

# Colony genetic structure and queen mating frequency in fire ants of the subgenus *Solenopsis* (Hymenoptera: Formicidae)

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Colony genetic structure was studied in natural populations of three fire ant taxa, *Solenopsis richteri* Forel, *S. geminata* (Fabr.), and hybrid *S. invicta/richteri*, using allozyme markers. All colonies studied exhibited arrays of female genotypes predicted under a model of monogyny (single functional queen) and monoandry (single insemination of queens). Males produced in the colonies appear to originate exclusively from the foundress queen, rather than from any virgin females present in the colonies. Thus these social insect colonies represent simple, albeit enormous, family groups. Single insemination and foundress parentage of males appear to be conserved reproductive traits in the subgenus *Solenopsis*, whereas another major determinant of colony genetic structure, the number of functional queens, is evolutionarily labile in this group.

KEY WORDS:—Social Hymenoptera – genetic structure – mating system – relatedness.

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## INTRODUCTION

Recent genetic studies of social Hymenoptera have often focused on colony genetic structure, which encompasses patterns of genetic relatedness among

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nestmates as well as the apportionment of maternity and paternity of offspring produced in a colony. The motivation for these studies is Hamilton's (1964, 1972) theory of kin selection, the recent formulations of which seek to explain social evolution in the Hymenoptera not only on the basis of elevated degrees of genetic relatedness among interactants, but also favourable cost/benefit ratios for individuals living in societies and appropriate patterns of colony sex ratio investment (e.g. West-Eberhard, 1975; Crozier, 1979; Starr, 1979; Andersson, 1984; Gadagkar, 1985). The intractability of quantitative cost/benefit studies in species with highly derived or complex social organizations, and increasing technical and theoretical concerns with sex ratio investment studies have led to a renewed emphasis on quantitative studies of relatedness among colony members (for which methods of data collection and analysis are well developed) to assess the importance of kin selection in the evolution of eusociality.

Analyses of colony genetic structure have been undertaken in a diversity of lineages of social Hymenoptera, particularly among the ants, but only a few of these studies have made comparisons between closely related groups (e.g. Metcalf, 1980; Pamilo, 1982a, 1983; Ward, 1983; Ross & Fletcher, 1985a; Ross 1986). Such comparisons are assuming increased importance, because a more complete understanding of the role of kin selection in social evolution will require greater knowledge of the evolutionary lability of key traits influencing colony genetic structure, such as number of matings by queens, number of functional queens in colonies, and male production by workers. For example, it has been suggested by several authors (e.g. Hamilton, 1972; West-Eberhard, 1975; Crozier & Page, 1985; Fletcher & Ross, 1985; Ross, 1986) that the inception of eusociality may have preceded the evolution of both polyandry (multiple mating by females) and polygyny (multiple functional queens per colony), characteristics which dilute relatedness among interacting females and thereby diminish the potential impact of kin selection (see also Page & Metcalf, 1982). Furthermore, much recent evolutionary conjecture ascribes changes in mating systems and social structure in social Hymenoptera to ecological factors (e.g. Hölldobler & Wilson, 1977; Brian, 1983; Cole, 1983; Crozier & Page, 1985). Implicit in much of this speculation is the assumption of a specific polarity of these character states (monoandry and monogyny being ancestral in social taxa) and the notion that the traits are labile, heritable, and responsive to selection. Yet few comparative data from well-defined contemporary lineages are available to support these basic assumptions. Such data, in addition to identifying plausible routes for the evolution of colony social and genetic structure, may further prove useful in clarifying any links between social evolution and cladogenesis in the social Hymenoptera (Wilson, 1971; Brian, 1983; West-Eberhard, 1983; Ross, 1988).

In this paper we extend our earlier comparative studies of colony genetic structure within the single fire ant species *Solenopsis invicta* Buren to include two additional congeners, as well as an interspecific hybrid population. The resulting sum of the comparative data spans several levels of taxonomic (genetic) affinity within the genus; from intraspecific variants, to closely related species in the same species complex, to more distantly related species in separate species complexes. These data represent an initial step toward a complete evolutionary analysis of factors influencing colony genetic structure in this group of ants.

