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Mating behavior of *Phyllopalpus pulchellus* Uhler (Orthoptera: Gryllidae: Trigonidiinae)

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Abstract

Phyllopalpus pulchellus Uhler (Trigonidiinae) is a small, diurnally-active cricket common throughout much of the eastern United States. Mating interactions in this species are here described based on observations of 14 mating pairs. Males produce two very different types of spermatophores: small, spermless microspermatophores which are removed and consumed by females shortly after transfer, and larger, sperm-containing macrospermatophores that are only produced following the successful transfer of a microspermatophore. A bell-shaped structure surrounding the sperm tube of macrospermatophores makes them more difficult for females to remove prematurely. Males' production and transfer of spermless microspermatophores likely serve as a test of female receptivity prior to investing in a relatively expensive macrospermatophore that must be used within a short period.

Key words

microspermatophore, macrospermatophore

Introduction

Ensiferan mating behavior is quite diverse (Alexander & Otte 1967) but generally involves the transfer of an external spermatophore which then inseminates the female under its own power. Between its transfer and the completion of insemination, the spermatophore is vulnerable to predation by the female, and some post- or inter-copulatory male behaviors as well as structural details of the spermatophore are thought to function at least partly to prevent the premature removal of spermatophores by females (Sakaluk 1984). Members of the Trigonidiinae have not been as well studied as other gryllid subgenera, but the occurrence of sequentially dimorphic spermatophores, with and without sperm, has been documented for several of its members (deCarvalho & Shaw 2010b). Here I describe the spermatophores and mating behavior of *Phyllopalpus pulchellus* Uhler.

P. pulchellus is a distinctive cricket found throughout much of the eastern U.S. (Blatchley 1920; <http://www.entnemdept.ufl.edu/walker/buzz/641a.htm>) and is common among course weeds around the margins of old fields in Chester County, Pennsylvania. There, adults appear in early August and are present through September and early October. The distinctive sputtery, metallic, broken trill of calling males is easily recognized among the cricket singers in this area. Walker (1962) described this song as an "irregular and 'ragged' trill". *P. pulchellus* is strikingly colored (Figs 1, 2) and primarily diurnal, and yet difficult to see or collect because of its rapid retreat (usually to the underside of a leaf) upon the approach of an observer. Males usually sing while concealed between leaves (Fig. 3) about 0.5-2 m above ground level. But on cool (less than 15 °C), sunny mornings in September and October in southeastern

Pennsylvania both males and females can be readily collected while sunning themselves on the upper surfaces of foliage.

The mating interactions of *P. pulchellus* have not been described in the literature. I have observed 14 mating pairs of this species, all following the pattern described below.

Methods

Laboratory observations were made on individuals collected in the field as last instar nymphs in southern Chester County, Pennsylvania, USA (39.8535°N, 75.7867°W) and reared individually to the imago in the laboratory. In order to quantify courtship and mating behaviors between individual pairs, uninterrupted tests were conducted on 28 individuals (14 of each sex), reared from nymphs and housed individually as adults for at least one week. Observations were made one pair at a time in 4 inch diameter plexiglass tubes covered with plastic petri dish lids during the daylight hours, between 9 and 11 A.M. Crickets were able to walk about freely on the smooth plastic surfaces, and the plexiglass tubes allowed observation of details of spermatophore production and transfer from a ventral view with a hand-held Wild M3 stereo microscope. For each pair, observations continued until the female tried to escape the male's attention.

Most interactions described here were also observed (and photographed) on various occasions in the field. An unrecorded number of additional interactions were staged, with some cases including individuals that had been used previously for the matings described above. These were used to supplement photographic illustration, document male-male interactions and to collect spermatophores for the enumeration of sperm. For the latter, spermatophores were removed from the female immediately after transfer, or in some cases intercepted as the male collected them following an aborted mating attempt. These were placed in ~100 µL saline (0.15M NaCl) where they were allowed to empty themselves, after which an equal volume of 5% buffered formalin was added as a fixative and preservative.

