Unusual Behavior of Polygyne Fire Ant Queens on Nuptial Flights

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This study reports previously undescribed behavior of fire ant queens (Solenopsis invicta) *on their nuptial flights. We captured large numbers of alate (winged) queens flying at low altitudes in dense swarms that were virtually devoid of males. We assayed the genotypes of these alate queens at the locus* Gp-9, *which exhibits strong genotype frequency differences between monogyne (single-queen) and polygyne (multiple-queen) populations, and found that almost all of these low-flying queens originated from polygyne colonies. Comparisons of mtDNA haplotype distributions of these queens to those of alates leaving polygyne nests suggest that the flying queens had not dispersed more than a few hundred meters. Moreover, the proportion of flying queens that were mated did not differ significantly from the proportion of reproductive queens that were mated within the same sites. Thus the flight behavior appears to occur subsequent to mating. We suggest that the flying queens are sampling the local environment in order to select a suitable landing site. Such a site would contain established polygyne nests into which the queens may be adopted as new reproductives.*

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INTRODUCTION

Variation in the number of reproductive queens within ant colonies frequently is associated with variation in the morphology, social behavior, and reproductive physiology of these queens (Hölldobler and Wilson, 1990; Ross and Keller, 1995a; Keller, 1993; Bourke and Franks, 1995; Crozier and Pamilo, 1996). For instance, new queens produced in polygyne (multiple-queen) colonies often are lighter and possess lower energy reserves than conspecific queens produced in monogyne (single-queen) colonies (Keller and Passera, 1989; Passera *et al.*, 1990; Bourke and Franks, 1995; Sundström, 1995). Polygyne queens also produce fewer sexual offspring (Vargo and Fletcher, 1989; Vargo, 1992) and often have a shorter lifespan than their monogyne counterparts (Hölldobler and Wilson, 1990; Bourke and Franks, 1995; Keller and Genoud, 1997). However, one of the most important differences between queens of the two social types relates to their reproductive behavior. Typically, monogyne queens engage in large-scale nuptial flights, with many nests in a local population simultaneously emitting numerous male and female sexuals. Monogyne males may form swarms at a location far from the natal nest, and monogyne queens may disperse widely before landing to found a new colony (Hölldobler and Bartz, 1985; Hölldobler and Wilson, 1990; Bourke and Franks, 1995). In contrast, polygyne queens frequently mate in their natal nests and disperse only short distances (Hölldobler and Wilson, 1990; Keller, 1991, 1993; Bourke and Franks, 1995; Heinze and Tsuji, 1995).

The introduced fire ant *Solenopsis invicta* presents an excellent opportunity to compare the mating activities of conspecific monogyne and polygyne queens. The mating behavior of monogyne *S. invicta*, which has been described previously, conforms to the description for monogyne ants given above (Markin *et al.*, 1971). However, indirect evidence arising from comparisons of genetic structure within the two social forms indicates that differences in the mating biology of monogyne and polygyne *S. invicta* likely exist (Shoemaker and Ross, 1996; Ross and Shoemaker, 1997). Here, we describe features of the behavior of polygyne *S. invicta* queens in their introduced range that are distinct from features described for conspecific monogyne queens. Large numbers of polygyne queens on nuptial flights were found to exhibit low-altitude flight after mating. We suspect that this behavior allows polygyne queens to explore the local environment and locate a suitable landing site. These findings underscore the dramatic differences in mating and dispersal biology between the two social forms of this species.

METHODS

We collected queens in late spring from a well-studied polygyne population of *S. invicta* in its introduced range (Walton Co., Georgia) (Ross and

Keller, 1995b). This population, which spans approximately 75 km^2, is surrounded by monogyne *S. invicta* and does not contain *S. invicta–S. richteri* hybrids (Shoemaker *et al.*, 1994). Samples were obtained in the late spring and early summer from five sites within this population, with the distance between individual sites ranging from 1 to 10 km. On all days when collecting took place, alate (winged) queens and males were seen leaving polygyne nests on nuptial flights (Markin *et al.*, 1971; Glancey and Lofgren, 1988; Hölldobler and Wilson, 1990). We collected preflight queens as they gathered on the tops of the nests.

At all sites we observed *S. invicta* female alates flying horizontally approximately 2 m above the ground. This behavior started at approximately 1300 h, reached peak levels between 1400 and 1500 h, and then declined steadily until 1600 h. These flying alates were evenly distributed in the air and sometimes confined to open areas of less than 100 m^2 that were naturally formed by the surroundings. For example, we observed many ants flying along a small road, the sides of which were heavily covered with vegetation and trees. The alates flew in straight lines, although their flight did not appear to be directed toward any discernible landmarks.