## MATERIAL AND METHODS

*Biological background*

Fire ants of the subgenus *Solenopsis* represent a diverse array of species occurring primarily in the new world tropics and subtropics (Creighton, 1930; Buren, 1972; J. Trager, unpublished). While some species are native to the southern U.S.A., two of the more conspicuous elements in the North American fire ant fauna, the species *S. invicta* and *S. richteri*, were introduced from South America early in this century (Buren, 1972). These taxa were initially considered intraspecific variants by Wilson (1953), but were elevated to species status by Buren (1972) on the basis of morphological criteria. Subsequent morphological study (J. Trager, unpublished), as well as characterization of biochemical phenotypes (Vander Meer, Lofgren & Alvarez, 1985) and enzyme genotypes (Ross, Vander Meer, Fletcher & Vargo, 1987a; Ross, Vargo & Fletcher, 1987b), indicate significant differentiation between the two species over most of their North American ranges, although substantial introgression is known to occur in some areas (Vander Meer *et al.*, 1985; Ross *et al.*, 1987a; S. Diffie, unpublished).

The native North American species *S. geminata* is sufficiently distinct in its morphology from *S. invicta* and *S. richteri* to have been placed in a separate species complex (Creighton, 1930). A recent study of the biochemical genetic differentiation which exists among the three species (Ross *et al.*, 1987b) fully supports their classification based on traditional criteria.

*Collection of samples*

Samples of *S. richteri* were collected from northeastern Mississippi, U.S.A. (Prentiss, Itawamba and Tishomingo Counties) in an area where genetically 'pure' populations of this species have previously been shown to occur (Buren, Allen, Whitcomb, Lennartz & Williams, 1974; Vander Meer *et al.*, 1985; Ross *et al.*, 1987a). Samples of hybrid *S. invicta/richteri* were collected from a previously characterized hybrid zone in east-central Mississippi (Lauderdale, Kemper, Noxubee and Lowndes Counties; Vander Meer *et al.*, 1985; Ross *et al.*, 1987a) as well as from a hybrid population in northwestern Georgia (Floyd County; S. Diffie, unpublished). Samples of the 'red form' of the native species *S. geminata* (*sensu* Wilson, 1971; Wojcik, Buren, Grissell & Carlisle, 1976) were collected from north-central Florida (Alachua County), where this species occurs in sympatry with *S. invicta*.

For all taxa, mature female and male alates (winged sexuals) were collected from individual nests by spreading the contents of the soil mounds in a large plastic tray and systematically searching the soil. Collected ants were immediately placed in liquid nitrogen until returned to the laboratory, where they were held at  $-60^{\circ}\text{C}$  until electrophoresis. For *S. richteri*, 58 nests were sampled, with 11–26 ( $\bar{x}=16.7$ ) female alates collected from each and 3–13 ( $\bar{x}=7.0$ ) males collected from each of the 54 nests in which they were found. For hybrid *S. invicta/richteri*, only nests confirmed as being the products of introgression on the basis of electrophoretic markers and/or gas chromatographic analysis (see Ross *et al.*, 1987a) were included in the present

study. Fifty-one such nests from Mississippi and 13 from Georgia were sampled (64 total), with 6–33 ( $\bar{x}=18.4$ ) females collected from each and 4–30 ( $\bar{x}=15.7$ ) males collected from each of the 50 nests in which they occurred. Thirty nests of *S. geminata* were sampled, with 5–39 ( $\bar{x}=17.3$ ) females collected from each and 2–29 ( $\bar{x}=15.4$ ) males collected from each of 20 nests.

