

Effect of a Founder Event on Variation in the Genetic Sex-Determining System of the Fire Ant *Solenopsis invicta*

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ABSTRACT

Effects of a recent founder event on genetic diversity in wild populations of the fire ant *Solenopsis invicta* were studied, with particular attention given to the genetic sex-determining system. Diploid males are far more common relative to haploid males in introduced populations than in native populations of fire ants, and queens that produce diploid males account for a significantly larger proportion of the mated queens in introduced than in native populations. Differences between native and introduced populations in attributes of the mating systems (*i.e.*, queen mating frequency or level of inbreeding) can be excluded as factors contributing to these different levels of diploid male production. Thus, we conclude that diploid males have increased in frequency in introduced populations because of a loss of allelic diversity at the sex-determining locus (*loci*). This loss of sex alleles has generated a substantial increase in the estimated segregational genetic load associated with production of sterile diploid males in introduced populations over the load in native populations. The loss of allelic diversity in the sex-determining system in introduced *S. invicta* is paralleled by a loss of electrophoretically detectable rare alleles at protein-encoding loci. Such concordance between these different types of markers is predicted because each of the many sex alleles present in the native populations is expected to be rare. Estimates of expected heterozygosity (H_{exp}) based on 76 electrophoretic loci do not differ significantly between the native and introduced fire ant populations, illustrating the lack of sensitivity of this measure for detecting many types of bottlenecks.

EVOLUTIONARY biologists have long been concerned with the effects of founder events and other types of population bottlenecks on genetic variation. Changes in the nature and extent of variation following bottlenecks may affect the subsequent ability of populations to respond to selection and so are of interest with respect to the microevolutionary potential of populations (LEWONTIN 1974). These changes also are of interest with respect to macroevolution, because they may create sufficient genetic incompatibility between parental and founder populations to constitute barriers to gene flow once the populations regain contact, one outcome of which may be speciation (MAYR 1963; TEMPLETON 1980; CARSON and TEMPLETON 1984; BARTON 1989; COYNE 1992). Theoretical studies have explored the effects of bottleneck size and duration on variation in both simple and complex genetic systems, emphasizing that the underlying genetic architecture and nature of selection play important roles in determining the effect of a bottleneck on trait variation (*e.g.*, WRIGHT 1931; NEI, MARUYAMA and CHAKRABORTY 1975; CARSON and TEMPLETON 1984; BRYANT, MCCOMMAS and

COMBS 1986; GOODNIGHT 1987; BARTON 1989). These theoretical findings thus suggest that the significance of a bottleneck for the subsequent evolution of a population may best be assessed by examining diverse traits that possess different genetic architectures and are under different selective regimes (*e.g.*, O'BRIEN *et al.* 1985; NEVO 1989).

Many empirical studies of bottlenecks have been conducted to determine the effects on genetic variation and to test the predictions of theory. These studies often involved either artificially manipulated and enclosed populations not subject to the vagaries of natural environments (*e.g.*, POWELL and RICHMOND 1974; BRYANT, MCCOMMAS and COMBS 1986; LEBERG 1992) or wild populations for which the history and nature of the bottlenecks were not well understood (*e.g.*, BERRY and MURPHY 1970; BARKER *et al.* 1985; O'BRIEN *et al.* 1987). Furthermore, many such studies surveyed only one type of marker (usually protein-encoding loci studied electrophoretically). Although useful for describing the effects of bottlenecks on presumably neutral Mendelian genes with modest variation, such studies can say little about the effects on other types of genetic systems, such as those with high levels of polymorphism, complex architectures or

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strong fitness effects (BRYANT, MCCOMMAS and COMBS 1986; BEN-SHLOMO *et al.* 1988).

We describe in this paper the effects of a well-documented founder event on variation in two different classes of genetic markers in wild populations of the fire ant *Solenopsis invicta*. This species, which is a native of South America (TRAGER 1991), was introduced via commerce to the port of Mobile, Alabama, some time in the early 1930s. Since that time it has expanded its range rapidly to encompass most of the southeastern and south-central part of the country (see LOFGREN, BANKS and GLANCEY [1975] and LOFGREN [1986] for detailed accounts of the introduction and subsequent spread of the species in the USA). This ant maintains high population densities in its introduced range, presumably due to the absence of natural enemies and competitors (JOUVENAZ 1983; WOJCIK 1986; PORTER and SAVIGNANO 1990), and it is therefore regarded as a significant pest in these newly colonized areas.

The marker that is the principal focus of our study is the locus or loci responsible for sex determination. In most species of the order Hymenoptera that have been appropriately studied (excluding Chalcidoidea), sex appears to be determined by genotype at one or a few loci (WHITING 1943; CROZIER 1971, 1975, 1977; BULL 1983; STOUTHAMER, LUCK and WERREN 1992; PERIQUET *et al.* 1993). Diploid individuals heterozygous at the sex-determining locus (or heterozygous at one or more loci in a multilocus system) develop into females, whereas diploid individuals homozygous at the locus (loci) or haploid individuals (with hemizygous genotypes) develop into males. In *S. invicta*, the common occurrence of diploid males in introduced populations suggests that one or a few loci determine sex in this ant (GLANCEY, ST. ROMAIN and CROZIER 1976; ROSS and FLETCHER 1985a). Regardless of the number of loci involved, important features of such a genetic sex-determining system are that it is under strong selection and that it is expected to harbor considerable genetic variation.

Diploid males of Hymenoptera typically have low viability and/or fertility (CROZIER 1971; STOUTHAMER, LUCK and WERREN 1992). In *S. invicta*, diploid males apparently are fully viable but, because of abnormalities in their reproductive physiology, they are functionally sterile (HUNG, VINSON and SUMMERLIN 1974; GLANCEY, ST. ROMAIN and CROZIER 1976; HUNG and VINSON 1976; ROSS and FLETCHER 1985a). Diploid males in social species are produced largely at the expense of workers, whose labor is essential to optimal colony growth, survival and reproduction (PAGE 1980). Diploid male fire ants, in addition to contributing no useful labor to the colony, consume inordinate amounts of nutriment as brood that would otherwise be used to rear workers or fertile sexuals

(ROSS and FLETCHER 1986). Because the production of diploid males appears to exact a substantial fitness cost, strong frequency-dependent selection is expected to maximize heterozygosity at the sex-determining loci in Hymenoptera so as to minimize the number of diploid males produced (CROZIER 1975; ADAMS *et al.* 1977; YOKOYAMA and NEI 1979).

