrap replicates of each data set in which estimates of y_{mm} derived from the heterozygote proportions exceeded both estimates derived from the homozygote proportions. The top six panels are based on data from this study, whereas the bottom four panels are based on data from a previous study (Ross 1992). Offspring females in 1993 were all worker pupae; classes of offspring females for the other years are indicated at the top of each panel. The dashed lines in each panel are placed at $y_{mm} = 1.0$.

the extent of interform mating between polygyne queens and monogyne males at each site: y_{mm} was calculated as the mean of the estimates from equations (4)–(6), with no fecundity or survival advantages assumed; y_{mm} was calculated as the mean of the estimates from equations (4)–(6) when a fecundity advantage of 1.67 to *Pgm-3*$^{b/b}$ queens was assumed; and y_{mm} was calculated as the mean of the estimates from equations (4)–(6) when a survival advantage of 1.17 to *Pgm-3*$^{a/b}$ offspring was assumed.

Estimates of y_{mm} calculated using these three procedures are presented for each of the sites in figure 5. Monogyne males seem to contribute exclusively, or nearly so, to the fertilization of polygyne queens at site B, at the windward boundary of the two social forms, a conclusion that is independent of the particu-

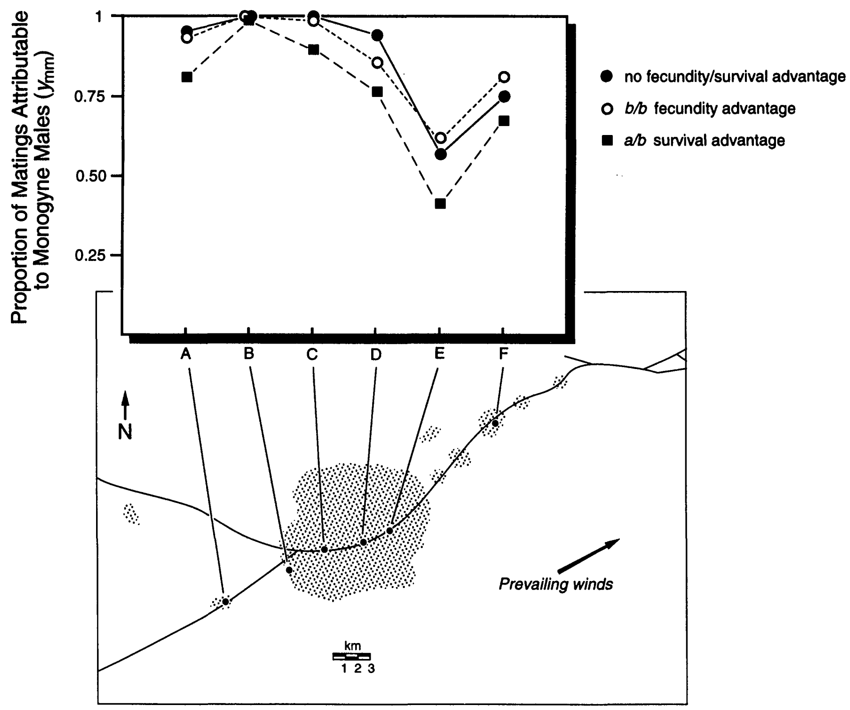

FIG. 5.—Proportions of matings of polygyne *Solenopsis invicta* queens attributable to monogyne males (y_{mm}) at six sampling sites, as estimated using three different procedures. The estimate that assumes no fecundity or survival advantages is simply the mean of the three estimates from eqq. (4)–(6) (see text), whereas the other two estimates are the means of the estimates derived from these equations assuming either a 1.67-fold fecundity advantage of *Pgm-3*$^{b/b}$ queens over *Pgm-3*$^{a/b}$ queens or a 1.17-fold survival advantage of *Pgm-3*$^{a/b}$ offspring over others. Values of y_{mm} that were calculated to be greater than one are shown as being equal to one.

lar estimation procedure employed. These immigrant males also have remarkable mating success at site C, the next site downwind. In fact, the proportionate success of monogyne males in securing matings is high throughout the study area, with over two-thirds of mated polygyne queens inferred by all procedures to have mated with monogyne males at every site but one. Only at site E, the site within the main polygyne population farthest downwind from the boundary between the forms, do indigenous polygyne males appear by all estimates to have success in mating with polygyne queens comparable to that of monogyne males. Estimates of the average proportionate mating success of monogyne males over all sites, y_{mm} = 0.77, 0.86, and 0.89 by the three estimation procedures, compare favorably with the value of y_{mm} = 0.91 obtained earlier for this population by a completely different method, that of direct progeny analyses and reconstruction of *Pgm-3* mating types (Ross and Shoemaker 1993).