#### *Gel electrophoresis and data analysis*

Horizontal gel electrophoresis was conducted using 14% starch gels, as described in Harris & Hopkinson (1976), May, Wright & Stoneking (1979), and Ross *et al.* (1987b). Staining followed Brewer (1970), Shaw & Prasad (1970), and Ross & Fletcher (1985a). Fire ants (and most other social Hymenoptera) are characterized by low levels of genetic variability as detected by protein electrophoresis, with this reduced variability being most pronounced in the introduced species *S. richteri* and *S. invicta* (Ross *et al.*, 1987b). In *S. richteri* only one locus exhibits sufficient polymorphism to be useful for the purposes of the present paper. This locus, *Est-2*, is best resolved on the amine-citrate ('C') buffer system of Clayton & Tretiak (1972) using alitrunks as the tissue source for the enzyme. Because *S. richteri* and *S. invicta* exhibit diagnostic differences at 15% of the loci studied to date (Ross *et al.*, 1987b), hybrid *S. invicta/richteri* possess considerably higher levels of polymorphism (heterozygosity) than either of the parental forms. The products of four polymorphic loci (*Agp-1*, *Est-2*, *Odh*, *Est-4*) exhibit sufficient resolution and consistency of expression in hybrid ants to be used as genetic markers in the present study. All four markers were studied using the conditions described above for *Est-2* in *S. richteri*. Five loci are polymorphic in *S. geminata* (*Ldh-1*, *Pgd-2*, *Odh*, *Gpi*, *Mpi*; Ross *et al.*, 1987b), however, *Mpi* and *Pgd-2* were polymorphic in only one or a few of the colonies studied, and are thus of limited use in examining colony genetic structure. Conditions for electrophoresis for the *S. geminata* markers were as described for *Est-2* in *S. richteri*, except for the locus *Mpi*, the products of which were studied using the lithium hydroxide buffer of Ridgway, Sherburne & Lewis (1970) and the gaster as the tissue source.

All loci exhibit co-dominant expression of the alleles and are diallelic in the study populations, with the exception of *Est-2* in hybrid *S. invicta/richteri* (three alleles) and *Ldh-1* in *S. geminata* (four alleles). Mendelian inheritance was previously demonstrated for the products of the variable loci in *S. richteri* and hybrid *S. invicta/richteri* (Ross & Fletcher, 1985a; Ross *et al.*, 1987a) and is confirmed here for the variable markers in *S. geminata* by, (1) presence of banding patterns in presumptive heterozygotes consistent with known quaternary structures of the enzymes, (2) presence of the expected hemizygous banding patterns for all (haploid) males, and (3) occurrence of predicted banding patterns among presumed progeny of queens, as discussed below. Hardy–Weinberg proportions of genotypes have been demonstrated for all of the populations studied here (Ross *et al.*, 1987a, b).

Single-locus genotype arrays for male and female sexuals within single nests were examined to infer properties of the social and genetic structure, including number of functional queens, number of matings by queens, and maternity of colony males. Relatedness of female nestmates was estimated from genotype distributions using the correlation procedure of Stanton (1960), as modified by

Crozier, Pamilo & Crozier (1984) and Pamilo (1984) (see Equation 4 of Pamilo (1984) and Equation 1 of Wilkinson & McCracken (1985)). For diallelic loci this procedure is equivalent to the regression method of Pamilo & Crozier (1982). Nests were weighted equally for the analyses, and standard errors of the estimates were generated using a jackknife procedure over colonies (Pamilo, 1984). These estimates of relatedness are sensitive to the number of nests sampled, population frequency of alleles, and expectation for the relatedness values, with the precision of the estimates dependent on the number of loci used, number of nests sampled, number of individuals sampled per nest, and population frequency of alleles (Pamilo & Crozier, 1982; Crozier *et al.*, 1984; Wilkinson & McCracken, 1985; Crozier, Smith & Crozier, 1987). For *S. geminata*, only the four most polymorphic loci (*Gpi*, *Odh*, *Ldh-1*, *Mpi*) were used in deriving these estimates, whereas all polymorphic markers were used for the other taxa studied.

Preliminary analyses revealed a significant association of alleles (gametic phase disequilibrium) at the loci *Est-2* and *Est-4* in hybrid *S. invicta/richteri* (K. Ross, unpublished), indicating that these two markers cannot be regarded as independent. However, because each is often polymorphic, and thus informative, in a colony where the other marker is invariant, the results for the two markers are presented independently for some of the analyses below. A more complete description of linkage disequilibrium in hybrid *S. invicta/richteri* populations will be presented elsewhere. Ross & Fletcher (1985a) have previously demonstrated gametic phase equilibrium for the products of the loci *Agp-1* and *Est-4* in *S. invicta*.