Levels of heterozygosity at sex-determining loci are governed by allelic diversity as well as by patterns of mating. The number of sex alleles that can be maintained by selection in a population depends upon the joint effects of mutation and drift (YOKOYAMA and NEI 1979), so that finite effective population size imposes an upper limit on allelic diversity. The level of heterozygosity that is actually generated from the multiple sex alleles present in a population is influenced by the extent of inbreeding and substructure in the population (CROZIER 1971; KUKUK and MAY 1990), as well as by any other processes affecting genome-wide heterozygosity. Because sex-determining loci typically are highly polymorphic in Hymenoptera (UNRUH and MESSING 1993; PERIQUET *et al.* 1993), variation at these loci can serve as a sensitive marker of events that reduce population size, such as founder events and other types of bottlenecks, if information on mating systems is also available. Furthermore, the fact that these loci are under strong selection means that the fitness consequences of a bottleneck can be studied with respect to these same markers, with the objective of evaluating the evolutionary fate of a bottlenecked population.

Allelic diversity at sex-determining loci cannot be assessed directly at present but must be inferred from the relative frequencies of queens that produce diploid males or from the frequencies of such males themselves. In the case of a single locus, diploid males are produced by a mother that has mated with a normal haploid male that shares one of her alleles at this locus (termed a "matched mating" by ADAMS *et al.* [1977]):

$$X^i/X^j \times X^j.$$

The probability of such a matched mating occurring (θ) is simply related to the effective number of alleles (K) at the sex-determining locus:

$$\theta = 2/K$$

(ADAMS *et al.* 1977), where $K = 1/\sum x_i^2$ and x_i is the frequency of the i th sex allele (YOKOYAMA and NEI 1979). The effective number of sex alleles should in turn closely approximate the actual number of alleles in a population if the estimate is based on a large sample size (YOKOYAMA and NEI 1979). This is because the expected equilibrium frequency of each allele under strong frequency-dependent selection is the reciprocal of the total number of alleles (LAIDLAW,

GOMES and KERR 1956; WOYKE 1976), that is, all x_i are expected to be equal. Thus, comparative measurements of the frequency of matched matings (θ) in parental and founder populations can provide a means of assessing any loss of allelic diversity at the sex-determining locus due to a bottleneck. Furthermore, because an increase in the frequency of matched matings is expected to lead also to an increase in the proportion of males that is diploid relative to the proportion that is haploid (ϕ), this latter parameter provides another potentially useful measure of sex-allele diversity in a population.

In addition to data for the genetic sex-determining system of *S. invicta*, we also have obtained data for a large number of genetic markers derived from protein electrophoresis. Results from these two sets of markers are complementary for studying the effects of a bottleneck because electrophoretic markers represent structural loci with modest variation that generally are not expected to be under strong selection, whereas sex-determining loci probably represent regulatory genomic elements (WHITING 1961; KERR 1975) that are highly polymorphic and under intense selection. The composite results from these two classes of markers thus may provide a unique perspective on the genetic consequences of a recent founder event.

MATERIALS AND METHODS

Study populations: Specimens of *S. invicta* representing native populations were collected from two sampling localities in northeastern Argentina. One of these is located in Corrientes Province just south of the city of Corrientes, while the other is located in Formosa province and includes the city of Formosa and its environs. The areas over which samples were collected at each locality encompass ca. 150 km², with the closest sampling sites between the two localities ca. 160 km apart. The populations found at these localities, which are separated by a major drainage basin (the Río Paraná), are rather distinct from one another genetically and morphologically (unpublished data). Polygyne nests (which contain multiple fertile queens) occur along with monogyne nests (which contain only a single fertile queen) in these Argentine populations of *S. invicta*, but only nests believed to be polygyne were collected for this study (see GREENBERG, FLETCHER and VINSON [1985] and VARGO and FLETCHER [1987] for criteria that distinguish these two types of nests). Polygyny was confirmed in most of the nests by collecting multiple fertile queens or by observing nestmate genotype distributions inconsistent with monogyny (ROSS and FLETCHER 1985b; ROSS 1993). The level of polygyny within the Argentine nests appears comparable to that seen in some polygynous populations in the USA, judging from the numbers of queens retrieved and a low estimate for the relatedness of female nestmates (unpublished data). Only polygyne nests were studied in Argentina because previous work has shown that reliable estimates of the frequencies of diploid males cannot be obtained from monogyne nests (ROSS and FLETCHER 1986).

Specimens of *S. invicta* representing introduced populations were collected from several localities throughout the southern USA. The most extensive collections came from two populations in northern Georgia, one located in Walton

County that contains predominantly polygyne nests and the other located in Putnam, Jasper and Morgan counties that contains predominantly monogyne nests. (The latter population was not used to estimate frequency of diploid males but rather to study properties of the mating system and heterozygosity at electrophoretic loci. The polygyne and monogyne populations from Georgia have been shown to be very similar genetically, as determined from allele frequencies at 13 polymorphic electrophoretic loci [ROSS and SHOEMAKER 1993].) Samples taken from the two Georgia populations were separated by distances of at least 40 km; samples within each population were taken from areas comparable in size to the sampling localities in Argentina.

To collect samples, large (mature) nests were excavated and the nest soil was spread in white plastic trays in the field to search for specimens of the different castes and life stages. Larvae, pupae, and adults of the worker caste were collected from each excavated nest in the Argentina and Georgia study populations. In addition, both adult winged queens (young, nonfertile individuals that have not mated) and wingless queens (fertile individuals that have mated), as well as pupal and adult males, were collected opportunistically from nests in which they were encountered.

Four additional populations of introduced *S. invicta* from the southern USA that contain predominantly polygyne nests were sampled, but only limited material was collected from each. Adult males were collected from Marion County, Florida; Jackson County, Mississippi; and Tangipahoa Parish, Louisiana (see also ROSS and FLETCHER 1985a); wingless (fertile) queens were collected from Travis County, Texas.

Samples from all of the study populations typically were frozen in the field and held in a dry cryogenic container until they could be transported to the laboratory, where they were stored at -75° in a freezer pending electrophoresis. However, some of the wingless queens collected in their nests at three of the polygyne study populations (Corrientes, Georgia, Texas) were kept alive and taken to the laboratory to study their brood production patterns or to rear their progeny for genetic analyses (see below). Also, newly mated queens not associated with nests were collected from the Georgia monogyne population on the ground immediately after their mating flights and taken alive to the laboratory for the same purposes.

The sample sizes for each part of this study (numbers of nests and numbers of individuals) are listed in the relevant sections below.