A remarkable pattern evident from the data in figure 5 is the steady decrease in the proportionate mating success of monogyne males within the main polygyne

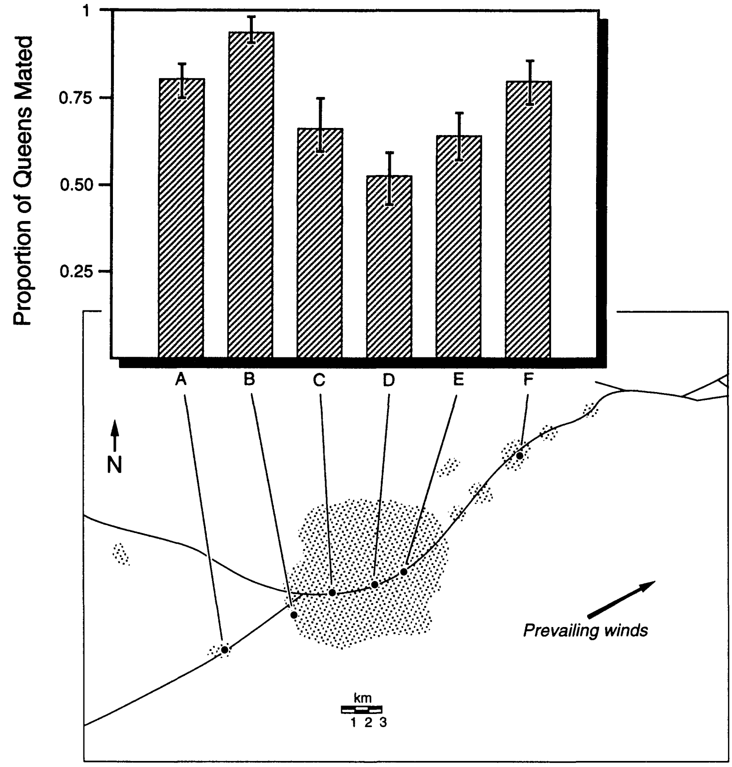

FIG. 6.—Proportions of reproductive polygyne *Solenopsis invicta* queens that are mated at six sampling sites. Bars around the point estimates indicate the 95% confidence intervals.

population (sites B–E) at increasing distances from the windward boundary of the social forms. Indeed, there is a striking correspondence overall between the proportion of matings attributable to monogyne males at a given site and the proximity and extent of monogyny upwind of that site.

The proportion of reproductive queens that is mated at each site is shown in figure 6. The highest proportion of such queens occurs at the windward boundary of the polygyne population with the monogyne form (site B), where about 95% of queens are mated. The lowest proportion occurs in the center of the main polygyne population (site D), where almost half of the queens are unmated. The general pattern in the main polygyne population is one of a decrease in the frequency of mated queens from the windward margin toward the population center, while the two outlying sites in the isolated pockets of polygyny (sites A and F) have proportionately more mated queens than any of the sites in the interior of the main population (sites C–E). The mating success of queens at a given site largely parallels the proportionate contribution to these matings by monogyne males (y_{mm}), with both patterns explicable in terms of the proximity and extent of monogyny upwind of a site. The only site at which concordance of the patterns breaks down is site E, where the proportionate mating success of monogyne males predictably reaches its lowest level, but queen mating success is higher than at the site immediately upwind (figs. 5 and 6). Apparently, fertile polygyne males are sufficiently abundant at this site that queen mating success is not strongly tied to the availability of immigrant monogyne males.

