

# Comparative study of genetic and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae)

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**Summary.** The genetic and social structures of polygyne and monogyne forms of the fire ant, *Solenopsis invicta*, are investigated in a comparative manner using allozyme data from two polymorphic loci. Foundress queens of the monogyne form are singly inseminated and appear to produce all males present in the colony during the major summer mating flights. The average regression coefficient of relatedness ( $b$ ) among female nestmates of the monogyne form is 0.714 (Fig. 2), statistically indistinguishable from the pedigree coefficient of relatedness ( $G$ ) of 0.75. We suggest that the evolution of obligate worker sterility in *Solenopsis* is associated with this high relatedness between workers and the queens they rear. Functional queens in polygyne nests also are singly inseminated and are no more closely related to nestmate queens than to other queens (within-nest  $b=0$ ). Within-nest relatedness of workers in the polygyne population is similarly low (Fig. 2). Both the monogyne and polygyne populations from north-central Georgia are in Hardy-Weinberg equilibrium at both allozyme loci and we found no evidence of significant population subdivision or inbreeding in the polygyne population. These results do not support the view that kin selection has promoted the evolution of polygyny in North American *S. invicta*. Rather, mutualism appears to be the most likely selective factor mediating queen associations in this ant.

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## Introduction

A fundamental problem in evolutionary biology is understanding the modes and mechanisms of speciation. This problem is particularly acute in the social Hymenoptera, where speciation may be

especially rapid (Crozier 1977) due to low effective population size, male haploidy, or inbreeding (Hartl 1971; Crozier 1979). Speciation in social Hymenoptera has frequently been found to be associated with changes in social structure (Wilson 1971; Crozier 1979; Brian 1983), the most notable cases being the evolution of social parasites from free-living forms and the evolution of multiple-queened from single-queened societies. While the ecological consequences of such events are rather well documented (Wilson 1971; Hölldobler and Wilson 1977; Elmes 1978), the accompanying genetic changes at the population and colony level are at present only poorly understood.

Among the ants (Formicidae), pairs or sets of sibling species exhibiting contrasting social organizations are widespread. Such sibling forms are presumed to represent the products of recent speciation events. Since its introduction to North America over 40 years ago, the fire ant, *Solenopsis invicta*, has apparently given rise to a new form on repeated occasions (Fletcher et al. 1980; Fletcher 1983). This new form differs most conspicuously in that nests are always permanently polygyne (many functional queens), whereas colonies of the common form of the ant are monogyne (one functional queen). The polygyne form differs in other attributes of its biology as well, including relatively close spacing of nests (Fletcher 1983) consistent with polycaly (occupation of multiple nests by a single colony). In order to better understand the genetic changes associated with this shift in social structure, we have undertaken a comparative study of the biochemical genetics of an isolated, recently established polygyne population of *S. invicta*, the history of which is well known, and a nearby monogyne population. In conjunction with behavioral and ecological studies these data should further our understanding of the evolution of social

structure and the process of speciation in ants and other social Hymenoptera.

## Methods

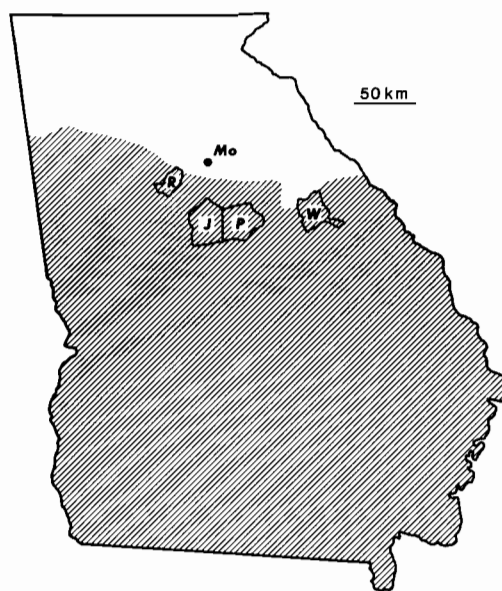
**Colony collection.** Colonies of the monogyne (M) form of *S. invicta* were collected during the period October 1983–June 1984 from four counties in north-central Georgia, USA (Fig. 1). Polygyne (P) nests were sampled during the same period from an isolated population also in north-central Georgia. Nests were collected at random from all areas of this population to secure a representative sample. The P population covers ca. 5 km<sup>2</sup> and is located ca. 15 km north of the limit of distribution of the M population (Fig. 1; Fletcher 1983). Colony inhabitants were removed from the soil by slow flooding (Jouvenaz et al. 1977) and transferred to artificial nests in plastic trays. These were maintained in a large rearing room (29 ± 2° C, 14/10 h light/dark regime) for periods of from one day to three months before sampling. Colonies were fed artificial diet (Banks et al. 1981) supplemented with mealworms, crickets, and baby food (mixed vegetable).