Electrophoresis: Electrophoresis was conducted on horizontal gels of 14% starch using previously published methods (ROSS, VARGO and FLETCHER 1987; SHOEMAKER, COSTA and ROSS 1992). Markers representing the products of 78 presumptive loci are included in the present study; the source materials for each of these (life stage, caste and body region) are listed in SHOEMAKER, COSTA and ROSS (1992). These 78 loci constitute a subset of the 110 loci studied by SHOEMAKER, COSTA and ROSS (1992) that encode products that are not unduly expensive or difficult to stain for. Their abbreviations are: *Aat-1*, *Aat-2*, *Acoh-1*, *Acoh-5*, *Acy1*, *Adh-1*, *Ak-1*, *Ak-3*, *Ak-4*, *Ao*, *Apk*, *Ca-1*, *Ddh-1*, *Ddh-2*, *Eno*, *Est-1*, *Est-2*, *Est-4*, *Est-6*, *Fbp-1*, *Fbp-2*, *Fk-1*, *Fumh-2*, *G3pdh-1*, *G3pdh-2*, β -*Gal-2*, β -*Gala*, *Gapdh*, *Gcdh*, β -*Glur*, α -*Glus-1*, α -*Glus-2*, *Gndh*, *Gp-0*, *Gp-1*, *Gp-2*, *Gp-3*, *Gp-4*, *Gp-5*, *Gp-6*, *Gp-7*, *Gp-8*, *Gpi*, *Gr*, *Gtdh-4*, *Hbdh-2*, *Hk-2*, *Hk-3*, *Hk-4*, *Hk-5*, *Iddh-1*, *Idh-1*, *Idh-2*, *Lap-1*, *Lap-3*, *Ldh-1*, *Ldh-2*, α -*Man*, *Mdh-1*, *Mdhp-1*, *Mdhp-2*, *Mpi*, *Odh*, *Pep(gl)*, *Pep(pap)-1*, *Pep(pap)-2*, *Per*, *Pgam-1*, *Pgam-2*, *Pgdh-2*, *Pgdh-3*, *Pgk*, *Pgm-1*, *Pgm-2*, *Pgm-3*, *Pk-1*, *Skdh-2*, and *Sod-2*. Among the 24 loci that are polymorphic in any of the study populations (frequency of the most common allele ≤ 0.95), all 14 that are

polymorphic in the USA and another one that is polymorphic in Argentina have been subjected to progeny studies and thus have been verified to encode products that are inherited in Mendelian fashion (ROSS and FLETCHER 1985b; ROSS *et al.* 1987; ROSS, VARGO and FLETCHER 1988; SHOEMAKER, COSTA and ROSS 1992; unpublished data). The product of one of these polymorphic loci (*Ca-1*) is unstable in storage, so this marker could not be surveyed in most of the study populations. Progeny studies revealed that alleles giving rise to "null" banding phenotypes were segregating at another polymorphic locus (*Est-6*) in one study population only (Corrientes), so this marker was not included in the comparative analyses of allozyme diversity. Finally, strong selection appears to affect genotype frequencies at a third polymorphic locus (*Pgm-3*) in polygyne populations only (ROSS 1992; KELLER and ROSS 1993), so this locus was not used to estimate effective numbers of matings or inbreeding in such populations.

Genetic variation at electrophoretic protein-encoding loci: Larvae, pupae and adults of the worker caste, as well as winged adults of the queen caste, were collected from 30–36 nests in each of the polygyne populations from Corrientes and Formosa and the monogyne population from Georgia. A single genotype per nest was scored at each of 76 electrophoretic protein loci. Single genotypes per nest were scored in order to avoid using nonindependent genotypes in the estimation procedures. (Fire ant colonies represent families of varying complexity [ROSS and FLETCHER 1985b; ROSS 1993], so that any two individuals from a single nest may be related and have correlated genotypes.) The sample sizes of genotypes scored for each locus in the two native populations (Corrientes, Formosa) were always equal to or less than those in the introduced population (Georgia).

Expected heterozygosity (gene diversity) was estimated for each locus (h_{exp}) and for the loci combined (H_{exp}) from the allele frequencies in each population using Equations 8.4 and 8.6 of NEI (1987). The sampling variances of H_{exp} were obtained from Equations 8.7 and 8.8 of NEI (1987), with the 95% confidence intervals about the mean values constructed from the variances by assuming the *t*-distribution. Estimates of H_{exp} were compared between pairs of populations using paired-sample *t*-tests on the h_{exp} values (see ARCHIE 1985). Zero values of h_{exp} were converted to $1/(4n)$, and all values were subsequently angular-transformed for the comparisons (SNEDECOR and COCHRAN 1980; ARCHIE 1985). The sequential Bonferroni procedure (HOCHBERG 1988) was used to evaluate the statistical significance of each pairwise comparison of the heterozygosity estimates.

Proportions of diploid males: For the native populations, 65 pupal males from six nests and 161 adult males from 17 nests were collected from the Corrientes population, whereas 75 pupal males from 10 nests and 326 adult males from 26 nests were collected from the Formosa population. The multilocus electrophoretic phenotype of each male was scored using five (Corrientes) or six (Formosa) polymorphic markers in pupae and nine (Corrientes) or 10 (Formosa) polymorphic markers in adults.

The proportion of males that was diploid relative to the proportion that was haploid (ϕ) was estimated from the electrophoretic data for adults using two methods. First, this proportion was estimated independently for each marker from the observed frequencies of the banding phenotypes. The observed frequency of heterozygote banding patterns, which is a minimum estimate of ϕ , can be adjusted upward to obtain an estimate of the true value of ϕ by the method of maximum likelihood (ROSS and FLETCHER 1985a; PACKER and OWEN 1990; OWEN and PACKER 1993). The log likelihood equation for ϕ was solved algebraically for

loci with two alleles using Equation 1 of OWEN and PACKER (1993). The log likelihood equation for loci with three alleles was solved numerically by means of a grid search algorithm (WEIR 1990), using a starting value derived from Equation 20 of OWEN and PACKER (1993). Because maximum likelihood estimators for ϕ have not been extended to loci with more than three alleles, rare alleles were pooled at such loci to create three allelic classes and the above numerical approach to solving the likelihood equations was employed. An assumption of the maximum likelihood approach used here is that only one offspring per mating is included in the sample (*i.e.*, the genotypes are independent), but this assumption is unrealistic for fire ants because males sampled from single nests may be the offspring of only one or a few queens. Thus, a resampling procedure was instituted whereby single male phenotypes from each nest were sampled at random (with replacement) 200 times, with the mean phenotype frequencies for the 200 resampled distributions then used for the maximum likelihood estimations. The individual-locus maximum likelihood values were jackknifed to give a mean value and variance for ϕ in the native populations; the 95% confidence intervals were constructed from the variances by assuming the *t*-distribution.

The second method employed to estimate proportions of diploid males was simply to examine the multilocus phenotypes of adult males and score individuals with heterozygote banding patterns at any of the loci as diploids. This method is expected to give a reasonable estimate of ϕ in the Argentine populations because of the large numbers of polymorphic markers scored from each adult male.

For the introduced populations, a total of 2779 adult males were collected from 61 nests in four polygyne populations of introduced *S. invicta* located in Florida, Georgia, Mississippi and Louisiana (see ROSS and FLETCHER [1985a] for sample sizes at each site). The electrophoretic phenotypes of each individual were scored using two polymorphic markers. Because examination of the multilocus phenotypes would lead to a considerable underestimate of ϕ with only two moderately variable markers, only the individual-locus maximum likelihood procedure was employed for estimating this parameter in introduced populations. Single male phenotypes from each nest were randomly sampled 200 times to generate mean phenotype frequencies (and their 95% confidence intervals) that were then used in the maximum likelihood equation.