In some areas only tens of individuals were seen displaying this behavior, but in other sites the alates numbered in the hundreds to thousands. The ants frequently flew up out of sight (>5 m height) and sometimes descended to land on the ground, only to take flight again after a few seconds. We used standard insect-collecting nets to capture ants in flight. In a few cases when the flying ants reached their highest densities, we were able to collect over 100 individuals by randomly sweeping a net through the air for approximately 5 min. Only three males were among the approximately 600 ants netted, and one of these males was in copulation with a female.

To ascertain when mating takes place in polygyne *S. invicta*, we determined the mating status of all flying queens, as well as a subset of 50 of the preflight queens. Sperm in mated queens was evident as an opaque, whitish mass in the spermathecae. Queen mating status may also serve as a tool for detecting structure in mating swarms if the distribution of fertile males varies spatially. We expect the frequency of mated queens to vary among sites if fertile males are not uniformly distributed across the population.

The genotypes of all queens were determined electrophoretically at the diallelic locus *general protein-9* (*Gp-9*). This locus (or one closely linked to it) apparently is under strong selection in introduced *S. invicta* (Ross, 1997). Prereproductive queens of the polygyne form possess one of three genotypes (*BB*, *Bb*, and *bb*), whereas reproductive polygyne queens are almost always (99.9%) heterozygotes (Ross, 1997; DeHeer *et al.*, 1999). In contrast, no queens of genotype *Bb* have been collected from a confirmed monogyne colony; rather, all females sampled from monogyne colonies are genotype *BB* (Shoemaker and Ross, 1996; Ross, 1997). Thus, a queen with at

least one copy of the *b* allele necessarily originates from a polygyne colony. Differences in the distributions of *Gp-9* genotypes of queens within and among sites were assessed using an exact test for population differentiation as implemented by the program GENEPOP (Raymond and Rousset, 1995).

Queen haplotypes at a specific 4-kb region of the mitochondrial DNA (mtDNA) also were assayed (Ross and Shoemaker, 1997). The mtDNA haplotype was obtained by PCR amplification of the region followed by digestion with the enzyme *Hin*I, which differentiates among the four haplotypes found in this population (Shoemaker and Ross, 1996; Ross and Shoemaker, 1997). The program WINAMOVA was used to measure and evaluate the significance of mitochondrial genetic differentiation within and among sites (Excoffier *et al.*, 1992).

In this study, we consider two types of mitochondrial genetic structure. The first type, quantified by the statistic ϕ_{ST}, is concerned with the genetic differentiation of a single class of queens (e.g., preflight queens) among sites within the total population. The second type of genetic structure, estimated by ϕ_{CS}, pertains to the genetic differentiation among different classes of queens within sites. Thus, while the statistic ϕ_{ST} examines variation in mtDNA haplotype frequencies over space, the statistic ϕ_{CS} is concerned with variation in mtDNA haplotype frequencies among age classes. Values of ϕ_{ST} or ϕ_{CS} were considered to be significantly different from zero ($P < 0.05$) if fewer than 5% of 9999 estimates obtained by random permutation of haplotypes across individuals were more extreme than the observed measure.

RESULTS

Genotypes of Queens at *Gp-9*

Table I gives the *Gp-9* genotype frequencies of preflight (PF) and inflight (IF) queens captured at the five sites. Most of the PF (97.6%) and IF (98.7%) queens were of genotype *Bb* or *bb*. This result confirms that PF queens were sampled from polygyne nests, and, more importantly, demonstrates that most, if not all, of the IF queens captured also originated from polygyne colonies. In three of the five sites, the genotype distribution of PF queens differed significantly from that of IF queens from the same site (exact test: $P = 1.000$, $P = 1.000$, $P < 0.0001$, $P < 0.005$, and $P < 0.05$ for sites A, B, C, D, and G, respectively). At all sites, a lower frequency of *bb* queens is found in the IF than in the PF samples. Therefore, it appears that relatively few *bb* queens participate in the low-altitude flight behavior.