## RESULTS

Examination of the arrays of single-locus genotypes for females from individual colonies reveals a simple pattern common to all three fire ant taxa studied. Only one or two genotypes are represented among the females; where two genotypes are present at least one of them is the heterozygous genotype (only one at diallelic loci) and the two genotypes occur in a 1:1 ratio in most instances. Where two genotypic classes of females are present the genotypes share an allele in common. Following simple rules of Mendelian inheritance, these are the predicted patterns if all females in a colony are the daughters of a single, once-mated queen (see also Crozier, 1973; Ward, 1983; Ross, 1988).

Given that the above is true, some deviations from a 1:1 ratio of genotypes in colonies with two genotypes should occur by chance in a large sample, so that it becomes important to compare the observed number of such deviations (estimated here from the binomial distribution at a 5% probability level) with those expected due to sampling error. In Table I the observed and expected numbers of deviations are presented for females of all three taxa. In all cases the observed number of significant deviations from a 1:1 ratio of genotypes is similar to the expected number, thus confirming the postulated model of monogyny (single functional queen) and monoandry (single insemination) in these ants.

In monogyne colonies headed by singly-inseminated queens all female nestmates are full sisters (=super sisters). Using this pedigree information, we estimate the pedigree coefficient of genetic relatedness ( $G$ ) among females in a

TABLE 1. Number of observed and expected cases of deviation from 1:1 ratio of genotypes in *Solenopsis* colonies with two genotypic classes. Only cases with  $\geq 6$  individuals are included

|          | <i>S. richteri</i> | <i>S. invicta/richteri</i> | <i>S. geminata</i> |
|----------|--------------------|----------------------------|--------------------|
| Females  |                    |                            |                    |
| Observed | 0                  | 6                          | 2                  |
| Expected | 1.4                | 5.3                        | 1.5                |
| <i>N</i> | 28                 | 105                        | 29                 |
| Males    |                    |                            |                    |
| Observed | 0                  | 8                          | 2                  |
| Expected | 1.1                | 3.6                        | 1.0                |
| <i>N</i> | 22                 | 72                         | 19                 |

In *S. invicta/richteri* colonies where both *Est-2* and *Est-4* are polymorphic the genotype distribution for only one is included since these markers are not independent.

nest to be 0.75 (Crozier, 1970; Pamilo & Crozier, 1982). Estimation of relatedness ( $r$ ) by the genotypic correlation method yields values very close to 0.75 for all taxa (Table 2).

An important component of colony genetic structure is the maternal origin of males, which are impaternal in the Hymenoptera. Inspection of male single-locus genotype arrays in individual colonies shows that, (1) if only one genotypic class of females is present then a single genotypic class of males is present as well, (2) if two genotypic classes of females are present then two genotypic classes of males are present, and (3) in both of the preceding situations the genotypes of the foundress queens, as inferred from the array of male genotypes in a colony are consistent with the identity and distribution of female (daughter) genotypes in the colony, presuming single mating of foundresses. These findings are consistent with the interpretation that the fire ants studied here form colonies which are simple family units, with all the colony's males, as well as females, the progeny of single, once-mated foundress queens.

Under the interpretation of exclusive foundress maternity of colony males, not only should colonies with two genotypic classes of females possess two genotypic

TABLE 2. Genetic relatedness ( $r \pm$  s.e.) among female nestmates for two species of *Solenopsis* and a hybrid *S. invicta/richteri* population estimated by the genotypic correlation method (Stanton, 1960; Crozier *et al.*, 1984; Pamilo, 1984)

| Locus        | <i>S. richteri</i><br>( <i>N</i> =58) | <i>S. invicta/richteri</i><br>( <i>N</i> =64) | <i>S. geminata</i><br>( <i>N</i> =30) |
|--------------|---------------------------------------|---|---------------------------------------|
| <i>Est-2</i> | 0.738 $\pm$ 0.043                     | 0.727 $\pm$ 0.030                             |                                       |
| <i>Agp-1</i> |                                       | 0.726 $\pm$ 0.050                             |                                       |
| <i>Est-4</i> |                                       | 0.709 $\pm$ 0.049                             |                                       |
| <i>Odh</i>   |                                       | 0.750 $\pm$ 0.041                             | 0.807 $\pm$ 0.055                     |
| <i>Gpi</i>   |                                       |   | 0.740 $\pm$ 0.071                     |
| <i>Mpi</i>   |                                       |   | 0.786 $\pm$ 0.133                     |
| <i>Ldh-1</i> |                                       |   | 0.812 $\pm$ 0.056                     |
| All          | 0.738 $\pm$ 0.043                     | 0.728 $\pm$ 0.023                             | 0.788 $\pm$ 0.030                     |

