Morphological Characteristics of Mating in Tarnished Plant Bug (Heteroptera: Miridae)[1]

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Abstract Tarnished plant bugs (Heteroptera: Miridae) were dissected and photographed to show the morphological characteristics of mated and unmated individuals. The presence of what appears to be a spermatophore, and/or the enlargement of the genital pouch and the seminal depository through infusion of fluids from the male was evidence of females having mated. Loss of relative size and white material from the accessory glands of males held from females for 7 d prior to mating was evidence of males having mated. Decreased size of seminal vesicles resulting from insemination was not seen to be an indicator of males having mated. The photographs provide a simple method for determining mating, and when used in conjunction with drawings of tarnished plant bug morphology provided by early authors, may facilitate the understanding of other aspects of tarnished plant bug reproductive morphology.

Key Words Miridae, mating, spermatophore, genital chamber, common oviduct, genital pouch, dorsal sack, accessory gland, seminal vesicle

The morphology of the reproductive system of female tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), and other Miridae was described by Davis (1955). He placed special emphasis on the structure of the common oviduct or genital chamber, which includes the dorsal sack, or genital pouch (Strong et al. 1970), and the seminal depository. Davis's (1955) drawings do not depict the dorsal sack, which he describes as a pouch-like structure attached to the roof of the genital chamber, but this structure appears to be outlined in a drawing of *Plagiognathus albatus* (Van Duzee). The Davis (1955) drawings are of unmated insects.

The development and morphology of the reproductive systems of male and female *L. hesperus* Knight were depicted by Strong et al. (1970). Their drawings show an unmated female and a mated female with expanded seminal depository and inflated dorsal sack which they refer to as the genital pouch, the term used in the current paper. The drawing of the male reproductive tract appears to be that of an unmated male.

The current study was conducted to simplify, by photographic depiction, the determination of whether a particular *L. lineolaris* is mated or unmated. The photographs complement the descriptions and illustrations of Davis (1955) and Strong et al. (1970). Use of the photographs should make it easier for those unfamiliar with the

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reproductive tracts of *L. lineolaris* and *L. hesperus* to quickly determine their mating status for field or laboratory studies.

Photographs of the seminal depositories and genital pouches of mated and unmated *L. lineolaris* females are shown. Additionally pictured is a structure contained within the seminal depository, not adequately described by either Davis (1955) or Strong et al. (1970), but apparently referred to by Strong et al. (1970) as the "center region." Blackmer et al. (2004) used the term "spermatophore" for this structure, observed in both *L. hesperus* and *L. lineolaris*, and that is the term we have found to be more suitable here. Photographs of the accessory glands and seminal vesicles of mated and unmated males also are provided here.

Materials and Methods

The tarnished plant bug colony used in this study originated from insects collected in weed fields near Starkville, MS, in 2005. They were reared in Rubbermaid® (Rubbermaid Home Products Division, Fairlawn, OH USA) 7.8 L Servin' Saver rectangular containers with organdy cloth tops during the early nymphal stages and mosquito netting from 5th instar through adulthood. Shredded copier paper was hand wrinkled and added to the rearing container to reduce opportunities for cannibalism by keeping the bugs separated. Packets of Parafilm®-sealed (American National Can, Chicago, IL) artificial diet and 4% carrageenan gel (Cohen 2000) were placed on the screen-mesh tops for feeding and oviposition, respectively. The packets were replaced on alternate days. The rearing room was maintained at a 14:10 (L:D) photoperiod, ≈28°C temperature, and unregulated humidity.

Test insects were narcotized with CO_2 and sexed as virgins. Males and females were kept in separate containers for 7-10 days before being paired in plastic Petri dishes (60 × 15 mm). Pairs were fed green bean, *Phaseolus vulgaris* L., pods and held in temperature cabinets (14:10 L:D; 25°C) for 24 h prior to dissection. Bugs were again narcotized with CO_2 and pinned through the thorax, ventral side up, in a 60 (diam.) by 11 (ht) mm glass Petri dish half-filled with lampblack impregnated beeswax over which enough phosphate-buffered saline (Wiygul et al. 1982) to cover the bugs was added. Partners were dissected side by side to ensure that a mated or unmated pair was being observed. A female was classified as mated if accumulations of white material were present in the seminal depository and genital pouch (Strong et al. 1970). A male was classified as mated if his partner was classified as mated.

