

## Short communication

# Evidence for mating plugs in the fire ant *Solenopsis invicta*

A. S. Mikheyev

Section of Integrative Biology, The University of Texas at Austin, 1 University Station C0930, Austin, TX 78712-0253, USA,  
e-mail: mikheyev@mail.utexas.edu

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**Summary.** Male inhibition of female re-mating is common in many insects. Mating plugs, used by males to control female re-mating, have been postulated in several ant species. Recent studies of bumblebees have described re-mating inhibition by male accessory gland secretions. Fire ants *Solenopsis invicta* possess accessory glands containing the same four fatty acids as do the bumblebees. Furthermore it appears that some of these acids are transferred to the female at mating. Thus, it is possible that single mating of fire ant females may be enforced by male mating plugs.

**Key words:** Fire ants, male accessory glands, mating plugs.

## Introduction

Single insemination of queens appears to be the norm in many hymenopteran social insects, despite numerous advantages postulated for polyandrous females (Strassmann, 2001). The lack of polyandry can be explained adaptively either by the absence of selection favoring multiple mating by females, or by selection on males to oppose polyandry. The latter appears to be the case in the bumblebee *Bombus terrestris*, where polyandrous females have higher fitness, but are typically prevented from re-mating by male ‘mating plugs’, which consist of a lipid cocktail secreted by male accessory glands (MAGs) (Duvoisin et al., 1999; Baer et al., 2000). The existence of mating plugs has also been speculated for several ant species (see Bourke and Franks (1995) for a review). The fire ants *Solenopsis invicta* possess MAGs that resemble those of bumblebees (Ball and Vinson, 1984), which raises the possibility that their single mating (Ross and Fletcher, 1985) may also be mating plug enforced.

Fire ant mating takes place high in the air and is difficult to observe, rendering manipulative mate choice studies, such as those performed on the bumblebees (e.g., Sauter et al.,

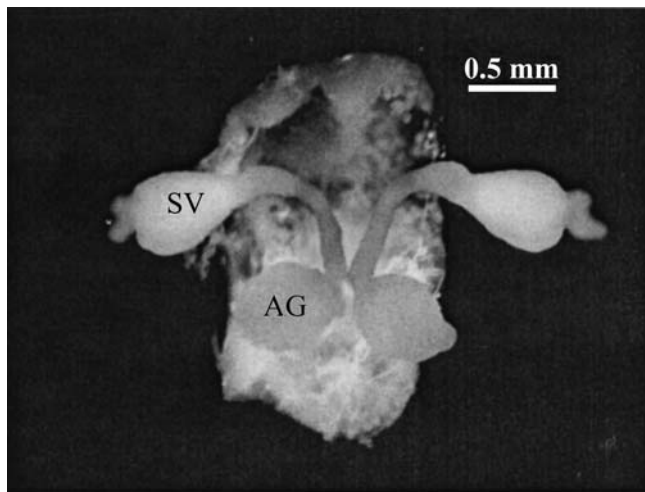
2001), impossible. Thus, evidence for the existence of mating plugs can only be indirectly inferred. Specifically, if the ant MAGs are homologous to those of bumblebees and serve the same function, one would expect their contents to be chemically similar, i.e. to contain fatty acids. Furthermore, it should be possible to detect the transfer of the secretion into the female after mating.

## Materials and methods

In order to determine the composition of fire ant secretions, MAGs were dissected, ruptured into hexane and analyzed by GC/MS, following methylation with methanolic hydrochloride. The methylation was also carried out on synthetic stearic acid to determine approximate yield for the reaction. All data presented are extrapolated to 100% theoretical yield based on this synthesis. In order to determine transfer of MAG secretions into the female, the internal reproductive organs (i.e. the bursa copulatrix and spermatheca) of 20 newly mated (1–3 h) and 20 virgin females were dissected and analyzed by GC/MS as above. The relative amount of secretion in mated and unmated females was analyzed by ANCOVA using queen live weight as a covariate.

## Results

The *S. invicta* MAGs contained a mixture of fatty acids (linoleic, oleic, palmitic and stearic acids), as well as small amounts of benzoic acid. Taken together, these fatty acids make up around 20% of the total dry MAG weight. The live weight of the queens did not correlate with the amount of fatty acids contained inside their copulatory organs ( $P > 0.20$ ). The amount of benzoic acid was not different in the mated and unmated queens ( $P > 0.15$ ). Although the mean amount of palmitic acid was higher in mated queens, this effect was suggestive but not significant ( $0.07 < P < 0.08$ ). However, mated queens contained much greater amounts of linoleic ( $P < 0.001$ ), oleic ( $P < 0.001$ ) and stearic ( $P < 0.003$ ) acids. The copulatory organs of newly mated queens contain about 16 ng of the latter three fatty acids, much more than those of unmated queens (3 ng). The amount of secretion transferred



**Figure 1.** Seminal vesicles (SV) and male accessory glands (AG) of *S. invicta*

to the females is on the same order as the total amount of lipids estimated in *S. invicta* MAGs (20 ng).

## Discussion

The contents of fire ant MAGs consists of the same three or four fatty acids that make up more than 90% of the bumblebee secretion's weight (Baer et al., 2000). In particular, the males transfer linoleic acid, which was experimentally demonstrated to inhibit re-mating by bumblebee queens (Baer et al., 2001). Though the present study cannot definitively rule out other male organs as sources of the fatty acids (e.g. the seminal vesicles), a study of several carpenter ant species has found accessory glands to contain major stores of carbohydrates (Wheeler and Krutzsch, 1992), suggesting that the lipid secretions are indeed MAG-derived. The observation that fire ant MAGs contain and transfer the same fatty acids as do those of the bumblebees makes it likely that they serve they both serve to inhibit female re-mating. Further circumstantial evidence linking the function of fire ant and bumblebee MAGs comes from reports that MAG secretions are not critical for the successful instrumental insemination of either bumblebees (Baer and Schmid-Hempel, 2000) or fire ants (Ball et al., 1983).

The mere presence of a mating plug may not be enough to guarantee single insemination. For example, mating plugs are present in the naturally polyandrous bumblebee *B. hypnorum* (Brown et al., 2002). Similarly, if mating plugs diminish re-mating in ants, they may not be completely successful in all cases. For instance, male secretions that block the vaginal orifice in the ant *Carebara vidua* do not prevent multiple mating (Robertson, 1995). Thus, much more work must be

done on the mechanism of re-mating suppression via lipids and the possibility of female counter-measures.

Based on structure, position and the content of their secretions, it appears that the MAGs of bumblebees and the fire ants are homologous. If mating plugs are found in both ants and bumblebees, MAG-mediated queen re-mating suppression may be widespread and ancient in the hymenopteran social insects and may have played an important role in the evolution of complex relatedness-based social systems.

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