

Genitalic stridulation during copulation in a species of crane fly, *Tipula (Bellardina) sp. (Diptera: Tipulidae)*

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Abstract: The male genitalia of many animal groups have elaborate and species-specific forms. One hypothesis to explain why this is so is that male genitalia function as stimulatory devices that are under sexual selection by cryptic female choice. This report is based on a videotaped observation of a single male of an unidentified species of *Tipula (Bellardina)* from San José Province, Costa Rica. A male crane fly stridulated (produced vibrations) during copulation using sustained and stereotyped movements of file and scraper structures on his genitalia. Males of related species have similar file and scraper structures on their genitalia, suggesting that they probably also stridulate during copulation. *Rev. Biol. Trop.* 57 (Suppl. 1): 251-256. Epub 2009 November 30.

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The male genitalia of many animal groups have elaborate and species-specific forms. One hypothesis to explain why this is so is that male genitalia function as stimulatory devices that are under sexual selection by cryptic female choice (Eberhard 1985). One prediction made by this hypothesis is that the male genitalia of some species will be found to move during copulation in ways that are appropriate to stimulate the female. One possible type of stimulation is vibration, and the structure of the male's genitalia in two groups suggests that he stridulates (produces vibrations) during copulation, apatelandid moths (Forbes 1941, Franclemont 1973) and the vespid wasp *Chartergellus* (Richards 1978). No behavioral observations of genitalic stridulation have ever been made, however, and stridulation prior to copulation, as occurs in the pyralid moth *Syntonarcha iriastis* using the male genitalia (Gwynne & Edwards 1986), was not ruled out in these groups.

Behavior by male genitalia that could stimulate the female, mainly involving rhythmic thrusting or squeezing, occurs in a variety of animals, is widespread, and has been observed in insects, spiders, mammals, pseudoscorpions, nematodes, reptiles and mites (Eberhard 1996). The functions of some of these behavior patterns are open to alternative interpretations, however. For example thrusting might provide the male with sensory information on female readiness to receive and accept his ejaculate, or help remove or immobilize the sperm from previous males (Waage 1984, Eberhard 1996). Thus further observations of apparent stimulatory movements in which such alternative interpretations can be ruled out are of interest. This note documents genitalic stridulation by the male genitalia of the tipulid fly *Tipula (Bellardina) sp.* It describes file and scraper structures in this and related species, and presents the first direct observations of the

behavior of such a genital structure, confirming that the file and scraper are used to stridulate during copulation.

MATERIALS AND METHODS

On the relatively cool morning of 27 December 2006 a copulating pair of *T. (Bellardina)* sp. was found by one of us (WGE) at about 11 AM clinging to the outer wall of a house near San Antonio de Escazú, 1 325m, San José Province, Costa Rica, with the male hanging below the female. The female was induced to climb onto a plant stem, and the stem was placed under a dissecting microscope, where the pair's genitalia were observed. Behavior was taped at 30 frames/sec through the microscope at room temperature using a SONY DCR

TRV50 digital video camera. The flies gave little sign of being disturbed by this treatment, and their large size permitted detailed observations. "Dorsal" and "ventral" in the descriptions below are determined with respect to the male. The specimens are deposited in the collection of the Academy of Natural Sciences, Philadelphia.

RESULTS

The female's cerci were flexed sharply dorsally, and inserted through the cylinder formed by the male's curved apical lobes of the ninth sternite; the tips of the female cerci protruded free in the air beyond (Fig. 1). For about 10-15min the pair was nearly completely immobile, except for one brief 2-3second burst