Numbers of colonies used in the calculations are shown in parentheses.

classes of males (because of the necessity of foundress heterozygosity at the locus in question under single mating), but the male genotypes should occur in a 1 : 1 ratio, presuming an absence of segregation distortion during oogenesis or genotype-specific mortality or dispersal from the nest. In Table 1 the observed number of deviations from a 1 : 1 ratio in colonies where two genotypic classes of males occur is compared with the number expected, using an approach analogous to that for females. Significant deviations are rare or nonexistent for *S. geminata* and *S. richteri*, but occur at approximately twice the frequency expected due to chance alone in hybrid *S. invicta/richteri* ( $\chi^2 = 5.66$ ,  $df = 1$ ,  $P < 0.05$ ). If the excess of deviations from a 1 : 1 ratio were due to some or all of the males in the exceptional colonies being the progeny of virgin daughters of the foundress, rather than of the foundress herself, then the male genotype overrepresented in such samples would bear the allele for which one-half of the colony females are homozygous\*. In all six hybrid colonies appropriate for such analysis the deviation in male genotype ratios is, in fact, in a direction consistent with some proportion of these males being derived from daughters of the foundress.

A more sensitive test for the presence of males originating from daughters of the foundress involves an examination of male genotypes in colonies where foundresses are homozygous at one or more marker loci and are inseminated by a male bearing an alternate allele (e.g. Crozier, 1974; Ward, 1983; Ross, 1986). In such colonies all sons of the foundress will possess the allele for which she is homozygous. Since all daughters of the foundress are heterozygotes at the relevant loci, any males produced by these will segregate in a 1 : 1 ratio for the two possible haploid genotypes. Thus one-half of these males can be distinguished from queen-derived males. For *S. richteri*, no such males were found in the 13 appropriate colonies studied (85 male genotypes determined). For *S. geminata*, ten colonies exhibited genotype arrays appropriate for conducting this analysis. Only a single male genotype, out of 119 examined, was consonant with that individual being the son of a female other than the foundress. Twenty-two hybrid *S. invicta/richteri* colonies exhibited appropriate genotype arrays, and not a single male from this subsample (267 genotypes studied) possessed a genotype inconsistent with maternity by the foundress queen (data from five such colonies are presented in Table 3). Thus, although the data bearing on maternity of males in hybrid colonies appear somewhat ambiguous, this latter, more sensitive analysis suggests that few, if any, males in these colonies are sons of females other than the foundress.

#### DISCUSSION

The results of this study, in conjunction with data from Ross & Fletcher (1985a) on *S. invicta*, show that single mating by queens is common to several species of varying taxonomic affinity within the subgenus *Solenopsis* and may well constitute a ground plan characteristic for the group. Further assessment of the evolutionary stability of this component of the mating system, as well as its polarity with respect to higher-level taxa, must await studies of more distantly related species in the genus *Solenopsis* and species in related genera such as

\*When the relevant marker locus has three or more alleles only colonies in which one of the two female genotypes present is homozygous can be used in this analysis.

TABLE 3. Genotype distributions for alate queens, males, foundresses, and male mates of foundresses in five *S. invicta/richteri* colonies appropriate for determining maternity of males. Genotypes of foundresses and their mates are inferred from offspring genotypes. Values at crucial loci are italicized