Table I. *Gp-9* Genotype Frequencies of Preflight (PF) and In-flight (IF) Queens from a Single Polygyne Population of Introduced *S. invicta*

Site		Genotype			Sample size
		BB	*Bb*	*bb*	
A	PF	0.048	0.838	0.114	272
	IF	0	1	0	6
B	PF	0.037	0.889	0.074	54
	IF	0.006	0.952	0.042	166
C	PF	0.011	0.629	0.359	259
	IF	0	0.981	0.019	52
D	PF	0	0.780	0.220	91
	IF	0.071	0.843	0.086	70
G	PF	0.017	0.903	0.080	237
	IF	0.006	0.968	0.025	314
All sites	PF	0.024	0.793	0.183	913
	IF	0.013	0.951	0.036	608

Mitochondrial Genetic Structure

Table II gives the mtDNA haplotype frequencies of PF and IF queens captured at the five sites, as well as those of reproductive (RP) queens sampled previously from three of the five sites (Goodisman and Ross, 1998). In accord with previous results for introduced polygyne *S. invicta* (Shoemaker and Ross, 1996; Ross and Shoemaker, 1997), only three haplotypes were found among queens in this population. We tested for the presence of mtDNA genetic structure using queens of single classes from different sites. The haplotype distributions for both PF queens and RP queens revealed highly significant differentiation among sites ($\phi_{ST} = 0.076$, $P < 0.0001$ for PF queens; $\phi_{ST} = 0.019$, $P < 0.001$ for RP queens). The IF queens also showed significant mitochondrial heterogeneity across sites ($\phi_{ST} = 0.013$, $P < 0.05$), although not to the extent seen in the other two queen classes. These results suggest that there may be some mixing of queens among sites while the queens are in flight.

We next compared the distributions of mtDNA haplotypes across the PF, IF, and RP queen classes within each of the five sites. If the IF queens originated mostly from the same nests from which the PF and RP queens were sampled, then estimates of ϕ_{CS}, which assesses differences in the distribution of mitochondrial haplotypes among classes of queens within sites, should not differ significantly from zero. Our first test nested mtDNA haplotypes of PF and IF queens within the five sites. The resulting calculations yield a nonsignificant value of $\phi_{CS} = 0.000$. The second test nested mtDNA haplotypes of IF and RP queens within the three sites from which RP queens

Table II. MtDNA Haplotype Frequencies of Preflight (PF), In-flight (IF), and Reproductive (RP) Queens from a Single Polygyne Population of Introduced *S. invicta*

Site		Haplotype			Sample size
		A	B	C	
A	PF	0.281	0	0.719	270
	IF	0.333	0	0.667	6
	RP	0.299	0	0.701	422
B	PF	0.407	0.037	0.556	54
	IF	0.325	0.012	0.663	163
	RP	0.419	0	0.581	597
C	PF	0.079	0.015	0.906	266
	IF	0.164	0.016	0.820	61
	RP[a]	—	—	—	—
D	PF	0.349	0.035	0.616	86
	IF	0.343	0.029	0.629	70
	RP	0.372	0.006	0.622	164
G	PF	0.215	0	0.785	223
	IF	0.234	0.024	0.742	295
	RP[a]	—	—	—	—
All sites	PF	0.219	0.010	0.771	899
	IF	0.266	0.020	0.714	595
	RP	0.369	0.001	0.630	1183

[a] No data available.

were sampled. Again, we obtain a nonsignificant value of $\phi_{CS} = 0.004$. Finally, we nested all three classes of queens within the three sites for which data from all of these queens were available. Not surprisingly, this value of ϕ_{CS} also does not differ significantly from zero ($\phi_{SC} = -0.001$). Therefore, the mtDNA haplotype composition of PF, IF, and RP queens at a single site are similar, indicating that IF queens mostly originate from nests in the sites where they were captured. This result is especially compelling because of the pronounced mtDNA differentiation found among sites within all three classes of queens.

Queen Matedness

None of the 50 PF queens that we dissected was mated, suggesting that polygyne *S. invicta* queens in this population do not mate in the nest before their nuptial flight. We next examined the proportions of mated and unmated IF queens captured at the five sites, as well as the proportions of mated and unmated RP queens sampled from three of the five sites (Table III) (Goodisman and Ross, 1998). We compared the proportions of mated queens of

Table III. Proportions of Mated and Unmated In-flight (IF) and Reproductive (RP) Queens from a Single Polygyne Population of Introduced *S. invicta*

Site		Mated	Unmated	Sample size
A	IF	0.500	0.500	6
	RP	0.469	0.531	431
B	IF	0.669	0.331	160
	RP	0.599	0.401	614
C	IF	0.610	0.390	59
	RP[a]	—	—	—
D	IF	0.493	0.507	67
	RP	0.467	0.533	165
G	IF	0.502	0.498	301
	RP[a]	—	—	—
All sites	IF	0.556	0.444	593
	RP	0.535	0.465	1210

[a]No data available.

each class across sites to determine if there is any evidence that queens mated in spatially distinct swarms. Both the proportions of mated IF queens and mated RP queens differed across the five sites (*G*-test of independence: $G_4 = 13.91$, $P < 0.005$ for IF queens; $G_2 = 20.99$, $P < 0.0001$ for RP queens). The variation in matedness among sites suggests that mating in this polygyne population does not occur in a single, panmictic swarm, and that the relative availability of fertile males (and hence the operational sex ratio) varies spatially (see below).