**Electrophoresis.** Electrophoresis was conducted on horizontal starch gels (12% starch, 1:1 ratio of Electrostarch and Connaught starch) using standard methodology (Utter et al. 1974; Harris and Hopkinson 1976; May et al. 1979; Ross et al. 1985). The gel and electrolyte buffer systems were those of Clayton and Tretiak (1972) (electrode buffer – 0.04 M citric acid adjusted to pH 6.1 with N-(3-aminopropyl)morpholine; diluted 1:9 for gel buffer).

Two enzyme loci, designated *Alpha-glycerophosphate dehydrogenase-1* (*Agp-1*, E.C. No. 1.1.1.8) and *Esterase-4* (*Est-4*, E.C. No. 3.1.1.1), are polymorphic in *S. invicta* (Ross et al. 1985; Ross and Fletcher 1985; Hung and Vinson 1976; Hung et al. 1979) and were used as genetic markers in this study. *Agp-1* was stained following the method of Shaw and Prasad (1970); the product of this locus in *S. invicta* behaves as a dimer and migrates anodally (at pH 6.1). *Est-4* was stained using the method of Johnson et al. (1969, their locus *Esh*). This esterase behaves as a monomer in *S. invicta* and migrates cathodally. Both loci are diallelic in the study populations, with the alleles co-dominant. This model was confirmed by the presence of only a single staining band at each locus for all haploid males ( $n = 3451$  genotypes observed). Mendelian inheritance of the alleles was confirmed by studying known male (haploid) and female (diploid) offspring of queens, as discussed below. The two alleles of *Agp-1* have previously been designated F and S (Hung et al. 1979), while we designate the alleles of *Est-4* as A and B. To test for linkage between the two loci, the recombination fraction ( $r$ ) was calculated for the gametes (sons) of five M queens heterozygous at both loci (Hartl 1980;  $\bar{x} = 14.0$  sons/queen). The mean value of  $r$  for the five queens is 0.517 (range 0.364–0.667), indicating no linkage.

**Sampling and progeny studies.** To study colony genetic structure, foundress queens and samples of female and male sexuals (when present) were removed from 55 M colonies for electrophoresis. Foundresses were recognized by their degree of physogastry. In instances of questionable identity, queens were dissected in ice water to confirm insemination, then immediately frozen. A mean of 26.5 alate females (range 11–78) and 57.5 males (range 7–278) was sampled from each M colony in which these were found. All sampled ants were held at –40° C until electrophoresis.

To determine whether P queens of *S. invicta* are singly or multiply inseminated, 27 dealate (functional) queens from 21



**Fig. 1.** Collection areas for monogyne and polygyne *S. invicta* in north-central Georgia, USA. Stippling indicates present range of the monogyne form in Georgia. Monogyne colonies were collected in four counties; Jasper (J), Putnam (P), Rockdale (R), and Warren (W). The polygyne population is located in Monroe, Georgia (Mo)

P nests were kept individually in artificial nests in the laboratory. Each queen was given 3 ml of brood and workers from the parent colony and the resulting units were maintained in the rearing room for at least 50 days. Teneral adults and pupae were then removed and held for another two weeks in smaller nest units, at which time enzyme activity was high. This procedure also assured that the individuals subjected to electrophoresis were the progeny of the isolated P queens, as the developmental period for sexuals under similar conditions is ca. 34 days (O'Neal and Markin 1975). Developmental periods for workers are considerably shorter. Alate females were sampled when these were present, but most units had produced only workers at the time of sampling. A mean of 50.5 females (range 11–141) from each unit and all P queens producing these females were subjected to electrophoresis.