Insects were photographed in saline through a Zeiss Stemi 2000-C microscope (Carl Zeiss Light Microscopy, P.O.B. 40 41, 37,030 Göttingen, Germany) equipped with a JVC KY-F55B color video camera (JVC, 1700 Valley Road, Wayne, NJ) and the AcQuis digital imaging system by Syncroscopy (SYNCROSCOPY USA; 5,108 Pegasus Court, Suite M; Frederick, MD). Photo quality was enhanced using Adobe® Photoshop® CS2 (Adobe Systems, Inc. 345 Park Avenue, San Jose, CA).

Results and Discussion

To show its relative size and location, the seminal depository of an unmated female is shown attached to the posterior end of the insect from which it was dissected (Fig. 1A). Most of the fat body obscuring the organ was removed. Following mating, the seminal depository becomes greatly enlarged and a bulbous Y-shaped bladder inside of it becomes clearly visible (Fig. 1B, upper arrow). Neither Davis

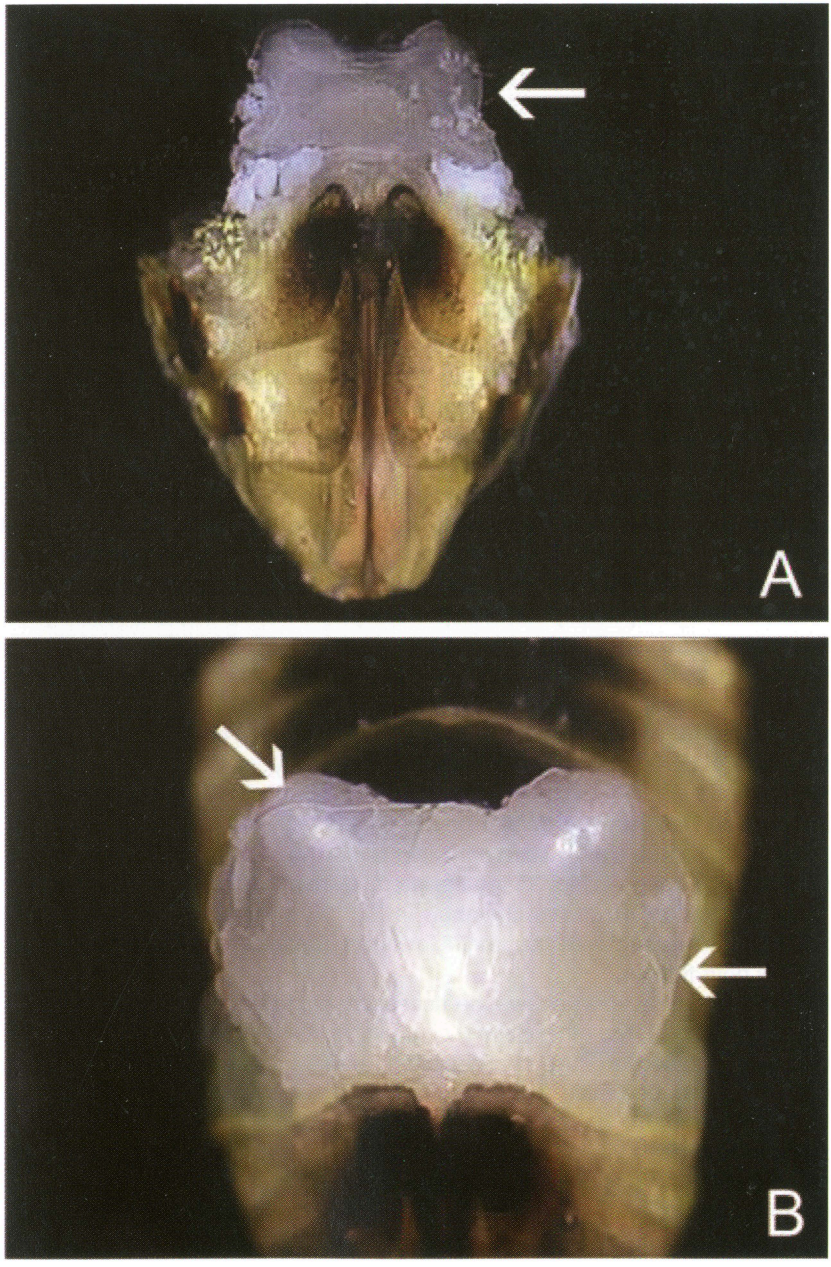

Fig. 1. Ventral views of seminal depositories of an unmated female tarnished plant bug (A), and a recently mated female (B). Upper arrow (B) indicates center region or spermatophore; lower arrow (B) indicates lateral portion.

(1955) nor Strong et al. (1970) depicted this structure, although the latter authors, citing Davis (1955), wrote that the "seminal depository is composed of two portions, a center region and a lateral portion." The center region apparently is the Y-shaped bladder in Fig. 1B (arrow), and Strong et al. (1970) indicated that immediately after mating, motile sperm are found there. The lateral portion of the seminal depository appears to be that portion containing the center region (Fig. 1B, lower arrow). Blackmer et al. (2004) also found sperm in the center region of *L. hesperus*, but they used the term "spematophore" instead of center region to describe "the paired structures that contained spermatozoa and were found inside the seminal depository." Blackmer et al. (2004) found multiple spermatophores in *L. hesperus* females, 5x more than in *L. lineolaris* females (avg. 2.16, 0.41, respectively). EJV (pers. observ.) found up to 6 spermatophores in females of a laboratory colony of *L. hesperus* from Stoneville, MS, with most females containing more than one spermatophore. Thus, the center region referred to by Strong et al. (1970) appears to be a spermatophore, as suggested by Blackmer et al. (2004), and multiple spermatophores can be found in *L. hesperus*. In the *L. lineolaris* from which specimens for the current study were taken and in hundreds more dissected during other studies (Villavaso and Snodgrass 2004, 2005), rarely was more than 1 spermatophore found inside a female, suggesting that *L. lineolaris* may tend to avoid multiple matings. The spermatophores of the two species, whereas similarly constructed, are easily differentiable (EJV, pers. observ.).