We next compared the proportions of mated IF and RP queens within sites. If these proportions did not differ between the two classes of queens, then the IF queens likely had completed their mating behavior [*S. invicta* queens do not mate with more than one male (Ross and Fletcher, 1985)]. The proportions of mated IF and RP queens within the three sites for which both types of queens were available did not differ significantly from each other (*G*-test of independence: $G_1 = 0.02$, 0.13, and 2.62 for sites A, B, and D, respectively). The lack of a difference in the proportions of mated IF and RP queens within sites, combined with the variation in the proportions of mated queens among sites, suggests that the IF queens had completed mating and were returning from localized mating swarms.

DISCUSSION

We observed *S. invicta* queens exhibiting an unusual and previously unreported type of flight behavior during large-scale nuptial flights. These

queens were captured flying at head level, but frequently flew up out of sight, or touched down briefly on the ground. The swarms consisted of females from local nests and were differentiated from other such swarms in the population with respect to frequency of queen matedness and mtDNA haplotype frequency. Most importantly, almost all queens displaying this behavior originated from polygyne colonies.

Nature of Polygyne Swarms

Two observations indicate that the swarms of flying queens were not actual mating swarms. First, of the ∼600 alates we captured, only 3 were males. In contrast, the mating swarms of monogyne *S. invicta* include many males (Markin *et al.*, 1971). Second, the proportion of queens captured in the air that were mated did not differ significantly from the proportion of mated egg-laying queens collected in nests at the same site, indicating that most of the queens captured in flight had completed their mating activity [*S. invicta* queens mate with no more than one male (Ross and Fletcher, 1985)]. It has been suggested that polygyne *S. invicta* queens may mate in the nest (Fletcher, 1983; Ross and Keller, 1995a), but our data imply that many queens of this form probably do not mate in the nest. Rather, the flying polygyne queens we collected appear to mate on the wing.

The collection of a single copulating pair suggests that the actual location of mating activity may not have been far from the locations where we netted the flying queens. Markin *et al.* (1971) captured monogyne queens and males at heights of 50–100 m, and it is possible that polygyne queens mate well out of visual range as well. Regardless of where mating takes place, differences across sites in the proportions of queens that were mated indicate that polygyne mating swarms are fairly localized. The fact that many queens remain unmated suggests that fertile males are in limited supply [presumably because most males are sterile diploids (Ross and Keller, 1995a)]. That is, the operational sex ratio is female biased. The heterogeneity in matedness across sites suggests that the extent of female bias varies due to differences in the density of either queens or fertile males within swarms.

The flight activity documented in this study may represent postmating dispersal or nest-site selection. Evidence already exists for site selection in *S. invicta*; monogyne queens are attracted to, and preferentially land on, open reflective or shiny surfaces following nuptial flights (Vinson and Greenberg, 1986). Such visual cues may allow monogyne queens to identify disturbed habitats that are suitable for the founding of new colonies. However, the flying polygyne queens observed in this study probably were not attempting to locate these types of sites. Most polygyne *S. invicta* queens lack sufficient

energy reserves to found colonies independently (Keller and Passera, 1989; Keller and Ross, 1993b). Rather, most new polygyne nests originate as buds of preexisting nests (Vargo and Porter, 1989). Newly mated polygyne queens normally enter established colonies and, if successful, are adopted as egg-layers (Glancey and Lofgren, 1988; Porter, 1991). It is possible that the flight behavior of polygyne queens observed in this study constitutes some type of searching behavior that may be related to locating suitable polygyne nests, which the queens subsequently attempt to enter.

The observed flight behavior of polygyne *S. invicta* queens is consistent with prior expectations derived from colony and population genetic studies of introduced populations. Queens within polygyne nests of introduced *S. invicta* are statistically unrelated to one another when either nuclear or mitochondrial markers are considered (Ross and Fletcher, 1985; Goodisman and Ross, 1997, 1998). However, polygyne populations display considerable mitochondrial genetic differentiation among sites (areas of ~1000 m^2) located only a few kilometers apart (Shoemaker and Ross, 1996; Ross and Shoemaker, 1997; Goodisman and Ross, 1998). Thus, although polygyne queens frequently enter foreign nests, they usually reproduce within their natal sites. In accord with this result, the distribution of mitochondrial haplotypes of the flying alates did not differ significantly from that of preflight queens collected from the tops of nests nor from that of reproductive queens within nests. Therefore, the in-flight queens appear to originate from local nests and may be attempting to enter these nests to become reproductives after their nuptial flights. We note that monogyne *S. invicta* queens have not been seen displaying the unusual flight behavior, an observation that is consistent with the lack of genetic differentiation that characterizes the monogyne social form on small geographic scales (Shoemaker and Ross, 1996; Ross and Shoemaker, 1997, Ross *et al.*, 1999).

