THE BREEDING SYSTEM OF THE FIRE ANT *SOLENOPSIS INVICTA*: EFFECTS ON COLONY GENETIC STRUCTURE

KENNETH G. ROSS

Department of Entomology, University of Georgia, Athens, Georgia 30602-2603

Abstract.—Genetic and observational data are combined to describe the breeding system in a polygyne population of *Solenopsis invicta* using a formal theoretical framework that links properties of the breeding system with colony genetic structure. Queens of *S. invicta* mate only once, and the study population is outbred. The number of mated queens per nest is variable but generally low, with the average relatedness of nest-mate queens indistinguishable from zero. The genetic data are sufficiently complete that worker relatedness in individual nests can be estimated accurately, and the values obtained are shown to be well accounted for by the number of queens present in each nest. Thus, variance in maternity apportionment among nest-mate queens or internest movement of ants need not be invoked as determinants of colony genetic structure. Average worker nest-mate relatedness results from the opposing effects of two groups of factors: single mating by queens and the apparent closed nature of the societies elevate relatedness, while the presence of multiple queens and their low relatedness to one another depress it. This study also reveals consistent differences within nests in the matrilineal composition of worker and queen brood. This constitutes further evidence for inequities among nest-mate queens in the allocation of their progeny to the two castes at a single point in time.

The amount of genetic variation and its distribution within and between social insect colonies has become the subject of widespread interest. The original impetus for this concern with "colony genetic structure" arose from the explicit genetic focus of kin selection theory as an explanation for social evolution. Thus, most studies of social insect colony genetic structure have been concerned solely with determining patterns of nest-mate relatedness (Hölldobler and Wilson 1990; Ross and Carpenter 1991*a*), relatedness constituting a measure of genetic variation within and between colonies (Wade 1982). More recently, however, attention has turned from simply describing patterns of relatedness to elucidating the factors responsible for generating them. The most frequently mentioned factors constitute what can collectively be termed the "breeding system" of a social insect (after Wade 1985). Elements of the breeding system affecting colony genetic structure can be grouped into three general categories: (1) elements influencing genetic variation across matrilines in nests (number of queens in a colony, variance in the apportionment of maternity among nest-mate queens, genetic relatedness of queens in a colony [or relatedness of their mates]), (2) elements influ-

encing genetic variation across patrilines in nests (number of matings by each queen, variance in the apportionment of paternity among mates of each queen, genetic relatedness of male mates of single queens), and (3) an element influencing genetic variation within both matrilines and patrilines (genetic relatedness of mating partners [inbreeding]).

The three matrilineal elements are similar conceptually to the three patrilineal elements in their effects on genetic structure. In general, an increase in the number of nest-mate queens (matrilines) or mates of single queens (patrilines) is expected to increase the ratio of within-colony to between-colony genetic variation, which leads to lower average genetic relatedness of nest-mates. An increase in variance in the apportionment of maternity among nest-mate queens or in the apportionment of paternity among the mates of single queens generally has the effect of decreasing this ratio (and increasing relatedness) compared to cases in which there is more even distribution of maternity or paternity. An increase in relatedness between reproducing nest-mate queens (or their mates) or between the multiple mates of single queens also decreases this ratio compared to the case of zero relatedness between same-sex reproductives. The final breeding system element, relatedness between mating partners, affects both matrilineal and patrilineal genetic diversity. Most patterns of inbreeding decrease the ratio of within-colony to between-colony genetic variation (increase nest-mate relatedness) compared to random mating by reducing within-matriline and within-patriline genetic variation (Wade and Breden 1981; Michod 1982; Pamilo 1985; but see Uyenoyama 1984). The importance of these breeding system elements in determining social insect colony genetic structure has been reviewed by Hamilton (1972), Trivers and Hare (1976), Crozier (1979), Page and Metcalf (1982), Wade (1982, 1985), and Page (1986), and in an earlier article that I coauthored (Ross and Carpenter 1991a).

One reason for increased attention to social insect breeding systems is that knowledge of these systems is crucial for reconstructing the pathways of social evolution (see, e.g., Carpenter 1989; Ward 1989; Ross and Carpenter 1991a, 1991b). Also, knowledge of breeding systems continues to be relevant for understanding the role of kin selection, aside from merely predicting values of nest-mate relatedness. For instance, kin discrimination, as well as nest-mate conflict over sex allocation and worker production of males, is a phenomenon that is expected to emerge when kin selection operates within the context of specific types of breeding systems (Trivers and Hare 1976; Michener and Smith 1987; Ratnieks 1988, 1990; Page et al. 1989; Pamilo 1991).

In this article I present a comprehensive description of colony genetic structure in a population of the fire ant *Solenopsis invicta* containing multiple-queen nests. These genetic data, combined with other information, are used to assess the role of each element of the breeding system in determining this structure, employing a theoretical framework that links properties of the breeding system to colony genetic structure. This study also addresses the issue of whether there are caste-specific differences in colony genetic structure, such as arise when patterns of maternity and/or paternity differ for workers and queens from the same colonies.

Recent studies have yielded a mathematical framework that links properties of
breeding systems to colony genetic structure. This framework is quite general in
its applicability to diverse social systems (see Queller 1993), but it is presented
here in a more restricted form that is oriented to my empirical data. For instance,
I am concerned only with colony genetic structure as it pertains to females.
Hymenopteran males are generally impaternate, so the patrilineal breeding sys-
tem elements are irrelevant to genetic structure as it pertains to offspring males.
Furthermore, the framework presented here is static; it describes genetic struc-
ture at only a single point in time and so does not explicitly consider the effects
of changes in breeding system elements as colonies develop (see Laidlaw and
Page 1984; Page 1986). Finally, the framework does not incorporate covariance
terms that may arise from the interaction of breeding system elements.

Values of genetic relatedness for particular classes of nest mates represent
descriptions of colony genetic structure, so the influence of the breeding system
on this structure is generally interpreted via its effects on relatedness. The mathe-
matical framework includes two systems of equations that correspond to the
first two groups of breeding system elements, those influencing variation across
matrilines and those influencing variation across patrilines. For the matrilineal
elements, Queller et al. (1988) showed that the average relatedness (r_f) of nest-
mate females of the filial generation in a population of multiple-queen (polygyne)
nests is

$$r_f = (r_s) \cdot (1/N) + (r_{ns}) \cdot [(N - 1)/N], \tag{1}$$

where N is the number of queens per colony, r_s is the average relatedness of
females belonging to the same matriline (sisters), and r_{ns} is the average relatedness
of females belonging to different matrilines (nonsisters). This equation ignores
two elements of variation at the population level that affect values of r_f, variation
in the number of queens (matrilines) from colony to colony and variation in the
apportionment of maternity among queens in single colonies. To account for this
variation, N in equation (1) is substituted by N_e, the effective mean queen num-
ber, where

$$N_e = N_h/(N_h \overline{n\sigma^2} + 1) \tag{2}$$

(Wade 1985; Queller 1993; P. Pamilo, unpublished data). Here, N_h is the harmonic
mean number of queens per nest and $\overline{n\sigma^2}$ is the population arithmetic mean of
the product of the number of queens (n) and variance about the mean maternity
apportionment (σ^2) in each nest. If there is no variance in the reproductive output
of nest-mate queens in any colony, then N_e becomes the harmonic mean number
of queens per nest in the population.