DISCUSSION

This study indicates that both selection and gene flow are important forces influencing the nature and extent of diversity at *Pgm-3,* a gene with important reproductive effects, in the polygyne social form of the fire ant *Solenopsis invicta.* The continuing action of strong negative selection on the allele *Pgm-3*^{a} in the polygyne form is documented by the fact that no reproductive queens, among the 1,160 whose genotypes were studied, were found to be *Pgm-3*$^{a/a}$ homozygotes. In contrast, one-quarter of the worker pupae studied possessed this genotype. The common occurrence of genotype *Pgm-3*$^{a/a}$ in nonreproductive females (workers) or prereproductive females (winged queens), coupled with the absence of this genotype in reproductive females (wingless queens), now has been observed over a 4-yr period. This recurring pattern in the field accords with experimental results indicating that young queens with the genotype *Pgm-3*$^{a/a}$ invariably are destroyed by workers before they can make the transition to the role of functional egg layer (Keller and Ross 1993*a*). The close match of the ratios of genotype *Pgm-3*$^{a/b}$ to genotype *Pgm-3*$^{b/b}$ that we observed between worker pupae and reproductive queens is an expected outcome under this scenario of selection.

Gene flow from the monogyne social form clearly also is an important force influencing the gene frequency dynamics at *Pgm-3* in the polygyne study population. Earlier studies of the progenies of individual polygyne queens established both that such gene flow is substantial and that it is likely to occur mostly by means of monogyne males dispersing into the polygyne population and mating with resident queens (Ross 1992; Ross and Shoemaker 1993). These conclusions are further supported by two independent and complementary lines of evidence from this study. First, analyses of the proportionate mating success of males of the two forms based on genotype and allele frequencies at *Pgm-3* in polygyne worker pupae and in reproductives of both forms suggest that monogyne males contribute exclusively or predominantly to the paternity of polygyne queens' offspring at all but one study site (site E, where 40%–60% of the matings are attributable to polygyne males). Second, mating opportunities for polygyne queens seem to be limited to a large extent by the availability of immigrant monogyne males, as suggested by the fact that there is a general correspondence between queen mating success at each site and the proximity and extent of monogyny upwind of the site. The sole site where queen mating success does not reflect the presumed availability of immigrant monogyne males, site E, is unique because the proportionate mating success of polygyne males here is on a par with that of monogyne males. Thus, the unexpectedly high proportion of mated queens at this site seems to be the result of fertile polygyne males accumulating to high densities in the leeward reaches of the main polygyne population. In sum, both genotype distributions at *Pgm-3* and patterns of queen mating success support a major role for gene flow from the monogyne form in opposing directional selection on *Pgm-3* in the polygyne form. These data further substantiate the earlier conclusion that the dispersal of monogyne males into the polygyne population is an important route for this interform gene flow.

The dispersal of mated monogyne queens into the polygyne population is an unlikely route for interform gene flow for two reasons. First, the most common genotype in monogyne queens, $Pgm\text{-}3^{a/a}$, is missing in reproductive queens from the polygyne population. Second, polygyne workers are likely to be completely intolerant of any immigrant monogyne queens because such queens resemble $Pgm\text{-}3^{a/a}$ polygyne queens with respect to the traits used by workers as cues for selective queen destruction (Keller and Ross 1993*a*, 1993*b*; Ross and Shoemaker 1993).

Gene flow from the monogyne form appears to have effectively counteracted the strong negative selection on $Pgm\text{-}3^{a}$ in the polygyne study population to prevent significant selection-mediated changes in allele frequencies or erosion of diversity at this locus over time. The frequencies of $Pgm\text{-}3^{a}$ in 1993 both in worker pupae and in reproductive queens are virtually identical to the frequencies in 1990 (workers, 0.55 vs. 0.52, respectively; queens, 0.40 vs. 0.38). Similarly, heterozygosities observed at this locus are closely similar between the two years for both castes (workers, 0.59 vs. 0.65; queens, 0.80 vs. 0.76) (see Ross 1992). The eventual equilibrium gene frequencies and heterozygosities in the polygyne population depend in a complex way on the size of the population, strength of selection, magnitude of interform gene flow, and dominance patterns of the *Pgm-3* alleles (see, e.g., Haldane 1930; Wright 1969; Felsenstein 1976). Data are now available concerning most of these factors, so that it soon may be possible to construct reasonable models to predict equilibrium conditions. However, based on the above empirical results showing stability of the gene frequencies over time, it appears as though the study population presently may be near equilibrium.