A dorsal view of a partially inflated spermatophore from a different recently mated *L. lineolaris* is seen in Fig. 2A (upper arrow) along with the inflated, chalky-white genital pouch (lower arrow). Strong et al. (1970) concluded that the genital pouch contains fluid "similar to that found only in the medial pair of male accessory glands." The medial pair of accessory glands of an unmated male may contain large amounts of this white material (Fig. 4A, left arrow). The partially inflated spermatophore dissected from the seminal depository shown in Fig. 2A is shown in Fig. 2B.

In unmated females, the genital pouch (Fig. 3A, top arrow; dorsal view) is not prominent and can be clearly seen only after removing clumps of fat body which tend to obscure it. Also seen in Fig. 3A are the lateral oviducts (bottom arrow) as they enter the common oviduct or genital chamber. In Fig. 3B a fluid-filled genital pouch (top arrow) is shown along with the lateral oviducts (bottom arrow). Mature eggs are present in both figures.

Figure 4A (ventral view) shows the accessory glands of two 9-d-old males - one unmated (left photo), the other paired with a virgin female for the 24-h period immediately before dissection. The chalky-white material seen in the median accessory glands of the unmated male (left arrow) is mostly absent from those of the recently mated male (right arrow). When a male has been held apart from females for $\geq$7d, and then paired with a virgin female for 24 h before dissection, depletion of accessory gland material is an accurate way to judge whether the male has mated. In a prior study, 97% of males that inseminated females had depleted accessory glands, and only 4% of males that had not inseminated females had depleted accessory glands (Villavaso 2007). In contrast, depletion of seminal vesicle material cannot be readily used to determine whether a male has mated or not. In general, mating did not cause the seminal vesicles of males held apart from females for $\geq$7d to lose enough material to easily distinguish size differences under a dissecting microscope (Fig. 4B; mated male, right arrow; unmated male, left arrow; dorsal view). The encircling of the seminal vesicles by the accessory glands is also evident (Fig. 4B, middle and left arrows).