The liquid portion and/or the spermatophore itself (after having been macerated) were sonicated using a sonicator at 50 watts for 30 seconds in ~5 mL volume (deionized water added) in Corex test tubes to disperse clumps of sperm. Test tubes were then vortexed and filtered onto 25 mm diameter, 0.80 micrometer pore size, Millipore type AA filters. The sperm was then stained (on the filter) for 10 minutes with 4',6-diamidino-2-phenylindole (DAPI) fluorescent nuclear stain. Filters were transferred to glass microscope slides and covered with a coverslip and observed under a Zeiss Universal microscope equipped for epifluorescence. The DAPI stains only the nuclei, allowing enumeration of sperm with relative ease (see Sakaluk 1984 for details of a similar method using Hoechst 33258 stain). The sonication/vortexing/filtering technique described here

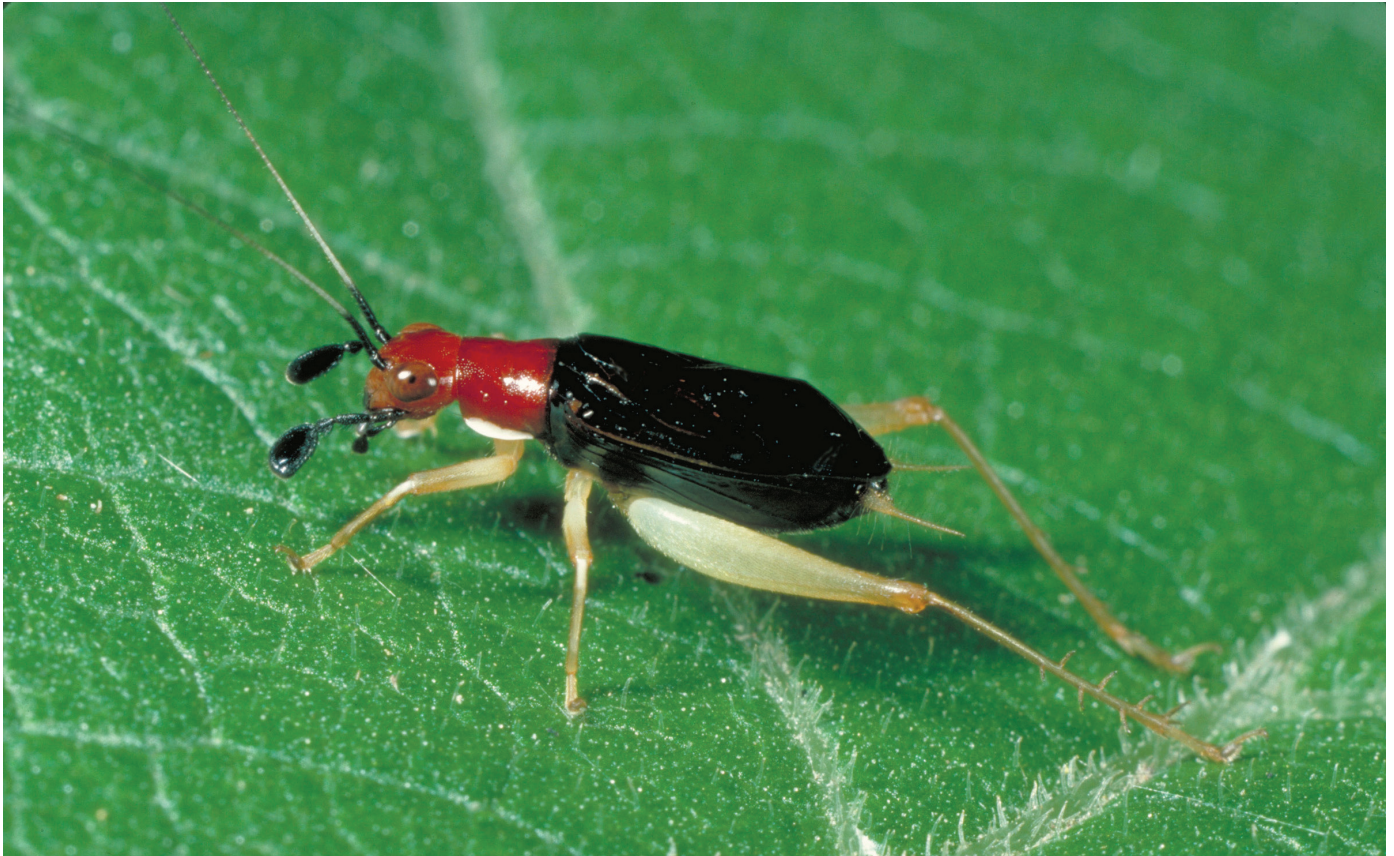


Fig. 1. Male *Phyllopalpus pulchellus*.