Mating Behavior in Polygyne Ants

Many studies have documented mating tactics in polygyne ants. One general conclusion of these studies is that polygyne queens frequently mate in or near their natal colonies. Queens of the unicolonial polygyne ant *Linepithime humile* almost always mate in the nest (Passera and Keller, 1990, 1992; Keller and Passera, 1992), as do reproductives in the genus *Ophthalmopone* (Peeters and Crewe, 1985) and queens of some *Lasius* species (Boomsma *et al.*, 1990; Van Loon *et al.*, 1990). Queens in other polygyne ants are polymorphic for mating behavior, with some queens mating on or near their natal nests and others undertaking nuptial flights. This is the case in some species of *Formica* (Passera *et al.*, 1990; Cherix *et al.*, 1991; Fortelius *et al.*, 1993;

Rosengren *et al.*, 1993), *Lasius* (Yamauchi *et al.*, 1981), *Leptothorax* (Leprince and Francoeur, 1986; Heinze, 1989, 1993; Franks *et al.*, 1991), *Myrmica* (Brian and Brian, 1955; Kasugai, 1983; Elmes, 1991), *Rhytidoponera* (Ward, 1981), and *Technomyrmex* (Yamauchi *et al.*, 1991). Although many studies have described mating activity of polygyne ants, postmating behavior similar to what we have documented in *S. invicta* has not been reported previously.

Phenotypic Correlates of Dispersal Strategies

Variation in queen morphology frequently correlates with mode of colony founding and dispersal strategy in ants (Hölldobler and Wilson, 1990; Keller, 1993; Bourke and Franks, 1995; Crozier and Pamilo, 1996). Queens in many species of ants that exhibit dependent colony founding (i.e., workers aid in the founding process) frequently are smaller or have lower reserves than queens in the same or related species that found colonies independently (without the aid of workers) (Keller and Passera, 1989; Passera and Keller, 1990; Sundström, 1995). Such correlations have been observed in the genera *Solenopsis* (McInnes and Tschinkel, 1995; Tschinkel, 1996; DeHeer and Tschinkel, 1998), *Leptothorax* (Heinze, 1989, 1993; Buschinger and Francoeur, 1991; Rüppell *et al.*, 1998), and *Myrmica* (Brian and Brian, 1955; Kasugai, 1983; Elmes, 1991; Ohkawara *et al.*, 1993). More exceptional examples of the association between queen morphology and colony-founding strategies are found in ants in which queens exhibit variation for the presence or size of wings (Buschinger and Heinze, 1992), as is the case in species of *Chelaner* (Briese, 1983), *Leptothorax* (Heinze and Buschinger, 1987; Heinze, 1989; Heinze and Tsuji, 1995), *Monomorium* (Bolton, 1986), *Rhytidoponera* (Ward, 1983), and *Technomyrmex* (Yamauchi *et al.*, 1991). In these cases, queens without wings (or with reduced wings) often seek acceptance into their natal nests, while queens with fully developed wings frequently partake in mating flights.

Polygyne *S. invicta* queens also display alternative reproductive behaviors related to their phenotype, which is directly influenced by genotype at the locus *Gp-9* (or a gene closely linked to it). In introduced populations, *Gp-9* genotype correlates significantly with queen mass, with mass ordered among the genotypes $BB > Bb > bb$ (DeHeer *et al.*, 1999). The heaviest queens (those with genotype *BB*) never become reproductives in North American polygyne *S. invicta* colonies because they are killed by polygyne workers as they become reproductively active (Keller and Ross, 1993a; Ross, 1997). These queens are similar in mass to monogyne queens (all of which are genotype *BB*) and likely possess sufficient energy reserves to engage in high-altitude nuptial flights and to found colonies independently. Therefore, these

queens may provide a rare route of gene flow between the two social forms (Ross and Shoemaker, 1993; Ross and Keller, 1995b). This important subject is treated elsewhere (DeHeer *et al.*, 1999).