| Colony    | <i>Est-2</i> |            |            |             |            |             | <i>Agp-1</i> |            |             | <i>Odh</i>  |            |             | <i>Est-4</i> |            |             |
|-----------|--------------|------------|------------|-------------|------------|-------------|--------------|------------|-------------|-------------|------------|-------------|--------------|------------|-------------|
|           | <i>F F*</i>  | <i>F M</i> | <i>F S</i> | <i>M M*</i> | <i>M S</i> | <i>S S*</i> | <i>F F*</i>  | <i>F S</i> | <i>S S*</i> | <i>F F*</i> | <i>F S</i> | <i>S S*</i> | <i>A A*</i>  | <i>A B</i> | <i>B B*</i> |
| 65-1BB    |              |            |            |             |            |             |              |            |             |             |            |             |              |            |             |
| Queens    |              |            | 8          |             |            | 8           | 16           |            |             |             | <i>14</i>  |             |              | 16         |             |
| Males     | 11           |            |            |             |            | 7           | 18           |            |             | <i>18</i>   |            |             |              | 18         |             |
| Foundress |              |            | 1          |             |            |             | 1            |            |             | <i>1</i>    |            |             |              | 1          |             |
| Mate      |              |            |            |             |            | 1           | 1            |            |             |             |            | <i>1</i>    |              | 1          |             |
| 65-1X     |              |            |            |             |            |             |              |            |             |             |            |             |              |            |             |
| Queens    |              | 6          | 11         |             |            |             |              | <i>17</i>  |             | 6           | 6          |             |              | 17         |             |
| Males     |              |            |            | 9           |            | 3           | <i>12</i>    |            |             | 8           |            | 4           |              | 12         |             |
| Foundress |              |            |            |             | 1          |             | <i>1</i>     |            |             |             | 1          |             |              | 1          |             |
| Mate      | 1            |            |            |             |            |             |              |            | <i>1</i>    | 1           |            |             |              | 1          |             |
| 65-1L     |              |            |            |             |            |             |              |            |             |             |            |             |              |            |             |
| Queens    |              | 4          |            | 9           |            |             | 18           |            |             |             | <i>18</i>  |             |              | 18         |             |
| Males     | 8            |            |            | 10          |            |             | 19           |            |             | <i>19</i>   |            |             |              | 16         |             |
| Foundress |              | 1          |            |             |            |             | 1            |            |             | <i>1</i>    |            |             |              | 1          |             |
| Mate      |              |            |            | 1           |            |             | 1            |            |             |             |            | <i>1</i>    |              | 1          |             |
| 65-1T     |              |            |            |             |            |             |              |            |             |             |            |             |              |            |             |
| Queens    |              |            |            |             | <i>18</i>  |             | 12           | 6          |             |             | 11         | 7           | 9            | 9          |             |
| Males     |              |            |            | <i>11</i>   |            |             | 4            |            | 7           | 5           |            | 6           | 5            |            | 6           |
| Foundress |              |            |            | <i>1</i>    |            |             |              | 1          |             |             | 1          |             |              | 1          |             |
| Mate      |              |            |            |             |            | <i>1</i>    | 1            |            |             |             |            | 1           | 1            |            |             |
| 65-1Z     |              |            |            |             |            |             |              |            |             |             |            |             |              |            |             |
| Queens    |              |            |            | 10          | 9          |             | 16           |            |             | 8           | 11         |             |              | <i>19</i>  |             |
| Males     |              |            |            | 6           |            | 8           | 14           |            |             | 7           |            | 7           | <i>14</i>    |            |             |
| Foundress |              |            |            |             | 1          |             | 1            |            |             |             | 1          |             | <i>1</i>     |            |             |
| Mate      |              |            |            | 1           |            |             | 1            |            |             | 1           |            |             |              |            | <i>1</i>    |

\*For males, these columns represent the corresponding hemizygote genotypes.

*Monomorium*, *Megalomyrmex*, and *Oxyepoecus* (Wilson, 1986; J. Trager, unpublished). Interestingly, *S. lou*, a member of the presumably more advanced subgenus *Diplorhoptrum*, is reported to exhibit multiple mating by queens (Cole, 1983), suggesting that this may represent a derived condition within the genus *Solenopsis*. It should be noted, however, that the value of such an evolutionary analysis of mating behaviour is dependent upon a well-founded phylogenetic framework which serves to identify relevant groups for study (such as outgroups for rooting character-state trees), and that sufficiently resolved phylogenies are currently not available for this purpose in this and in many other groups of ants. The limited comparative genetic studies of queen mating frequency undertaken in other ants and in wasps suggest that this property of the mating system may often be relatively conserved at the level of the genus (Metcalf & Whitt, 1977; Metcalf, 1980; Pamilo, 1982b; Ward, 1983; Ross, 1986), whereas the larger comparative data base for social Hymenoptera based on less reliable techniques (e.g. observation and sperm counts) would appear to provide less support for this hypothesis (see Page & Metcalf, 1982; Cole, 1983; Starr, 1984; Page, 1986).