Fig. 2. Female ovipositing in trunk of an apple tree.



Fig. 3. Male singing from between leaves.

gave very even dispersal over the entire filter. Ten fields (area = 1.072 mm²) at 160× were counted when total numbers were low (generally under 60/field). When densities were higher, 10 fields (area = 0.610 mm²) were photographed using Kodak TMax 400 film. Negatives were then placed in a photographic enlarger and projected onto an 8-1/2 × 11 inch sheet of white paper where accurate counts could be made by checking off each sperm nucleus as it was counted.

Results

Figure 9 is a flow chart of behaviors observed, with time statistics shown for particular events in the 14 uninterrupted virgin pairings.

Male *Phyllopalpus* sang when calling for mates, or in the presence of another member of their species of either sex. Courtship songs seemed softer and less melodic than calling songs, but attempts to quantify these differences were not made. Males caged together sang almost continuously and frequently tremulated (by moving their body violently in a forward to backward cyclical movement, usually consisting of two or three cycles for a given tremulation). Their behavior among other males seemed to be a territorial display, but only once was actual physical aggression between males observed; two males courting the same female briefly turned back-to-back and kicked at each other with their hind legs — it was not very effective as neither one dislodged the other, and the exchange lasted only a few seconds, after which they resumed courtship.

In the presence of a female, males kept their tegminae raised almost continuously, but sang intermittently. During courtship, males faced directly away from their potential mate, and (especially during

the early stages of courtship) tremulated frequently. Tremulation by males was accomplished by rocking the body forward and backward vigorously while maintaining a firm foothold on the substrate. Attentive females (*i.e.*, those that did not walk away) remained about one antenna-length behind the male, maintaining contact with the tip of one antenna touching the males' tergites (Fig. 4). Sometimes a female approached a male and mouthed one of his hind tibia, but males seemed to discourage this by moving away slightly. Occasionally males turned around, apparently to check the female's location. In the majority of cases this occurred after the female had wandered off. Both the male and female antennated continuously and palpated the substrate. After 5 to 10 minutes of uninterrupted courtship, the male extruded his genitalia and produced a spermatophore (Fig. 5).

Two distinctly different types of spermatophores were produced by *Phyllopalpus* males, differing in both the size of the ampulla and in the structure of the spermatophore tube (Fig. 6). One type, the microspermatophore, was always produced first. The microspermatophore usually appeared about five minutes after a female became attentive. The male first extruded his genitalia and within seconds a spermatophore ampulla became visible beneath them. In two to three minutes the male suddenly dropped his tegminae and attempted to back under the female (Fig. 7). Receptive females raised their bodies and allowed the male to back under them and transfer the spermatophore. Transfer of the spermatophore was very brief — usually taking only a second or two, after which the male immediately crawled back out and resumed courtship (singing, tremulating, and palpating the substrate) in the same position as he had done before the transfer.



Fig. 4. Male singing and female approaching from behind.



Fig. 5. Extruded macro-spermatophore in male's genitalia.

