Male accessory gland size and the evolutionary transition from single to multiple mating in the fungus-gardening ants

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Abstract

Polyandry in hymenopteran social insects is surprisingly rare, despite its likely colony-level fitness benefits. Ordinarily, a male's fitness will be at odds with that of a colony when the genetic representation within it is diluted by multiple mating by the queen. Consequently, males are expected to be under selection to limit female re-mating, for example via secretions of their accessory glands. I hypothesized that if accessory glands in some way regulate female mating frequency, an evolutionary transition from single to multiple mating would likely be accompanied with a change in the morphology of the accessory glands. The accessory gland morphology was examined in the fungus-gardening ants, which have made a single transition from single to multiple mating. The evolution of polyandry within this clade corresponds to the loss of male accessory glands, lending tentative support to the idea that they may be involved in regulating mating frequency.

Abbreviation:
MAG   Male accessory gland

Introduction

Polyandry, multiple mating by females, occurs frequently in animals. By mating with several males, females gain benefits such as reduced harassment (Wilcox 1984), nuptial gifts (Zeh and Smith 1985), fertilization insurance (Ridley 1983), or genetic variety among offspring (Keller and Reeve 1994). However, it is generally believed that the proportion of eggs fertilized due to polyandry, is not in the in the interests of a male. Indeed, males have evolved numerous tactics preventing females from re-mating, from mate guarding (Birkhead and Parker 1997), to mating plugs (Devine 1977).

Hymenopteran social insects (ants, bees and wasps) are a notable exception to the general prevalence of polyandry, with most females mating just once and using sperm from that copulation for the rest of their lives. To date polyandry has been documented in only about a half-dozen genera of ants, bees and wasps (Boomsma and Sundström 1998, Strassmann 2001). Although their mates do not provide nuptial gifts or parental care, as they die shortly after mating, social insect females are nonetheless expected to benefit from polyandry. In particular, females may either acquire a potentially advantageous mixture of genes for their offspring (Page and Metcalff 1982; Crozier and Page 1985; Sherman *et al*. 1988; Keller and Reeve 1994), obtain a quantity of sperm necessary for long term production of workers and large colony sizes (Cole 1983) or reduce kin conflict with daughters over sex ratios and male parentage (Starr 1984; Moritz 1985). Indeed, genetic advantages of polyandry have been demonstrated both in naturally polyandrous harvester ants (Cole and Wiernasz 1999) and, by artificial insemination, in singly mating bumblebees (Baer and Schmid-Hempel 1999).

Mating frequency in social insects determines the relatedness of workers and thus the genetic structure of the colony, which affects numerous aspects of behavior. For example, changes in relatedness brought upon by polyandry may be involved in resolving conflicts over male parentage and offspring sex ratios (Ratnieks 1988; Boomsma and Grafen 1990). Accordingly, knowledge of the forces underlying the evolution of multiple mating by queens is fundamental to our understanding of social evolution.

The interests of males have been largely ignored when considering social insect polyandry, probably because of the secondary roles males appear to play in these societies, but see Hölldobler and Bartz (1985) and Boomsma and Sundström (2000), for alternative perspectives. However, secretions of male accessory glands (MAGs), which attenuate re-mating by females, have been documented in numerous non-social insects (Chen 1984). More recently mating plugs were discovered in a bumblebee species (Sauter *et al*. 2001). These plugs, which consist principally of a mixture of four fatty acids, were found to inhibit re-mating by females (Baer *et al*. 2001). Male fire ants transfer at least two of the same four fatty acids to females during mating (Mikheyev, 2003) suggesting that re-mating suppression of polyandry may occur in many hymenopteran social insects (Sauter *et al*. 2001), and may involve the same chemical mechanism as the bumblebees.

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The fungus gardening ants have been the focus of several phylogenetic (Schultz and Meier 1995, Wetterer *et al*. 1998) and paternity studies (Kerr 1961, Moser 1967, Corso and Serzedello 1981, Villesen *et al*. 1999, Murakami *et al*. 2000). While the basal species are singly mating, leaf cutters in the genera *Atta* and *Acromyrmex*, made a single transition to polyandry (Villesen *et al*. 2002). Thus, these ants provide a means of examining the possible association between MAGs and the evolution of polyandry. Specifically, if MAG secretions in some way affect female mating frequency, then the evolutionary transition from monandry to polyandry would likely be correlated with morphological changes in the glands themselves.