Our demonstration from genetic data of single mating in fire ants accords well with the known details of the mating behaviour. In *S. invicta*, males take flight

shortly before females and stay aloft at an altitude of 50–100 m (Markin, Dillier, Hill, Blum & Hermann, 1971). Female alates ascend, mate on the wing, and descend rather quickly (Markin *et al.*, 1971; D. Fletcher, unpublished), shedding their wings immediately upon landing. It is intriguing that monoandry universally characterizes not only the monogyne forms, which exhibit nuptial flights and independent colony founding, but is also characteristic of the polygyne form of *S. invicta*, in which mating may take place in the nest and colony reproduction likely occurs via fission or budding (Fletcher, 1983; Greenberg, Fletcher & Vinson, 1985).

The evolution of multiple mating in social Hymenoptera has recently received considerable attention, as its occurrence in the absence of non-random sperm utilization diminishes inclusive fitness effects favouring worker-like behaviour (Page & Metcalf, 1982; Cole, 1983; Page, 1986). Several hypotheses invoking selection have been put forth to explain the evolution of polyandry in this group (see Crozier & Page, 1985, for a review); our data bear directly on two.

Cole (1983) has suggested that multiple mating arises as an adaptation for maintaining large colonies, in that several matings are necessary for queens to be able to store sufficient sperm to sustain colony development. Colonies of the *Solenopsis* species we have studied are among the most populous social insect colonies known, with adult populations of *S. invicta* often exceeding several hundred thousand adult individuals at a given time (Markin, Dillier & Collins, 1973; Lofgren, Banks & Glancey, 1975). It is clear from our results and from sperm count data for newly-inseminated *S. invicta* and *S. richteri* queens (Glancey & Lofgren, 1985; Tschinkel, 1987) that a single male indeed provides sufficient sperm to a female to last through the normal 4–7 year development of a monogyne colony (Tschinkel, 1987).

Crozier & Page (1985; see also Page, 1980; Page & Metcalf, 1982) argue that the occurrence of multiple mating in social Hymenoptera may reflect an important association between mating frequency and individual fitness in these organisms which possess a sex determination system based on heterozygosity at one or a few major regulator genes. Polyandry minimizes the between-colony variance in diploid male production over the population so that individual queens are unlikely to produce a high proportion of diploid male offspring (which may represent as much as 50% of brood production for single-mating queens). High levels of diploid male production by queens can result in a substantial fitness loss through diminished colony reproductive success, because these males are effectively sterile (Crozier & Page, 1985; Ross & Fletcher, 1985b) and the colony produces them at some expense to worker production.

In the model of Page (1980) for the evolution of polyandry in honey bees, specific fitness components affected by diploid male production were not identified, whereas Crozier & Page (1985) generalized the model by introducing as a major fitness component the timing of the production of sexuals during colony ontogeny. In North American *S. invicta*, 15–20% of newly-mated queens in monogyne populations produce high proportions of diploid male offspring during the initial 'claustral' phase of colony founding. These queens invariably fail to establish successful colonies, presumably because the worker force does not reach a sufficient size to aggressively compete with other incipient colonies or to excavate the nest to an adequate depth to avoid winter freezing (Ross & Fletcher, 1985b, 1986). This finding implicates diploid male production during

the claustral period, rather than the timing of production of sexuals, as the most significant fitness component to be considered when assessing the evolution of queen mating frequency in *S. invicta*. (Data on diploid male production are presently not available for *S. richteri* or *S. geminata*.)