The variable r_{ns} in equation (1) can be broken down into the sum of two terms
specifying how nonsisters are related through their mothers and through their
fathers. For male-haploid species, $r_{ns} = (r_q/4) + (r_{ml}/2)$, where r_q is the average
relatedness of reproducing nest-mate queens and r_{ml} is the relatedness of the

males inseminating these queens. (In the extreme, $r_{ml} = 1$ if a single male mates with all of the nest-mate queens.) If it is assumed that females of different matrilines are related only through their mothers (as is likely for *Solenopsis invicta;* see below), then $r_{ns} = (r_q/4)$, and equation (1) can be modified to

$$r_f = (r_s) \cdot (1/N_e) + (r_q/4) \cdot [(N_e - 1)/N_e] . \tag{3}$$

The system of equations for the patrilineal elements is analogous to that for the matrilineal elements. The average relatedness, r_s, of sisters (female filial generation) in a population of single-queen (monogyne) colonies is

$$r_s = (r_{fs}) \cdot (1/M) + (r_{hs}) \cdot [(M - 1)/M] , \tag{4}$$

where M is the number of mates per queen, r_{fs} is the relatedness of sisters belonging to the same patriline (full or super sisters), and r_{hs} is the relatedness of sisters belonging to different patrilines (half sisters) (Crozier 1970; Laidlaw and Page 1984). As before, this equation ignores two elements of variation, variation among queens in the number of matings and variation in the apportionment of paternity among the mates of single queens. Such variation can be accounted for by substituting M_e, the effective mean number of mates, for M in equation (4), which leads to

$$r_s = (r_{fs}) \cdot (1/M_e) + (r_{hs}) \cdot [(M_e - 1)/M_e] , \tag{5}$$

where

$$M_e = M_h/(M_h \overline{m\sigma^2} + 1) \tag{6}$$

(Wade 1982; Laidlaw and Page 1984; Page 1986; Queller 1993; P. Pamilo, unpublished data). Analogous to the derivation for matrilineal elements, M_h is the harmonic mean number of mates per queen, and $\overline{m\sigma^2}$ is the population mean of the number of mates per queen (m) times the variance in paternity apportionment for her offspring (σ^2).

Half sisters may be related through their fathers as well as through their mother if the multiple mates of a queen are related to one another. Thus, the variable r_{hs} can be broken down into the sum of two terms, which leads to $r_{hs} = 0.25 + (r_{m2}/2)$, where r_{m2} is the relatedness of mates of single queens. If the mates of single queens are unrelated to one another, then the value of r_{hs} is that for sisters related only through their mothers, $r_{hs} = 0.25$. If queens are invariably singly mated, then $M_e = 1$ and $r_s = r_{fs}$.

The final breeding system element, relatedness between mating partners, acts by influencing values of r_{fs}. If relatedness between a queen and her mate(s) is negligible, that is, there is no inbreeding, then $r_{fs} = 0.75$ under male haploidy. A shift from random mating to inbreeding will lead to an increase in the value of r_{fs} (see, e.g., Hamilton 1972; Aoki 1981; Pamilo 1985).

In overview, this system of equations can be seen to take into account the effects of all elements of the breeding system on colony genetic structure. The numbers of matrilines and patrilines, as well as variance in their representation, are encompassed in the terms N_e and M_e. The relatedness between same-sex parents, that is, between nest-mate queens or between the mates of single or

TABLE 1

Symbols Used to Describe Breeding Systems and Colony Genetic Structure

Symbol	Definition
N_e	Effective mean number of queens per nest in a population of polygyne nests.
N_h	Harmonic mean number of queens per nest in a population of polygyne nests.
n	Number of queens in a single nest.
M_e	Effective mean number of mates per queen in a population of multiply mated queens.
M_h	Harmonic mean number of mates per queen in a population of multiply mated queens.
m	Number of mates of a single queen.
σ^2	Variance about the mean maternity or paternity apportionment for a single nest or queen.
r_f	Average relatedness of nest-mate females of the filial generation in a population of polygyne nests.
r_s	Average relatedness of nest-mate females belonging to the same matriline, that is, having the same mother (sisters).
r_{ns}	Average relatedness of nest-mate females belonging to different matrilines, that is, having different mothers (nonsisters).
r_q	Average relatedness of reproducing nest-mate queens.
r_{fs}	Average relatedness of sisters belonging to the same patriline, that is, having the same father as well as mother (full or super sisters).
r_{hs}	Average relatedness of sisters belonging to different patrilines, that is, having different fathers but the same mother (half sisters).
r_{m1}	Average relatedness of males inseminating the multiple nest-mate queens in single polygyne nests.
r_{m2}	Average relatedness of males inseminating each multiply mated queen.

multiple queens, is encompassed in the terms for relatedness between matrilines or patrilines, r_{ns} and r_{hs}. Relatedness between mating partners (inbreeding) is reflected in the value of the term r_{fs}. A final feature of note is that the equation describing the effects of the patrilineal elements and of inbreeding is nested within the equation for the matrilineal elements through the term r_s. A compilation of the symbols used in this article to describe breeding systems and colony genetic structure is provided in table 1.

REPRODUCTIVE AND SOCIAL BIOLOGY OF *SOLENOPSIS INVICTA*

The fire ant *Solenopsis invicta* is a highly eusocial species with complete reproductive division of labor between the female castes—workers have vestigial ovaries so that only queens lay eggs (Goetsch 1953). Two social forms of *S. invicta* exist. Colonies in monogyne populations are genetically simple families with a single mother queen (Ross and Fletcher 1985a), whereas colonies in polygyne populations are genetically complex societies with up to several hundred reproductive queens (Glancey et al. 1973; Fletcher et al. 1980; Ross and Fletcher 1985a; Vargo and Fletcher 1987). Because of the richness in complexity of its breeding system, the polygyne social form is the subject of this study.

Mating in *S. invicta* is only known to take place during mating flights, although the possibility that mating also occurs within nests in the polygyne form has been raised (Ross and Fletcher 1985b; Porter 1991). Mating flights are triggered by

specific weather conditions and typically involve individuals from large numbers of colonies distributed over wide areas (Markin et al. 1971; Morrill 1974). Mating occurs at an altitude of 300 m or more during these flights. This mating behavior, if the rule, suggests panmixis, and indeed, previous studies of two genetic markers have found no evidence of significant local inbreeding (Ross and Fletcher 1985*a;* Ross et al. 1987).

Detailed genetic studies of families have shown that queens of both social forms mate only once (Ross and Fletcher 1985*a;* Ross 1992; Shoemaker et al. 1992). Similarities in spermatozoon counts from mature males and newly mated queens, coupled with the improbability that males are able to fly more than once, suggest that male fire ants also mate only once (Ball and Vinson 1983; Glancey and Lofgren 1985; Tschinkel and Porter 1988).

Queens of *S. invicta* shed their wings at the onset of ovarian development; thus, winglessness serves to distinguish reproductive from nonreproductive queens. The origin of queens recruited as new reproductives in polygyne colonies is not entirely clear, although most seem to be adopted into nonnatal nests following mating flights (Glancey and Lofgren 1988; Porter 1991). If this is the predominant mode of queen recruitment, then neither nest-mate queens nor their mates are expected to be closely related to one another.

Reproductive queens in polygyne nests generally are mated and capable of producing diploid offspring, although up to half of the reproductive queens in any given colony may be unmated (Vargo and Fletcher 1987, 1989). A substantial fraction of the mated queens in polygyne nests in the United States produce infertile diploid sons as well as diploid daughters (Ross and Fletcher 1985*b*), presumably as the result of reduced allelic diversity at the sex-determining locus. Because of the very low number of fertile haploid males present in polygyne *S. invicta* populations, the present study of colony genetic structure is concerned with female colony members only.

METHODS

Thirty-one nests of *Solenopsis invicta* were collected from a polygyne population in north-central Georgia over a 2-d period in spring 1990. Nests were widely spaced (>20-m separation between any two) to reduce the chance that any of the nests were part of the same polydomous colony (Vargo and Porter 1989). Excavation and collection procedures were planned so that all or nearly all of the wingless reproductive queens could be retrieved from each nest. Nests were excavated on sunny days following cool nights when the ants were concentrated in the tops of the mounds. The entire mounds and galleries below were dug up and placed into buckets. The ants were then slowly flooded out of these buckets in the laboratory (Jouvenaz et al. 1977) and the colonies transferred into laboratory rearing units.