Excess heterozygosity is predicted at *Pgm-3* in polygyne offspring if substantial interform gene flow occurs as a result of polygyne queens mating predominantly with monogyne males. However, the level of heterozygosity observed in this and previous studies appears to be higher than can be explained even by the greatest possible extent of interform mating (i.e., all polygyne queens mating with monogyne males), which leads to our conclusion that a second form of selection, distinct from the well-documented negative selection on $Pgm\text{-}3^{a}$ in queens, also may act on *Pgm-3* in the polygyne form. Further evidence for such supplemental selection comes from the fact that the magnitude of interform mating (y_{mm}) estimated from offspring heterozygosity at *Pgm-3* invariably exceeds that estimated from offspring homozygosity, which should not be the case if only gene flow is contributing to heterozygosity excess. Modest levels of differential fecundity or survival among *Pgm-3* genotypes can adequately explain the unexpectedly high heterozygosity that we have observed, but as yet no data are available to implicate these or any other supplemental selective forces. Although this putative second form of selection cannot yet be identified, by accounting for its effects we are likely to have extracted a clearer signal of the pattern of interform mating in our study area.

Our conclusion of extensive ongoing gene flow from the monogyne to the polygyne form raises questions concerning the mode of inheritance of social organization in these ants and the manner in which the characteristic social organization of the polygyne form is maintained. Our earlier studies employing cross-fostering of brood into the alternate social environments suggest that individual phenotypic traits associated with monogyny or polygyny are influenced largely by the social environment in which a young adult matures (Keller and Ross 1993*b*). Thus, ongoing interform gene flow is not expected to have a direct effect on the expression of social organization in *S. invicta*.

Our data on the proportions of polygyne queens that are mated and on the fractions of these matings attributable to males of each social form can be combined to provide a detailed profile of the abundance of males of each form throughout the study area. A substantial proportion of polygyne queens remains unmated throughout most of the study area, so receptive females generally do not appear to be in limited supply. Thus, it is reasonable to assume both that the total abundance of fertile males at each site is indexed by the proportion of queens that is mated and that the relative abundance of males of each form is reflected in their proportionate mating success. When the proportion of matings attributable to monogyne males (y_{mm}) is estimated to be 1.0 on the basis of the genetic data, as is invariably the case at site B, then the abundance of monogyne males is represented simply by the proportion of queens that is mated (*upper bound of shading* in fig. 7). Otherwise, the frequency of mated queens must be factored by y_{mm} to give an approximation of the abundance of monogyne males. The region of lighter shading in figure 7 represents the abundance of monogyne males inferred throughout the study area by using the average of our three different estimates of y_{mm} from each site. The remaining, darker-shaded area under the queen mating success curve represents the inferred abundance of polygyne males.

The greatest abundance of dispersing monogyne males available for mating with polygyne queens is inferred to be at the site on the abrupt windward boundary of the polygyne population with the monogyne form (site B), which is gratifying given that this polygyne site is the one most closely associated with an upwind source of monogyne males. The abundance of monogyne males drops precipitously as one moves progressively further along the study transect into the main polygyne population, as is predicted on the basis of ever-increasing distances of these sites from an upwind source of such males. The abundance of monogyne males apparently increases again at site F, the leeward outlying site, presumably because monogyne nests become interspersed with polygyne nests immediately upwind of this site. It is significant that the abundance of monogyne males at this outlying site appears to be not as great as at the windward outlying site (site A), which is immediately downwind of extensive tracts of monogyny (see fig. 2).

It is clear from these results that kilometer-scale distances represent biologically significant obstacles to the dispersal of fire ant males. The sharp and consistent decline in abundance of dispersing monogyne males from the boundary between the two forms (site B) to the area just downwind of center of the polygyne population (site E) corresponds to a greater than 50% decrease in the absolute level of mating of resident females with immigrant males over this 10-km distance (fig. 7). These findings are compatible with previously published observational