Proportions of diploid-male-producing queens: Live wingless queens were collected from three polygyne populations, the Corrientes population (95 queens from 15 nests), the Georgia polygyne population (172 queens from 53 nests), and the Texas population (88 queens from six nests). All of these nests were confirmed to be polygyne by virtue of containing multiple wingless queens. The queens collected were isolated in small rearing units containing several hundred worker brood and adults and were maintained in the laboratory for 6 weeks to study their brood production patterns (see ROSS and FLETCHER 1985b). Diploid-male-producing queens (DMP queens) produce diploid males among their progeny arising from fertilized eggs because they have had a "matched mating"; that is, they have mated to a male that has an allele in common with them at the sex-determining locus (loci) (ADAMS *et al.* 1977). DMP queens of *S. invicta* can be recognized when held in small laboratory rearing units because a substantial proportion of their brood consists of males after 6 weeks. Queens that have not had a matched mating produce only worker brood in such units (ROSS and FLETCHER 1985a, 1986). The distinctive brood production patterns were used to determine the proportions of DMP queens in the polygyne study populations from

Corrientes, Georgia and Texas. The 95% confidence intervals for the proportions of DMP queens were generated by drawing 200 bootstrap samples from the original data sets, estimating the proportions of DMP queens in each sample, and eliminating the five extreme low and five extreme high values from the ordered array of bootstrap sample estimates (e.g., WEIR 1990).

The proportion of DMP queens was assessed also for the monogyne study population from Georgia, but by somewhat different means. Newly mated queens ($n = 648$) were collected immediately after their mating flights and isolated in the laboratory in small rearing units without workers. Because such queens normally found colonies without the assistance of workers in the wild, they are able to rear their first brood in complete isolation in the laboratory. The appearance of sexualized larvae and male pupae in this first brood indicates that the queen has had a matched mating and is producing diploid sons along with worker daughters (ROSS and FLETCHER 1985a, 1986). The 95% confidence interval about the estimated proportion of monogyne DMP queens was generated from 200 bootstrap samples, in the same way as for the polygyne populations.

That the appearance of male brood in either of the circumstances described above is diagnostic for DMP queens of introduced *S. invicta* has been confirmed previously by genotyping males produced in subsets of the rearing units (ROSS and FLETCHER 1985a, 1986). In the present study, 15 males produced by each putative DMP queen from the native Corrientes population, as well as 15 of each of the queen's daughters, were genotyped at seven polymorphic loci to ascertain that the males were the diploid, biparentally produced sons of the isolated queens.

Effective numbers of queen matings: Forty wingless (fertile) queens collected from 15 polygyne nests in the Corrientes population were established individually in the laboratory in small rearing units containing worker brood and adults. These units were maintained for 6 weeks, at which time 30 worker larvae and 30 worker pupae were collected from each (all brood were certain to be the offspring of the resident queens by this point). Larval genotypes were determined at three polymorphic electrophoretic loci and pupal genotypes at an additional three loci. Average genetic relatedness of the offspring of single queens (r) was estimated from the distribution of genotypes at these six loci using the procedure of QUELLER and GOODNIGHT (1989), and the effective mean number of matings per queen (M_e) was estimated from the relatedness values using Equation 5 of ROSS (1993).

Worker larvae and pupae, as well as adult winged (non-fertile) queens, were collected from 55 nests in the Georgia monogyne population. Larval genotypes were scored at three loci (1188 individuals from 44 nests), pupal genotypes at an additional three loci (868 individuals from 31 nests) and adult queen genotypes at another six loci (943 individuals from 41 nests). Because each nest was monogynous (had a single fertile queen), the individuals collected from single nests are presumed to be the offspring of the same mother. Thus, M_e was calculated from the values of genetic relatedness (r) estimated for female nestmates in this population. The mean values and variances of r and M_e in both the Corrientes and Georgia populations were estimated by jackknifing over the single-locus values, with the 95% confidence limits generated by assuming the t -distribution.

Inbreeding coefficients: Inbreeding coefficients (F) were estimated for the two Argentine populations using the genotypic data from which heterozygosity was calculated (single genotype scored per locus per nest). Data used to estimate F for the two study populations from Georgia included

multiple genotypes per locus per nest. Sample sizes for the Georgia monogyne population are as given above for the estimation of effective number of matings. For the Georgia polygyne population, genotypes of 2728 individuals from 31 nests were determined (see ROSS [1993] for additional information on these samples). To avoid using nonindependent nestmate genotypes to estimate F in the Georgia populations, single genotypes from each nest were randomly sampled 50 times (with replacement) and values of F were calculated independently for each allele for each of the resampled data sets using the method of WEIR and COCKERHAM (1984). Overall values of F and its variance were obtained by summing variance components and jackknifing over loci, as suggested by WEIR and COCKERHAM (1984), using the means of the 50 estimates of F for each allele in the computations for the Georgia populations. Only $n - 1$ of the n alleles present at a particular locus at frequencies of 0.05–0.95 were used in the analyses for each population (to account for the nonindependence of estimates of F derived from different alleles at a single locus). Values of F are based on data from 19 alleles at 14 loci in the Corrientes population, 18 alleles at 15 loci in the Formosa population, 13 alleles at 12 loci in the Georgia monogyne population, and 11 alleles at 10 loci in the Georgia polygyne population.

RESULTS

Genetic sex-determining system: Estimates of the proportions of *S. invicta* males that are diploid (ϕ) in the native and introduced study populations are presented in Figure 1. The mean individual-locus maximum likelihood estimates of ϕ for adult males in the two native populations are 9.8% in Corrientes and 16.4% in Formosa. Estimates of ϕ derived from examination of multilocus genotypes are similar to the maximum likelihood estimates (7.7% in Corrientes and 20.5% in Formosa). In sharp contrast to these relatively low proportions in native populations, diploid males predominate among the males surveyed in the four introduced populations in the USA. The maximum likelihood estimates of ϕ for the two marker loci surveyed range from 73.3–100%, with a mean value of 90.9% across all four populations and both loci.