All wingless reproductive queens were collected within 48 h of field excavation by systematically searching all of the laboratory rearing units. Samples of worker larvae, pupae, and adults, as well as of queen pupae and winged adult queens, were collected from the laboratory units within 5 d of field excavation. Among

TABLE 2

ALLELE FREQUENCIES AT 11 POLYMORPHIC LOCI IN A POLYGYNE POPULATION OF *SOLENOPSIS INVICTA*

	Aat-2 (2.6.1.1)	Acoh-1 (4.2.1.3)	Acoh-5 (4.2.1.3)	Acy1 (3.5.1.14)	Ddh-1 (1.8.1.4)	Est-4 (3.1.1.-)	Est-6 (3.1.1.-)	G3pdh-1 (1.1.1.8)	Pgm-1 (5.4.2.2)	Pgm-3* (5.4.2.2)	Pro-5 (-.-.-.-)
Workers:											
Larvae	.924 .076 (32.6) T	...	...	...	.832 .168 (33.1)	.708 .292 (32.1)	...	...	.845 .155 (32.8)	...	...
Pupae	...	.817 .183 (32.8) G	.725 .275 (32.7)	...	...	...	.596 .215 .189 (32.6)	...	.814 .186 (32.9)	.562 .438 (32.9)	...
Adults	.923 .077 (32.7) T	...	...	...	...	.630 .370 (32.0) T	...	.724 .276 (28.3) T	.835 .165 (32.9) T	...	...
Queens:											
Pupae	...	.829 .171 (32.3)	.757 .243 (32.4)	...	...	...	.590 .206 .204 (29.9)	...	.834 .166 (32.4)	.463 .537 (32.4)	...
Winged nonreproductives	.940 .060 (31.6) G	.794 .206 (29.3) G	...	.853 .147 (31.6) G	...	.625 .375 (31.6) T	...	.693 .307 (31.8) T	.821 .179 (31.8) T	...	.793 .207 (28.7) T
Wingless mated reproductives†	.933 .067 G	.909 .091 G	.782 .218 G	...	...	.737 .263 T	...	.715 .285 T	.836 .164 T	...	...

NOTE.—Enzyme Commission numbers are in parentheses below locus designations. The number of nests studied was 31 for all classes of individuals except queen pupae; only 29 of the nests contained queen pupae. The mean number of individuals studied per nest, for each locus and class of individual, is indicated in parentheses below the allele frequencies. For adults, a G indicates that the marker was scored from the gaster, whereas a T indicates that it was scored from the thorax. Ellipses indicate that a marker was not scored in that particular class of individuals.

* This locus was not used in the estimation of relatedness or inbreeding because it is under strong selection (see Ross 1992).

† See table 4 and fig. 1 for numbers of mated wingless reproductive queens retrieved and subjected to electrophoresis.

worker larvae, only those in the fourth instar were collected, because workers cannot be distinguished from sexuals until this final larval stage (O'Neal and Markin 1975a). One-third of the collected larvae were inspected at random under a microscope to ensure that their mandibles were sclerotized, a diagnostic feature of the fourth instar (Petralia and Vinson 1979). Ages of the worker and sexual pupae collected were standardized by selecting only unpigmented individuals whose eyes were beginning to develop pigmentation. All collected material was stored at −70°C until electrophoresis.

An attempt was made to score the genotypes of at least 30 workers and nonreproductive queens of each life stage from each nest. However, because of limited material in some nests and occasional inconsistent staining for some markers, final sample sizes are in some instances less than 30 per nest for each of the caste/life stage/locus combinations (see table 2 for sample sizes). The terminalia of the gasters (''abdomens'') of all wingless queens were dissected to determine mating status, and the remainder of the gaster and the thorax were subjected to electrophoresis separately (some markers were best or only expressed in a single body region; table 2).

Electrophoresis was conducted on 14% horizontal starch gels using standard procedures (Shoemaker et al. 1992). Eleven polymorphic loci were studied; the identity of these markers, the material from which they were scored, and population allele frequencies are listed in table 2. Mendelian inheritance of the 11 markers is demonstrated elsewhere (Ross and Fletcher 1985a; Ross 1992; Shoemaker et al. 1992). Because of strong selection acting on $Pgm-3$ in polygyne queens (Ross 1992), this locus was not used in the estimation of inbreeding coefficients or relatedness.

Genetic relatedness of nest mates (r) was estimated using the procedure of Queller and Goodnight (1989). Colonies generally were weighted equally for this procedure, although estimates for reproductive queens were obtained also by weighting each colony by the total number of reproductives present. Variances of the estimates were obtained by jackknifing across colonies, and 95% confidence intervals about the estimates were constructed from these variances by assuming the t-distribution. Estimates of r that were negative were in most instances assumed to equal zero if the 95% confidence intervals overlapped with zero. Values of r were estimated for the entire population, for subsets of the population, and, in some cases, for individual colonies.

To obtain unbiased estimates of population allele and genotype frequencies, a resampling procedure was instituted whereby single genotypes were drawn at random from each of the nests (with replacement) 50 times. Population allele and genotype frequencies were taken as the arithmetic mean frequencies from these 50 genotype distributions. The resampling procedures were undertaken to ensure the independence of the sampled genotypes on which estimates of these population frequencies (and the inbreeding coefficients derived from them) were based. Colonies of these ants represent families of varying complexity, so the genotypes of multiple individuals sampled from a single nest are unlikely to be completely independent owing to recent pedigree connections between some nest mates.

The inbreeding coefficient F was estimated for each locus and class of individu-

TABLE 3

VALUES OF THE INBREEDING COEFFICIENT F IN POLYGYNE *SOLENOPSIS INVICTA*

	Aat-2	Acoh-1	Acoh-5	Acy1	Ddh-1	Est-4	Est-6	G3pdh-1	Pgm-1	Pro-5	Multilocus F
Workers:											
Larvae	.003 (−.110–.482)	...	...	...	.127 (−.155–.439)	−.125 (−.334–.124)	...	...	−.084 (−.249–.364)	...	−.043 (−.178–.092)
Pupae	...	−.070 (−.276–.191)	−.074 (−.334–.232)	...	...	...	−.043 (−.276–.224)	...	.085 (−.202–.459)	...	−.028 (−.101–.045)
Adults	−.013 (−.132–.529)	...	...	...	...	.139 (−.111–.450)	...	−.153 (−.364–.141)	−.009 (−.276–.364)	...	.005 (−.178–.187)
Queens:											
Pupae	...	−.135 (−.304–.148)	−.106 (−.332–.157)	...	...	...	−.013 (−.273–.321)	...	−.037 (−.232–.206)	...	−.075 (−.137–−.014)
Winged nonre-productives	.032 (−.110–.482)	−.115 (−.290–.210)	...	−.072 (−.222–.364)	...	.035 (−.345–.335)	...	.021 (−.313–.285)	.166 (−.132–.526)	−.024 (−.249–.299)	.006 (−.062–.074)
Wingless mated reproductives	−.023 (−.132–.279)	−.084 (−.178–−.043)	−.070 (−.304–.191)	...	...	−.028 (−.276–.278)	...	.039 (−.276–.334)	.166 (−.015–.395)	...	.005 (−.066–.076)

NOTE.—The 95% confidence intervals about the values are in parentheses.

als independently, as well as for the loci combined within and across classes, using the procedure of Weir and Cockerham (1984). For the single-locus estimates, values of F were taken as the arithmetic means of the values from the 50 resampled genotype distributions; 95% confidence intervals about the means were constructed by eliminating the extreme high and low values from the resampled distributions (Weir 1990). Weighted unbiased averages of F and estimates of their variances were obtained for groups of loci by summing the variance components and jackknifing across loci (Weir and Cockerham 1984). The 95% confidence intervals were estimated from the variances by assuming the t distribution.

Genotype distributions were compared between different classes of females within nests using contingency-table χ^2 analysis. The least frequent genotypes were grouped together when expected cell counts fell below five.

<div align="center">RESULTS</div>

<div align="center">Effects of the Breeding System on Colony Genetic Structure</div>

A description of the breeding system of polygyne *Solenopsis invicta* is obtained by characterizing in turn each of its constituent elements. Considering first the patrilineal elements, previous work has revealed that fire ant queens are singly mated. This simplifies the remaining analyses by eliminating the need to consider the other patrilineal elements. Because queens mate only once, $M_e = 1$ and equation (5) reduces to $r_s = r_{fs}$; that is, all fire ant sisters are full sisters.