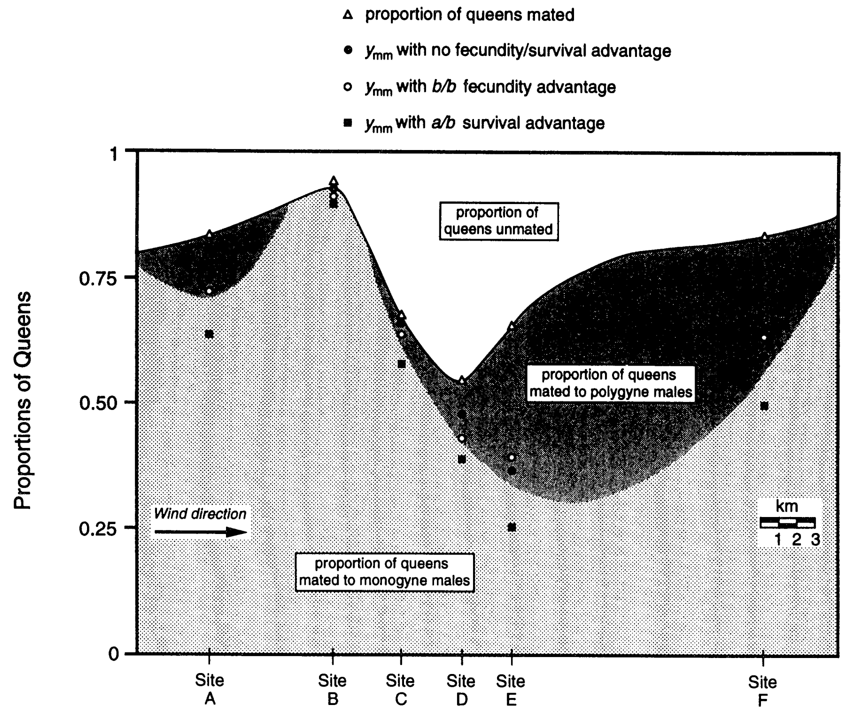

FIG. 7.—Proportions of polygyne queens of *Solenopsis invicta* that are unmated and that have mated to males of each social form throughout the study area. The continuous distributions illustrated are interpolated from the queen mating data and estimates of y_{mm} (the proportion of matings attributable to monogyne males) obtained from six sampling sites, taking into account the local distributions of the two social forms and the prevailing wind direction during mating flights. The combined abundance of males of the two forms determines the proportion of queens that becomes mated at a given locale (this proportion is indicated by the triangular symbol at each site). The variable levels of shading represent the abundances of males of the two social forms inferred from the average value of y_{mm} calculated at each sampling site by three different procedures (see text for explanation). The three point estimates of y_{mm} at each site, scaled to the local proportion of mated queens, are indicated by the circular and square symbols.

data on fire ant dispersal that indicated that queens of *S. invicta* usually disperse less than 2 km during their mating flights but occasionally are carried distances of up to 10 km on air currents (Markin et al. 1971).

An assumption implicit in the foregoing analysis is that the observed patterns in proportions of mated queens and in male contributions to matings result only from differences in male abundance along the study transect—that is, that the abundance of receptive queens is constant. Monogyne nests in northeastern Georgia produce an average of three to four times as many winged queens as do polygyne nests over the course of a year (Vargo and Fletcher 1987; E. L. Vargo, unpublished results). On the other hand, polygyne nests occur at higher densities than do monogyne nests, with recent comparative studies showing a greater than twofold disparity in nest densities of the two forms in both Texas and Florida (Porter et al. 1991; Porter 1992). Assuming that these reported densities are realistic estimates for our study area, the total abundance of receptive queens of the two forms is likely to be relatively constant across our sampling transect or actually may decrease somewhat from the windward boundary of the main polygyne population to the interior sites. If queen abundance decreases in this way, then the decline in abundance of immigrant monogyne males along the transect that we have inferred represents a conservative estimate of the actual decline.

Our results reveal that fertile polygyne males often are uncommon relative to immigrant monogyne males in our study population (fig. 7), although there clearly is important microgeographical variation in this regard. Fertile polygyne males also seem to be uncommon relative to receptive polygyne queens, since many polygyne queens remain permanently unmated at sites with a reduced abundance of monogyne males. This latter conclusion is consistent with the finding that operational sex ratios within nests in the polygyne study population are highly female biased (six females to one male) (Vargo and Fletcher 1987; Ross and Shoemaker 1993; E. L. Vargo, unpublished results). The apparent scarcity of polygyne males suggests that the mating success of immigrant monogyne males is not limited by the loss of receptive queens from the mating pool after they mate with polygyne males. Rather, the ability of monogyne males to inseminate polygyne queens seems to be limited simply by the distances they often must traverse to reach these queens.