To study the relatedness of dealate queens in P colonies, a mean of 48.5 (range 24–150) of these was sampled from 26 colonies and subjected to electrophoresis. The within-nest regression coefficients of relatedness ( $b$ ) were calculated (Pamilo and Crozier 1982) using allozyme data from the two polymorphic loci with the nests weighted equally. The relatedness of workers in P nests was similarly calculated using major workers sampled from 20 colonies (mean 41.3 per nest, range 32–44). Within-nest relatedness coefficients were also calculated for alate females from all M colonies collected ( $n = 55$ ). Relatedness values were compared by calculating the 95% confidence limits for the regression coefficients from the standard error of the estimates based on the  $t$  distribution (Snedecor and Cochran 1980; see Pamilo and Crozier 1982 for method of calculating variance of  $b$ ). Relatedness values are considered significantly different when no overlap of the confidence limits occurs.

Genetic structure was further analyzed by calculating the genotype fixation index ( $F_{IS}$ ), population inbreeding coefficient ( $F_{IT}$ ), and standardized genetic variance ( $F_{ST}$ ) for each locus (Nei 1977; Wright 1978; Pamilo and Crozier 1982; Pamilo 1982, 1983).  $F_{IS}$  and  $F_{IT}$  are measures of the deviations of

genotypic frequencies from Hardy-Weinberg proportions in subpopulations (nests) and the entire population, respectively, while  $F_{ST}$  is a measure of the extent of genetic differentiation of subpopulations (nests) (Nei 1977).

Genotype and allele frequencies for the two populations were calculated from the genotypes of the Mendelian (reproductive) portions of the populations only. For the M population this included only newly mated queens ( $n=106$ ) and foundresses from mature colonies whose genotypes were known or could be inferred from progeny analyses ( $n=54$  for the locus *Agp-1* and 45 for the locus *Est-4*). Newly mated M queens were collected immediately following three nuptial flights at two widely separated locations. For the P population the genotypes of all dealate queens sampled from 31 P nests were used in the calculations ( $n=1460$  queens for *Agp-1* and 1053 queens for *Est-4*).

## Results

Genotype distributions for female alates from M colonies indicate that foundress queens of this form are singly inseminated. Only one or two genotypic classes of female alates are present in each of the 55 study colonies for each marker locus. In colonies with two genotypic classes, one is always the heterozygote class and the two classes segregate in a ratio indistinguishable from a 1:1 ratio in 51 of 52 (98.1%) instances ( $n=26$  colonies for *Agp-1* and 26 colonies for *Est-4*, binomial confidence probability  $>0.95$  for each). Genotype distributions for female alates sampled from four representative M colonies are presented in Table 1.

Genotype distributions for the female progeny of individual P queens resemble those for the progeny of M queens. Only one or two genotypic classes are represented among the progeny of each queen; when two classes are present they occur in a 1:1 ratio ( $n=12$  for *Agp-1* and 15 for *Est-4*). Thus, P queens are also singly inseminated.

Twenty-three of the 33 M colonies with males contain foundresses which are heterozygous at one or both marker loci. The two genotypic classes of male progeny at each locus are present in a 1:1 ratio in 27 of 28 (96.4%) instances ( $n=14$  colonies for *Agp-1* and 14 colonies for *Est-4*), further confirming the Mendelian inheritance of the allozyme markers.