Turning next to the breeding system element that influences variation within both matrilines and patrilines, estimated values of the inbreeding coefficient, F, are typically indistinguishable from zero (table 3). For only one of the 29 single-locus estimates (*Acoh-1* in reproductive queens) and one of the six multilocus estimates (queen pupae) do the 95% confidence intervals fail to overlap zero, and, in both cases, the point estimates are negative. Taking the weighted average of all of the loci over all of the classes yields $F = -0.004$, with a 95% confidence interval of -0.050 to 0.042. Thus, inbreeding is unlikely to be important in the study population, and r_s, the relatedness of sisters, is expected to equal 0.75.

Considering next the matrilineal elements, it is useful to begin by evaluating the factors that affect the relatedness of nest-mate females belonging to different matrilines (r_{ns}). It is unlikely that such females are related through their fathers, because fire ant males probably mate only once after dispersing from their natal nests. Thus, r_{ns} can be assumed to be equal to $r_q/4$, where r_q is the relatedness of the mother queens. In estimating r_q it is important to exclude unmated reproductive queens, which cannot produce daughters. Estimates of r_q based on mated queens only are 0.048 when nests are weighted equally and -0.017 when nests are weighted by the total number of mated queens in each, with the 95% confidence intervals including zero in both cases. Thus, mated reproductive queens are, on the average, no more closely related to their nest mates than they are to queens present in any other nests in the population.

In considering the numbers of mated queens (matrilines) present per nest, two independent means of quantifying these numbers are possible. The first is a direct count of mated queens retrieved from each nest, giving observed values such as
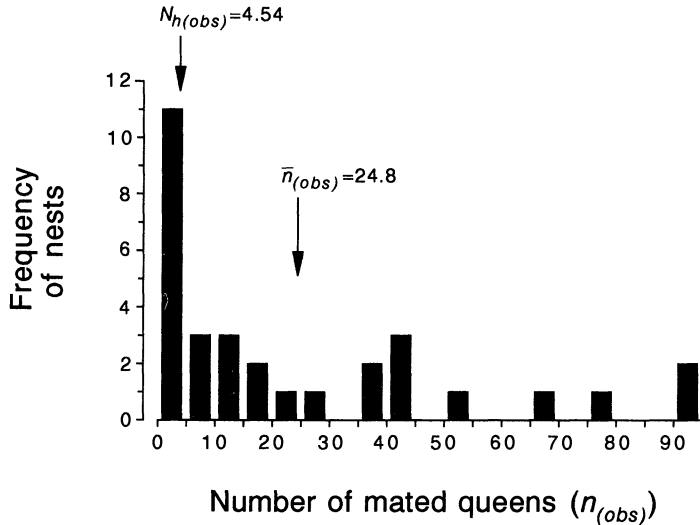
FIG. 1.—Distribution of the number of mated reproductive queens ($n_{[obs]}$) captured in each of 31 nests in a polygyne population of *Solenopsis invicta*. Also shown are the harmonic mean ($N_{h[obs]}$) and arithmetic mean ($\bar{n}_{[obs]}$) numbers of mated queens per nest.

$n_{(obs)}$ and $N_{h(obs)}$. The second involves calculating the expected numbers of queens using equation (3) and the empirical relatedness values. The value obtained is the expected effective mean queen number and has notation $N_{e(exp)}$.

The distribution of numbers of mated queens retrieved from each nest is shown in figure 1. Queen number ranges from one to 92, with an arithmetic mean ($\bar{n}_{[obs]}$) of 24.8 and a harmonic mean ($N_{h[obs]}$) of 4.54. Many of the nests had relatively few queens, which explains the substantial difference between the harmonic and arithmetic means (the former statistic is strongly influenced by low values). Only a single mated queen was found in four of the 31 nests. Distributions of genotypes for offspring females in two of these nests were consistent with these individuals' being the daughters of the lone captured reproductive and a single father, which indicates that these colonies were indeed monogynous. Genotype distributions in the other two nests were not characteristic of such simple families, which suggests that these colonies had only recently become monogynous or, alternatively, that some reproductive queens were not captured.

If variance in maternity apportionment and internest movement of ants are ignored as determinants of within-nest genetic variation, then a positive relationship between colony queen number and such variation is anticipated, given that within-matriline relatedness greatly exceeds between-matriline relatedness in these ants (e.g., eq. [1]). Since the average relatedness of workers in the nest serves as a measure of within-nest genetic variation, values of r_f estimated for single nests can be compared to the number of queens collected in the same nest ($n_{[obs]}$) to see if worker relatedness decreases as queen number increases. Significant negative correlations between r_f and $n_{(obs)}$ were obtained when each
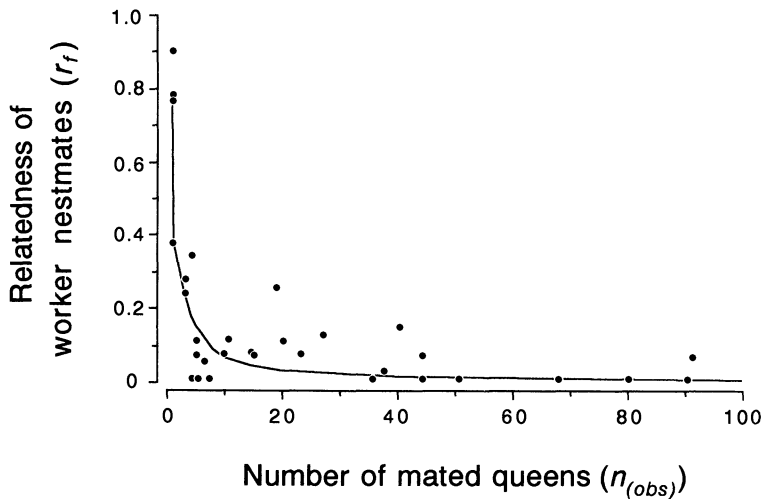
FIG. 2.—Relationship between the number of mated reproductive queens captured in individual nests of *Solenopsis invicta* ($n_{[obs]}$) and the relatedness of nest-mate workers (r_f) measured in each nest. Values of r_f were obtained by pooling genetic data from all worker life stages. Negative values not significantly different from zero were assumed to equal zero. The plotted line shows the theoretical relationship between number of mated queens and worker relatedness when queens are singly mated, relatedness of nest-mate queens is zero, there is no variance in maternity apportionment, and nests are closed to immigration. The plotted line provides a significant fit to the observed data ($R^2 = 0.917$, $F = 322$, df = 1,29, $P < .001$).

of the worker life stages was considered separately, when both brood stages were considered together, and when all of the worker life stages were considered together (Spearman rank-correlation tests, $-0.61 \leq r \leq -0.34$, $.001 < P < .05$). Thus the amount of genetic variation within each nest reflects the number of mated queens actually residing in that nest. More specifically, the relationship between $n_{(obs)}$ and the single-nest r_f values for the combined worker life stages matches well the theoretical relationship between queen number and worker relatedness, if one assumes that there is no variance in maternity apportionment and nests are closed to immigration (fig. 2).