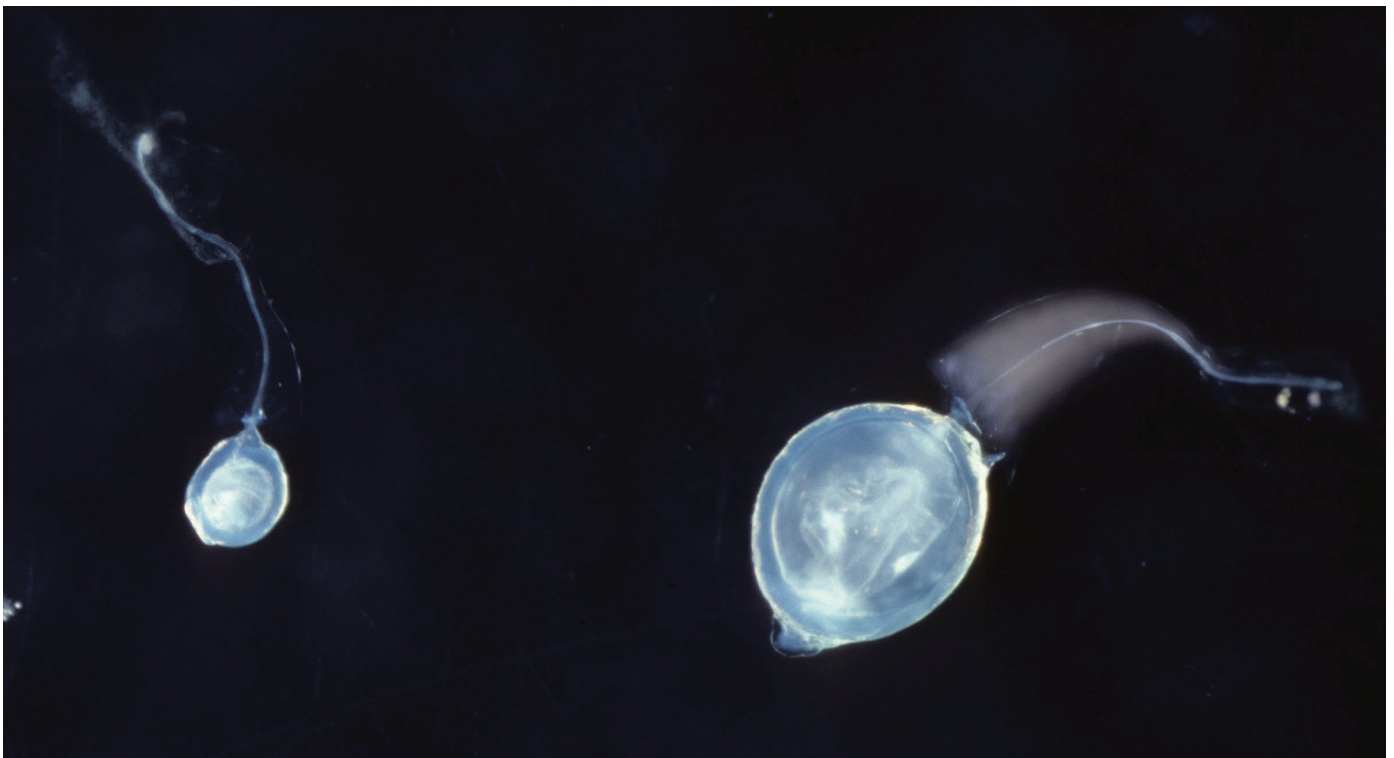


Fig. 6. Micro-spermatophore and macro-spermatophore.



Fig. 7. Male singing with attentive female moments before the male backed under her and transferred a microspermatophore.

Females removed the microspermatophore, usually within seconds of its transfer, always within three minutes. *Phyllopalpus* females were apparently unable to reach the spermatophore directly with their mouthparts the way some other crickets (such as the oecanthines) do. Instead, they used the hind tibial spurs to "pluck" it out. This was accomplished by cradling the exposed portion of the spermatophore tube in the V-shaped notch formed by the two apical spurs of the hind tibia and subsequent rearward movement of that leg. This notch was run along the sperm tube until the bulbous ampulla portion of the spermatophore was reached. The ampulla then became lodged in the notch and the spermatophore was plucked out, after which the female removed it from her tibia with her mouthparts and ate it. The microspermatophore was usually completely consumed within two minutes.