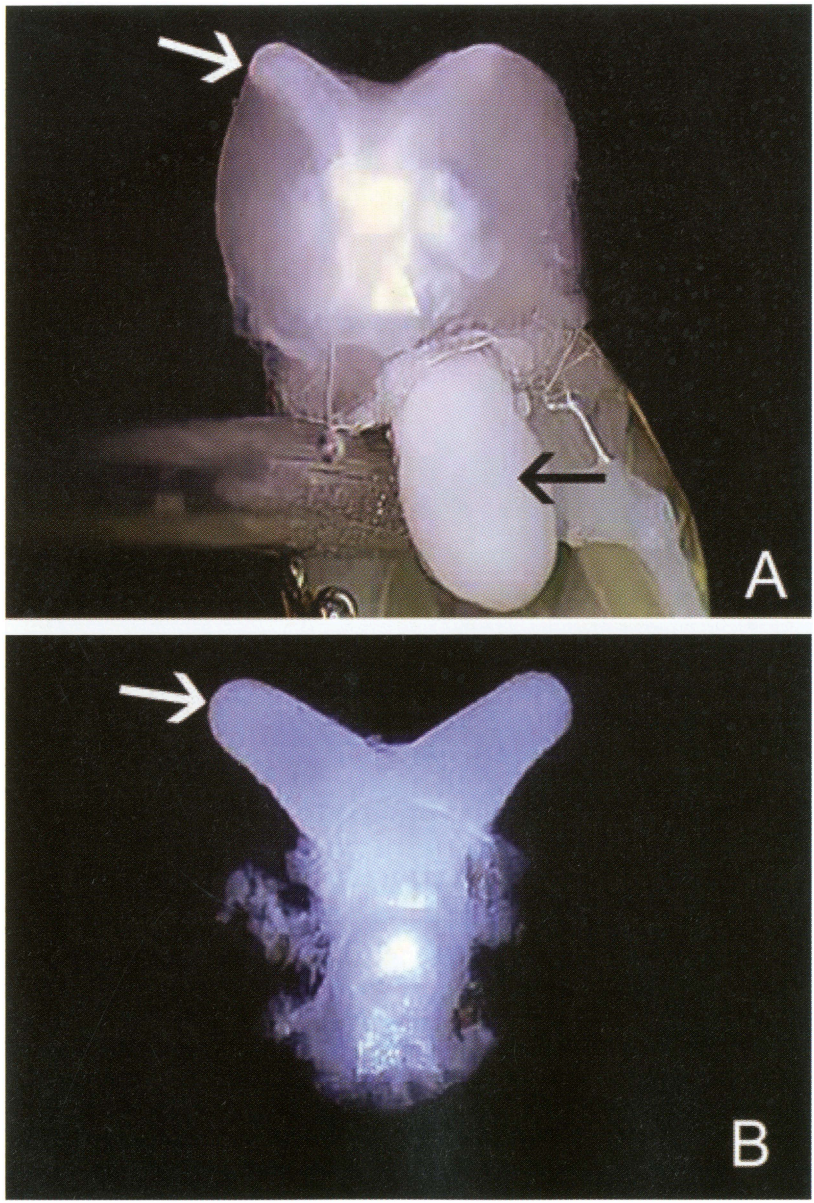

Fig. 2. (A) Dorsal view of seminal depository and genital pouch of recently mated female tarnished plant bug; upper arrow indicates center region or spermatophore, lower arrow indicates the inflated genital pouch (object left of genital pouch is an insect pin). (B) Spermatophore or center region dissected from seminal depository of mated female.

