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Source: Florida Entomologist, 99(3) : 566-568

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.099.0339>

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Evidence of selective mating and triploidy among two social forms of *Solenopsis invicta* (Hymenoptera: Formicidae)

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A number of important behavioral and physiological traits distinguish both social forms of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) and correlate with 2 categories of alleles (B-like and b-like alleles) at the *Gp-9* locus within a supergene complex; these traits include fat body content, number of colony queens, dispersal, ability to initiate new colonies, selective mortality of sexuals, and size of workers (Ross 1997; Goodisman et al. 1999; Keller & Ross 1999; Krieger & Ross 2002; Fritz et al. 2006). All females in colonies with single queens have the BB genotype and males are haploid B. Alternatively, queens in multiple-queen colonies (polygyne) are exclusively heterozygous Bb or homozygous bb; in these colonies, there is also selection against male and female alates that are homozygous BB (Fritz et al. 2006). Most males in polygyne colonies are sterile, diploid, and have the Bb genotype for the *Gp-9* gene (Hung et al. 1974; Fritz et al. 2006). Furthermore, gene flow between both social forms is thought to be unidirectional and limited to males from monogyne colonies mating with polygyne queens (Ross 1992; Shoemaker & Ross 1996; Ross et al. 1997). Fritz et al. (2006) proposed, however, that gene flow directionality between both social forms may be dependent on the relative frequencies of both social forms in a given area and that female alates from polygyne colonies mate disproportionately with males from their own social form. Fritz et al. (2006) also found evidence that some queens mate with more than one male.

The purpose of this study was to test the hypothesis of mating bias in an area of high polygyne colony density, and examine the possibility that some alates with the BB genotype for the *Gp-9* locus copulate with polygyne males. To this end, we examined the genotypes of queens and their stored sperm during a mating flight in Florida where polygyne colonies comprised over 90% of the colonies in the area (Fritz & Vander Meer 2003). The results from this study provide strong evidence of non-random mating, a relatively high frequency of triploidy, and some degree of mating between BB alates and males from polygyne colonies.

Newly mated queens were collected during a mating flight at the confluence of highways 441 and 301 in Marion County, Florida, previously surveyed for both social forms, and where polygyne colonies were determined to comprise approximately 96% of colonies in the area (Fritz & Vander Meer 2003). Queens were collected on the ground during a mating flight and stored in 100% ethanol. The spermatheca of each queen was removed and the sperm isolated according to the protocols of Fritz et al. (2006). DNA was isolated and amplified for the *Gp-9* locus by using the protocols of Fritz et al. (2006) and Krieger & Ross (2002). DNA was also amplified for 3 microsatellite loci (*Sol 11*, *Sol 42*, and *Sol 49*) with fluorescently labeled primers (D4 WellRED Dye, Sigma-Aldrich®). These 3 loci have high levels of heterozygosity and are detailed in Krieger & Keller (1997). Fragment analysis of amplicons was

accomplished by capillary gel electrophoresis on a Beckman Coulter™ CEQ 8000XL Sequence Analyzer with the CEQ DNA Size Standard-400.

In total, 101 newly mated queens were collected and analyzed for the *Gp-9* locus and 3 microsatellite loci (Table 1). Fifteen queens had at least 1 copy of the b allele indicating they originated from polygyne colonies (Table 1). Of these, 2 were aspermic and 84.6% of the remainder were mated to males of their own social form (males with b sperm). In contrast, of all BB queens collected, 7 were aspermic and 92.4% of the remainder stored only B sperm. Approximately 7.6% of inseminated BB queens were storing sperm from polygyne males compared with 84.6% of inseminated Bb queens.

As expected, all sperm samples heterozygous for the *Gp-9* gene (Bb genotype) also produced 2 different amplicons for microsatellite loci. Single microsatellite amplicons were observed for 71 of 75 samples of sperm with a B genotype consistent with these sperm originating from haploid males; the remaining 4 sperm samples with the B genotype for the *Gp-9* locus produced 2 microsatellite amplicons. Five of 79 inseminated BB alates (6.3%) stored sperm that produced 2 microsatellite amplicons in comparison with 7 of 13 inseminated polygyne alates (53.8%). Of those sperm samples with only the b allele for the *Gp-9* locus, all but 1 had single microsatellite amplicons. Of 101 female alates sampled, the percentage of triploid individuals for the 3 *Gp-9* genotypes was the following: 23 of 86 BB (26.7%), 5 of 14 Bb (35.7%), and 0 of 1 bb individuals.

A previous study on the *Gp-9* genotype of polygyne queens and their stored sperm, sampled from colonies at various sites in Florida, suggested this social form mates disproportionately with males from its own social form (Fritz et al. 2006). In our study, and in an area where the proportion of polygyne versus monogyne colonies is high, almost all polygyne queens (84.6%) mated with a male from their own social form. Of these queens, 53.8% were also storing B sperm; we believe this pattern of sperm storage is best explained as the result of some polygyne alates mating with diploid males whose sperm was unreduced. This conclusion is consistent with data re-

Table 1. The *Gp-9* genotype for 101 female alates and their stored sperm. Numbers in parentheses are sperm that exhibited 2 microsatellite amplicons.