The expected effective mean numbers of queens per nest can be calculated from equation (3) by inserting values of r_f, r_s, and r_q estimated from the genetic data. The relatedness values used are shown in table 4, along with the resulting estimates of $N_{e(exp)}$. Differences in the values of r_q arise because of the different weighting schemes used in the estimation procedure. Values of r_f differ according to the class of female used for the estimation. Only workers are considered in the present analysis, so differences in r_f stem from the use of different life stages. In all instances there is close agreement between the estimated $N_{e(exp)}$ values and the harmonic mean number of queens captured in the nests ($N_{h[obs]} = 4.54$). Such agreement is expected if nests in the study population constitute relatively closed societies. Furthermore, because the expected number $N_{e(exp)}$ is an effective mean,

TABLE 4

EXPECTED NUMBERS OF MATED QUEENS OR MATRILINES PER NEST ($N_{e[exp]}$) ESTIMATED FROM
RELATEDNESS VALUES IN A POLYGYNE POPULATION OF *SOLENOPSIS INVICTA*

RELATEDNESS OF OFFSPRING NEST-MATE FEMALES	$N_{e(exp)}$	
	$r_q = 0*†$	$r_q = .048*$
Worker larvae ($n = 1{,}027$), $r_f = .179$	4.19	4.42
Worker pupae ($n = 1{,}023$), $r_f = .147$	5.10	5.47
All worker brood ($n = 2{,}050$), $r_f = .161$	4.66	4.95
Worker adults ($n = 1{,}023$), $r_f = .160$	4.69	4.99
All worker life stages ($n = 3{,}073$), $r_f = .161$	4.66	4.95

NOTE.—These expected values are calculated under the assumptions that there is no variance among nest-mate queens in their apportionment of maternity and that $r_s = 0.75$. The numbers of individuals used in calculating relatedness are indicated by n. These individuals are from all 31 nests in the study population. The harmonic mean number of queens collected from these nests is 4.54.

* The different values for the relatedness of mated queens (r_q) depend on whether nests were weighted equally for the estimation (right column) or by the number of reproductive queens collected (left column); $n = 765$ queens.

† The negative value actually estimated is assumed to be zero for this analysis.

it represents the harmonic mean queen number when there is no variance among nest mates in their offspring production (e.g., eq. [2]). Thus the close agreement between $N_{e(exp)}$ and $N_{h(obs)}$ further suggests that variance in maternity apportionment, the final breeding system element to be considered, may not play a major role in determining colony genetic structure.

Even if such variance were important, however, similarities between the values of $N_{e(exp)}$ and $N_{h(obs)}$ may arise simply because many nests with very few queens were included in the analysis. The similarities may come about for two reasons. First, included among such nests are the four with single queens, for which there is no opportunity for variance in maternity apportionment. Second, nests with few queens have an inordinate influence on estimates of $N_{e(exp)}$, in parallel with their strong influence on the observed harmonic mean number of queens. Thus, a situation is conceivable in which nests with few queens have little variance in maternity apportionment while nests with many queens have considerable variance, yet the values of $N_{e(exp)}$ and $N_{h(obs)}$ obtained for the population as a whole are similar.

To explore this possibility, $N_{e(exp)}$ and $N_{h(obs)}$ were compared for a subset of 18 nests with high queen numbers (10 or more queens in each nest). The observed harmonic mean number of queens in these nests is 25.5. All $N_{e(exp)}$ values except those estimated from worker adults are less than this value of $N_{h(obs)}$, and often considerably so (table 5), a pattern consistent with the presence of substantial maternity variance. The extent of variance in matriline representation required to explain the disparities between observed and expected queen numbers can be quantified using equation (2), with $N_e = N_{e(exp)}$ and $N_h = N_{h(obs)}$. However, the variance estimated in this fashion actually comprises two sources of variance, that arising from real variance in maternity apportionment and that attributable

TABLE 5

EXPECTED NUMBERS OF MATED QUEENS OR MATRILINES PER NEST ($N_{e[exp]}$) IN 18 POLYGYNE NESTS OF
SOLENOPSIS INVICTA WITH HIGH QUEEN NUMBERS

RELATEDNESS OF OFFSPRING NEST-MATE FEMALES	$N_{e(exp)}$	
	$r_q = 0^{*}\dagger$	$r_q = .009^{*}$
Worker larvae ($n = 598$), $r_f = .038$	19.7	20.9
Worker pupae ($n = 594$), $r_f = .106$	7.08	7.21
All worker brood ($n = 1,192$), $r_f = .079$	9.49	9.74
Worker adults ($n = 594$), $r_f = .026$	28.8	31.5
All worker life stages ($n = 1,786$), $r_f = .062$	12.1	12.5

NOTE.—These expected values are calculated under the assumptions that there is no variance among nest-mate queens in their apportionment of maternity and that $r_s = 0.75$. The numbers of individuals used in estimating p_x for calculating relatedness are indicated by n (see Queller and Goodnight 1989). These individuals are from 18 nests in which 10 or more mated queens were present. The harmonic mean number of queens collected from these nests is 25.5.

* The different values for the relatedness of mated queens (r_q) depend on whether nests were weighted equally for the estimation (right column) or by the number of reproductive queens collected (left column); $n = 724$ queens.

† The negative value actually estimated is assumed to be zero for this analysis.

to sampling errror. The real maternity variance can be estimated from the total variance by the formula

$$\sigma_{mat}^2 = [x/(x - 1)] \cdot [\sigma_{tot}^2 - (n - 1)/(x \cdot n^2)],$$

where σ_{mat}^2 is the actual variance in maternity apportionment, σ_{tot}^2 is the total observed variance, x is the number of workers sampled per nest, and n is the number of queens per nest (P. Pamilo, unpublished data). The term σ_{tot}^2 can be extracted from the empirical values of $n\sigma^2$ (estimated using eq. [2]) by dividing by reasonable values of n. Here, values of n are systematically varied between 10 and 90 to estimate a range of values of σ_{mat}^2 for the subset of nests with high queen number. For estimates based on samples of worker larvae or worker adults, σ_{mat}^2 is invariably less than zero so that all of the observed variance results from sampling error. For the estimates from worker pupae, combined worker brood, or all worker life stages combined, σ_{mat}^2 constitutes only 27%–76% of the total variance. Thus, the proportion of the measured variance in matriline representation that actually can be attributed to differences in nest-mate queen reproduction is often small or even negligible.

Another problem in the above analysis of maternity variance in high-queen-number nests stems from the fact that estimates of $N_{e(exp)}$ are exceptionally volatile over the low end of the range of r_f values. This can be seen from the curve in figure 2, which illustrates that the relationship between queen number and worker relatedness in polygyne *S. invicta* flattens out dramatically with more than 15 queens. Thus, small changes in low relatedness values lead to large differences in the expected numbers of queens. As an example of this effect, modest differences in r_f values for different classes of workers from the same nests translate into as much as four-fold differences in estimated colony queen numbers

(table 5). The estimates of r_f obtained for high-queen-number nests are unlikely to be sufficiently precise to attach great confidence to the resulting $N_{e(exp)}$ estimates.

Caste-specific Differences in Colony Genetic Structure

Patterns of maternity and/or paternity may differ between the worker and queen offspring in colonies of social insects with well-developed caste dimorphism, which leads to different colony genetic structures for the two castes (Ross 1986, 1988). The above description of colony genetic structure refers exclusively to the worker caste, so it is of interest to compare that structure with one that refers to the offspring queens. The questions of importance here are, first, whether queens producing workers are the same individuals producing queen offspring and, second, whether variance in apportionment of maternity is distributed in equivalent fashion for offspring of each caste. These questions are addressed using two approaches. First, the effective numbers of matrilines represented among worker and queen offspring, as assessed from relatedness values, are compared to see if there are differences in the effective numbers of queens contributing to production of the two castes. Second, genotype distributions for worker and queen offspring within single colonies are compared to see whether there is evidence for differential matrilineal composition of the two castes.

For either approach care must be taken to standardize ages across the castes so that temporal variation in patterns of maternity is not confounded with caste-specific differences in maternity at a single point in time. For instance, it would be inappropriate to compare genotypes of worker and queen adults. Workers are likely to remain within their natal colonies longer than do queens, many or most of which depart on mating flights once they are mature (Morrill 1974). Thus, if there is a regular turnover of mother queens in a nest, the adult workers would represent more matrilines than the offspring queens simply on the basis of their longer residence times in nests. One means to minimize this problem of temporal variation is to compare only the pupal stage of workers and offspring queens.

