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Selective Male Mortality in the Red Imported Fire Ant, *Solenopsis invicta*

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ABSTRACT

Males in polygyne populations of *Solenopsis invicta* are primarily sterile diploids and thought to not express the *Gp-9* gene coding for a pheromone-binding protein affecting complex social behavior. We examined an aspect of the breeding system hitherto not considered—male *Gp-9* genotypes in relation to sperm stored in queens. Four sites with varying frequencies of sympatric monogyne and polygyne colonies were sampled, including sexuals, workers, and broods from four colonies. Most queens were heterozygotes storing *B* sperm. Although predicted to be common, only 14 of 504 males were *B* or *BB* genotypes, suggesting strong selection. Increased frequency of polygyne colonies at each site paralleled increases in queens with *b* sperm (1.9–32.8%) and of noninseminated queens. The presence of both *B* and *b* sperm in 1.9–18.9% of queens, genotype profiles of colonies, and genotypes of offspring from individual queens suggest some frequency of multiple mating. The *bb* genotype, rather than an obligate, developmental lethal, was present in some queens and common in alates, workers, and brood. Selective mortality of sexuals may affect multiple aspects of the breeding system, including female-mediated dispersal, mating success, and gene flow.

EUSOCIAL Hymenoptera with multiple queens present a particular challenge to kin selection theory and the evolution of social systems insofar as an additional layer of relatedness dynamics (*e.g.*, queens to queens, workers to queens) must be considered in formulating models of sociality, estimating costs and benefits, and predicting particular social outcomes (Ross and Keller 1995; Hannonen and Sundström 2003). The effect of polyandry has similar theoretical implications regarding the balance between reproductive cooperation and conflict (Ratnieks 1990; Keller 1995; Crozier and Pamilo 1996), although obligate polyandry is relatively rare in the social Hymenoptera (Strassmann 2001).

Outside of honeybees, the red imported fire ant, *Solenopsis invicta* Buren, is perhaps the best-known and most intensely studied species of eusocial insect. Over the past 20 years, studies have provided a detailed description of the population genetic structure and breeding system of this species, demonstrating that complex social behavior can have a simple genetic basis and be affected by novel environmental/genetic contexts due to introduction (*e.g.*, Ross 1988, 1993; Keller and Ross 1993, 1999; Ross *et al.* 1996; Ross and Keller 1998; Krieger and Ross 2002). The red imported fire ant has two social forms: single-queen (monogyne) and multiple-queen (polygyne) colonies apparently determined by a single, diallelic locus (*Gp-9*) that may code for a pheromone-binding protein (Keller and Ross 1999; Krieger and Ross 2002). All polygyne queens are reportedly heterozygous *Bb* at this locus and monogyne colonies lack the *b* allele altogether. The *Gp-9* locus is thought to affect worker recognition of conspecifics and is responsible for the selective killing of *BB* queens in polygyne colonies (a "green beard" gene); *bb* queens and workers are thought to perish as brood or as young adults (Ross 1997; Keller and Ross 1998). Males are haploid (fertile) or homozygous (sterile diploids, Hung *et al.*1974) for one or more sex-determining loci (Ross and Fletcher 1985), although some fertile diploid males have been reported (Hung *et al.* 1974; Krieger *et al.* 1999). Reduced allelic diversity at the sex gene(s) (due to the genetic bottleneck-associated introduction into the United States) is thought to be responsible for a high percentage of sterile (aspermic), diploid males (average between 85 and 95%) in polygyne populations (Ross and Fletcher 1985, 1986; Ross 1992; Krieger *et al.* 1999). Diploid males are absent in mature monogyne colonies, since the high "cost" of rearing sterile males causes incipient monogyne colonies to perish (Ross and Fletcher 1985, 1986). Female alates are reported to mate once (Ross and Fletcher 1985; Ross *et al.* 1988; Ross 1992, 1993; Shoemaker *et al.* 1992).

Gene flow between both social forms has been reported to be almost exclusively by males from monogyne colonies mating with polygyne queens (Ross 1992; Ross and Shoemaker 1993; Shoemaker and Ross 1996; Ross *et al.* 1997). A formal assessment of this hypothesis

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by Goodisman *et al.* (2000), however, found that the genetic structure of polygyne populations is explained most parsimoniously by the absence of gene flow from monogyne colonies. As Goodisman *et al.* (2000) pointed out, conclusions regarding singular gene flow from monogyne males to polygyne colonies have come from indirect evidence. One explanation invoked by Goodisman *et al.* (2000) for the apparent contradiction between their results and those of previous studies was the possibility of polygyne male selection (weak male selection was suggested by their data).