Materials and Methods

Males from seven attine genera (seventeen species) were dissected under magnification, their internal reproductive systems were photographed and the volumes of their male accessory glands (MAGs) and seminal vesicles measured by approximating them as either cylinders or spheroids. In addition, a photograph of the male reproductive system (Hung and Vinson 1975) of the polyandrous (Moser 1967) *At. texana* was included in the analysis. See Table 1 for a complete list of species.

Males of the fire ant *Solenopsis invicta*, a known singly mated species (Ross and Fletcher 1985) with a well-studied reproductive system (Ball and Vinson 1984), were dissected and included as an outgroup. *S. invicta*, which belongs in the same subfamily as the attines (Myrmicinae), illustrates the internal reproductive morphology common to most ants studied previously (Beck 1972; Hung and Vinson 1975; Wheeler and Krutzsch 1992), which appears to be the primitive condition in ants.

The seminal vesicles and MAGs of the multiply mating genera *Acromyrmex* and *Atta*, and a few singly mates species, were stained and sectioned. All the specimens were stored in 70% ethanol. The MAGs and seminal vesicles were dissected out, fixed for 16–24 h with either Bouins solution or Z-5, then dehydrated with a succession of 95% ethanol (15 min), 100% ethanol (15 min), methyl salicylate (30 min) and two changes of xylene. The tissues were then infiltrated with two changes of Paraplast Plus (Fisher Scientific,

www.fishersci.com/) mixed with a few drops of honeybee wax (1 hour each infiltration). Tissues were sectioned at 10 µm, mounted on slides and dried overnight. After being deparaffinized and hydrated with distilled water, the slides were stained with hematoxylin (30 sec) and eosin-Y (15 sec). The estimates of MAG volumes for *Acromyrmex* and *Atta* species were made from stained sections mounted on slides. In this manner sperm could be clearly visible in the seminal vesicles, while MAG contents stained a solid red.

Statistical analysis

Male ants are highly specialized for locating and mating with females. A male's gaster contains the bare minimum of life support organs with the rest being occupied by the reproductive structures; MAGs and the seminal vesicles. As there appeared to be a trade-off between allocating space to MAGs versus seminal vesicles, the relative changes in their morphology were expressed as a ratio of MAG volume over seminal vesicle volume.

To eliminate any potential problems caused by phylogenetic non-independence due to unequal genetic distance between species, phylogenetic information derived from (Wetterer *et al*. 1998) and T. R. Schultz, (pers. comm. 2002) was incorporated to compute a set of phylogenetically independent contrasts (Felsenstein 1985) using the CONTRAST module of PHYLIP (version 3.5).

Results

The loss or extreme reduction of MAGs exactly corresponds with the appearance of polyandry in the fungus gardening ants. While the MAGs of the basal attines and fire ants are prominent, with conspicuous lobes protruding laterally off the ejaculatory duct, *Atta* possess minute accessory glands, relative to the size off their seminal vesicles, and *Acromyrmex* appear to lack MAGs entirely. Figure 1 shows the presence or absence of large MAGs mapped onto the attine phylogeny (Schultz and Meier 1995, Wetterer *et al*. 1998), as well as characteristic MAG morphologies. The reproductive system of attine males, excluding the leaf cutter clade, is remarkably similar to that of fire ants and to that of most ants studied to date in that they all possess slender seminal vesicles and prominent lobed MAGs. In the singly mating species, MAGs

Table 1. Species used for morphological and histological study. Numbers in parentheses indicate sample size.

Genus	Species
Acromyrmex	**echinatior** (1)**, octospinosus** (1**)**, *volcanus* (3)
Apterostigma	*auriculatum* (1), **collare** (1)
Atta	*cephalotes* (3), **colombica** (3)**, sexdens** (3)**, texana**
Cyphomyrmex	**costatus** (1), **longiscapus** (1)**, rimosus** (1)
Myrmicocrypta	*buenzlii* (1)
Sericomyrmex	**amabilis** (1)
Trachymyrmex	*septentrionalis* (10)*, cornetzi* (1)

Boldface type indicates species where the presence or absence of polyandry has been confirmed (Strassmann 2001, Villesen et al. 2002).