In the first approach, matrilineal diversity was compared between worker and queen pupae in subsets of nests with differing numbers of reproductive queens. While no differences in relatedness (r_f) between the two castes were detected in subsets of nests with few mother queens (data not shown), relatedness of worker pupae was consistently lower than that of queen pupae in nests with higher queen numbers, a pattern that became increasingly evident as nests with the highest numbers of reproductive queens were considered (table 6). Additionally, the observed harmonic mean queen numbers ($N_{h[obs]}$) in the highly polygyne nests invariably were greater than the expected numbers ($N_{e[exp]}$) calculated from worker pupa relatedness (e.g., table 6). This pattern suggests that differences in r_f between worker and queen pupae actually arise from differential patterns of worker and queen offspring production among resident reproductive queens. An alternative explanation for differences in r_f values, that these arise because worker brood are moved from nest to nest to a greater extent than sexual brood, would be more tenable if $N_{e(exp)}$ values derived from workers consistently and substantially exceeded $N_{h(obs)}$.

The second approach to assess caste-specific differences in colony genetic

TABLE 6

EXPECTED NUMBERS OF MATRILINES ($N_{e(exp)}$) ESTIMATED FROM PUPAL RELATEDNESS VALUES AND HARMONIC MEAN NUMBERS OF QUEENS OBSERVED ($N_{h(obs)}$) FOR THREE SUBSETS OF POLYGYNE *SOLENOPSIS INVICTA* NESTS

RELATEDNESS OF OFFSPRING NEST-MATE FEMALES	$N_{e(exp)}$		$N_{h(obs)}$
	$r_q = .007*$ ($n = 630$)	$r_q = .062*$ ($n = 630$)	
All polygyne nests			8.94
Worker pupae ($n = 825$), $r_f = .099$	7.69	8.80	
Queen pupae ($n = 808$), $r_f = .116$	6.55	7.31	
	$N_{e(exp)}$		
	$r_q = .001*$ ($n = 589$)	$r_q = .027*$ ($n = 589$)	$N_{h(obs)}$
High-queen-number nests			23.8
Worker pupae ($n = 528$), $r_f = .126$	5.95	6.23	
Queen pupae ($n = 511$), $r_f = .147$	5.10	5.30	$N_{h(obs)}$
	$N_{e(exp)}$		
	$r_q = 0†$ ($n = 450$)		$N_{h(obs)}$
Highest-queen-number nests			50.3
Worker pupae ($n = 264$), $r_f = .079$	9.49		
Queen pupae ($n = 247$), $r_f = .142$	5.28		

NOTE.—Values of $N_{e(exp)}$ are calculated under the assumptions that there is no variance among nest-mate queens in their apportionment of maternity and that $r_s = 0.75$. The numbers of individuals used in estimating p_x for calculating relatedness are indicated by n. These individuals are from all 25 polygyne nests that contained queen pupae, from the 16 nests with 10 or more mated queens that contained queen pupae (high-queen-number nests), or from the eight nests with more than 40 mated queens that contained queen pupae (highest-queen-number nests).

* The different values for the relatedness of mated queens (r_q) depend on whether nests were weighted equally for the estimation (right column) or by the number of reproductive queens collected (left column).

† Estimated values of r_q were negative regardless of whether nests were weighted equally or by the number of reproductive queens collected. These values are assumed to be zero for this analysis.

structure is to compare single-locus genotype distributions between worker and queen pupae within individual colonies. The null expectation here is not that differences in genotype distributions are nonexistent between the two castes but that the frequency of such differences does not exceed some background level. This background level arises because, first, the queen and worker pupae collected were not exactly the same age (time of development to the stage at which pupae were harvested may differ by up to 1 wk between the castes [O'Neal and Markin 1975b]), and, second, there may be regular changes in patterns of maternity due to turnover of reproductive queens or other causes (Ross 1988). Again, temporal sources of variation in colony genotypic composition must be distinguished from

TABLE 7

ASSOCIATION OF GENOTYPIC DIFFERENCES ACROSS WORKER LIFE STAGES WITH GENOTYPIC DIFFERENCES
BETWEEN WORKER AND QUEEN PUPAE IN INDIVIDUAL POLYGYNE *SOLENOPSIS INVICTA* NESTS

	DIFFERENCES ACROSS WORKER LIFE STAGES	
	Yes	No
Differences between worker and queen pupae:		
Yes	4	8
No	5	8

variation arising from caste-specific differences in maternity apportionment at a single point in time.

The background level of temporal variation was quantified by determining the frequency at which significant differences in genotypic ratios at one or more loci were found between worker life stages (χ^2 tests, $\alpha = 0.05$). Twenty-five nests with more than one mated queen produced queen pupae and thus are appropriate for this analysis. Each nest can be classified as to whether significant genotypic differences existed across worker life stages and whether significant genotypic differences existed between the worker and queen pupae (table 7). If temporal differences in maternity can explain genotypic differences between pupae of the two castes, then nests in the Yes/Yes and No/No cells of table 7 are expected to be relatively overrepresented. Instead, such nests make up only half of the total number. Significantly, almost one-third of the study nests exhibit genotypic differences between the worker and queen castes in the absence of detectable differences among worker life stages. This analysis thus suggests that the observed differences in caste-specific colony genetic structure cannot be wholly accounted for by turnover of reproductive queens in a nest or other causes of temporal change in patterns of maternity. Rather, nest-mate queens frequently appear to differ from one another, at a single point in time, in the relative allocation of their offspring production to the two castes.

DISCUSSION

The description of colony genetic structure in polygyne *Solenopsis invicta* provided by this study is fairly comprehensive in that it is based on analyses of the different life stages and castes, it employs large sample sizes, and it makes use of a large number of genetic markers. On the other hand, understanding exactly how properties of the breeding system give rise to this genetic structure remains a difficult task because of limitations of the data and the complexity of fire ant reproductive behavior. Thus, while this discussion focuses on what can be concluded with some certainty about the breeding system of this ant, difficulties with the interpretations of the data are also addressed.

Analysis of the breeding system of *S. invicta* is facilitated by two attributes of the mating biology, single mating by queens and the absence of inbreeding. Not

only can variance in paternity apportionment and relatedness of a single queen's mates be disregarded as relevant patrilineal elements of the breeding system, but relatedness between sisters can confidently be assigned the pedigree value for full sisters under male haploidy and outbreeding, $r_s = 0.75$ (Crozier 1970). Because r_s is nested in the equation that describes the effects of the matrilineal elements, a well-supported value for r_s plays a pivotal role in the analysis of breeding systems by providing a bridge between the patrilineal and matrilineal elements.

The number of reproductive queens is an important determinant of colony genetic structure in polygyne social insects (see, e.g., Pamilo and Varvio-Aho 1979; Ross and Fletcher 1985a; Stille et al. 1991; Strassmann et al. 1991; Seppä 1992), so it is imperative to obtain reliable information on the distribution of queen numbers when describing breeding systems. Two independent data sets based on queen collections show that the number of mated queens in polygyne *S. invicta* is remarkably variable from nest to nest. The range observed in this study was 1–92, while an earlier study of the same population revealed the range in queen numbers to be 1–178 (Vargo and Fletcher 1987; E. Vargo, unpublished data). An important point is that the harmonic means are relatively low for both data sets (4.5 and 8.7, respectively), and this statistic is the relevant one for predicting how colony queen number will affect average nest-mate relatedness in a population (Wade 1985).

With relatively low numbers of singly mated queens per nest, average worker nest-mate relatedness is expected to be greater than zero, even if there is no variance in maternity apportionment and nest-mate queens are not closely related. In fact, all of the r_f values listed in table 4 for the different worker life stages are significantly greater than zero, with average worker nest-mate relatedness for the pooled stages estimated to be about equal to the relatedness of first cousins ($r_f = 0.161$). Lower values of worker relatedness that did not differ statistically from zero were reported earlier for this same polygyne population (Ross and Fletcher 1985a), but large nests with high queen numbers were preferentially sampled in that study.