From 10 seconds to 1 minute after successful transfer of a microspermatophore males everted their genitalia (which were always retracted between spermatophores) and produced a second, much larger macrospermatophore (Fig. 5). Structurally, this spermatophore differed from the microspermatophore in two important respects: the ampulla was about five times the volume of the microspermatophore, and the spermatophore tube was surrounded by a bell-shaped structure (Fig. 6). This macrospermatophore was transferred in the same manner as the microspermatophore, within five minutes of its first appearance beneath the male's spermatophore mold. Figure 8 shows a female with macrospermatophore attached. Often the female attempted to remove the macrospermatophore immediately (the same way she had done with the microspermatophore), but she was usually unsuccessful. This was because the bell-shaped struc-

ture surrounding the tube of the macrospermatophore effectively prevented her from catching the ampulla in the notch between her hind tibial spurs. The bell-shaped structure at its widest end (toward the ampulla) was nearly the same diameter as the ampulla. This meant there was effectively no sudden widening at the ampulla to catch in her tibial spur notch, and her method for removal was relatively ineffective. As many as 11 unsuccessful attempts by females to remove macrospermatophores were observed (over a period of 8-15 minutes) before the final successful removal. Removal of the macrospermatophore usually required the simultaneous use of both hind tibiae.

After successful transfer of a macrospermatophore, males resumed courtship. If the female remained attentive, or at least did not wander off, the male produced another microspermatophore. In all cases I observed, at least 15 minutes passed between the transfer of a macrospermatophore and the production of a second microspermatophore. If this microspermatophore was transferred successfully, the male quickly produced another macrospermatophore. On one occasion I observed the transfer of six spermatophores (including 3 micro- and 3 macrospermatophores) in a single mating bout which lasted 2-1/2 hours. In all my observations, if a microspermatophore was not transferred successfully the male did not produce a macrospermatophore. Instead, he removed the microspermatophore himself and consumed it. If the female became attentive again, the male would often produce another microspermatophore. Macrospermatophores were produced only following the successful transfer of a microspermatophore.

The difference in morphology between the micro- and macrospermatophores appeared to be the result of how much material was extruded by the accessory glands of the male during the formation of the spermatophore. Unlike the oecanthines and some other cricket groups, but similar to the nemobiines, in *Phyllopalpus* only the spermatophore tube was formed within the male's "mold" and the ampulla was extruded below the mold. The final size of the ampulla appeared to be controlled by how much material was injected into the mold and the lack of the bell-shaped structure in microspermatophores appeared to be the result of the male's "holding back" of accessory gland secretions so that the mold was incompletely filled.

There was another important difference between micro- and macrospermatophores. Microspermatophores did not contain (or dispense) sperm. Sperm counts performed on four successive spermatophores from one male, plus one from another male (a total of three micro- and two macrospermatophores) revealed no sperm in the microspermatophores, but 35,330 ($\pm 1,145$ S.E.) for the first macrospermatophore and 12,013 (± 851) for the second.

Females were never observed to successfully remove a macrospermatophore on the first try ($n=7$), whereas microspermatophores were always successfully removed on the female's first attempt ($n=5$). Two of the macrospermatophores, which I removed from the female seconds after their transfer and placed in normal saline, were observed to discharge sperm for between 4 and 5 minutes. It seemed therefore that five minutes in the female may be adequate for complete transfer of sperm. Females removed the microspermatophores 0.70 \pm 0.63 minutes after transfer ($n=5$), compared with 10.13 \pm 2.85 minutes ($n=4$) for macrospermatophores. Spermatophores apparently had a short period of usability and could not be resorbed or stored by males. If males were unable to transfer either micro- or macrospermatophores in a timely manner they removed them (using the hind tibial spurs in a manner similar to females) and ate them.



Fig. 8. Female with attached macrospermatophore.

Discussion

Dimorphism in spermatophores is found in at least some other trigonidiines [*Anaxipha* spp. in North America (Walker & Funk 2014) and *Laupala* and *Prolaupala* in Hawaii (deCarvalho & Shaw 2005, 2010b)]. A similar pattern is apparent in at least one nemobiine (*Nemobius sylvestris*; Prokop & Maxwell 2008, 2011). In other nemobiines males produce no spermatophore at all for the initial copulation (*Allonemobius*, *Neonemobius*, *Eunemobius*; Mays 1971 and D.H. Funk unpublished).