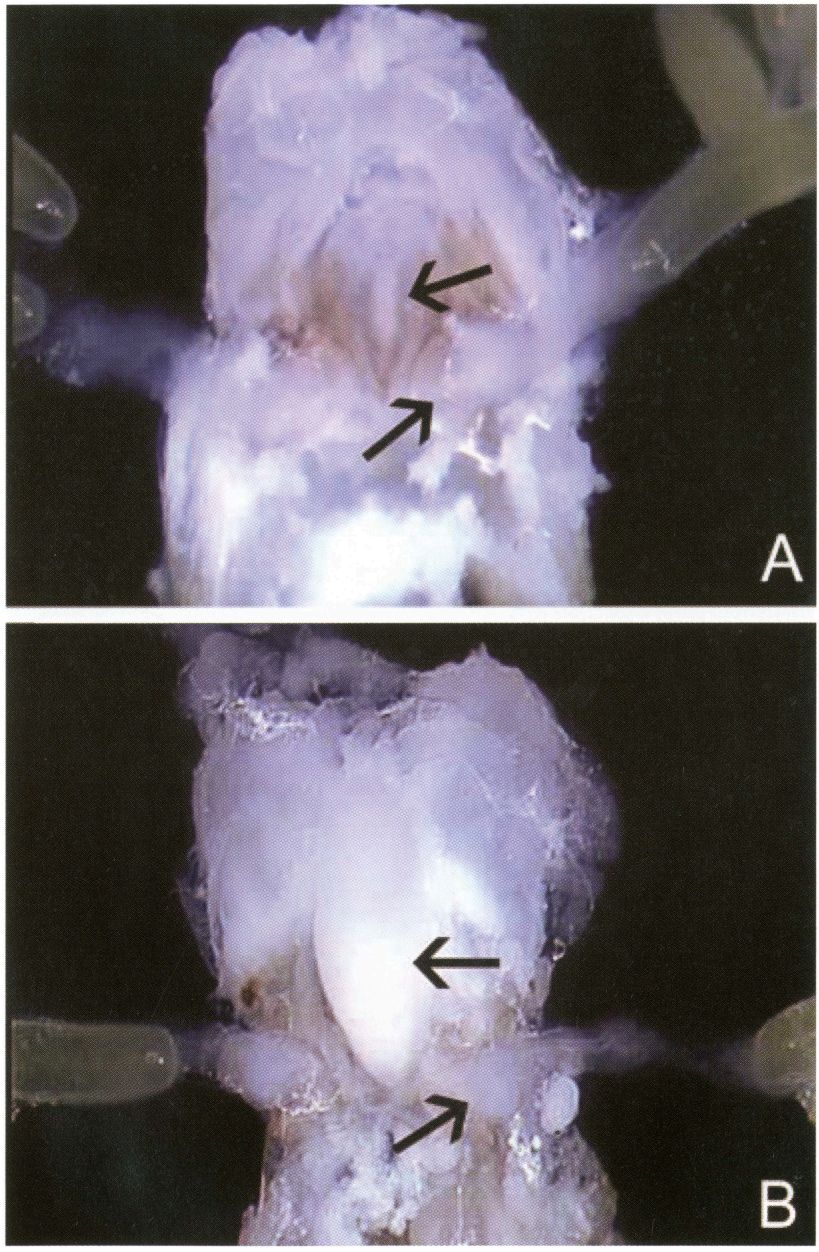

Fig. 3. Dorsal view of genital pouches of unmated (A) and recently mated (B) female tarnished plant bugs (A and B, upper arrows) and lateral oviducts entering common oviducts or genital chambers (A and B, lower arrows).

