Gene Flow or Heterozygote Advantage?

K. G. Ross (*1*) and L. Keller and Ross (*2*) describe a relationship between colony social structure and allele frequencies at the enzyme locus *Pgm-3* in the red imported fire ant *Solenopsis invicta*. In polygynous colonies, queens homozygous for *Pgm-3*^{a} (genotype *aa*) are killed by workers. A population consisting entirely of polygynous colonies should therefore have a low frequency of *Pgm-3*^{a}, because in females this allele (or a closely linked gene for which it serves as a marker) is effectively a recessive lethal. Yet *Pgm-3*^{a} is common ($P \approx 0.37$) in the population studied by Ross (*1*), leading Keller and Ross (*2*) to conclude that *Pgm-3*^{a} is probably flowing into the population at a high rate through males from a neighboring monogynous population where queens of all genotypes survive equally well and *Pgm-3*^{a} is by far the most common allele ($P \approx 0.75$).

Gene frequency and fecundity data presented by Keller and Ross (*2*) suggest a possible alternative explanation for the high frequency of *Pgm-3*^{a} in polygynous colonies. In a laboratory experiment, heterozygous *Pgm-3*^{a}/*-3*^{b} (*ab*) queens laid eggs more than three times more frequently than homozygous (*bb*) queens [table 2 in (*2*)]. Keller and Ross (*2*) do not comment on this difference because it is not statistically significant by a conservative multiple unplanned comparisons criterion and because the egg-laying rates of *ab* and *bb* queens were both far below that of *aa* queens (the ones attacked by workers in polygynous colonies). But the difference between *ab* and *bb* egg-laying rates is itself nearly three standard errors in magnitude and might well be real. If so, then heterozygous females may be fitter than either type of homozygote in polygynous populations where *aa* queens are killed by workers. All else being equal, both alleles should then be present at equilibrium within an entirely closed polygynous population. For example, genotype frequencies virtually identical to those found by Ross [table 1 in (*1*)] are predicted when one assumes that the relative fitnesses of the three genotypes (in queens) are 0:1:0.4. The assumed 2.5-fold advantage of *ab* over *bb* queens is smaller than that indicated by the egg-laying data and vastly smaller than the observed 10- to 25-fold advantage of *aa* over the other two genotypes.

If this explanation is even partly correct, then *Pgm-3*^{a} should be common where colonies of polygynous *S. invicta* occur far from the nearest monogynous populations, and the polymorphism may represent an unusually dramatic instance of heterozygote advantage in nature. If not, and if massive gene flow is needed to maintain *Pgm-3*^{a} in polygynous populations, then there can be little genetic differentiation between the polygynous and monogynous forms, and their distinctness must be maintain by cultural transmission or by responses to some environmental factor or factors. Two lines of evidence support this environmental explanation. First, the frequency of *Pgm-3*^{a} in males from polygynous colonies is nearer to the frequency expected according to the environmental model (with migration) than according to the heterozygote advantage model. Second, a cross-fostering experiment using queens from polygynous and monogynous colonies suggests that their differences in size and fat content have a predominately environmental basis (*3*). Further work aimed at distinguishing between these alternatives should help to clarify the puzzling relationship between *Pgm-3* and the social structure of *S. invicta* colonies.

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Response: The issue of whether gene flow or heterozygote advantage opposes the strong selection against *Pgm-3*^{a} that we have observed in polygynous *S. invicta* (*1*) is important because this case constitutes an unusually clear example of how fundamental evolutionary forces can interact to determine the extent of genetic variation in wild populations. Any force hypothesized to explain the maintenance of *Pgm-3*^{a} in the face of strong negative selection must explain another striking feature of the genetic data from polygynous *S. invicta* as well: the consistent excess of heterozygotes among nonreproductive females (*2*, *3*).

The strongest evidence for gene flow comes from progeny studies of 69 polygynous queens in which the *Pgm-3* mating types were reconstructed. The distribution of reconstructed mating types suggests that about 91% of these polygynous queens mated with monogynous rather than polygynous males, if one assumes no assortative mating according to *Pgm-3* genotype (*2*, *3*). Population genetics theory predicts that if alleles differ in frequency between the sexes, as the *Pgm-3* alleles do between polygynous queens and monogynous males, then excess heterozygosity will be generated in the offspring (*4*). The heterozygosity expected at this locus when 91% of polygynous queens mate with monogynous males ($H_{exp} = 0.57$) is very close to the high level of heterozygosity observed in our polygynous study population in northern Georgia ($H_{obs} = 0.62$; 95% confidence interval, 0.48 to 0.76). Thus the gene flow that results from polygynous queens mating primarily with immigrant monogynous males can explain not only how *Pgm-3*^{a} is maintained in the polygynous population but also the existence and magnitude of the elevated heterozygosity observed at *Pgm-3* in this population.

The operational sex ratio observed in our polygynous study population is highly female-biased (14:1) (*3*, *5*). This strong sex ratio bias suggests that the mating success of polygynous queens often may be limited by the availability of males and, indeed, a large proportion of the egg-laying queens in this population remains permanently unmated (*2*, *6*). If, as we suggest, most polygynous queens mate with immigrant monogynous males, then the proportion of permanently unmated queens at various sites in polygynous populations is predicted to increase with increasing distance of a site from a source of such males. We have found just such a pattern in our polygynous study population, with the proportion of egg-laying queens that is unmated equal to about 5% at the periphery of the population, about 47% in the center, and about 34% in intermediate positions (*7*).