Although the *average* relatedness of worker nest mates in polygyne *S. invicta* may approximate that of first cousins, the breeding system is such that one rarely would encounter a pair of individuals within a colony that actually were related to one another by this amount. Instead, any pair of nest mates would likely be very closely related (members of the same matriline) or completely unrelated (members of different matrilines), with the relative frequency of the two types depending on the number of reproductive queens in the nest and the degree of inequity in their offspring production. This example illustrates how knowledge of average relatedness may often shed little light on the details of colony genetic structure, or the determinants of that structure, in the absence of other information. The average relatedness reported here for polygyne *S. invicta* could be observed in a population in which nests contained many multiply mated queens that were full sisters to one another. A large proportion of the daughter-generation females in such nests would be first cousins, and there would be relatively little difference in the within- and between-matriline relatedness values. Differences between this hypothetical colony genetic structure and that actually found in this

study clearly have important consequences for kin selection (e.g., in terms of kin discrimination and nepotism) that transcend the similarities in values of average nest-mate relatedness.

The results of this study are equivocal with respect to the effect that variance in maternity apportionment has on colony genetic structure. Unequal representation of matrilines in a nest can affect average offspring relatedness, compared to the absence of such maternity variance, by altering the relative frequencies of within- and between-matriline pedigree links. The existence of significant maternity variance should have profound consequences for average nest-mate relatedness in polygyne fire ants because of the large difference in relatedness values within and between matrilines. However, changes in patterns of maternity apportionment in other polygyne social systems in which there is less of a relatedness differential may have little effect on overall nest-mate relatedness. Indeed, extreme variance in maternity apportionment may be indistinguishable from its absence if nest-mate queens are closely related and mate frequently.

Yet even in systems such as the one under study, in which small changes in maternity apportionment can translate into substantial differences in relatedness patterns, assessment of variance in maternity apportionment by indirect means remains a challenging task. In the approach taken here, the expected harmonic mean number of queens was estimated from three relatedness terms, and the extent to which this expected number was lower than the observed harmonic mean queen number was assumed to reflect variance in matriline representation. Difficulties with this approach arise because variance in maternity apportionment can be confused with variance arising from sampling error, and estimates of offspring relatedness (r_f) at the low end of the range of values do not return reliable estimates of expected queen numbers (discussed above). Aside from these statistical concerns, factors not explicitly studied may inflate within-colony variation and thus depress r_f below the value appropriate for assessing maternity variance.

The first such factor is the presence within a nest of individuals that are the offspring of queens residing in different nests. The occurrence of such "extramural matrilines" results from the movement of brood or adults among nests. Internest movement is known in polygyne *S. invicta* (Vargo and Porter 1989; Porter 1991), but it is difficult to reconcile such movement with the close match of the empirical data to the theoretical relationship between queen number and relatedness when nests are assumed to be closed to immigration. Nonetheless, because maternity variance and internest movement have opposing effects on genetic variation within nests, it is possible that both occur but that their effects equal out, which would render both undetectable. Internest movement seems to be common in many social insects, especially primitively social wasps and polygyne ants (Crozier 1980; Pamilo 1983; Hölldobler and Wilson 1990; Reeve 1991; Ross and Carpenter 1991*a*), and so can be expected frequently to complicate analyses of queen number and maternity variance.

Another factor increasing within-colony genetic variation above the level appropriate for assessing maternity variance is a relatively high turnover of reproductive queens, which leads to the presence of individuals whose mothers are no

longer resident in a particular nest. The problem posed by high queen turnover is that a temporally dynamic aspect of the breeding system is imposed on the static framework erected to describe it. Its importance in confounding estimates of maternity variance depends both on the rate of queen mortality relative to the age of workers used to estimate r_f and on the regularity with which expired queens are replaced. The problem can be alleviated by estimating r_f from young worker brood, which improves the prospects that their mothers are still present when nests are collected. On the other hand, such sampling does not allow further differential mortality to occur among the matrilines as individuals develop, and this ontogenetic source of variance in maternity apportionment may be of interest. Previous laboratory studies of *S. invicta* revealed a fairly constant mortality of queens in polygyne nests (Ross 1988), but comparison of r_f values for worker larvae and adults in tables 4 and 5 offers no evidence that queen turnover led to inflated genetic variation in the adult stage in the present study. Turnover of queens in polygyne insect societies has received relatively little attention previously (see Elmes 1980; Nonacs 1988; Keller and Passera 1990), but clearly it is an important factor to be considered in studies of colony genetic structure.

A final issue addressed by this study concerns the existence of caste-specific genetic structures. Two lines of evidence suggest that the matrilineal compositions of the pools of worker and queen pupae in single nests are not equivalent. One is the high frequency with which genotype distributions of nest-mate queen and worker pupae differ. The other is the consistently higher values of relatedness for queen pupae than for worker pupae in highly polygyne nests. The latter finding suggests that effectively fewer mother queens in these nests are contributing to the production of new queens than to the production of new workers at a given point in time. These differences in relatedness for the two castes could come about if worker brood were moved among nests to a greater extent than are sexual brood. However, the fact that effective numbers of matrilines are invariably lower than the numbers of queens found in the nests does not support a major role for worker brood movement in creating the observed caste-specific genetic structures.

The discovery of differences in genotypic composition of the two castes in a field population of polygyne *S. invicta* parallels the results of a laboratory study, in which such differences were shown to be caused by greater variance in the apportionment of maternity of sexuals than of workers (see Ross 1988 for a discussion of these results and possible mechanisms). The implication of these two studies is that nest-mate queens are not faring equally well in terms of their direct reproductive success, as some appear to be achieving disproportionate success in having their female offspring reared as queens. Because direct reproduction seems to be the only route for fitness returns to mated queens—low relatedness of nest-mate queens precludes the existence of significant indirect fitness effects—those queens producing relatively few female sexual offspring would appear to be at a severe fitness disadvantage, although their role in producing fertile males remains to be determined. While it may be argued that lifetime success in producing sexual offspring cannot be assessed by a study in which samples were collected at a single point in time, the earlier laboratory study

revealed that differences among nest-mate queens in allocation of their offspring production to the two castes often persist over considerable periods. Inequities in sexual offspring production may represent one of the many facets of reproductive competition in fire ant societies (Willer and Fletcher 1986; Vargo and Ross 1989; Ross 1992). Basic information on breeding systems and colony genetic structure can be expected to yield many further insights into the nature of reproductive competition in this and other social insects.

LITERATURE CITED

Aoki, K. 1981. Algebra of inclusive fitness. Evolution 35:659–663.

Ball, D. E., and S. B. Vinson. 1983. Mating in the fire ant, Solenopsis invicta Buren: evidence that alates mate only once. Journal of the Georgia Entomological Society 18:287–291.

Carpenter, J. M. 1989. Testing scenarios: wasp social behavior. Cladistics 5:131–144.

Crozier, R. H. 1970. Coefficients of relationship and the identity of genes by descent in the Hymenoptera. American Naturalist 204:216–217.

———. 1979. Genetics of sociality. Pages 223–286 in H. R. Hermann, ed. Social insects. Vol. I. Academic Press, New York.

———. 1980. Genetical structure of social insect populations. Pages 129–146 in H. Markl, ed. Evolution of social behavior: hypotheses and empirical tests. Chemie, Weinheim.

Elmes, G. W. 1980. Queen numbers in colonies of ants of the genus Myrmica. Insectes Sociaux 27:43–60.

Fletcher, D. J. C., M. S. Blum, T. V. Whitt, and N. Temple. 1980. Monogyny and polygyny in the fire ant Solenopsis invicta. Annals of the Entomological Society of America 73:658–661.

Glancey, B. M., and C. S. Lofgren. 1985. Spermatozoon counts in males and inseminated queens of the imported fire ants, Solenopsis invicta and Solenopsis richteri (Hymenoptera: Formicidae). Florida Entomologist 68:162–168.

———. 1988. Adoption of newly mated queens: a mechanism for proliferation and perpetuation of polygynous red imported fire ants, Solenopsis invicta Buren. Florida Entomologist 71:581–587.

Glancey, B. M., C. H. Craig, C. E. Stringer, and P. M. Bishop. 1973. Multiple fertile queens in colonies of the imported fire ant, Solenopsis invicta. Journal of the Georgia Entomological Society 8:237–238.