The large differences in proportions of diploid males between native and introduced populations may have been inflated if monogyne nests were included inadvertently among the nests surveyed in Argentina. The reason is that monogyne colonies headed by DMP queens invariably die during the founding stage, so that all males produced by mature monogyne nests are necessarily haploid (ROSS and FLETCHER 1985a, 1986). Therefore we reestimated ϕ in the Formosa population by considering only the 15 nests from which two or more fertile queens (mean = 7.0 queens) were collected—that is, those nests that could unquestionably be confirmed to be polygyne (an insufficient number of such nests occurred in the Corrientes sample to warrant reanalysis). The estimates of ϕ for this subset of nests from Formosa are slightly lower than for the entire set of nests using either the

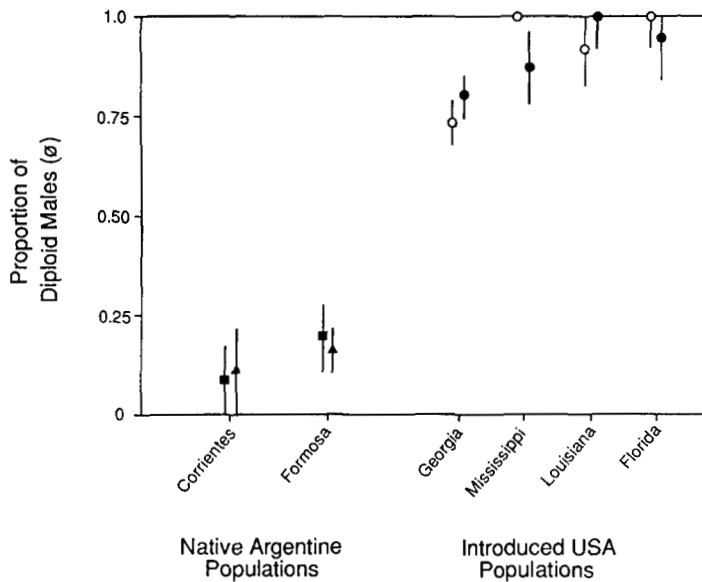


FIGURE 1.—Proportions of males that are diploid (ϕ) in two native and four introduced populations of polygyne *S. invicta*. Lines represent the 95% confidence intervals about the point estimates. The number of polymorphic markers used to assess male ploidy was 9 in Corrientes, 10 in Formosa and 2 in each of the USA populations. Data for the USA populations are modified from ROSS and FLETCHER (1985a). ■, estimates based on examination of multilocus genotypes; ▲, means of maximum likelihood estimates from single loci; ○, maximum likelihood estimates from *G3pdh-1*; ●, maximum likelihood estimates from *Est-4*.

maximum likelihood procedure or examination of the multilocus genotypes, indicating that the differences in frequency of male diploidy between native and introduced populations are not artifacts attributable to the inclusion of monogyne nests in the Argentine samples.

Male pupae were not collected from a sufficient number of nests in the Argentine populations to generate sound estimates of ϕ for the pupal stage. Nonetheless, comparison of male pupal and adult genotypes in nests containing both stages revealed that nests with confirmed adult diploids invariably also contained diploid pupae, whereas other nests contained only haploid pupae. Thus, estimates of ϕ for adult males appear to reflect the proportions of diploid males actually reared in polygyne populations (that is, any selective destruction of diploid males by workers as these males mature would not seem to affect the estimates greatly; see also ROSS and FLETCHER 1986). We conclude that the disparity between native and introduced populations in the proportions of diploid males produced relative to haploid males is likely to signal a corresponding disparity in genetic variation maintained at the sex-determining locus (loci) in the two types of populations.

A more direct measure of variation in the genetic sex-determining system is the proportion of mated queens that produces diploid males in a population (proportion of DMP queens). This measure is especially useful if certain properties of the mating system, such as effective number of matings by queens and level of inbreeding, are known. Data concerning both of these aspects of the mating system are available for native and introduced *S. invicta*. Estimates of genetic relatedness of the female offspring of single queens indicate that the effective number of matings per queen (M_e) in both native and introduced populations

TABLE 1

Estimates of genetic relatedness and effective number of matings by queens for native (Argentina) and introduced (USA) populations of *S. invicta*

	Corrientes, Arg.	Georgia, USA (monogyne pop.)
Relatedness of female offspring of single queens (r)	0.726 (0.675–0.777)	0.749 (0.710–0.788)
Effective number of matings by queens (M_e)	1.05 (1.00–1.17)	1.00 (1.00–1.09)

Values are estimated on the basis of genotypic data from six (Corrientes) or 12 (Georgia) polymorphic loci. The 95% confidence intervals about the means are shown in parentheses. The lower limits of these intervals are truncated to one for effective number of matings.

is one or very nearly one (Table 1). This result, which substantiates earlier findings that fire ant queens typically mate only once (ROSS and FLETCHER 1985b; ROSS, VARGO and FLETCHER 1988), is useful for two reasons. First, proportions of DMP queens can be compared directly between the two types of populations to assess relative amounts of genetic variation in the sex-determining system, without concern for the confounding effects of differing numbers of matings (*e.g.*, PAGE 1980). Second, the proportion of DMP queens in a population is identical to the proportion of matched matings (θ) if queens effectively mate only once.

Proportions of DMP queens (or θ in the case of $M_e = 1$) are shown for four study populations in Figure 2. Only 3 of 95 queens (3.2%) from the native Corrientes population produced diploid males. Genotype distributions of these males corresponded completely to those of their sisters at seven polymorphic loci, confirming that these males were diploid and were produced biparentally via normal sexual reproduction. In contrast to the rarity of DMP queens in the

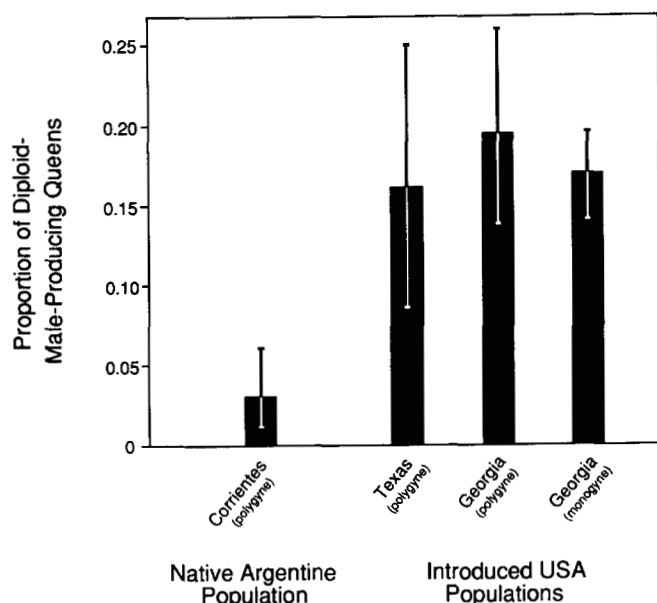


FIGURE 2.—Proportions of DMP queens in one native and three introduced populations of *S. invicta*. Bars represent the 95% confidence intervals for the proportions. The data are derived from newly mated queens collected immediately after their mating flights in the single monogyne population and from fertile, actively reproducing queens collected within their nests in the polygyne populations. A portion of the data for the two Georgia populations is from ROSS and FLETCHER (1985a, 1986).

native population, 154 of 908 queens (17.0%) from the introduced populations produced diploid males. The variation in proportions of DMP queens across the introduced populations is small and statistically insignificant ($\chi^2 = 1.18$; d.f. = 2; $P > 0.5$), whereas the difference between the native and introduced populations is highly significant ($\chi^2 = 13.7$; d.f. = 3; $P < 0.005$). Thus, matched matings are considerably more likely to occur in introduced than in native populations of fire ants. These data again point to the conclusion that there is a substantially lower diversity of sex alleles in the introduced than in the native populations.