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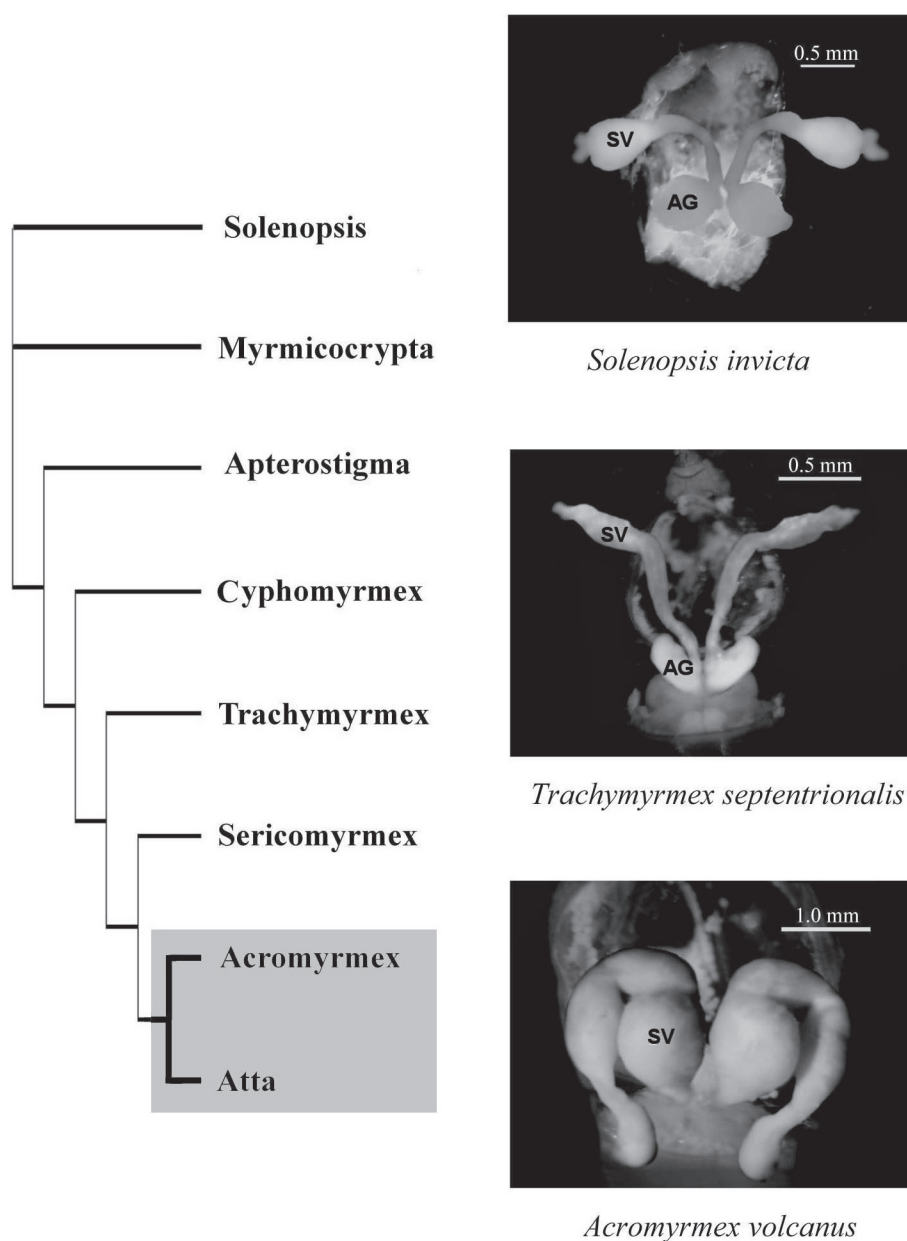
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Figure 1. Trait mapping on the attine phylogeny. The transition indicated on the phylogeny corresponds to both the evolution of polyandry, as well as to the reduction of MAGs in the monophyletic leafcutter clade including *Acromyrmex* and *Atta*. Representative dissections of males, illustrating the primitive and derived morphologies, are shown alongside the phylogeny. An outgroup, the fire ants (*Solenopsis*) has been included for an informal comparison. AG = accessory glands, SV = seminal vesicles. Note that *Ac. volcanus* does not have accessory glands.

were about equal in volume to the seminal vesicles. However, a major transition in internal morphology occurs in the leaf cutting genera with the extreme reduction of MAGs in *Atta* and their complete disappearance in *Acromyrmex*. Figure 2 illustrates the inverse relationship between the volumes of MAGs relative to seminal vesicle volume and a species' effective paternity, which is a measure of the genetic diversity within a colony due to multiple matings. The effective mating frequency controls for unequal sperm usage by weighing the relative contribution to fertilizations of the different mates of the queen. The phylogenetically independent contrasts of relative MAG volume and effective paternity were negatively correlated ($r=-0.77$, $N=6$) for all the species listed in Figure 2, except *Ac. echinatior* and *At. colombica,* which lacked phylogenetic distance information and were not included in the analysis.