Many of the previous studies that examined the role of the *Gp-9* locus in fire ant behavior used phenotype (protein electromorphs for *Gp-9*) to infer genotype. In this study, we surveyed *Gp-9* genotypes of two components of the breeding system hitherto not examined: male *Gp-9* genotypes in polygyne populations and sperm stored in the spermatheca of queens. These two sources of genetic information interrelate and elucidate the breeding system of *S. invicta*, especially since polygyne colonies produce primarily diploid males (*i.e.*, are the outcome of both sperm and egg). Thus, the interpretation and significance of male genotype frequencies in colonies or populations necessitates knowing the genotypes of queens and of the sperm they store.

High rates of diploid males in polygyne colonies and the relatively small numbers of sexuals produced in these colonies (Vargo and Fletcher 1987) predicts that fertile males are a limiting resource during mating flights where monogyne colonies are absent or rare. Recently, Fritz and Vander Meer (2003) found that the large polygyne population of north-central Florida reported by Porter (1992, 1993) is not homogenous, but composed of sympatric polygyne and monogyne colonies at frequencies that vary across this region. In this study, we examined the *Gp-9* genotypes of polygyne males, queens, and their stored sperm at four sites along a transect of north-central Florida where frequencies of monogyne colonies varied. We also "profiled" genotypes of four colonies in one site with the highest frequency of polygyne colonies. On the basis of the prevailing model of the red imported fire ant breeding system, we hypothesized that queens at all sites would be storing almost exclusively *B* genotype sperm (the genotype of males from monogyne colonies) and that increases in the frequency of queens without sperm would coincide with decreases in the frequency of sympatric monogyne colonies. We also expected genotype frequencies of males to match those predicted by the genotypes of queens and of sperm stored in their spermathecae (since most males are diploids). The results of our study expand the multiple effects of the *Gp-9* locus on complex behaviors in this species.

MATERIALS AND METHODS

Field collections: Queens were collected from polygyne colonies at four sites (sites A, B, E, and D, respectively) in north-central Florida surveyed previously and shown to have different proportions of both social forms in sympatry (Fritz and Vander Meer 2003). Site D was chosen for a detailed analysis of queens, sperm, males, and colony genetic profiles, since this site had the highest frequency of polygyne colonies (96.1%). Males were collected from 14 colonies at site D. In addition, four polygyne colonies from site D were profiled for the *Gp-9* locus by sampling queens, spermathecae, adult major workers, last instar sexual larvae, sexual pupae, and adult alates. Colonies were unearthed with a shovel, and the soil was spread on a flat surface; the soil was sifted manually and all queens encountered were collected along with a sample of workers, sexuals, and brood. Colonies were considered "separate" only if they were at least 10 m apart from any other colony. Colonies were designated as polygyne only if they contained multiple, inseminated queens of the *Bb* genotype.

Sperm isolation: Ants were stored in 100% ethanol and kept at −10° at least 2 weeks prior to dissection of spermathecae. This storage treatment dehydrates the sperm within the spermatheca into a single, solid mass that separates easily from the spermathecal capsule (spermathecal capsules also maintain their integrity during dissection, are pliable, but easily torn open with micropins to release the ball of sperm). Spermathecae were removed from queens with flamed dissection micropins under a stereomicroscope. Spermathecae were then transferred to a drop of sterile water (200 μl) on a clean microscope slide. The spermathecal capsule was separated from the sperm mass with fresh dissection micropins and subsequently rinsed twice in sterile, deionized water to ensure no contamination from the female genome. Sperm masses were treated with proteinase-K for ~15 hr in a water bath maintained at 55°.

DNA extraction, polymerase chain reaction, and endonuclease digestion: All ants were stored in 100% ethanol until genotyped. A portion of the *Gp-9* locus was amplified by PCR using the primers and protocols described by Krieger and Ross (2002). The amplicons were then cut with the restriction enzyme *Bsa*AI, which produces genotype-specific banding patterns for the *Gp-9* locus (Krieger and Ross 2002) on agarose gel electrophoresis; the *B* allele produces two amplicons (545 and 283 bp), whereas the *b* allele produces three amplicons (428, 283, and 117 bp). DNA of sperm and ants was isolated with QIAGEN DNeasy kits (QIAGEN, Valencia, CA) using the protocol for animal tissue.