Proper interpretation of the parameter θ , the proportion of matched matings, in terms of sex-allele diversity requires knowledge of any departures from panmixis in the populations. Estimated values of the inbreeding coefficients (F) derived from all of the polymorphic markers in two introduced and two native populations are shown in Table 2. These values are small and do not differ significantly from zero except for the Corrientes population. The significant positive value of F estimated for this native population suggests the presence of local inbreeding due to population subdivision or consanguineous matings. Its importance for the present analyses stems from the fact that it may cause sex-allele diversity in Corrientes to be underestimated when the proportions of diploid males or DMP queens are used to assess this diversity. Inbreeding increases the homozygosity of offspring

TABLE 2

Estimates of inbreeding coefficients (F) for native (Argentina) and introduced (USA) populations of *S. invicta*

Corrientes, Arg.	Formosa, Arg.	Georgia, USA (monogyne pop.)	Georgia, USA (polygyne pop.)
0.165	0.003	0.036	-0.016
(0.067-0.262)	(-0.162-0.168)	(-0.033-0.105)	(-0.057-0.026)

The 95% confidence intervals about the estimates are shown in parentheses.

because genotypes of mates are correlated; thus, inbred populations are expected to have higher frequencies of matched matings than outbred populations with equivalent allelic diversity at the sex-determining locus (loci).

The extent to which sex-allele diversity in Corrientes is underestimated can be measured by making use of the fact that inbreeding increases genetic relatedness within families relative to the case in which there is no inbreeding. This is shown by the relationship:

$$r^* = [r - 2F/(1 + F)] / [1 - 2F/(1 + F)],$$

where r and F are the empirically determined values of relatedness and inbreeding, respectively, and r^* represents the relatedness expected within families in the absence of inbreeding (PAMILO 1991). The value of $r^* = 0.618$ that we obtain when our empirical estimates of r and F for Corrientes are used in this formula corresponds to a corrected value for the effective number of queen matings of $M_e^* = 1.36$. The proportion of matched matings (θ) is no longer equivalent to the proportion of DMP queens when $M_e > 1$, but θ can be estimated in this case by dividing the proportion of DMP queens by M_e^* . The estimate of θ so obtained for Corrientes is 2.3%, a small drop from the value of 3.0% obtained when inbreeding is not taken into account (Table 3). Thus, correction of θ for inbreeding in Corrientes further increases the disparity between this native population and the introduced populations in this measure of variation in the genetic sex-determining system.

Potentially more useful measures of such variation, and of its significance for the adaptive capacity of populations, are the effective number of alleles (K) segregating at the sex locus (loci) and the segregational genetic load (L) imposed on a population by the presence of diploid males. Estimates of these parameters for single-locus and two-locus sex-determining systems are presented along with their corresponding θ values in Table 3 for the Corrientes population and three introduced populations in the USA. Estimates of K for the two-locus system, where K is now the effective number of alleles at each locus, assume that the loci are in linkage equilibrium, that the numbers of alleles are the same at each locus, and that the frequencies of these alleles are $1/K$. The segregational

TABLE 3

Estimates of proportion of matched matings, effective number of sex alleles and segregational genetic load for native (Argentina) and introduced (USA) populations of *S. invicta*

	Corrientes, Arg.	Corrientes, Arg. ^a	Texas, USA	Georgia, USA (monogyne pop.)	Georgia, USA (polygyne pop.)
Proportion of matched matings (θ)	0.030 (0.010–0.060)	0.023 (0.008–0.046)	0.159 ^b (0.080–0.250)	0.164 (0.136–0.193)	0.198 ^c (0.134–0.267)
No. of sex alleles (K , single locus)	66.5 (33.3–199)	86.1 (43.1–258)	12.6 (8.00–25.1)	12.2 (10.4–14.7)	10.1 (7.48–15.0)
No. of sex alleles (K , two loci)	12.0 (8.62–20.5)	13.6 (9.75–23.3)	5.45 (4.43–7.55)	5.38 (4.99–5.87)	4.93 (4.29–5.91)
Segregational load (L , single locus)	0.010 (0.003–0.020)	0.008 (0.003–0.015)	0.053 (0.027–0.083)	0.055 (0.045–0.064)	0.066 (0.045–0.089)
Segregational load (L , two loci)	0.005 (0.002–0.009)	0.003 (0.001–0.007)	0.022 (0.012–0.034)	0.023 (0.019–0.027)	0.027 (0.019–0.036)

The 95% confidence intervals about the estimates are shown in parentheses. These are derived for all parameters from the confidence intervals generated for the proportion of DMP queens by means of bootstrapping (see text). The proportion of matched matings by queens (θ) is equal to the proportion of DMP queens when the effective number of matings per queen (M_e) is one. Otherwise, θ is equal to the proportion of DMP queens divided by M_e .

^a Parameter estimates are corrected for the effects of inbreeding.

^b Assumes $M_e = 1$ and no inbreeding, as has been shown for other polygyne *S. invicta* populations in the USA (ROSS and FLETCHER 1985a, 1985b; ROSS, VARGO and FLETCHER 1987; ROSS 1993; this study).

^c Queens in this polygyne population typically mate with single males (ROSS and FLETCHER 1985a, 1985b), so M_e is assumed to equal one.

load, which represents the proportionate reduction in fitness of the average genotype relative to the most fit genotype in solitary organisms (SPIESS 1989), is calculated as the probability of an individual being homozygous at the sex locus (loci), multiplied by two-thirds, the selection coefficient suggested by YOKOYAMA and NEI (1979) in the case where haploid males are not subject to selection and diploid males have zero fitness. It should be noted that the concept of genetic load is difficult to interpret for highly social organisms such as ants because the reduced fitness of an individual affects the fitness of other colony members in complex ways. Nonetheless, we assume that this parameter has some biological meaning since increased production of diploid males in polygyne nests must lead to a corresponding reduction in the number of fertile sexuals produced (the situation is different in monogyne nests—see ROSS AND FLETCHER 1986). Here we calculate L with the aim of comparing the relative cost of producing diploid males among populations that differ in the number of sex alleles.

The magnitude of the founder effect resulting from colonization of the USA on sex-allele diversity in *S. invicta* is considerable. The introduced populations appear to have suffered a loss of at least 80% of their allelic diversity if a single major locus determines sex, and at least 50% if two loci are involved (Table 3). This loss of alleles generates a six- to eight-fold increase in the estimated segregational load associated with diploid male production in the introduced populations over that in the native population, regardless of whether one or two loci are assumed to be involved.