Fifteen M colonies containing males are appropriate for studying their maternity because foundresses are homozygotes at one or both marker loci and are mated to males possessing the alternate allele(s). Thus, all daughter alates are heterozygotes and any males produced by these should occur in a 1:1 ratio of the two possible haploid genotypes (Crozier 1974). By contrast, all males produced by the queen will possess the allele for which she is homozygous. The 15 colonies were collected in October 1983 ( $n=7$ ) and June 1984

**Table 1.** Genotype distributions for alate female (♀) and male (♂) progeny, foundress queens (Q), and inferred mates (♂) of foundresses from four monogyne *S. invicta* colonies collected in June 1984 in north-central Georgia, USA

Colony		<i>Agp-1</i>			<i>Est-4</i>		
		<i>FF</i> <sup>a</sup>	<i>FS</i>	<i>SS</i> <sup>a</sup>	<i>AA</i> <sup>a</sup>	<i>AB</i>	<i>BB</i> <sup>a</sup>
731	♀		13	12		26	
	♂	110		113			223
	Q		1				1
732	♂		26		14	12	
	♀	1		244	111		134
	Q			1		1	
7157	♂		26			15	11
	♀	164			74		88
	Q	1				1	
7158	♂		14	13		27	
	♀	45		47	92		
	Q		1		1		
	♂			1			1

<sup>a</sup> For males, these columns represent the corresponding hemizygote genotypes *Agp-1*<sup>F</sup>, *Agp-1*<sup>S</sup>, *Est-4*<sup>A</sup>, and *Est-4*<sup>B</sup>

( $n=8$ ), the second group within one week of the major mating flights of the season in north-central Georgia (unpublished data; see Table 1 for genotype data from four of these colonies). All males but one ( $n=1565$ ) possess the allele for which the foundress is homozygous, indicating that she produces the males in M colonies such as these. The single male whose genotype is inconsistent with production by the queen (colony 732) may have resulted from contamination of the assay colony in the laboratory.

The above data indicate that M colonies of *S. invicta* represent simple family units with singly inseminated foundresses producing all colony members. This social structure results in a pedigree coefficient of relatedness ( $G$ ) of 0.75 among females within a colony (Crozier 1970; Pamilo and Crozier 1982). The calculated within-nest regression coefficients of relatedness ( $b$ ) among female alates using the allozyme data are statistically indistinguishable from 0.75 ( $b \pm SE = 0.747 \pm 0.050$  for *Agp-1* and  $0.680 \pm 0.057$  for *Est-4*,  $t=0.06$  and  $1.21$ , both  $P > 0.05$ , mean  $b$  is 0.714, see Fig. 2).

The social structure of polygyne *S. invicta* is more complex by virtue of the presence of multiple functional queens. Figure 3 shows the genotype proportions for dealate queens sampled from ten representative P nests. All three genotypic classes at each locus are represented in each sampled nest

$F_{IS}$ , for the P population is  $-0.019$  for *Agp-1* and  $-0.018$  for *Est-4*. Neither value is significantly different from zero ( $\chi^2 = 0.53$  and  $0.38$ , both  $P > 0.05$ ; see Li and Horvitz 1953 for statistical method). These low values indicate no significant deficiency of heterozygotes in P nests, suggesting a lack of local inbreeding. Values of  $F_{IT}$  for the P population are  $-0.001$  for *Agp-1* and  $-0.003$  for *Est-4*, confirming the lack of significant subdivision or inbreeding at the population level.

## Discussion

The basis for an evolutionary discussion of our results is the hypothesis that polygyne *S. invicta* populations have repeatedly arisen and diverged from monogyne *S. invicta* since the introduction of the ant to North America. This hypothesis is predicated on several lines of evidence. (1) The M form has spread rapidly and widely since its introduction, yet multi-queened colonies were not discovered until relatively recently (Glancey et al. 1973; Hung et al. 1974; Morrill 1974; Miranda and Vinson 1982). (2) The P form always occurs in association with the M form, yet P populations appear to be geographically discrete and temporally stable (Fletcher 1983; Greenberg et al. 1985), suggesting restriction of gene flow between the two forms. (3) The two forms have virtually identical morphologies except for the conspicuous downward shift in size of P workers (W. F. Buren, personal communication). (4) Both M and P queens are readily transferred to colonies of the alternate type in the laboratory (Fletcher and Blum 1983a and unpublished data). (5) Closely related pairs of polygyne and monogyne forms are common in several other ant genera (Wilson 1971; Brian 1983). (6) M and P *S. invicta* possess identical alleles at 27 biochemical loci (Ross et al. 1985).