Discussion

The change in MAG volume, which decreases in multiply mating species, suggests that these glands may be involved in regulating multiple mating by females. Ideally, behavioral assays using instrumentally inseminated females should be used to test the function of MAGs, as has been done in bumblebees (Baer *et al.*
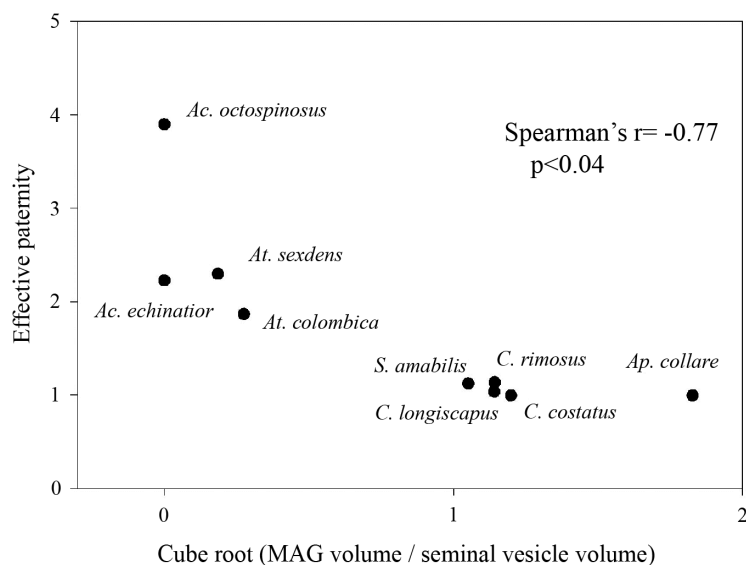
Figure 2. Relationship between relative MAG volume and the number of matings by queens of attine ants (Strassmann 2001, Villesen *et al*. 2002). The polyandrous species (*Acromyrmex* and *Atta*) have much smaller MAGs relative to their seminal vesicles than the monandrous genera (*Apterostigma*, *Cyphomyrmex* and *Sericomyrmex*).

2001). However, to the best of my knowledge, no attine ant has ever been instrumentally inseminated or mated in a laboratory setting. Thus, for now, morphological correlations, somewhat corroborated by evidence for fatty acid transfer from fire ant MAGs (Mikheyev, 2003), provide the best, though inconclusive evidence that MAGs regulate multiple mating in attine ants.

However, as is the case for all correlative evidence, these observations are consistent with a number of other alternative hypotheses. For instance, MAGs could be the source of nuptial gifts, which are no longer offered by males of *Acromyrmex* and *Atta* due to some unknown change in the direction of sexual selection accompanying polyandry. Though Sauter *et al*. (2001) argued that the bumblebees' mating plug is too small to provide measurable nutritional support and the fire ants secretion also appears miniscule (Mikheyev, 2003), there are no data on size or composition of MAG secretions in the attine ants and this hypothesis cannot be excluded. Likewise, MAG secretions may be used for intersexual signaling that happens differently in singly and multiply mated species. Additionally, the evolution polyandry and subsequent sperm competition may have reduced the adaptive value of large MAGs relative to the need for large seminal vesicles. Doubtless, more hypotheses could be offered.

Assuming that MAG secretions do enforce monandry, we can speculate about forces causing the evolution of polyandry. For instance, polyandry may have evolved when higher attine females, through an unknown mechanism, were able to overcome the male's manipulative strategy. If so, males would not be expected reduce MAG size, but to pursue a coevolutionary arms race, perhaps even leading to MAG hypertrophy. More likely, the evolution of polyandry

in the fungus gardeners was driven not solely by female preference for multiple mating but by selection on the males themselves. In theory, polyandry (*i.e.* increased genetic diversity) amplifies the fitness of a multiply mated colony, relative to a singly mated colony (Page and Metcalff 1982; Sherman *et al*. 1988). If a queen's multiple mating confers a colony-level fitness boost sufficient to overcompensate her mates for effects of genetic dilution due to polyandry, males will be selected to stop monopolizing females. The beneficial effects of polyandry in harvester ants described by Cole and Wiernasz (1999) appear sufficient to give the necessary fitness amplification, especially considering the high rates of mortality among the more genetically homogeneous colonies. Given the relative rarity of polyandry in the social insects, more needs to be known about the specific benefits of this behavior. Comparative studies may yield clues about why it evolved in only a few lineages.

The inhibition of female re-mating via MAG secretions may be a widespread phenomenon in insects (Chen 1984). The high relatedness within social insect colonies, which has been proposed for the evolution of eusociality through kin selection (Hamilton 1964), presupposes monandry. Thus, MAG-enforced single mating by females in some insect groups may be yet another factor facilitating the evolution of complex kin-based social systems.

Acknowledgements

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