Alate <i>Gp-9</i>	Sperm <i>Gp-9</i>			
	B	b	Bb	Aspermic
BB	73(4)	2(1)	4(4)	7
Bb	2	3	7(7)	2
bb	0	1	0	0

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ported by Hung et al. (1974) indicating a 10% occurrence of diploid males with testicular development. In addition, Krieger et al. (1999) reported a 12% occurrence of triploid workers in polygyne colonies from a location in Georgia where 2.4% of the diploid males had functional reproductive tracts. The study in Georgia also reported the presence of triploid female alates in polygyne colonies, though triploid polygyne queens have not been found (Lawson et al. 2012). Thus, triploidy in alates and workers is best explained as the result of queens that have mated with diploid males with unreduced sperm (diploid sperm). Queens mated with diploid males would also explain the high frequency of triploid female alates found in our study and that by Lawson et al. (2012).

The high frequency of triploid BB queens was surprising, because monogyne queens are presumed to mate almost exclusively with haploid B males. Some of the BB alates collected in our study, however, may have originated from polygyne colonies, because Fritz et al. (2006) reported that 7.2% of female alates in polygyne colonies had the BB genotype for the *Gp-9* locus. An additional explanation for some of the triploid BB alates in our study is the possibility that monogyne queens might occasionally mate with diploid BB males from polygyne colonies with unreduced sperm—perhaps at a frequency that depends on the relative abundance of polygyne colonies in the area. For example, we found 4 BB alates that had only B sperm in their spermatheca but produced 2 microsatellite amplicons (perhaps diploid sperm?).

The second reason we think polygyne queens may mate with diploid males carrying unreduced sperm is the unusual pattern of mating that would need to be invoked in order to explain our data otherwise. For example, if polygyne alates storing 2 genotypes of sperm are the outcome of an alate having first mated with a b genotype male (Lawson et al. 2012), why is the second male always B? Because polygyne alates mated disproportionately with males of their own social form in our study (84.6%), one would expect a high frequency of second matings with other b males; however, there are no examples of this outcome whatsoever from our microsatellite data.

Past studies have maintained that gene flow between both social forms is through monogyne males mating with polygyne female alates (Ross 1992; Shoemaker & Ross 1996; Ross et al. 1997). From this study and others (Fritz et al. 2006; Lawson et al. 2012), it is clear that BB alates will mate with polygyne males (at a rate of almost 8% in our study). If some of these BB female alates originate from monogyne colonies, then bi-directional gene flow between both social forms occurs and exclusively through males; this conclusion is consistent with data indicating certain mitochondrial haplotypes are specific to each social form (Ross et al. 1997) because female alates of one social form never become queens of the other social form.

Our data suggest the degree of gene flow between both social forms, though mediated exclusively by males, may be more tenuous for *S. invicta* in the USA than previously thought. The mating bias observed in this study suggests that intra-social-form mating behavior may be under positive selection and perhaps driving this species further on its way to incipient speciation.

We thank James Castner and Bob Vander Meer and the Council for Faculty Research, EIU.

Summary

A number of important behavioral and physiological traits distinguish both social forms of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) and correlate with the *Gp-9* locus within a supergene com-

plex. These traits include fat body content, number of colony queens, dispersal, ability to initiate new colonies, size of workers, selective mortality, etc. Previous studies suggested gene flow between both social forms is unidirectional, with monogyne males mating almost exclusively with polygyne females. This study examined the genotypes of queens and their stored sperm during a mating flight in Florida where polygyne colonies predominate, and provides evidence of non-random mating; 84.6% of inseminated polygyne queens were mated to males of their own social form, and 92.4% of inseminated monogyne queens were mated by haploid males from monogyne colonies. Fertile, diploid males with unreduced sperm may be a cause for the high frequency of triploid BB alates (26.7%) and triploid polygyne alates (33.3%), and for some portion of all alates storing sperm that exhibited 2 microsatellite alleles (15.8%).

Key Words: red imported fire ant; selective mating; triploidy

Sumario

Varias características importantes de comportamiento y fisiología diferencian los dos tipos sociales de *Solenopsis invicta* Buren (Hymenoptera: Formicidae) y correlacionan con el locus *Gp-9* vinculado en un complejo de genes; estas características incluyen el contenido de grasa, número de reinas, dispersión, iniciación de nuevas colonias, tamaño de obreras, y mortalidad, etc. Estudios previos indicaron un intercambio unidireccional de genes entre las dos formas sociales mediante machos de colonias con una reina (colonias monoginas) copulando con reinas de colonias con reinas múltiples (colonias poliginas). En este estudio examinamos los genotipos de reinas y su cúmulo de espermatozoides durante un vuelo nupcial en un local en el estado de Florida donde colonias poliginas predominan; proveemos evidencia que los apareamientos son predisuestos al mismo tipo social; el 84.6% de reinas poliginas y el 92.4% de reinas monoginas fueron apareadas con machos del mismo tipo social. Especulamos que machos diploides y fértiles con espermatozoides diploides fueron la causa de la frecuencia alta de reinas monoginas (26.7%) y poliginas (33.3%) triploides, y un porcentaje de los espermatozoides exhibiendo dos alelos microsatélites (15.8%) en las espermatecas de algunas reinas.

Palabras Clave: *Solenopsis invicta*; triploide; apareamiento selectivo; hormiga roja de fuego

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