

Introduction

Phyllopalpus pulchellus Uhler (Trigonidiinae) is a distinctive cricket found throughout much of the eastern U.S. (<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. Their 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". *Phyllopalpus pulchellus* is strikingly colored (Fig. 1 and 2) and primarily diurnal, and yet difficult to see or collect because of its extremely 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 meters above ground level. But on cool (less than 15°C), sunny mornings in September and October in



Figure 1. Male *Phyllopalpus pulchellus*.

southeastern Pennsylvania both males and females can be readily collected while sunning themselves on the upper surfaces of foliage. The mating behavior of *Phyllopalpus pulchellus* has 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 which had been caged individually for some time prior to the test. Observations were made in 4 inch diameter plexiglass tubes covered with plastic petri dish lids during the daylight hours, usually around 9 to 11 A.M. Crickets were able to walk about freely on the smooth plastic surfaces, and the plexiglass tubes allowed me to observe details of spermatophore production and

transfer from a ventral view with a hand-held Wild M3 stereo microscope (sans base and post). For enumeration of sperm, spermatophores were collected and placed in ~100 uL saline (0.15M NaCl) where they were allowed to empty themselves, after which an equal volume of 5%



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

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 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 gave very even dispersal over the entire filter. Ten fields (area = 1.072 mm²) at 160X 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 x 11 inch sheet of white paper where accurate counts could be made by checking off each sperm nucleus as it was counted.

Results and Discussion

Male *Phyllopalpus* sing when calling for mates, or in the presence of a another member of their species of either sex. Courtship songs seem softer and less melodic than calling songs, but attempts to quantify these differences were not made. Males caged together

sing almost continuously and frequently tremulate (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 seems to be a territorial display, but only once have I seen actual physical aggression between males; two males courting the same female briefly



Figure 3. Male singing from between leaves



Figure 4. Male singing and female approaching from behind.

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 keep their tegminae raised almost continuously, but sing intermittently. During courtship, males face directly away from their potential mate, and (especially during the early stages of courtship) tremulate frequently. Tremulation by males is accomplished by rocking the body forward and backward vigorously while maintaining a firm foothold on the substrate. Attentive females (i.e., those that do not walk



Figure 5. Extruded macrospermatophore in male's genitalia.

away) remain about one antenna-length behind the male, maintaining contact with the tip of one antenna touching the males' tergites (Fig. 4). Sometimes a female will approach the male and mouth one of his hind tibia, but males discourage this by moving away slightly. Occasionally males turn around, apparently to check the female's location. This usually occurs after the female has wandered off. Both the male and female antennate continuously and palpate the substrate. After 5 to 10 minutes of uninterrupted courtship, the male extrudes his genitalia and produces a spermatophore (Fig. 5).

Two distinctly different types of spermatophores are produced by *Phyllopalpus* males, differing in both the size of the ampulla and in the structure of the spermatophore tube (Fig. 7). One type, the

microspermatophore, is always produced first. The microspermatophore usually appears about five minutes after a female becomes attentive. The male first extrudes his genitalia and within seconds a spermatophore ampulla becomes visible beneath them. In two to three minutes the male suddenly drops his tegmina and attempts to back under the female (Fig 6). Receptive females raise their bodies and allow the male to back under them and transfer the spermatophore. Transfer of the spermatophore is very brief—usually taking only a second or two, after which the male immediately crawls back out and resumes courtship (singing, tremulating, and palpating the substrate) in the same position as he did before the transfer.

Females then remove the microspermatophore, usually within seconds of its transfer, always within three minutes.



Figure 6. Male singing with attentive female moments before the male backed under her and transferred a microspermatophore.