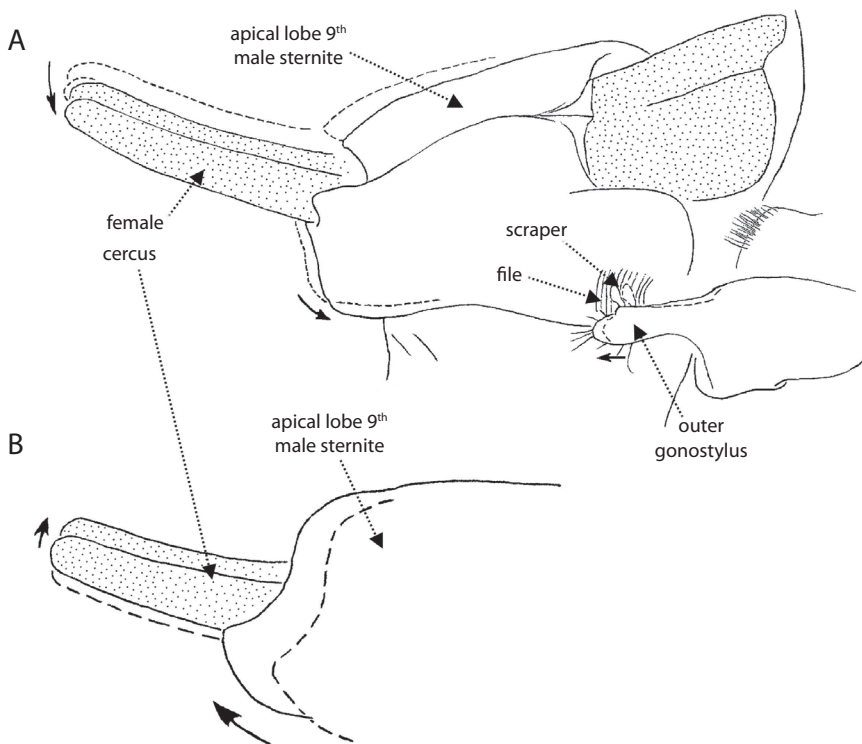


Fig. 1. Movements of the genitalia of copulating male and female of *Tipula (Bellardina)* sp. (drawn from frames of a video tape). a) Male stridulates as he holds the female cerci in the curved apical lobes of his ninth sternite, moving the scraper of his outer gonostylus and the file on the apical lobes of his ninth sternite in opposite directions (solid arrows); b) The movement (curved arrow) of the male apical lobe of the ninth sternite along the female cerci seen during the "exceptional interval" (see Fig. 3).

of rhythmic genital movements. Then, however, the male abruptly began to vibrate his genitalia in bursts, and continued to do so nearly continually for more than 10min (just over 8.5min were taped). Each vibration consisted of two movements: movements of his ninth sternite apical lobes (and thus of the female's cerci which they held) (two curved arrows at left of Fig. 1A); and a smaller, simultaneous movement of the outer gonostylus in approximately the opposite direction (arrow at right in Fig. 1A). The female cerci shifted little if at all in the grip of the male ninth sternite apical lobes during these movements.

During each movement the tip of the strong, dark-tipped, flattened extension of the outer gonostylus ("scraper" in Fig. 1A, 2B) scraped across a striated region at the lateral base of the male apical lobes of the ninth sternite ("file" in Figs. 1A, 2C). The scraper typically moved across about four ridges of the file

with each stroke (Fig. 1A). The scraper was probably in contact with the file during these movements, as the setae on the more basal portion of the outer gonostylus were sometimes deflected by contact with the file during a scraping movement.

The rate of scrapes during a single burst of vibration was on the order of 15-30cycles/sec. The rhythm of bursts of scraping and pauses between them was very regular, except during one 33.9sec burst of scraping (Fig. 3). During the regular phase, the duration of a burst averaged 2.02 ± 0.39 sec (N=83), and bursts were separated by pauses that averaged 2.91 ± 0.90 sec (N=79). During the exceptional 33.9sec interval, both the rhythm of bursts of scraping and the movements themselves changed. Stridulatory movements were nearly continuous, but an additional movement was imposed, in which the male's ninth sternite apical lobes periodically slid distally along the female cerci