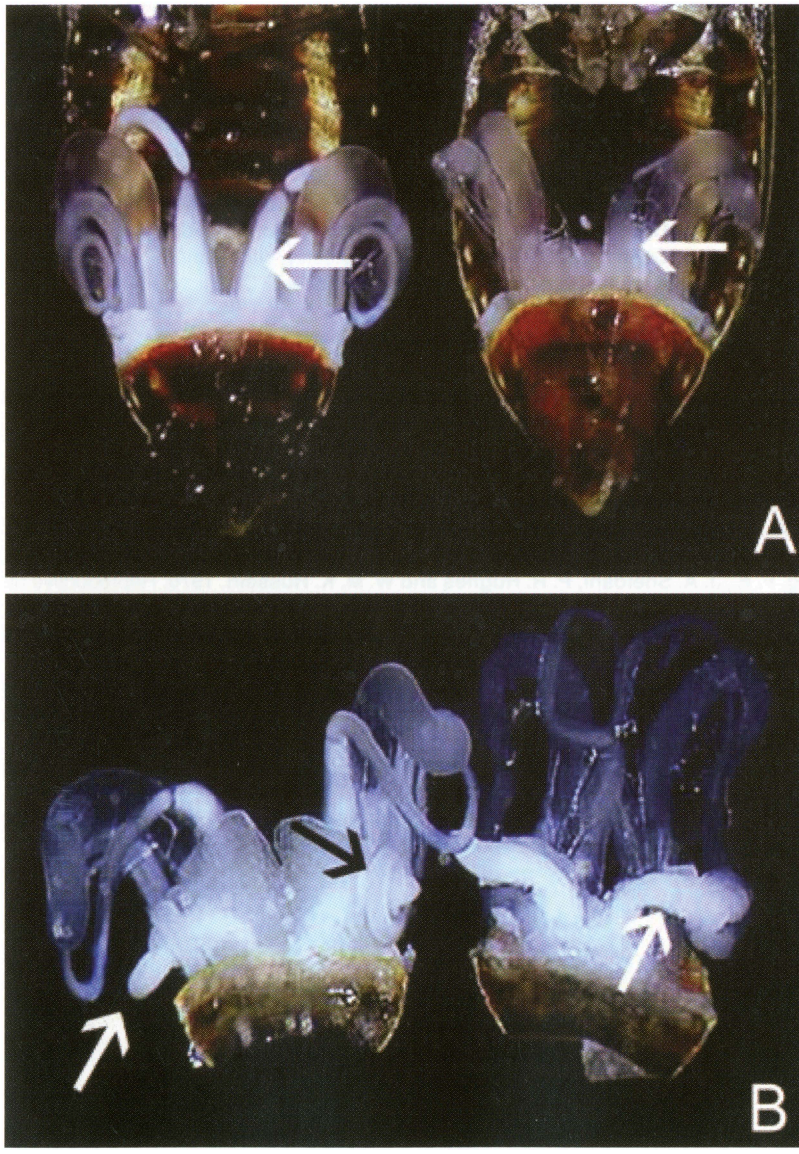

Fig. 4. (A) Ventral view of accessory glands of recently mated (right) and unmated (left) 9-d-old male tarnished plant bugs. Medial pair in unmated male contains large quantities of white fluid; medial pair in mated male is depleted of white fluid. (B) Dorsal view of seminal vesicles of recently mated (right) and unmated (left) 9-d-old male tarnished plant bugs. Middle and left arrows depict encircling of seminal vesicle by accessory gland.

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References Cited

Blackmer, J. L., S. E. Naranjo and L. H. Williams. 2004. Tethered and untethered flight by *Lygus hesperus* and *Lygus lineolaris* (Heteroptera: Miridae). Environ. Entomol. 33: 1389-1400.

Cohen, A. C. 2000. New oligidic production diet for *Lygus hesperus* Knight and *L. lineolaris* (Palisot de Beauvois). J. Entomol. Sci. 35: 301-310.

Davis, N. T. 1955. Morphology of the female organs of reproduction in Miridae (Hemiptera). Ann. Entomol. Soc. Am. 48: 132-150.

Strong, F. E., J. A. Sheldahl, P. R. Hughes and W. M. K. Hussein. 1970. Reproductive biology of *Lygus hesperus* Knight. Hilgardia 40: 105-147.

Villavaso, E. J. 2007. Attractiveness, responsiveness, mating, and flight of irradiated tarnished plant bug (Heteroptera: Miridae). J. Entomol. Sci. 42: 286-295.

Villavaso, E. J. and G. L. Snodgrass. 2004. Diapause in tarnished plant bug (Heteroptera: Miridae) reared in dynamic photoperiod environmental cabinets. J. Agric. Urban Entomol. 21: 87-97.

2005. Diapause in tarnished plant bug (Heteroptera: Miridae): Simulated seasonal occurrence in a Mississippi population. J. Entomol. Sci. 40: 10-15.

Wiygul, G., M. W. MacGown, P. P. Sikorowski and J. W. Wright. 1982. Localization of pheromone in male boll weevils. Entomol. Exp. Appl. 31: 330-331.