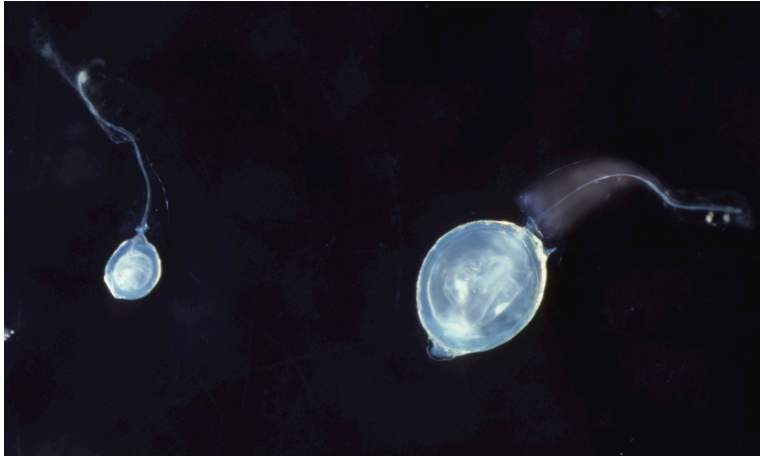


Figure 7. Microspermatophore and macrospermatophore.

Phyllopalpus females are apparently unable to reach the spermatophore directly with their mouthparts the way some other crickets (such as the oecanthines) do. Instead, they use the hind tibial spurs to “pluck” it out. This is 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 is run along the sperm tube until the bulbous ampulla portion of the spermatophore is reached. The ampulla becomes lodged in the notch and the spermatophore is then plucked out, after which the female removes it from her tibia with her mouthparts and eats it. The microspermatophore is usually completely consumed within two minutes.

About 10 seconds to 1 minute after successful transfer of a microspermatophore males evert their genitalia (which are always retracted between

spermatophores) and produce a second, much larger macrospermatophore (Fig. 5). Structurally, this spermatophore differs from the microspermatophore in two important respects: the ampulla is about five times the volume of the microspermatophore, and the spermatophore tube is different (Fig. 7). The macrospermatophore is transferred in

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Figure 8. Female with attached macrospermatophore.

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 attempts to remove the macrospermatophore immediately (the same way she did the microspermatophore), but she is usually unsuccessful. This is because the bell-shaped structure surrounding the tube of the macrospermatophore effectively prevents her from catching the ampulla in the notch between her hind tibial spurs. The bell-shaped structure at its widest end (toward the ampulla) is nearly the same diameter as the ampulla. This means there is effectively no