Results of the present study are also relevant to the question of genetic relationship between the two forms. The M and P populations from Georgia differ significantly in allele frequencies at both loci ( $t = 2.97$  for *Agp-1* and  $4.83$  for *Est-4*, both  $P < 0.05$ ; see Spiess 1977 for statistical method), however, neither locus is diagnostic for the two forms (Ayala and Powell 1972). While the use of allele frequency data in deducing phylogenetic relationships remains controversial (Felsenstein 1981; Buth 1984; Rogers 1984), our data are not inconsistent with the view that the Georgia P population is recently derived from the nearby M population and that incipient reproductive isolation has occurred. Our further results are thus best interpreted in terms of the comparative social and genetic

structure of a social insect in the initial stages of speciation.

Our results indicate strongly that both M and P queens are singly inseminated, in contrast to the prediction of Cole (1983) based on colony population. Despite the dramatic difference in social structure between the two forms, they have not diverged in this fundamental attribute of the mating systems. This may be taken as further evidence of a close relationship between the two forms. Single insemination of *S. invicta* queens has previously been suggested based on preliminary or inappropriate data (Hung and Vinson 1976; Ball and Vinson 1983).

The number of matings by queens is known for very few social Hymenoptera (Page and Metcalf 1982; Cole 1983), yet this information is crucial in assessing social and genetic structure within colonies and patterns of relatedness. These are in turn requisite to determining the importance of inclusive fitness effects on workers in the maintenance of eusociality (Hamilton 1972). The mating system and social structure of monogyne *S. invicta* confer a relatedness among female nestmates of  $0.75$ , the theoretical upper limit for outbred, male-haploid species. Thus, kin selection can be assumed to play an important role in the maintenance of eusociality in this form. In *Solenopsis* and a few other ant genera, workers are obligately sterile, not simply infertile as in most highly eusocial groups. It is tempting to conjecture that such sterility is likely to evolve only when workers stand to gain the greatest genetic payoff via an indirect fitness component; that is, relatedness between workers and the queens they rear is near the maximum possible value. That worker sterility does not inevitably evolve under such circumstances is indicated by the studies of Crozier (1973, 1974) on the ant, *Aphaenogaster rudis*.

Males present in M *S. invicta* colonies during the period of the major mating flights were shown to be the progeny of the foundress queens, not of virgin alate queens present in the colonies. Thus queen control over reproduction by female nestmates seems to be absolute during this period. It is possible that male progeny of virgin queens are present in M colonies in early spring since foundress production of inhibitory pheromones is low at this time and overwintered virgin queens lay non-embryonated (trophic) eggs and a few embryonated eggs (Fletcher and Blum 1983b). However, because such males would not participate in the major mating flights it is unlikely that they represent a significant source of gene flow or that selection would favor the production of these as

a viable alternative reproductive strategy for virgin queens.

During the period of geographical isolation from the M population presumed to have given rise to it, the P population in north-central Georgia has diverged not only in social structure but in the genetic structure of the population and nests. The population is not an assemblage of nests representing simple family units as in the M form; rather, queens in P nests seem to represent a random association of individuals showing no greater relatedness to nestmate queens than to other queens in the population. This low intra-nest relatedness, the lack of heterozygote deficiency, and the low genetic variance among nests suggests that a substantial amount of gene flow occurs throughout the P population and that nests are not closed genetic units. Thus, there is little genetic structuring of the population at the level of the nests, the most conspicuous level of organization in social insects. We cannot rule out the possibility that the P population is structured at some level higher than the nest, but the close approximation of observed genotypes to Hardy-Weinberg ratios suggests that this is not the case. Whether the observed genetic homogeneity is due to significant vagility of males, queens, or both remains to be determined, as do the precise mechanisms of dispersal.