Although deCarvalho and Shaw (2010a) provided some evidence that transfer of microspermatophores enhances subsequent sperm transfer from the macrospermatophore, in all the cases cited above initial copulation without sperm transfer probably functions as a test of the female's receptivity before a male produces a fully functional spermatophore that must be used quickly and cannot be stored. Once a functional spermatophore is transferred, all male crickets face the challenge of preventing premature removal and consumption by the female. A variety of behaviors, structures and nuptial gifts have evolved in this context (Funk 1989). In the case of *Phyllopalpus*, the bell-shaped structure around the sperm tube on the macrospermatophore serves to thwart the females' attempts at early removal.

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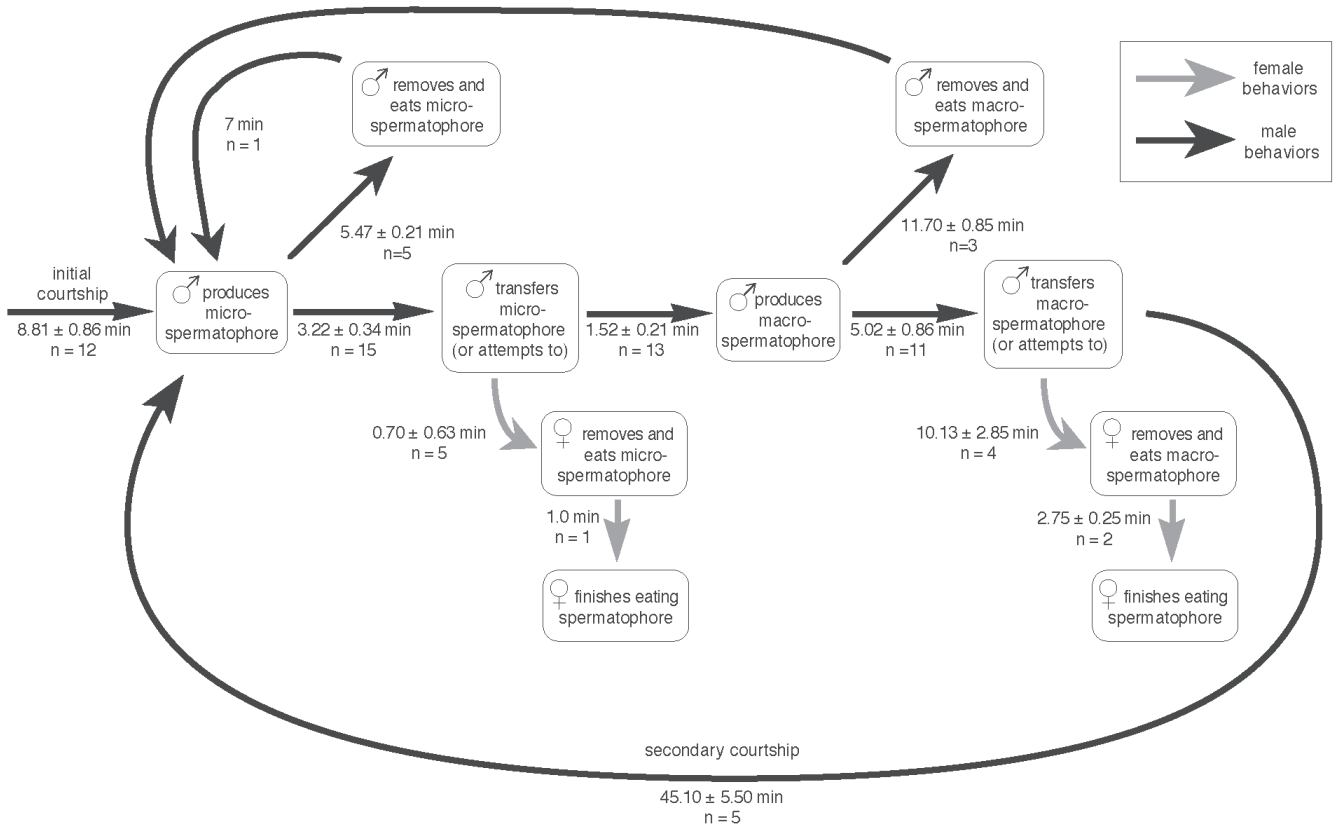


Fig. 9. Flow chart of behaviors observed during 14 pairings between virgin crickets. Times shown (means ±S.E, n) include only those instances for which an accurate estimate was recorded.