We were unable to detect a cline in which *Pgm-3*^{a} decreases in frequency from the upwind periphery to downwind of center of the main polygyne population. A plausible explanation is that the effective gene flow distance associated with immigration of monogyne males is sufficiently large compared to the distance over which the altered selection regime is present (roughly the diameter of the main polygyne population) that gene flow effectively homogenizes allele frequencies throughout the polygyne population (see, e.g., Slatkin 1973; May et al. 1975). This proposed facility of gene flow to homogenize allele frequencies within the polygyne population is compatible with our finding that immigrant monogyne males typically are involved in 50% or more of the matings of polygyne queens, even at sites quite remote from a source of such males. Any cline created under these circumstances presumably would be so flat as to remain undetectable even with sample sizes as large as ours.

The several lines of evidence presented in this and earlier studies combine to provide a compelling case for directional selection and gene flow acting jointly to strongly influence the gene frequency dynamics at *Pgm-3* in polygyne *S. invicta*. Although the interaction of these fundamental evolutionary forces long has been realized to have theoretically important consequences for the extent of genetic variation and its distribution within and among populations (Haldane 1930; Levene 1953; Nagylaki 1975; Felsenstein 1976; Karlin 1976; Maynard Smith and Hoekstra 1980; Barton and Clark 1990), the case of *Pgm-3* in polygyne *S. invicta* represents an unusually clear example of how gene frequencies can be altered, variation preserved, and population differentiation constrained by gene flow occurring in the face of strong directional selection. The extensive gene flow we document swamps local selection in the polygyne form to reset gene frequencies every generation at levels far from their selective equilibria and, in so doing, maintains a store of variation at *Pgm-3* that might otherwise be lost or severely depleted.

Other compelling examples of the joint influence of gene flow and selection on the extent and distribution of genetic variation include color-pattern variation in mainland- and island-inhabiting water snakes (Camin and Ehrlich 1958; King 1993), heavy metal tolerance in grasses on and around mines (Antonovics et al. 1971; Macnair 1981), melanism in the peppered moth in industrialized and rural areas (Mani 1982), and variation in frequencies of an aminopeptidase polymorphism in oceanic and estuarine mussels (Koehn et al. 1983). The selective pressures in each of these cases are rather well understood and result largely from extrinsic biotic or abiotic factors that affect survival or reproductive output (e.g., predation, environmental toxins, fluctuating salinity). In contrast, although the proximate cause of selection on *Pgm-3* in polygyne fire ants is clear, the ultimate reasons underlying the evolution of worker discrimination against queens with the genotype *Pgm-3*$^{a/a}$ remain obscure (see Keller and Ross 1993*a*). The behavior appears not to be of recent origin in the introduced populations, because *Pgm-3*$^{a/a}$ queens are absent also among reproductives in polygyne nests in two genetically distinct native populations of *S. invicta* in Argentina (K. G. Ross, E. L. Vargo, and L. Keller, unpublished data).

The preponderance of monogyne males among the mates of polygyne queens raises the issue of whether polygyne fire ant populations in the United States are ''parasitic'' on neighboring monogyne populations, in the sense that immigrant monogyne males may be required to ensure that a sufficient number of fertile, mated queens can be recruited to replace aging or lost queens in polygyne nests. If polygyne populations are dependent in this way, then they are expected invariably to be associated with the monogyne form and, perhaps, to occupy only a certain maximal area. For instance, it appears on the basis of our data as though no more than one-third of receptive polygyne queens would find a mate in areas sufficiently remote from monogyne nests that immigrant monogyne males are rare (e.g., site E in fig. 7). A gradual transformation to monogyny might ensue in such areas through a steady erosion in the number of mated queens per nest and/or through the occasional successful colonization of depleted areas by newly mated immigrant monogyne queens. The expected result under this scenario is a gradual fragmentation of polygyne populations once they extend more than 10–20 km from a source of monogyne males. It will be of interest to follow existing polygyne populations that appear to substantially exceed this size (Porter et al. 1991; Porter 1992) to learn whether they maintain their integrity through time.

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