Electrophoretic protein-encoding loci: Estimates of the expected heterozygosity (H_{exp}) based on 76 loci

TABLE 4

Estimates of expected heterozygosity (H_{exp}) for native (Argentina) and introduced (USA) populations of *S. invicta*

Corrientes, Arg.	Formosa, Arg.	Georgia, USA (monogyne pop.)
0.062 (0.056–0.067)	0.062 (0.057–0.067)	0.048 (0.043–0.053)

The 95% confidence intervals about the estimates are shown in parentheses.

are presented for two native and one introduced population in Table 4. The values for the two native populations are identical, while H_{exp} is somewhat less in the introduced population. Although the 95% confidence intervals derived from the sampling variances do not overlap between the introduced and native populations, the differences in H_{exp} are not judged to be significant for either comparison using one-tail paired-sample t -tests (both $P > 0.1$ with the sequential Bonferroni procedure for multiple comparisons).

The numbers of electrophoretic alleles that occur at various frequencies are depicted graphically for these same three populations in Figure 3. The total numbers of alleles detected at all 76 loci are 120 and 115 in the native Corrientes and Formosa populations, respectively, whereas only 96 alleles were found at the same loci in the introduced Georgia monogyne population. These differences in protein allele richness between the native and introduced ants are attributable primarily to a decreased abundance of rare alleles in the introduced population compared with the native populations. Differences between the Argentine and USA ants in proportions of alleles that are rare are statistically significant, regardless of whether rare

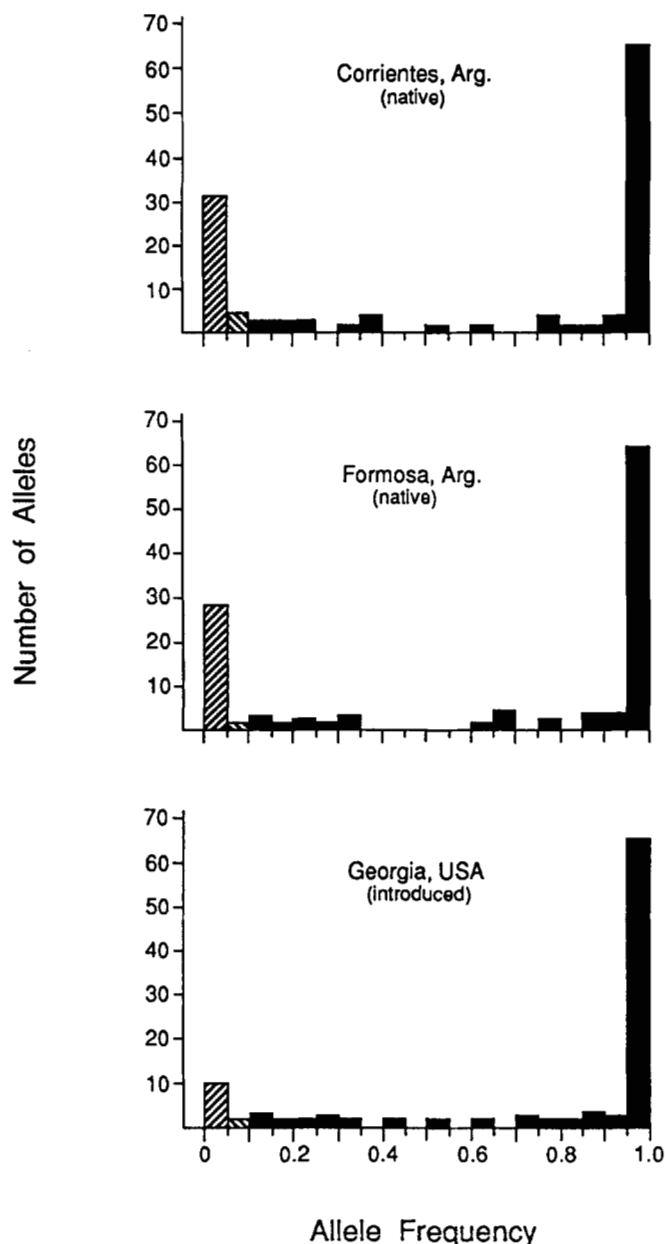


FIGURE 3.—Numbers of alleles of different frequency classes detected in two native populations and one introduced population of *S. invicta*. Rare alleles (present at frequencies of 0.10 or less, or at 0.05 or less) are indicated by cross-hatching. The alleles occur at 76 protein-encoding loci surveyed electrophoretically in 30–36 individuals from each population.

alleles are defined as those present at frequencies of 0.05 or less (Corrientes *vs.* Georgia: $\chi^2 = 8.20$, d.f. = 1, $P < 0.005$; Formosa *vs.* Georgia: $\chi^2 = 6.90$, d.f. = 1, $P < 0.01$) or are defined as those present at frequencies of 0.10 or less (Corrientes *vs.* Georgia: $\chi^2 = 9.98$, d.f. = 1, $P < 0.005$; Formosa *vs.* Georgia: $\chi^2 = 6.45$, d.f. = 1, $P < 0.02$). The proportions of rare electrophoretic alleles in the study populations inversely reflect the proportions of diploid males and matched matings (Figure 4); this relationship between number of rare protein alleles and frequency of dip-

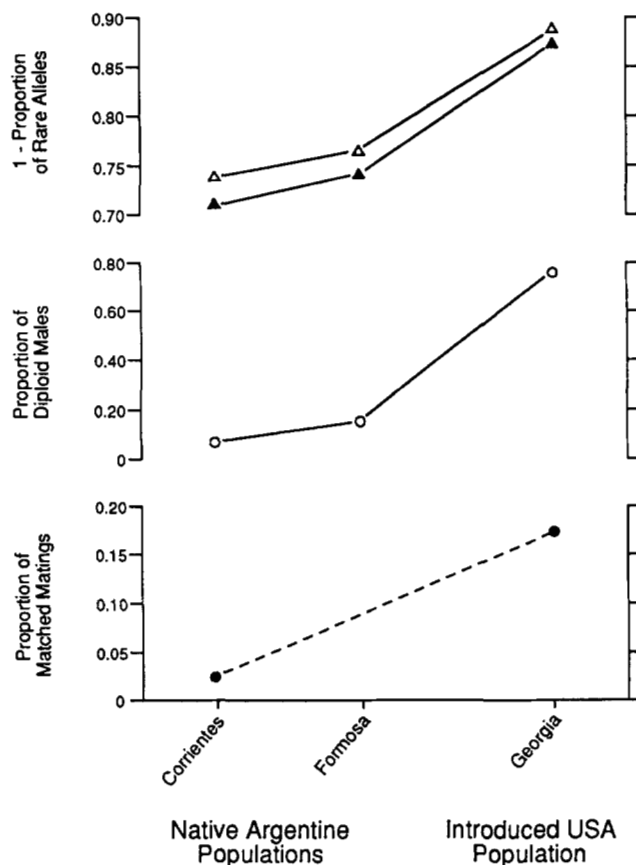


FIGURE 4.—Relationship between proportion of rare electrophoretic alleles (expressed as 1 - the proportion of rare alleles), proportion of males that are diploid and proportion of matings that are matched in two native and one introduced population of *S. invicta*. Data on proportion of matched matings are not available for the Formosa population. Δ , electrophoretic alleles present at a frequency of 0.05 or less; \blacktriangle , electrophoretic alleles present at a frequency of 0.10 or less.

loid males is apparent even within Argentina, where the differences between the populations are slight. Such a relationship may be explained by the fact that losses of rare protein alleles and of sex alleles (each of which is rare) should be similarly determined by effective population size.