Assuming a single major sex locus, the relative frequency of 'matched' matings (those in which a queen mates with a male bearing an allele in common with the queen at the sex locus) is equivalent to the frequency of diploid-male-producing queens for monoandrous species such as the *Solenopsis* studied here. From the frequency of matched matings can be calculated the effective number of alleles at the sex locus as well as the population-wide average proportion of viable workers (Adams, Rothman, Kerr & Paulino, 1977; Page & Marks, 1982). Estimates for these parameters for *S. invicta* are presented in Table 4. Given that survival during the claustral period represents a major fitness component, selection favouring or disfavouring multiple mating by queens can be simply analysed with reference to this component (R. Page, unpublished). If queens mate with a large number of males then all will produce diploid males at a frequency close to the population mean (low variance for this trait), that is, the proportion of viable workers produced by each queen will be similar to the population mean. If the proportion of viable worker production needed to ensure survival during founding exceeds the proportion actually produced (0.900–0.925 in this example for *S. invicta*, see Table 4), then no queens would succeed in establishing colonies. On the other hand, if all queens are singly inseminated then some proportion (80–85% for *S. invicta*) will produce all of their brood as viable workers, while the remainder will exhibit viable brood production of only 0.5. Thus in this example for North American *S. invicta*, polyandry would likely be favoured only if a loss of 7–10% of worker production during colony founding did not adversely affect the prospects for colony survival, an unlikely event given the rigorous conditions which exist during the claustral phase (see discussion in Ross & Fletcher, 1986). A necessary caveat for this discussion, however, is that *S. invicta*, as a recently introduced species, is likely to have suffered a significant population bottleneck during colonization so that South American populations may exhibit greater allelic diversity at the sex locus than is seen for North American populations (Ross & Fletcher, 1985b). Such a circumstance in the native populations would lead to a higher mean proportion of viable workers, perhaps relaxing conditions for the spread of polyandry.

The results of this study indicate that a diversity of taxa in the subgenus *Solenopsis* form colonies which are simple, albeit enormous, family groups in which females are closely related to one another and all colony members are

TABLE 4. Relevant parameters for interpreting the action of selection favouring or disfavouring polyandry in *S. invicta* in North America

| Proportion of matched matings ( $\theta$ ) | Effective number of sex alleles ( $k = 2/\theta$ ) | Population-wide mean proportion of viable workers ( $\bar{W} = 1 - [1/k]$ ) |
|--|--|---|
| 0.15–0.20                                  | 13.3–10.0  | 0.925–0.900   |

Based on data from Ross & Fletcher (1985b, 1986). A single sex locus is assumed.

offspring of the foundress queen. Workers of *Solenopsis* are obligately sterile so that the option of direct production of some or all of the colony males is not available to these individuals, as it is to workers in most social Hymenoptera (Fletcher & Ross, 1985). On the other hand, virgin alate females are present in nests during a substantial portion of the year, and in *S. invicta* such females are known to oviposit under appropriate conditions (Fletcher & Blum, 1983). Our data reinforce the conclusion of Ross & Fletcher (1985a) that oviposition by virgin alate fire ant queens in their natal colonies is unlikely to constitute a significant alternative reproductive strategy for these individuals, but rather serves some trophic function or simply represents 'noise' in the physiological regulation of reproduction in the colony (Fletcher & Blum, 1983).

Exceptions to the simple colony genetic structure we describe are known to exist in fire ants. These occur not as a result of variability in mating frequency, but rather as a result of adoption of alternative social structures. Polygyny is well known in *S. invicta* (Glancey, Craig, Stringer & Bishop, 1973; Fletcher, Blum, Whitt & Temple, 1980; Fletcher, 1983; Ross & Fletcher, 1985b), having been described from several populations distributed widely over the southeastern U.S.A. The genetic structure of polygyne colonies of this species contrasts sharply with that of monogyne colonies, in that female nestmates exhibit exceptionally low relatedness to one another in polygyne populations (Ross & Fletcher, 1985a). Interestingly, polygyny has also been reported for *S. geminata* from Florida (Banks, Plumley & Hicks, 1973; Adams, Banks & Plumley, 1976) and Texas (E. Vargo, unpublished), although we detected no evidence for this in our samples. This may be due to polygyny in this species being a localized phenomenon, as appears to be the case for *S. invicta*, or, perhaps less likely, may be due to *S. geminata* colonies with multiple inseminated queens being functionally monogynous (e.g. Tschinkel & Howard, 1978, for *S. invicta*). Detailed geographic surveys of colony genetic structure in this species should help distinguish between these two possibilities.

Plasticity in colony social structure among closely related populations of ants is a widespread phenomenon (Wilson, 1971; Brian, 1983). The preliminary data presented here and available in the literature may suggest that shifts in social structure are more important elements in the dynamics of colony genetic structure than are changes in mating behaviour in this group of social Hymenoptera. Further comparative genetic studies within well-defined lineages are required to evaluate this speculation.

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