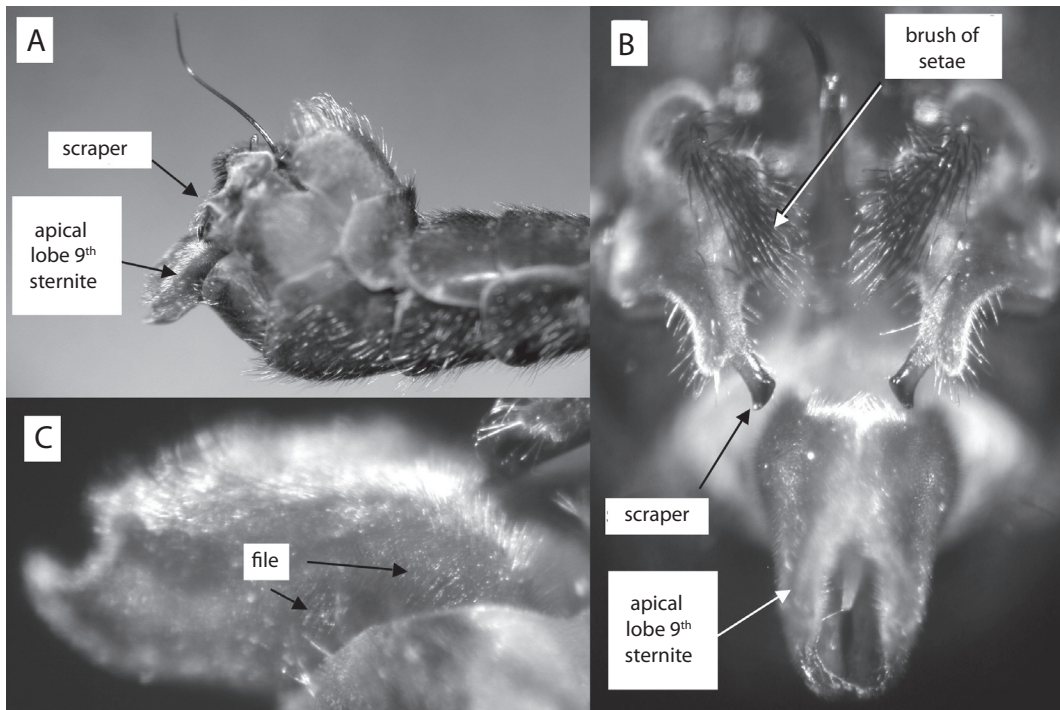


Fig. 2 Male genitalia of *Tipula (Bellardina)* sp. a) lateral view of tip of male abdomen; b) posterior view of scraper structure and the brush of stout setae on the outer gonostylus; c) lateral view of apical lobes of ninth sternite showing the ridges of the file.

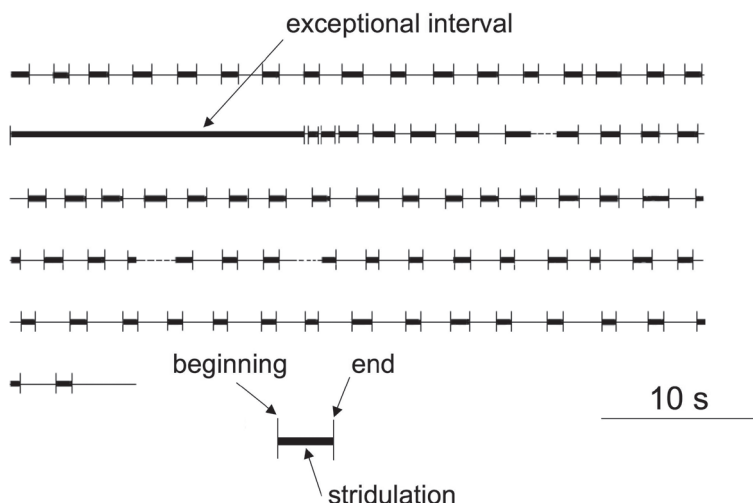


Fig. 3. Stereotyped stridulation behavior during several minutes of copulation. Dotted lines in X axis represent brief periods during which behavior was not filmed.

and also moved slightly dorsally (Fig. 1B), and then returned to the previous position. This movement was also very rhythmic, occurring on average once every 1.34 ± 0.36 sec.

The pair separated when an attempt was made to flash-freeze them with ethyl chloride spray, and they were placed in 80% ethyl alcohol. Any lingering doubt that the rhythmic movements just described were due to movements of the male rather than of the female genitalia was dispelled by the fact that the male outer gonostyli continued to execute the scraping movements in slow motion as the male expired.