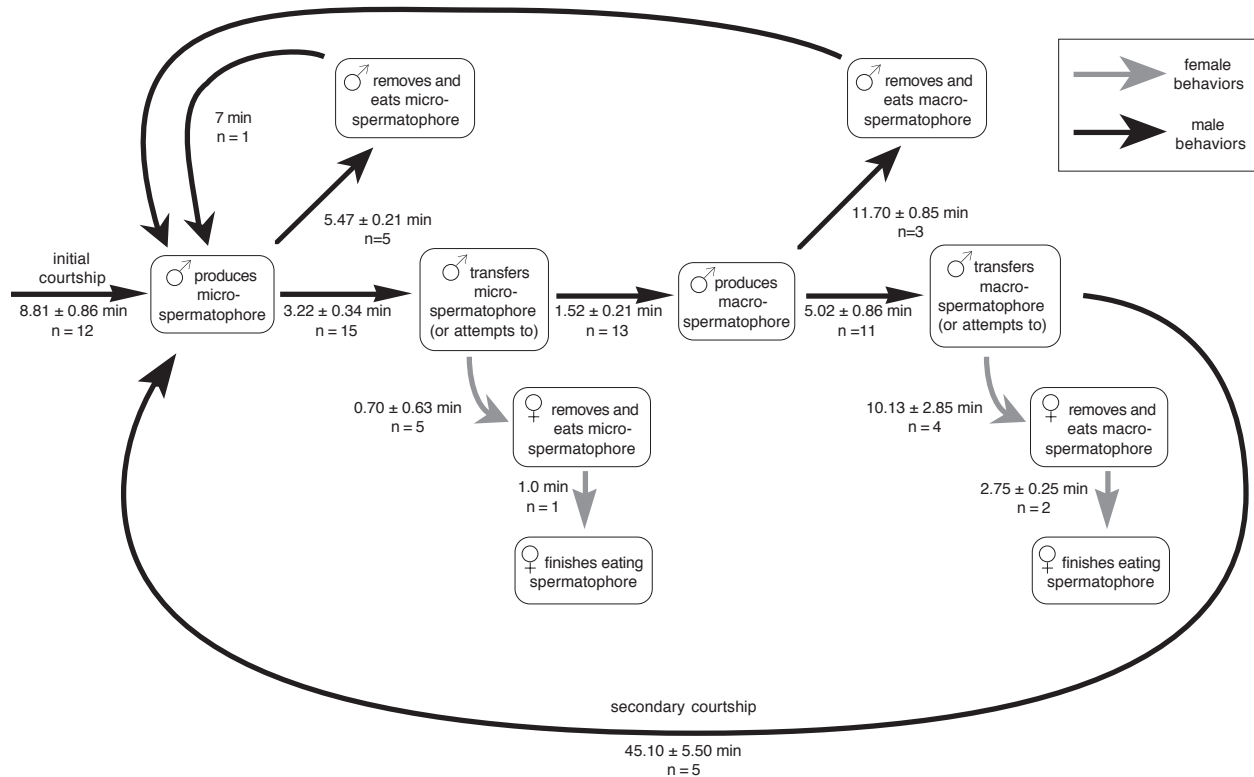


Figure 9. Flow chart of mating behaviors. Times shown ± S.E.

sudden widening at the ampulla to catch in her tibial spur notch, and her method for removal is relatively ineffective. I have observed as many as 11 unsuccessful attempts by females to remove macrospermatophores (over a period of 8-15 minutes) before the final successful removal. Removal of the macrospermatophore usually requires the simultaneous use of both hind tibia.

After successful transfer of a macrospermatophore, males resume courtship. If the female remains attentive, or at least does not wander off, the male will produce another microspermatophore. In all cases I observed at least 15 minutes passed between the transfer of an macrospermatophore and the production of a second microspermat-

ophore. If this microspermatophore is transferred successfully, the male quickly produces another macrospermatophore. On one occasion I observed the transfer of six spermatophores (including 3 micro- and 3 macro-spermatophores) in a single mating bout which lasted 2-1/2 hours. In all my observations, if a microspermatophore is not transferred successfully the male will not produce a macrospermatophore. Instead, he removes the microspermatophore himself and consumes it. If the female becomes attentive again, the male will often produce another microspermatophore. Macrospermatophores are produced only following the successful transfer of a microspermatophore.

The difference in morphology between the micro- and macro-spermatophores appears to be the result of how much material is 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 spermatopore tube is formed within the male's "mold" and the ampulla is extruded below the mold. The final size of the ampulla appears to be controlled by how much material is injected into it and the lack of the bell-shaped structure in microspermatophores appears to be the result of the male's "holding back" of accessory gland secretions so that the mold is incompletely filled.

There is another important difference between micro- and macro-spermatophores. Microspermatophores do 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 macro-spermatophores) 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.

I have never seen a female 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 seems therefore that five minutes in the female may be adequate for complete transfer of sperm. Females removed the microspermatophores 0.70 ± 0.63 minutes after transfer ($n=5$), compared with 10.13 ± 2.85 minutes ($n=4$) for macrospermatophores. Spermatophores apparently have a short period of usability and cannot be resorbed or stored by males. If males are unable to transfer either micro- or macro-spermatophores in a timely manner they remove them (using the hind tibial spurs the same way females do) and eat them.

Dimorphism in spermatophores is found in at least some other trigonidiines (*Anaxipha* spp. in North America [personal observation] and *Laupala* in Hawaii [deCarvalho and

Shaw 2005]). A similar pattern is apparent in at least one nemobiine (*Nemobius sylvestris*; Prokop and Maxwell 2008, 2011). In other nemobiines males produce no spermatophore at all for the initial copulation (*Allonemobius*, *Neonemobius*, *Eunemobius*; Mays 1971 and DHFunk unpublished). In all these cases, 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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