RESULTS

Genotype survey at four sites: The estimated frequency of monogyne colonies at our collection sites ranged from ~4–69% (Table 1) (Fritz and Vander Meer 2003). Nearly all polygyne queens were heterozygotes for *Gp-9*, but four queens had the *bb* genotype and two of these were inseminated. Among inseminated queens, the majority of the spermathecae contained sperm with the *B* genotype, and its estimated frequency at four sites ranged from 0.74 to 0.99 (Table 1) (these estimates assume approximately equal quantities of sperm in each queen). A high percentage of spermathecae at sites B, E, and D, however, contained *b* sperm (26.5, 24.8, and 32.8%, respectively) even though two of these sites had relatively high densities of monogyne colonies (56.3 and 23.9% for sites B and E, respectively). The percentage of queens storing both *B* and *b* sperm was 1.9, 18.9, 18.0, and 13.3% at sites A, B, E, and D, respectively. We considered that these data could, in

TABLE 1

Polygyne queen and sperm *Gp-9* genotypes at four sites in Florida

Site	Queen genotypes			Sperm genotypes[a]					
	BB	*Bb*	*bb*	*B*[b]	*B*/*b*	*b*[b]	% M	f(*B*)	% XS
A (12)	0	61	0	53	1	0	69.0	0.99	7.0
B (34)	0	157	3	97	25	10	56.3	0.83	14.3
E (28)	0	107	0	88	21	8	23.9	0.84	10.0
D (43)	0	187	1	86	17	25	3.9	0.74	25.0

Sites had different percentages of sympatric monogyne colonies (% M). Numbers of colonies sampled at each site are in parentheses. f(*B*) is the estimated frequency of the *B* allele in spermathecal sperm and % XS is the percentage of queens without sperm.

[a] Sites have significantly different frequencies of sperm genotypes (*G*-test of heterogeneity: $P < 0.02$) except site B with E.

[b] Diploid *BB* and *bb* males cannot be distinguished from their haploid counterparts.

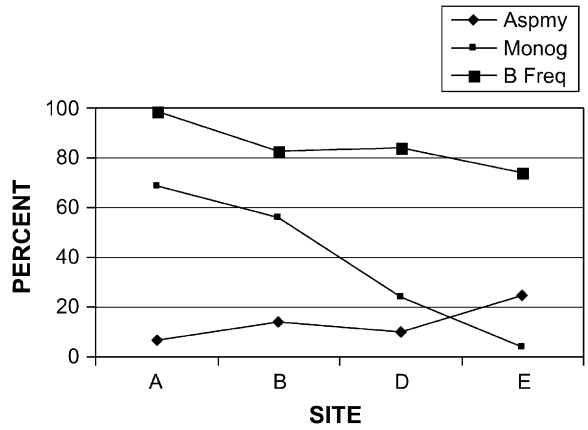

FIGURE 1.—The percentage of each of three variables at four sites (A, B, D, and E) in north-central Florida with differing frequencies of monogyne and polygyne colonies in sympatry: spermathecae without sperm (Aspmy), monogyne colonies (Monog), and *B* sperm (*B* Freq).

part, be the outcome of queen DNA contaminating sperm (in the PCR), but we do not think our protocol led to a systematic error of this kind. For example, we sampled the sperm from 53 heterozygous queens at site A and recorded only a single queen with both *B* and *b* sperm. Since this site was primarily inhabited by monogyne colonies (69%), the majority of polygyne queens were expected to have mated with *B* males (consistent with our data). Furthermore, contamination amplicons from queens should be proportional to their initial copy number in the PCR reaction. Since spermathecae contain as many as 5–6 million sperm, only a relatively large quantity of contaminating queen DNA would be detected on an agarose gel against the backdrop of sperm amplicons; our careful removal and rinsing of sperm balls from the spermathecae minimized this possibility.

Because we examined a single, diallelic gene, both haploid males and homozygous diploid males produced single amplicons for the *Gp-9* locus. Thus, except for *Bb* males, the ploidy of males with single amplicons (corresponding to genotype *B* or *b*) was indeterminate; hereafter, therefore, we have designated these male genotypes with a single letter for brevity. Although not significant (Spearman's rank correlation, $P = 0.08$), the data suggest a trend whereby decreases in the frequency of queens with *B* sperm coincide with increases in the percentage of queens without sperm (Table 1, Figure 1). For example, site D had significantly fewer monogyne colonies than other sites (*G*-test of heterogeneity: $P < 0.01$), exhibited the lowest frequency of *B* sperm in queens, and had the highest frequency of queens devoid of sperm (25%).