In contrast to *BB* queens, polygyne queens of genotype *bb* may lack the energetic resources needed to partake in mating flights, thus explaining the low proportion of *bb* queens observed in flight relative to such queens amassing on the tops of nests. Alternatively, these queens may go on nuptial flights, but not display the low-altitude flight behavior of the heterozygous queens because they lack sufficient reserves to support sustained flight. Remarkably, *bb* queens almost never become egg-layers in polygyne *S. invicta* colonies, apparently because they die of intrinsic defects before becoming reproductively active (Ross, 1997). Thus, the genotype of pre-reproductive polygyne queens strongly influences their morphological phenotype, which in turn mediates the mating and dispersal behaviors and the process of queen recruitment in introduced populations.

ACKNOWLEDGMENTS

REFERENCES

Bolton, B. (1986). Apterous females and shift of dispersal strategy in the *Monomorium salomonis*-group (Hymenoptera: Formicidae). *J. Nat. Hist.* **20:** 267–272.

Boomsma, J. J., Brouwer, A. H., and Van Loon, A. J. (1990). A new polygynous *Lasius* species (Hymenoptera: Formicidae) from Central Europe II. Allozymatic confirmation of species status and social structure. *Insectes Soc.* **37:** 363–375.

Bourke, A. F. G., and Franks, N. R. (1995). *Social Evolution in Ants*, Princeton University Press, Princeton, New Jersey.

Brian, M. V., and Brian, A. D. (1955). On the two forms macrogyna and microgyna of the ant *Myrmica rubra* L. *Evolution* **9:** 280–290.

Briese, D. T. (1983). Different modes of reproductive behaviour (including a description of colony fission) in a species of *Chelaner* (Hymenoptera: Formicidae). *Insectes Soc.* **30:** 308–316.

Buschinger, A., and Francoeur, A. (1991). Queen polymorphism and functional monogyny in the ant, *Leptothorax sphagnicolus* Francoeur. *Psyche* **98:** 119–133.

Buschinger, A., and Heinze, J. (1992). Polymorphism of female reproductives in ants. In Billen, J. (ed.), *Biology and Evolution of Social Insects*, Leuven University Press, Leuven, pp. 11–23.

Cherix, D., Chautems, D., Fletcher, D. J. C., Fortelius, W., Gris, G., Keller, L., Passera, L., Rosengren, R., Vargo, E. L., and Walter, F. (1991). Alternative reproductive strategies in *Formica lugubris* Zett. (Hymenoptera Formicidae). *Ethol. Ecol. Evol.* **S1:** 61–66.

Crozier, R. H., and Pamilo, P. (1996). *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*, Oxford University Press, Oxford.

DeHeer, C. J., and Tschinkel, W. R. (1998). The success of alternate reproductive tactics in monogyne populations of the ant *Solenopsis invicta*: Significance for transitions in social organization. *Behav. Ecol*. **9:** 130–135.

DeHeer, C. J., Goodisman, M. A. D., and Ross, K. G. (1999). Queen dispersal strategies in the multiple-queen form of the fire ant *Solenopsis invicta*. *Am. Nat*. **153:** 660–675.

Elmes, G. W. (1991). Mating strategy and isolation between the two forms, macrogyna and microgyna, of *Myrmica ruginodis* (Hym. Formicidae). *Ecol. Entomol*. **16:** 411–423.

Excoffier, L., Smouse, P. E., and Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131:** 479–491.

Fletcher, D. J. C. (1983). Three newly-discovered polygynous populations of the fire ant, *Solenopsis invicta*, and their significance. *J. Georgia Entomol. Soc*. **18:** 538–543.

Fortelius, W., Rosengren, R., Cherix, D., and Chautems, D. (1993). Queen recruitment in a highly polygynous supercolony of *Formica lugubris* (Hymenoptera, Formicidae). *Oikos* **67:** 193–200.

Franks, N. R., Sendova-Franks, A. B., Sendova-Vassileva, M., and Vassilev, L. (1991). Nuptial flights and calling behavior in the ant *Leptothorax acervorum* (Fabr.). *Insectes Soc*. **38:** 327–330.

Glancey, B. M., and Lofgren, C. S. (1988). Adoption of newly-mated queens: A mechanism for proliferation and perpetuation of polygynous red imported fire ants, *Solenopsis invicta* Buren. *Florida Entomol*. **71:** 581–587.

Goodisman, M. A. D., and Ross, K. G. (1997). Relationship of queen number and queen relatedness in multiple-queen colonies of the fire ant *Solenopsis invicta*. *Ecol. Entomol*. **22:** 150–157.

Goodisman, M. A. D., and Ross, K. G. (1998). A test of queen recruitment models using nuclear and mitochondrial markers in the fire ant *Solenopsis invicta*. *Evolution*, in press.