DISCUSSION

The introduction of the fire ant *S. invicta* to the USA represents a well-documented founder event in which the founder population, presumably consisting initially of only a few reproductive individuals, successfully established itself, underwent tremendous subsequent population growth, and rapidly expanded its new range. Some 60 years have passed since the inception of this event (LOFGREN 1986), which corresponds to 30–60 generations in this ant (MARKIN, DILLIER and COLLINS 1973; VARGO 1988). To better understand the nature and extent of the founder effect associated with this recent introduction, we have compared genetic diversity at two classes of

markers between native and introduced populations.

Our estimates of the proportions of males that are diploid (ϕ) reveal that diploid males are far more common in introduced than in native populations. Barring consistent differences in the tendencies of native and introduced ant workers to destroy such males, this pattern is most reasonably attributed to differences in allelic variation at the sex-determining locus (loci). If differences in sex-allele diversity are indeed the explanation for higher frequencies of male diploidy in introduced than in native fire ant populations, then the proportion of matched matings by queens (θ) also is expected to be significantly elevated in introduced populations, as we have found. The increased frequencies of matched matings in the USA do not result from inbreeding or population subdivision in the introduced populations (that is, via mates having correlated genotypes), reinforcing the conclusion that they stem from a loss of sex alleles during the colonization process. This loss of sex alleles may have occurred principally during the initial stages of establishment of *S. invicta* in the USA, rather than as a result of secondary founder events associated with the rapid spread of the ants. The introduced populations in the USA are relatively continuous across the new range and gene flow rates appear to be high (ROSS, VARGO and FLETCHER 1987), which would tend to equalize sex-allele diversity across large regions. The low variances in estimates of ϕ and of θ across several widely separated populations in the USA support this conclusion.

The loss of sex alleles in introduced fire ants has been paralleled by a loss of electrophoretically detectable rare alleles at protein loci. The explanation for these parallel results is that there are many sex alleles in the native populations, each of which is expected to be maintained at low frequency due to frequency-dependent selection (the equilibrium frequency of each of K alleles is $1/K$ —WOYKE 1976; YOKOYAMA and NEI 1979). Thus, both rare electrophoretic alleles and alleles at sex-determining loci are particularly susceptible to loss during population bottlenecks because of their low frequencies in the source population.

The bottleneck associated with establishment of fire ants in the USA did not produce a statistically detectable drop in overall heterozygosity (H_{exp}) at the electrophoretic loci that were surveyed. One reason may be that the 18 or 19 polymorphic loci surveyed in the native populations are not enough to have a high probability of detecting a drop in estimated heterozygosity for anything but the most severe of bottlenecks (see MCCOMMAS and BRYANT 1990; LEBERG 1992). This problem is compounded by the fact that heterozygosity is relatively low in all of the study populations (a general feature of populations of social

Hymenoptera—see SHOEMAKER, COSTA and ROSS 1992). Aside from these statistical problems, variant alleles present at moderate frequencies, which contribute substantially to heterozygosity, are less likely to be lost during a bottleneck than are rare alleles, which contribute little to heterozygosity, if population size rebounds quickly (NEI, MARUYAMA and CHAKRABORTY 1975). Thus, it is possible with even a large number of variable markers to observe the loss of a large fraction of rare alleles but only a modest or no drop in estimates of heterozygosity. The requirements for this scenario, that the bottleneck was not too severe (*i.e.*, was not less than a few reproductive individuals) and that post-bottleneck population expansion was rapid, may be fulfilled in the case of the introduction of *S. invicta* to the USA (LOFGREN 1986).

These data reinforce the conclusion of LEBERG (1992) and others that average heterozygosity can be a relatively insensitive measure for detecting many bottlenecks or other types of changes in effective population size. On the other hand, counts of rare alleles at electrophoretic loci or at other loci harboring large numbers of alleles (such as sex-determining loci, major histocompatibility complex loci, or self-incompatibility loci in plants) are expected to be very sensitive measures for this purpose. The sensitivity of rare alleles as indicators of effective population size may explain the parallel results for proportions of diploid males and numbers of rare electrophoretic alleles in the two Argentine populations; both measures suggest a somewhat higher current or historical effective population size in Corrientes than in Formosa.

The loss of rare alleles following a bottleneck, although perhaps not influencing greatly the average heterozygosity of individuals, may substantially deplete the allelic richness in a population that is available for natural selection to draw upon and thus is of concern with regard to the adaptive evolutionary potential of founder populations. In the case of loci encoding structural proteins such as enzymes, it is difficult to evaluate the fitness effects of a reduction in allelic richness, given that the extent to and manner in which these loci respond to selection is largely unknown. On the other hand, the fitness consequences of a loss of alleles at the sex-determining loci of Hymenoptera are expected to be important. The segregational load associated with the genetic sex-determining system of *S. invicta* apparently increased substantially following establishment of this ant in the USA, an increase that in some circumstances might be expected to jeopardize the long-term persistence of colonizing populations. Indeed, the failure of many attempts to establish non-native parasitoid Hymenoptera as agents of biological control has been attributed to such an increase in genetic load stemming from the loss of sex alleles (STOUTHAMER, LUCK and WERREN

1992). The success of *S. invicta* as a colonizer in the face of increased genetic load caused by the loss of sex alleles may be related to its release from natural enemies and competitors that would normally check the growth of native populations (JOUVENAZ 1983), as well as to the abundance of disturbed habitats available for this ant to colonize in the USA (TSCHINKEL 1986).

This study reveals how a recent population bottleneck has affected genetic variation at two different types of genetic markers. It is of special interest because it presents estimates of the numbers of sex alleles segregating in native and colonizing populations of a eusocial hymenopteran, thereby allowing calculation of the relative cost to recently bottlenecked populations of losing variation in the sex-determining system. It is of significance also in that it further demonstrates the usefulness of rare alleles as sensitive markers of changes in effective population size, changes that are of foremost importance with respect to both the evolutionary history and evolutionary potential of natural populations.

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