Little genetic differentiation should exist between polygynous and monogynous populations of *S. invicta* at loci that are not under differential selection if gene flow is responsible for the maintenance of *Pgm-3*^{a} at substantial frequencies in the polygynous form. A general absence of such differentiation has been confirmed in northern Georgia with the use of data from 12 presumably neutral electrophoretic loci and from the sex-determination locus (*3*).

The heterozygote advantage hypothesis receives little support from analyses of the available data. First, we remain unable to detect a significant difference in the fecundity of *ab* and *bb* queens using a two-way analysis of variance conducted only on these two genotypes ($F = 3.16$, df = 1, $P = 0.08$), a less conservative test than was applied in our report [table 2 in (*1*)]. Thus there is no compelling evidence that queens of these two genotypes really differ in their reproductive phenotypes, which supports our earlier statement that *Pgm-3*^{a} acts as a completely recessive allele with respect to

such traits (*1*). Second, as noted by Berrigan *et al.*, *Pgm-3* genotype frequencies in the sons of polygynous queens are not consistent with any reproductive advantage of *ab* over *bb* queens. Such an advantage presumably would be revealed by an excess of *Pgm-3*^{a} haploid male genotypes relative to the frequency expected if queens of different genotypes reproduced equally. In fact, *Pgm-3*^{a} is slightly (but significantly) *less* frequent in polygynous males than in their mothers (*2*, *3*). Finally, heterozygote advantage in queens does not provide an explanation for the consistent excess heterozygosity observed in their female offspring. We have constructed a simple genetic model that predicts genotype distributions at *Pgm-3* in the polygynous population when *ab* queens have a 2.5-fold reproductive advantage over *bb* queens, using as a starting point the observed genotype frequencies in polygnous queens and males. The proportion of heterozygous offspring expected after 20 generations of random mating between polygynous queens and polygynous males, that is, in the absence of gene flow, is far lower than the proportion actually observed (H_{exp} = 0.28; 95% confidence interval of H_{obs} = 0.48 to 0.76).

Our conclusion that gene flow is the dominant or sole force opposing selection against *Pgm-3*^{a} in polygynous *S. invicta* raises the issue of what factors maintain the social distinctiveness of the polygynous and monogynous forms, if not genetic isolation and differentiation. We argue elsewhere (*3*, *8*) that the different pheromonal environments in colonies of the two forms influence the behavioral and physiological phenotypes of workers and new queens reared in these societies and that these phenotypic differences act both to constrain the characteristic social organization that develops and to perpetuate it from generation to generation.

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Examining Langmuir-Blodgett Films with Atomic Force Microscopy

In their recent report (*1*), L. Bourdieu *et al.* examine the surface structure of barium arachidate Langmuir-Blodgett (LB) films with atomic force microscopy (AFM). They conclude that the films as deposited were disordered, but after annealing at ≥50°C, a lattice structure developed that was determined to be centered rectangularly with a lattice modulation every three rows in the [10] direction. Our observations [(*2*), published concurrently] however, show that the deposited films consisted of coexisting regions of three types of surface structure: (i) the disordered structure mentioned above, (ii) a structure (which we labeled 3 × 1) effectively identical to that noted by Bourdieu *et al.* after annealing, and (iii) a third lattice structure (which we labeled 2 × 2) with a similar area per molecule (density) as the 3 × 1 structure and the disordered structure. Although we appreciate the conclusion of Bourdieu *et al.* that the 3 × 1 structure is likely to be the equilibrium one (it covered the majority of the surface, 70%, in our studies as well), we were able to analyze the AFM images in detail. We determined a structure consistent with the entire Fourier pattern (including the reflection labeled a modulation by Bourdieu *et al.*, which, in our structural assignment, is labeled the [10] spot), the height of the modulation, previous infrared spectroscopic studies (*3*), and well-established lattice structures of alkanes based on geometric principles of close packing (*4*). Our conclusion is that the lattice observed by Bourdieu *et al.* consists of a local tilted triclinic packing {Kitaigorodskii's *T*[1/2,1] (*4*)} with a specific type of packing defect (analogous to a stacking fault in three dimensions) every third row. This regular series of defects reduces the tilt angle from 36°, as predicted by Kitaigorodskii, to 26°, which is consistent with the corresponding bilayer thickness (5.0 nm) that we measured. In addition, infrared spectroscopy (*3*) of barium arachidate LB films made in the pH range used in both studies is consistent with a local triclinic packing.

Bourdieu *et al.* oversimplified their analysis by assuming that the third harmonic of the [10] spot corresponds to a simple modulation of the molecular row spacing similar to that which we observed in cadmium and manganese arachidate films (*5*). However, even by duplicating their analysis with our data, we cannot justify their conclusion quantitatively, as we have measured distinctly left-handed and right-handed surface lattices, which rules out the possibility that the structure is centered-rectangular (although the deviation is subtle and may be smaller than the errors quoted by Bourdieu *et al.*).

As mentioned above, we also observed a minority structure that can be described as a tilted centered-rectangular "herringbone" arrangement (Kitaigorodskii's *R*[01]) with every other molecular row displayed vertically by the height of a single methylene group (0.25 nm). The dimensions of this structure could not be confused with the one observed by Bourdieu *et al.* It is quite reasonable, given the relative rarity of this structure in our "unannealed" films, that it would not be observed after annealing.

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Response: The comment of Schwartz *et al.* is based on a comparison between systems that are really different. Schwartz *et al.* (*1*) used a three-layered barium arachidate film deposited onto mica, whereas we discussed the case of a bilayer deposited onto a silanated wafer (*2*).

Contrary to the statements of Schwartz *et al.*, the molecular scale structure is, in our study, everywhere the same on the whole sample. We have checked this point on 20 images obtained on three samples (*2*). Het-