From a sample of 241 males from 14 colonies in site D, only 6 males (2.5%) were found with the *B* genotype, 72 (29.9%) were *b*, and 163 (67.6%) were *Bb*. Since ~90% of all males in polygyne populations are diploids (Ross and FLETCHER 1985, 1986; ROSS 1992), we assume that ~22.4% of our sample included diploid *BB* and *bb* males.

Genetic profiles of colonies at site D: The paucity of males with the *B* genotype was also evident from the genetic profiles of four colonies from site D (Table 2). Of 263 males sampled, only 8 (3.04%) were genotype *B*. All queens were heterozygotes except for a single individual with the *bb* genotype (inseminated with *B* sperm). The spermathecae of most queens contained sperm with the *B* genotype, although some also contained *b* sperm or both genotypes (Table 2). Most sexuals in all colonies had one or two copies of the *b* allele and this phenomenon was not confined to adults, but was also observed for sexual pupae and larvae. From 34 to 82% of all males in three colonies (1, 2, and 3) had the *b* genotype and most of the remainder were *Bb*. The genotype frequencies of workers in all four colonies were, overall, consistent or marginally different from those expected from the genotypes of the queens and their stored sperm (Table 2).

DISCUSSION

Queen and sperm *Gp-9* genotypes: Previous studies estimating gene flow concluded that fertile polygyne males were too rare to contribute significantly to the gene pool of either social form where monogyne colonies occur in sympatry (ROSS and SHOEMAKER 1993; SHOEMAKER and ROSS 1996). At three of our sites, however, a quarter to one-third of queens stored sperm of males from their own social form. Since most polygyne males are aspermic, and polygyne colonies produce a small fraction of males in comparison to monogyne colonies (VARGO and FLETCHER 1987), the high frequency of queens with *b* sperm, particularly at sites B and E (comprising 56 and 24% of monogyne

TABLE 2

Genotype profiles of four colonies at site D

	Q	MA[a]	MP[a]	FA	FP	SL	W	WE	P^b
Colony 1									
BB	0	1	0	5	2	1	10	14.29	0.500
Bb	6(2,1,0)	13	1	21	34	20	33	24.50	
bb	0	12	24	2	5	8	6	10.21	
Colony 2									
BB	0	2	0	0	0	3	16	17.50	0.823
Bb	6(0,2,0)	35	19	27	43	17	23	21.00	
bb	0	16	13	2	3	5	3	3.50	
Colony 3									
BB	0	2	0	3	2	—	13	15.38	0.056
Bb	16(2,3,2)	6	1	30	7	—	27	20.50	
bb	0	28	14	4	7	—	1	5.13	
Colony 4									
BB	0	1	2	2	1	—	11	17.29	0.043
Bb	9(0,1,3)	43	28	43	25	—	33	25.14	
bb	1(0,0,0)	1	1	0	0	—	0	1.57	

Gp-9 genotypes are shown for queens (Q), male adults (MA), male pupae (MP), adult female alates (FA), female sexual pupae (FP), sexual larvae (SL), and workers (W).

In parentheses, the first number refers to queens with only *b* sperm, the second number to queens with both *B* and *b* sperm, and the third number to queens without sperm; the remainder of queens all had *B* sperm. WE, expected number of workers based on queen sperm and equal contribution to workers by queens.

[a] Diploid *BB* and *bb* males cannot be distinguished from their haploid counterparts.

[b] Chi-square probability.

colonies, respectively), suggests that polygyne females mate disproportionately with males of their own social form. We estimate that 96–99% of queens at sites B and E should be mated by *B* genotype males, since monogyne colonies produce approximately four to eight times the number of males produced by polygyne colonies (Vargo and Fletcher 1987) and ~90% of the latter are expected to be aspermic. The formal genetic analysis of polygyne colonies by Goodisman *et al.* (2000) led to the conclusion that the genetic structure of polygyne colonies was best explained by an absence of substantial gene flow from monogyne colonies. Although this conclusion was dismissed in favor of unknown factors (*e.g.*, selection against males), our data indicate that mating bias and selection of males may, in fact, help explain their results.

