ECLOSION, MATING, AND GROOMING BEHAVIOR OF THE PARASITOID FLY *PSEUDACTEON CURVATUS* (DIPTERA: PHORIDAE)

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Abstract

Phorid flies from the genus *Pseudacteon* are parasitoids of *Solenopsis* ants. Recent efforts of controlling imported fire ants in the United States have focused on rearing and releasing these flies as biocontrol agents. We studied eclosion, mating, and grooming behavior of *Pseudacteon curvatus* Borgmeier in an effort to increase understanding of its biology. The sex ratio of eclosing flies in the lab was 1:1. The flies emerged only in the morning and were protandrous. Mating in the lab occurred on the substrate and did not require disturbed ants. Males and probably also females mated multiply.

Key Words: Biocontrol, *Solenopsis invicta*, Phoridae, Mass Rearing

RESUMEN

Las moscas del género *Pseudacteon* (Phoridae) son parasitoides de las hormigas *Solenopsis.* Esfuerzos recientes para controlar a la hormiga de fuego importada (*Solenopsis invicta*) en los Estados Unidos han estado enfocados en la cria y liberación de estas moscas como agentes de control biológico. Nosotros estudiamos la eclosión, apareamiento y el comportamiento de acicalamiento de *Pseudacteon curvatus* Borgmeier en un esfuerzo para aumentar nuestro entendimiento de su biología. La proporción de nacimiento de hembras y machos en el laboratorio fué 1:1. Las moscas emergieron solamente en la mañana y fueron protandrosas (los machos nacen más temprano que las hembras). El apareamiento en el laboratorio sucedió sobre el substrato y no requeria que las hormigas fueran perturbadas. Los machos y probablemente las hembras se aparearon varias veces.

Until recently, attempts at controlling the red and black imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, respectively, have been almost entirely by chemical means. The consequences of early practices were quite disastrous in some cases (Carson 1962). Fortunately, more ecologically sound and sustainable methods have been developed (Drees et al. 1996). One of the methods currently under investigation is the use of *Pseudacteon* phorid flies as agents of biological control, first suggested for the control of attine ants in Weber (1972).

The female *Pseudacteon* phorid fly attacks and lays an egg in an adult worker ant. The larva ecloses, consumes its host's internal organs, and then pupates in the ant's head capsule (e.g., Porter 1998). The supposed biological control effect lies not so much in direct mortality, for each mature *Solenopsis* nest contains hundreds of thousands of worker ants, but in the indirect effect the fly's presence has on normal fire ant behavior: fire ants stop foraging and defending resources in the presence of phorid flies, which potentially has a detrimental effect on the colony's fitness (e.g., Orr et al. 1995, 1997).

Progress has been made in development and evaluation of 2 species (*Pseudacteon tricuspis* Borgmeier and *P. curvatus* Borgmeier) for mass-rearing and release (Porter & Alonso 1999, Porter 2000, Porter 1998a, Porter 1998b, Morrison et al. 1997, Gilbert & Morrison 1997, Porter et al. 1997, Pesquero et al. 1996, Porter et al. 1995a), and initial investigations into suitability of several other species have also been published (Porter 2000, Porter & Alonso 1999, Morrison & Gilbert 1999, Porter 1998a, Porter 1998b, Gilbert & Morrison 1997, Morrison et al. 1997, Orr et al. 1997, Pesquero et al. 1996, Orr et al. 1995, Pesquero et al. 1995, Porter et al. 1995a, Porter et al. 1995b).

There are 18 described species of phorid flies that attack fire ants in South America (Porter & Pesquero 2001). Such diversity can only be supported if the species of parasitoids have differing habitat preferences, foraging ecologies, and host preferences. Indeed, this has been shown to be the case (Porter & Briano 2000, Orr et al. 1997, Gilbert & Morrison 1997, Pesquero et al. 1996, Porter 1998a, Porter et al. 1995a, Fowler et al. 1995, Borgmeier 1922). For instance, some phorid flies attack fire ant minors, whereas others attack the majors; some attack during the heat of the day, and others attack during the cooler parts of the day; and some attack at fire ant foraging trails, whereas others attack at disturbed mounds.