Heinze, J. (1989). Alternative dispersal strategies in a North American ant. *Naturwissenschaften* **76:** 477–478.

Heinze, J. (1993). Habitat structure, dispersal strategies and queen number in two boreal *Leptothorax* ants. *Oecologia* **96:** 32–39.

Heinze, J., and Buschinger, A. 1987. Queen polymorphism in a non-parasitic *Leptothorax* species (Hymenoptera, Formicidae). *Insectes Soc*. **34:** 28–43.

Heinze, J., and Tsuji, K. (1995). Ant reproductive strategies. *Res. Pop. Ecol*. **37:** 135–149.

Hölldobler, B., and Bartz, S. H. (1985). Sociobiology of reproduction in ants. In: Hölldobler, B., and Lindauer, M. (eds.), *Experimental Behavioral Ecology and Sociobiology*, Sinauer Associates, Sunderland, Massachusetts, pp. 237–257.

Hölldobler, B., and Wilson, E. O. (1990). *The Ants*, Belknap Press of Harvard University Press, Cambridge.

Kasugai, M. (1983). Some observations of the microgyne form of the ant *Myrmica ruginodis* Nylander (Hymenoptera, Formicidae) in Sapporo. *Kontyu* **51:** 73–79.

Keller, L. (1991). Queen number, mode of colony founding, and queen reproductive success in ants (Hymenoptera Formicidae). *Ethol. Ecol. Evol*. **3:** 307–316.

Keller, L. (1993). *Queen Number and Sociality in Insects*, Oxford University Press, Oxford.

Keller, L., and Genoud, M. (1997). Extraordinary lifespans in ants: A test of evolutionary theories of ageing. *Nature* **389:** 958–960.

Keller, L., and Passera, L. (1989). Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). *Oecologia* **80:** 236–240.

Keller, L., and Passera, L. (1992). Mating system, optimal number of matings, and sperm transfer in the Argentine ant *Iridomyrmex humilis*. *Behav. Ecol. Sociobiol*. **31:** 359–366.

Keller, L., and Ross, K. G. (1993a). Phenotypic basis of reproductive success in a social insect: Genetic and social determinants. *Science* **260:** 1107–1110.

Keller, L., and Ross, K. G. (1993b). Phenotypic plasticity and "cultural transmission" of alternative social organizations in the fire ant *Solenopsis invicta*. *Behav. Ecol. Sociobiol*. **33:** 121–129.

Leprince, D. J., and Francoeur, A. (1986). Hilltop swarming by ants (Hymenoptera: Formicidae) in southwestern Quebec and interspecific competition at the swarm marker. *Ann. Entomol. Soc. Am*. **79:** 865–869.

Markin, G. P., Dillier, J. H., Hill, S. O., Blum, M. S., and Hermann, H. R. (1971). Nuptial flight and flight ranges of the imported fire ant, *Solenopsis saevissima richteri* (Hymenoptera: Formicidae). *J. Georgia Entomol. Soc*. **6:** 137–144.

McInnes, D. A., and Tschinkel, W. R. (1995). Queen dimorphism and reproductive strategies in the fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol*. **36:** 367–375.

Ohkawara, K., Ito, F., and Higashi, S. (1993). Production and reproductive function of intercastes in *Myrmecina graminicola nipponica* colonies. *Insectes Soc*. **40:** 1–10.

Passera, L., and Keller, L. (1990). Loss of mating flight and shift in the pattern of carbohydrate storage in sexuals of ants (Hymenoptera; Formicidae). *J. Comp. Biochem. Syst. Environ. Physiol*. **160:** 207–211.

Passera, L., and Keller, L. (1992). The period of sexual maturation and the age at mating in *Iridomyrmex humilis*, an ant with intranidal mating. *J. Zool*. **228:** 141–153.

Passera, L., Keller, L., Grimal, A., Chautems, D., Cherix, D., Fletcher, D. J. C., Fortelius, W., Rosengren, R., and Vargo, E. L. (1990). Carbohydrates as energy source during the flight of sexuals of the ant *Formica lugubris* (Hymenoptera: Formicidae). *Entomol. General*. **15:** 25–32.

Peeters, C., and Crewe, R. (1985). Worker reproduction in the ponerine ant *Ophthalmopone berthoudi*: An alternative form of eusocial organization. *Behav. Ecol. Sociobiol*. **18:** 29–37.

Porter, S. D. (1991). Origins of new queens in polygyne red imported fire ant colonies (Hymenoptera: Formicidae). *J. Entomol. Sci*. **26:** 474–478.