Our data are consistent with other studies reporting queens devoid of sperm in polygyne populations, as well as their increase in frequency with distance from monogyne populations (Fletcher *et al.* 1980; Vargo and Fletcher 1987; Porter *et al.* 1988; Ross and Keller 1995); these data suggest that males are a limiting resource in mating flights where populations are primarily polygyne. If some frequency of multiple mating occurs in polygyne queens (see discussion below), availability of males probably affects this frequency.

As many as 18.9% of queens at our sites stored both *B* and *b* sperm. These data can be interpreted in two ways that are not mutually exclusive. The first is that some queens mated with fertile diploid males. Supporting this possibility are the following: (1) queens are reported to be strictly monandrous (Ross and Fletcher 1985; Ross *et al.* 1988; Ross 1992, 1993; Shoemaker *et al.* 1992); (2) diploid males take part in mating flights (Ross and Fletcher 1985); (3) Hung *et al.* (1974) reported testicular lobe development in ~10% of diploid males from a single polygyne colony; and (4) Krieger *et al.* (1999) reported triploid workers (12%) in polygyne colonies from a Georgia location and suggested that these workers were the result of copulations involving fertile diploid males (2.4% of all diploid males sampled) producing unreduced sperm.

The second interpretation of our results is that some queens mated with multiple, haploid males. We have evidence that some queens store and use haploid sperm of two different genotypes. Progeny and sperm were genotyped from two queens collected from a mating flight during a study on incipient colony formation (C. A. Preston, R. K. Vander Meer and G. N. Fritz, unpublished data). These queens were reared separately in the laboratory and each was sacrificed along with a sample of her larvae, pupae, and workers. Although we did not know the colony social form (polygyne or monogyne) from which these two queens originated, both had the *BB* genotype and both stored *B* and *b* sperm and produced 6.9% ($n = 58$) and 50% ($n = 50$) *Bb* offspring, respectively (remaining offspring were *BB*). Because fertile diploid males are rare, our results suggest that multiple mating occurs at some frequency with fertile haploid males. Alternatively, if queens are strictly monandrous, as previously reported (Ross and Fletcher 1985; Ross *et al.* 1988; Ross 1992, 1993; Shoemaker *et al.* 1992), then our data, and that of Krieger *et al.* (1999) on triploid workers, suggest that *S. invicta* may have four categories of fertile males in polygyne colonies: haploids, diploids with reduced sperm, diploids with unreduced sperm, and possibly diploids with both unreduced and reduced sperm. Although this array of fertile males may be unlikely, Cowan and Stahlhut (2004) recently reported that the vespid wasp *Euodynerus foraminatus* has functionally reproductive diploid and haploid males.

Male and colony Gp-9 genotypes: Goodisman *et al.* (1999) found some evidence of weak selection acting on haploid males in polygyne colonies on the basis of a study correlating male weights to an enzyme gene (linked to *Gp-9*). Our data support their inference, since we demonstrate strong selection against males (haploid and/or diploid) that lack the *b* allele at the *Gp-9* locus. To our knowledge, this is the first instance of intracolony male selection at a single gene locus.

Assuming that the *Gp-9* genotype of sperm in the spermatheca of a queen does not affect her relative contribution to males in a colony, we expected equal frequencies of haploid *B* and *b* males and primarily *BB* and *Bb* diploid males in our colonies. For example, at site D, where the frequency of the *B* allele in sperm stored in queens was 0.74, we expected 37, 13, and 50% of all diploid males to be *BB*, *bb*, and *Bb*, respectively. If one assumes that ~90% of all the males that we sampled were sterile diploids (Ross and Fletcher 1985, 1986; Ross 1992), and that polygyne queens produced *B* and *b* eggs in a 1:1 ratio, then the expected percentage of *BB* males should have been ~38%. However, only 2.5% of males sampled at site D were *B* (and/or *BB*). Males lacking the *b* allele were also rare in all colonies genotyped from site D, where the sperm in queens predicted that they should be common (Table 2). The presence of *BB* diploid males in many incipient monogyne colonies (Ross *et al.* 1988) argues against this genotype being lethal. Thus, unless queens are controlling the genotypes of eggs they lay with respect to the *Gp-9* locus (which is not supported by our worker genotype frequencies), the paucity of *B* males is due to selective mortality (culling by workers?) early in development.