Goetsch, W. 1953. Vergleichende Biologie der Insektenstaaten. Geest & Portig, Leipzig.

Hamilton, W. D. 1972. Altruism and related phenomena, mainly in social insects. Annual Review of Ecology and Systematics 3:193–232.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Harvard University Press, Cambridge, Mass.

Jouvenaz, D. P., G. E. Allen, W. A. Banks, and D. P. Wojcik. 1977. A survey for pathogens of fire ants, Solenopsis spp., in the southeastern United States. Florida Entomologist 60:275–279.

Keller, L., and L. Passera. 1990. Fecundity of ant queens in relation to their age and the mode of colony founding. Insectes Sociaux 37:116–130.

Laidlaw, H. H., and R. E. Page. 1984. Polyandry in honey bees (Apis mellifera L.): sperm utilization and intracolony genetic relationships. Genetics 108:985–997.

Markin, G. P., J. H. Dillier, S. O. Hill, M. S. Blum, and H. R. Hermann. 1971. Nuptial flight and

flight ranges of the imported fire ant, *Solenopsis saevissima richteri*. Journal of the Georgia Entomological Society 6:145–156.

Michener, C. D., and B. H. Smith. 1987. Kin recognition in primitively eusocial insects. Pages 209–242 *in* D. J. C. Fletcher and C. D. Michener, eds. Kin recognition in animals. Wiley, New York.

Michod, R. E. 1982. The theory of kin selection. Annual Review of Ecology and Systematics 13:23–55.

Morrill, W. L. 1974. Production and flight of alate red imported fire ants. Environmental Entomology 3:265–271.

Nonacs, P. 1988. Queen number in colonies of social Hymenoptera as a kin-selected adaptation. Evolution 42:566–580.

O'Neal, J., and G. P. Markin. 1975a. The larval instars of the imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society 48: 141–151.

———. 1975b. Brood development of the various castes of the imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society 48: 152–159.

Page, R. E. 1986. Sperm utilization in social insects. Annual Review of Entomology 31:297–320.

Page, R. E., and R. A. Metcalf. 1982. Multiple mating, sperm utilization, and social evolution. American Naturalist 119:263–281.

Page, R. E., G. E. Robinson, and M. K. Fondrk. 1989. Genetic specialists, kin recognition and nepotism in honey-bee colonies. Nature (London) 338:576–579.

Pamilo, P. 1983. Genetic differentiation within subdivided populations of *Formica* ants. Evolution 37:1010–1022.

———. 1985. Effect of inbreeding on genetic relatedness. Hereditas 103:195–200.

———. 1991. Evolution of colony characteristics in social insects. II. Number of reproductive individuals. American Naturalist 138:412–433.

Pamilo, P., and S.-L. Varvio-Aho. 1979. Genetic structure of nests in the ant *Formica sanguinea*. Behavioral Ecology and Sociobiology 6:91–98.

Petralia, R. S., and S. B. Vinson. 1979. Developmental morphology of larvae and eggs of the imported fire ant, *Solenopsis invicta*. Annals of the Entomological Society of America 72:472–484.

Porter, S. D. 1991. Origins of new queens in polygyne red imported fire ant colonies (Hymenoptera: Formicidae). Journal of Entomological Science 26:474–478.

Queller, D. C. 1993. Genetic relatedness and its components in polygynous colonies of social insects. *In* L. Keller, ed. Queen number and sociality in insects. Oxford University Press, Oxford (in press).

Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution 43:258–275.

Queller, D. C., J. E. Strassmann, and C. R. Hughes. 1988. Genetic relatedness in colonies of tropical wasps with multiple queens. Science (Washington, D.C.) 242:1155–1157.

Ratnieks, F. L. W. 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. American Naturalist 132:217–236.

———. 1990. Assessment of queen mating frequency by workers in social Hymenoptera. Journal of Theoretical Biology 142:87–93.

Reeve, H. K. 1991. *Polistes*. Pages 99–148 *in* K. G. Ross and R. W. Matthews, eds. The social biology of wasps. Cornell University Press, Ithaca, N.Y.

Ross, K. G. 1986. Kin selection and the problem of sperm utilization in social insects. Nature (London) 323:798–800.

———. 1988. Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 23:341–355.

———. 1992. Strong selection on a gene that influences reproductive competition in a social insect. Nature (London) 355:347–349.

Ross, K. G., and J. M. Carpenter. 1991a. Population genetic structure, relatedness, and breeding systems. Pages 451–479 *in* K. G. Ross and R. W. Matthews, eds. The social biology of wasps. Cornell University Press, Ithaca, N.Y.

———. 1991b. Phylogenetic analysis and the evolution of queen number in eusocial Hymenoptera. Journal of Evolutionary Biology 4:117–130.

Ross, K. G., and D. J. C. Fletcher. 1985a. Comparative study of genetic and social structure in two forms of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 17:349–356.

———. 1985b. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance. Evolution 39:888–903.

Ross, K. G., E. L. Vargo, and D. J. C. Fletcher. 1987. Comparative biochemical genetics of three fire ant species in North America, with special reference to the two social forms of *Solenopsis invicta* (Hymenoptera: Formicidae). Evolution 41:979–990.

Seppä, P. 1992. Genetic relatedness of worker nestmates in *Myrmica ruginodis* (Hymenoptera: Formicidae) populations. Behavioral Ecology and Sociobiology 30:253–260.

Shoemaker, D. D., J. T. Costa, and K. G. Ross. 1992. Estimates of heterozygosity in two social insects using a large number of electrophoretic markers. Heredity 69:573–582.

Stille, M., B. Stille, and P. Douwes. 1991. Polygyny, relatedness and nest founding in the polygynous myrmicine ant *Leptothorax acervorum* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 28:91–96.

Strassmann, J. E., D. C. Queller, C. R. Solís, and C. R. Hughes. 1991. Relatedness and queen number in the Neotropical wasp, *Parachartergus colobopterus*. Animal Behaviour 42:461–470.

Trivers, R. L., and H. Hare. 1976. Haplodiploidy and the evolution of the social insects. Science (Washington, D.C.) 191:249–263.

Tschinkel, W. R., and S. D. Porter. 1988. Efficiency of sperm use in queens of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Annals of the Entomological Society of America 81:777–781.

Uyenoyama, M. K. 1984. Inbreeding and the evolution of altruism under kin selection: effects on relatedness and group structure. Evolution 38:778–795.

Vargo, E. L., and D. J. C. Fletcher. 1987. Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta*. Physiological Entomology 12:109–116.

———. 1989. On the relationship between queen number and fecundity in polygyne colonies of the fire ant, *Solenopsis invicta*. Physiological Entomology 14:223–232.

Vargo, E. L., and S. D. Porter. 1989. Colony reproduction by budding in the polygyne form of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Annals of the Entomological Society of America 82:307–313.

Vargo, E. L., and K. G. Ross. 1989. Differential viability of eggs laid by queens in polygyne colonies of the fire ant, *Solenopsis invicta*. Journal of Insect Physiology 35:587–593.

Wade, M. J. 1982. The effect of multiple inseminations on the evolution of social behaviors in diploid and haplo-diploid organisms. Journal of Theoretical Biology 95:351–368.

———. 1985. The influence of multiple inseminations and multiple foundresses on social evolution. Journal of Theoretical Biology 112:109–121.

Wade, M. J., and F. Breden. 1981. Effect of inbreeding on the evolution of altruistic behavior by kin selection. Evolution 35:844–858.

Ward, P. S. 1989. Genetic and social changes associated with ant speciation. Pages 123–148 *in* M. D. Breed and R. E. Page, eds. The genetics of social evolution. Westview, Boulder, Colo.

Weir, B. S. 1990. Genetic data analysis. Sinauer, Sunderland, Mass.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Willer, D. E., and D. J. C. Fletcher. 1986. Differences in inhibitory capability among queens of the ant *Solenopsis invicta*. Physiological Entomology 11:475–482.

Associate Editor: Jon Seger