Because the ecology of the various species of parasitoids differ, the introduction of more than one species is likely to have a greater effect than if a single species is introduced. Further, to have the greatest effect, the suite of species released should have complementary biologies. This paper adds to what is known about the basic biology of *P. curvatus*. Much of this information will assist mass rearing and release efforts.

MATERIALS AND METHODS

A general description of eclosion behavior was obtained by observing five newly-eclosed flies from the time that the pupal cap opened until the fly was able to fly. For these observations, flies were kept in individual containers (capped 1.2 × 7.5 cm test tubes) and observed through a dissecting microscope.

A general description of mating was generated from observations of >20 matings. The mean duration of mating was timed from mounting until dismounting. Actual duration of copulation was probably less than "mating duration," but we were not able to discern when actual intromission occurred.

To determine if multiple mating occurred, five virgin females and one virgin male were put into a container and left together for 30 h (n = 3). At the end of the 30 hours, all living flies were squashed and a phase contrast microscope was used to look for sperm.

The description of grooming was generated from observations of flies as they groomed while in the mating observation arena or during eclosion. We observed >20 grooming bouts.

RESULTS

In the lab, *P. curvatus* flies have been observed to emerge between 0300 and 1000 h. Emergence in the late morning was probably due to exposure to artificial light the evening before they emerged. In the field, emergence probably occurs within a few hours of dawn depending on the temperature and light cycle. In the lab, flies emerged over a period of about 2 h with males emerging about 30 ± 5 (SE) min earlier on average than females (ANOVA, $F_{1,2} = 35.3$, $p < 0.0001$, n = 45 and 59, respectively). Emerging flies required about 10 min to expand their wings. When temperatures were warm (26°C) flight can occur within an hour of emergence and mating about an hour later. However, cool temperatures (<22°C) can delay flight and mating until late morning or even into the afternoon. The sex ratio of males to females did not differ significantly from 1:1 (Porter 2000).

Average duration of mating was 22.3s ± 8.6 (mean ± SD, n = 10). There was considerable variation in mating time, ranging from 12 to 39 s. Because mating was terminated by the female kicking the male off of her back, the variation was probably not due to actual time required for mating, but to changes in female motivation during mating.

The following is a detailed description of eclosion and post-eclosion behavior of one female fly. The emerging fly popped open the pupal cap and climbed out of the ant head capsule onto the substrate, a process that usually only requires a few seconds. On first emerging, the fly's abdomen was distended and elongated, and the ovipositor was extended. The cuticle of the legs, head and thorax was a dusty tan, the abdomen was a pale whitish color, and the wings were white and dull. The eyes and ovipositor appeared to be sclerotized. Almost immediately, the fly started pumping her abdomen to fill and expand her wings. After about 10 min, the wings appeared to be fully expanded and the abdomen had reduced considerably in size but was still elongated. About this time, the abdominal pumping stopped. Over the next several minutes, fluid returned to her abdomen, which became distended again, and she began pumping her abdomen again. About 15 min after eclosion, the costal margins of her wings began to sclerotize and her entire body began darkening about 10 min later. She continued to pump her abdomen from time to time and her cuticle became darker and darker until about 75 min after eclosion, when she finally retracted her ovipositor. Although her body seemed sclerotized and she began walking about 10 min later, her wings were still somewhat opaque, and she did not fly until almost 2h 15 min after eclosion.

Before mating, the females remained stationary for the most part, usually moving only if disturbed by another fly. Females groomed themselves while sitting. Males were also stationary and groomed some of the time, but were much more mobile than females; often walking about on the substrate and flying around the arena. While males were in flight, they sometimes tussled briefly with one another, but there were no discernible territories, nor were males particularly aggressive.

Visual cues and probably chemical cues were important to males for locating a mate. Males investigated small bumps in the substrate and were attracted to empty head capsules. One male vigorously attempted to copulate with an empty ant head capsule.

Females that had sat unnoticed for some time suddenly became attractive to males. We could not discern any difference in behavior of females that were attractive versus those that were not, so presumably they emitted some mate attraction chemical. There did not seem to be any mate choice by females. Mating was determined by scramble competition. Often, 2 or 3 males simultaneously attempted to copulate with the same female.