Our data demonstrate that male mortality, like that of female sexuals, correlates with genotype at the *Gp-9* locus. If this gene is involved in the dynamics of colony pheromonal recognition, then the pattern of gene expression in males, as well as in female sexuals, must play a role. Perhaps the absence of previous studies on the *Gp-9* locus in males was due to the assumption that "*Gp-9* is not expressed in males" (Shoemaker and Ross 1996; Goodisman *et al.* 1999). The absence of visible *Gp-9* protein in electrophoresis, however, does not necessarily indicate lack of gene expression. Recently, Liu and Zhang (2004) demonstrated that *Gp-9* is expressed in adult males.

The near absence of sexual larvae with the *B* genotype in our study (Table 2) indicates that differential mortality of sexuals occurs before the last instar of larval development. This conclusion is consistent with data indicating that workers may cull sexual larvae in response to pheromonal cues (Vargo and Fletcher 1986) and with recent data indicating that worker ants in polygyne colonies (*Formica fusca*) selectively rear broods in favor of close kin (Hannonen and Sundström 2003). Selective mortality of inseminated *S. invicta* queens lacking the *b* allele in polygyne colonies involves culling by workers that share the *b* allele (the "green-beard" gene) (Keller and Ross 1998; Ross and Keller 1998). Although other studies have reported *BB* female alates from polygyne colonies in Georgia (Ross 1997; Ross and Keller 1998), the near absence of *B* and/or *BB* sexuals in our study suggests that most of these individuals met a similar fate. Since *BB* workers were relatively common in our polygyne colonies, genotype-specific mortality appears to affect sexuals primarily.

The genotype profiles of four colonies from site D are not consistent with those reported in other studies and summarized in the most recent review of the genetics of social behavior in *S. invicta* (Bourke 2002). Not only are *B* males rare in our study, but also the *BB* genotype is rare in female sexuals. On the other hand, the *bb* genotype is present in workers, males (*bb or b*), female alates, and even in some queens. These data are contrary to those reported by Ross (1997) in which 20% of female alates in a Georgia population had the *BB* genotype and none had the *bb* genotype. The *bb* genotype was found in only one worker ($n = 406$) and was absent from mated queens ($n = 1535$). Ross (1997) reported an identical absence of *bb* females for polygyne colonies in Argentina and for polygyne colonies from a site in Texas. In addition, Ross (1997) reared progeny from 60 queens and noted that *bb* progeny were rare for 5 of the 6 queens that had obviously mated with *b* genotype males. From these data, Ross (1997) concluded that the *bb* genotype is a developmental, recessive lethal affecting individuals primarily before adulthood.

The lethality of the *bb* genotype has been echoed in subsequent literature on fire ant behavior and genetics (Keller and Ross 1998; Ross and Keller 1998) and in the most recent articles and reviews concerning the genetics of *S. invicta* social behavior (Goodisman *et al.* 2000; Ross 2001; Bourke 2002; Ross and Keller 2002). DeHeer *et al.* (1999), however, reported that 20% of the female alates leaving colonies in mating swarms at a particular polygyne population in Georgia were *bb*. Thus, the *bb* genotype has recently been described as intrinsically lethal for female alates, but "early in adult life" (Ross and Keller 2002), whereas the *bb* genotype in workers is still reported as a lethal recessive (Bourke 2002; Ross and Keller 2002). Our data are not consistent with an early developmental demise of *bb* workers. Rather, frequencies of *bb* workers were approximately those predicted by the genotypes of the queens and the sperm they stored (Table 2). Furthermore, *b* males and *bb* female alates were common in our colonies. And our data demonstrate that *bb* female alates, although underrepresented in colonies, are capable of mating and can subsequently become queens (Table 1) (we have also collected *bb* queens with sperm in mating flights; G. N. Fritz, unpublished data). Thus, we think it premature to dismiss this genotype as inconsequential in the breeding system of *S. invicta*; in polygyne populations devoid of monogyne colonies, for example, *bb* queens might be relatively common.

Since polygyne colonies persist beyond the life span of a single queen and have continuous queen turnover, the breeding system of this social form is quite complex/dynamic and may explain some of the variation in genotype frequencies within and among colonies (Table 2). The frequencies of workers with the *b* allele probably vary, depending on the influx of newly mated queens and the genotype of the sperm that they store.