Raymond, M., and Rousset, F. (1995). GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J. Hered*. **86:** 248–249.

Rosengren, R., Sundström, L., and Fortelius, W. (1993). Monogyny and polygyny in *Formica* ants: The result of alternative dispersal tactics. In Keller, L. (ed.), *Queen Number and Sociality in Insects*, Oxford University Press, Oxford, pp. 308–333.

Ross, K. G. (1997). Multilocus evolution in fire ants: Effects of selection, gene flow and recombination. *Genetics* **145:** 961–974.

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

Ross, K. G., and Keller, L. (1995a). Ecology and evolution of social organization: Insights from fire ants and other highly eusocial insects. *Annu. Rev. Ecol. Syst*. **26:** 631–656.

Ross, K. G., and Keller, L. (1995b). Joint influence of gene flow and selection on a reproductively important genetic polymorphism in the fire ant *Solenopsis invicta*. *Am. Nat*. **146:** 325–348.

Ross, K. G., and Shoemaker, D. D. (1993). An unusual pattern of gene flow between the two social forms of the fire ant *Solenopsis invicta*. *Evolution* **47:** 1595–1605.

Ross, K. G., and Shoemaker, D. D. (1997). Nuclear and mitochondrial genetic structure in two social forms of the fire ant *Solenopsis invicta*: Insights into transitions to an alternate social organization. *Heredity* **78:** 590–602.

Ross, K. G., Shoemaker, D. D., Krieger, M. J. B., DeHeer, C. J., and Keller, L. (1999). Assessing genetic structure with multiple classes of molecular markers: A case study involving the introduced fire ant *Solenopsis invicta*. *Mol. Biol. Evol*. **16:** 525–543.

Rüppell, O., Heinze, J., and Hölldobler, B. (1998). Size-dimorphism in the queens of the North American ant *Leptothorax rugatulus* (Emery). *Insectes Soc*. **45:** 67–77.

Shoemaker, D. D., and Ross, K. G. (1996). Effects of social organization on gene flow in the fire ant *Solenopsis invicta. Nature* **383:** 613–616.

Shoemaker, D. D., Ross, K. G., and Arnold, M. L. (1994). Development of RAPD markers in two introduced fire ants, *Solenopsis invicta* and *S. richteri*, and their application to the study of a hybrid zone. *Mol. Ecol*. **3:** 531–539.

Sundström, L. (1995). Dispersal polymorphism and physiological condition of males and females in the ant, *Formica truncorum. Behav. Ecol*. **6:** 132–139.

Tschinkel, W. R. (1996). A newly-discovered mode of colony founding among fire ants. *Insectes Soc*. **43:** 267–276.

Van Loon, A. J., Boomsma, J. J., and Andrasfalvy, A. (1990). A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe I. Description and general biology. *Insectes Soc*. **37:** 348–362.

Vargo, E. L. (1992). Mutual pheromonal inhibition among queens in polygyne colonies of the fire ant *Solenopsis invicta*. *Behav. Ecol. Sociobiol*. **31:** 205–210.

Vargo, E. L., and Fletcher, D. J. C. (1989). On the relationship between queen number and fecundity in polygyne colonies of the fire ant *Solenopsis invicta*. *Physiol. Entomol*. **14:** 223–232.

Vargo, E. L., and Porter, S. D. (1989). Colony reproduction by budding in the polygyne form of *Solenopsis invicta* (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am*. **82:** 307–313.

Vinson, S. B., and Greenberg, L. (1986). The biology, physiology, and ecology of imported fire ants. In Vinson, S. B. (ed.), *Economic Impact and Control of Social Insects*, Praeger, New York, pp. 193–226.

Ward, P. S. (1981). Ecology and life history of the *Rhytidoponera impressa* group (Hymenoptera: Formicidae) II. Colony origin, seasonal cycles, and reproduction. *Psyche* **88:** 109–126.

Ward, P. S. (1983). Genetic relatedness and colony organization in a species complex of ponerine ants I. Phenotypic and genotypic composition of colonies. *Behav. Ecol. Sociobiol*. **12:** 285–299.

Yamauchi, K., Kyoichi, K., and Miyake, S. (1981). Sociobiological studies of the polygynic ant *Lasius sakagamii* I. General features of its polydomous system. *Insectes Soc*. **28:** 279–296.

Yamauchi, K., Furukawa, T., Kinomura, K., Takamine, H., and Tsuji, K. (1991). Secondary polygyny by inbred wingless sexuals in the dolichoderine ant *Technomyrmex albipes*. *Behav. Ecol. Sociobiol*. **29:** 313–319.