The male flew toward the female, approaching her posteriorly. The male sat on top of the female and struggled to hold onto the back of the female's abdomen with his hind legs. Once the male had a hold on the female's abdomen, the adeagus was extended and curved around the back of the female's abdomen to one side of the ovipositor. The female's cloaca was just in front of the ovipositor. Toward the end of the mating, the female began to walk around on the substrate and sometimes pushed off the male with her hind legs.

Following mating, the male and female sat within a few cm of each other and groomed, first the abdomen, then the hind legs, thorax, head, and legs. They did not groom their wings. After grooming, the female became more active and flew about the arena. The male also sometimes flew about, but tended to become less active.

Squashes of single virgin males kept with five virgin females clearly showed multiple mating by males. Of the female flies recovered alive after 30 h, 5/5, 3/3 and 2/4 were mated. All 3 males were still positive for sperm at the end of the test.

While making observations for mating duration, it was common to see more than one male attempting to mount a female both simultaneously and sequentially. However, whether females functionally mate more than once is not clear.

The abdomen, ovipositor, and wings are all groomed with the third pair of legs. To groom the abdomen, the inside surfaces of the hind tibiae and fibiae are drawn over the ventral and lateral surfaces of the abdomen. The wings are drawn to the side of the abdomen with the hind legs and the ventral surface of the hind leg is rubbed over the dorsal surface of the wing. The underside of the wing is groomed by rubbing the dorsal surface of the third leg along the ventral surface of the wing.

The posterior portion of the thorax is groomed using the femur of the third leg, whereas the forelegs are used to groom the posterior portion of the thorax and the head. The head is turned to each side and ventrally in order for each of the surfaces to be rubbed. Much more care is given to grooming the aristae than to any other part of the body. The fly often goes over each one with its forelegs several times.

The legs are groomed by rubbing against other legs. The forelegs are rubbed together, starting at the base, and moving toward the tarsi. The hind legs are rubbed together in similar fashion. To groom the middle legs, the forelegs are drawn back to the side to be groomed and rubbed over the middle leg. The hind legs are also used to groom the middle legs in similar fashion. It is possible that these grooming movements also groom the fore and hind legs respectively. Similar leg-cleaning movements are described and illustrated for *D. melanogaster* in Szebenyi (1969).

DISCUSSION

Our observations revealed that *P. curvatus* flies mate on the ground in the morning several hours after eclosion from the puparium. This information will be useful to researchers interested in releasing *P. curvatus* flies for biocontrol of imported fire ants because field releases of this fly should be timed for late morning or afternoon, as mating does not occur in the air while females are trying to oviposit.

Males readily and successfully mated multiple times in our tests, indicating that lab-reared females will rarely lack sufficient mates for mating.

Very little is known about mating behavior of other *Pseudacteon* flies. Published reports of mating behavior for this genus are primarily field studies reporting male *Pseudacteon* presence at aggregations or trails of *Solenopsis* ants (Williams 1980, Feener 1987, Feener & Brown 1992, Porter et al. 1995a). The most detailed account is for *P. tricuspis* (Porter et al. 1997). From this, we know that mating behavior differs considerably between *P. tricuspis* and *P. curvatus*. Although males of *P. tricuspis* are attracted to disturbed fire ants, males of *P. curvatus* are not. Mating for *P. curvatus* takes place on the ground and takes more than 20 seconds on average; whereas for *P. tricuspis*, mating is initiated on the wing and is extremely brief (<1s, Porter et al. 1997).

Mating in other kinds of phorids often occurs in mating swarms (reviewed in Disney 1994). Not surprisingly, the most common type of mating swarm was composed of lekking males. However, there are species in which there are both sexes in one swarm, another with both sexes forming separate swarms, and one species with female mating swarms to which males were attracted (reviewed in Disney 1994). The preponderance of species using swarms for mating probably only indicates the ease with which swarms can be observed versus other mating strategies that are less likely to be stumbled upon.

The duration of mating among phorids ranges from <1s (*P. tricuspis*, Porter et al. 1997) to 8 minutes (*Dohrniphora cornuta*, Barnes 1990). How this interspecific variation relates to sperm competition and/or mate selection by females would be an interesting research direction.

ACKNOWLEDGMENTS

We thank D. Kelly, B. Mayfield, C. Vann, and J. Saunders for help in completing this project. The State of Texas Fire Ant Research and Management Committee (FAR-MAAC) provided partial funding for this research.

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