Pairs or sets of closely related species exhibiting contrasting social organizations and reproductive strategies appear to be common among ants (Wilson 1971; Brian 1983). Comparative data suggest that polygyne forms represent the derived state and that their origin from monogyne forms is linked to habitat specialization (Brian and Brian 1955; Hölldobler and Wilson 1977; Elmes 1978; Ward 1983; Pamilo and Rosengren 1984; Fletcher and Ross 1985). Reproductive strategies of P forms appear to be conducive to occupation of habitats in which related M forms cannot persist. Ecological factors have thus been envisioned as the driving force behind the evolution of polygyny in ants. *S. invicta* appears to constitute an exceptional case in this regard, in that the P form does not occupy habitats distinct from those in which the M form is found (Greenberg et al. 1985; unpublished data).

When analyzed in genetic terms, the evolution of polygyny is counterintuitive given the predictions of individual and kin selection theories (Fletcher and Ross 1985). This difficulty was recognized early by Hamilton (1964, 1972) who suggested that the evolution of polygyny should be favored in highly structured populations, i.e. those in which related individuals live in close proximity. The search for evidence of such structure among

ant populations has thus become of primary importance.

Behavioral studies of P forms in several genera have revealed reproductive patterns consistent with significant structuring of populations (e.g. Talbot 1948; Scherba 1961; Higashi 1979; Yamauchi et al. 1981). These include mating in or near the parental nest, readoption of newly mated queens, and budding as the mode of colony founding. Closely allied M forms typically undergo long distance mating flights and claustral founding, reproductive patterns which promote gene flow throughout a population.

Population genetic studies have in several cases confirmed the above behavioral evidence for viscous population structures among P forms. Significant relatedness among nestmates and between ants in neighboring nests has been found in the genera *Myrmica* (Pearson 1982), *Formica* (Pamilo and Varvio-Aho 1979; Pamilo 1981, 1982; Pamilo and Rosengren 1984), and *Rhytidoponera* (Ward 1983; Crozier et al. 1984). On the other hand, our data and results of other studies (Craig and Crozier 1979; Pamilo 1981, 1982; Pearson 1982) show that nestmate relatedness in some P forms is insignificant and variable, suggesting that kin selection cannot wholly explain the evolution and maintenance of polygyny in these groups.

Even though no genetic structuring can be discerned in the Georgia P population of *S. invicta*, it is nonetheless possible that kin selection may have been involved in the origin of polygyny here. This population was only recently established, probably 5–6 years prior to this study (Fletcher 1983), and if founded by one or very few colonists then the early generations would represent an extended kin group. It can be argued that kin selection can promote queen associations in such an unstructured group (e.g. Tschinkel and Nierenberg 1983), particularly if there has been erosion of variability in the genetic determinants of pheromonal systems that normally enforce monogyny. However, by using the sex locus as a marker locus (Ross and Fletcher 1985), we have shown that the Georgia P population did not suffer a detectable loss of genetic diversity during founding and thus probably does not comprise an extended kin group.

A reasonable alternative explanation for polygyny, in the light of our results, invokes mutualism (Lin and Michener 1972; West-Eberhard 1981) as the primary selective force mediating stable queen associations. Mutualism has received considerable attention in discussions of temporary associations of queens in a diversity of social insects (e.g. Bartz and Hölldobler 1982; Thorne 1984), but its action

in permanent polygyne associations is more problematic (see for example Hölldobler and Wilson 1977; Pamilo and Rosengren 1984). For mutualism to operate in the evolution of permanent polygyny, the lifetime benefits to a queen of associating with non-related queens must outweigh the costs in loss of personal reproduction. The advantages of associating with other reproductives in forms such as *P. S. invicta* are yet to be identified, although our studies of the production of diploid males suggest that this source of genetic load may be involved (Ross and Fletcher 1985). In species exhibiting habitat partitioning between social forms, queen association through mutualism and the consequent changes in reproductive strategies may explain the persistence of P populations in habitats where related M forms do not survive (Pamilo 1981; Pamilo and Rosengren 1984), this without recourse to explanations invoking selection at higher levels (i.e. interdemic selection). In structured populations, mutualism may act in concert with kin selection to promote association of foundresses. Only through further comparative studies of related M and P forms in ants and other social Hymenoptera can the relative importance of these competing explanations for the evolution of polygyny be assessed.

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