We suggest, speculatively, that the relative frequencies of *BB* workers to those that are *Bb* and *bb* determines the differential mortality rates of the various genotypes of sexuals in polygyne colonies (see Table 2 and Ross 1988); in the case of female sexuals, however, biasing of brood sexualization by workers (in favor of certain genotypes) might also be possible (Vargo and Fletcher 1986). This dynamic, which invokes frequency-dependent effects, is supported by the observation of Ross and Keller (2002) that colonies can be manipulated to behave more like one social form or the other (in terms of accepting queens) by changing the percentage of workers with the *b* allele. In our colonies at site D, where the frequency of queens with *b* sperm is relatively high, the *b* allele is common among workers and female alates, and both castes have *bb* genotypes present; furthermore, *b* males are present, too (Table 2). Very few sexuals had the *BB* or *B* genotype whether as last instar larvae, pupae, or adults. The reverse was true for the polygyne colonies sampled by Ross (1997), where the *b* allele was relatively rare. These observations suggest that workers that are *BB* tend to destroy *bb* and *b* broods and that workers that are *bb* or *Bb* destroy *BB* and *B* broods. Although the sensitivity of workers to subtle differences in nest-mate recognition cues may ultimately be under queen control (Deslippe 2002; Klubachar and Deslippe 2002; Vander Meer and Alonso 2002), the relative fates of particular genotypes in a colony may depend on the frequencies of worker genotypes being produced, which in turn depends on the frequency of *B* and *b* sperm present in queens.

The phenomenon that we propose would explain the "unexpected" results obtained by DeHeer *et al.* (1999) (20% *bb* female alates). Frequency-dependent worker effects also predict underrepresentation of some queen progeny in a colony, at least insofar as their contributions to sexuals; a female producing *BB* and *Bb* alates, for example, may not fare as well in a colony where most queens are mated to *b* males (and producing *Bb* and *bb* alates) and producing relatively high frequencies of *bb* and *Bb* workers. Unequal contributions to offspring by polygyne queens have been reported (Ross 1988, 1993) as perhaps due to differential egg production; we suggest that differential mortality may also contribute to unequal fitness.

The genotype of female alates at the *Gp-9* locus correlates with alate weight (Keller and Ross 1993), dispersal potential (DeHeer *et al.* 1999), and the probability of founding a colony (DeHeer 2002). DeHeer *et al.* (1999) found that *bb* female alates are the least vagile genotype (DeHeer *et al.* 1999), *Bb* alates have a mixed dispersal, and *BB* alates disperse the farthest from natal colonies. More recently, DeHeer (2002) demonstrated that newly mated *BB* and *Bb* female alates from polygyne colonies reared in isolation may produce enough first workers to found colonies on their own. Heterozygote queens, however, are the least likely genotype to found colonies individually, since most produce few to no workers. Our data indicate that even in areas where monogyne colonies are common and in sympatry with the polygyne form, polygyne colonies produce few, if any, homozygous or hemizygous sexuals for the *B* allele. It is probable, then, that *B* males and *BB* females are absent in 100% polygyne areas. Alternatively, where sympatric monogyne colonies are common, polygyne colonies should produce relatively high frequencies of *BB* female alates (and perhaps *B* males too?). If some percentage of *BB* queens from polygyne colonies initiate single-queen colonies successfully, then the *Gp-9* locus can be said to affect dispersal and colony initiation success in at least two ways: by affecting fat-body reserves (Keller and Ross 1993; DeHeer *et al.* 1999; DeHeer 2002) and by selective mortality of sexuals.

Intraspecific variation in dispersal, polyandry, sex ratio, and other components of social animal breeding systems are often interpreted within the broad framework of evolutionary theory as different, complex strategies that maximize inclusive fitness outcomes. Over 60 years of research into the biology of *S. invivcta*, particularly population genetics studies over the past 20 years, has led to a model indicating that the breeding system and other complex social dynamics of *S. invicta* are highly influenced by a single gene or by closely linked gene(s). Because the *Gp-9* locus may be associated with pheromonal cues involved in the recognition and regulation of social behavior, alleles that change the expression of this gene invariably lead to a multitude of direct and indirect effects. Our results and those of other studies illustrate that variation observed in multiple components of breeding systems can have a simple genetic basis: *Gp-9* alleles affect the social form of queens, may cause differential execution of sexuals, affect the potential for dispersal and incipient colony formation, and perhaps affect female mating success (by reducing fertile male frequency).

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