Species identification: The subgenus *Tipula (Bellardina)* is an entirely New World group of 15 species distributed from the Central and Southwestern USA to the central Andes (Oosterbroek 2006); the aquatic larval stage is described in Gelhaus (1986) and biogeographic distribution is discussed by Contreras Ramos & Gelhaus (2002). Although identification to subgenus can be made by keys (Alexander & Byers 1981 for North America; Gelhaus, 2009, for Central America), the group is otherwise poorly known, with no keys or comprehensive

reviews of species, and the identity of several described species is still uncertain. Gelhaus (2009), provides a summary of knowledge for this group in Central America; there are no described species recorded for Costa Rica.

One of us (JKG) attempted to identify the species whose behavior was observed using the literature, and specimens, including types, in the Alexander Collection of the Smithsonian Institution and in The Academy of Natural Sciences. At least four species occur in Costa Rica, with none clearly matching any identified species in the material from the Smithsonian or Academy collections. Based on scraper and file morphology, three groups of species were identified:

The *T. rupicola* species group including at least *T. cydippe* (Guatemala, Venezuela), *T. fuscolimbata* (Mexico), *T. larga* (Mexico), *T. rupicola* (Arizona, USA) and two undescribed species from Costa Rica, including the Costa Rican specimens whose behavior is described here. The male genitalia of these species all show the following traits. The apical lobe of the outer gonostylus (scraper) is flat, of variable length, with a slightly expanded, darkened truncate to spatulate apex (Fig. 2b). The mesal

aspect of the outer gonostylus is expanded into a ridge near the base covered with a distinct brush of strong setae. The apical lobes of the ninth sternite show distinct ridges on their outer surfaces at the base (Fig. 2c); the number and height of the ridges is variable among the species. The species which is the subject of this paper is closest to *T. rupicola*, differing in minor details such as the lobe of the outer gonostylus.

The *T. schizomera* species group including at least *T. flinti* (Mexico), *T. praeluta* (Southwest USA), *T. pura* (Southern Rockies, USA), *T. schizomera* (Southwest USA to Mexico) and two undescribed species from Costa Rica. In the male genitalia of these species, the apical lobe of the outer gonostylus (scraper) forms a strong, black, apical spine, often separated from an equally long fleshy lobe. The mesal aspect of the outer gonostylus is not expanded into a ridge and lacks strong setae, although scattered hairs are sometimes present. The apical lobes of the ninth sternite lack distinct strong striae or ridges at the base, but have a roughened surface of fine bumps extending from midlength to near the apex, sometimes set in a depressed region.

The *T. theobromina* species group includes only this single species (distributed in the Central to Northern Andes). The male genitalia of this species are intermediate with respect to the above two groups. The apical lobe of the outer gonostylus (scraper) is flat, with a rounded, not expanded, darkened apex. The mesal aspect of the outer gonostylus is slightly expanded into a ridge, but has only scattered setae that are not concentrated in a distinct brush. The apical lobes of the ninth sternite lack distinct strong striae at the base, but show a set of very fine lines at midlength which are seen at high magnifications (400X) to be a roughened surface of fine bumps.

DISCUSSION

This is apparently the first direct observation of stridulatory behavior in male genitalia; previous examples of male genital stridulation

were based only on observations of apparent file and scraper morphology in dead specimens. Although the observation described here concerns only a single pair, the sustained and highly stereotyped movements that caused the process of the outer gonostylus to scrape repeatedly across the low, closely spaced ridges on the ninth sternite apical lobes, and the appropriate designs of these two genitalic structures for stridulation leave little doubt that these portions of the male's genitalia function to produce stridulation during copulation. It seems reasonable to conclude that the function of this behavior and of these structures is to stimulate the female, because stridulation behavior is ill-designed for any of the alternative functions that have been mentioned previously for genital movements, such as sensing the female, removing sperm, forcing open female passages, or penetrating more deeply.

An additional structure on the male genitalia of *T. (Bellardina)* sp. is the brush of robust pointed setae projecting medially from the male's outer gonostylus (Fig. 2B). These setae probably press on the female's 10th segment pleural membranous area, just basal to her cerci during copulation, and the setae may scrape against the female during male stridulatory movements. These setae may thus constitute a second structure that produces copulatory stimulation of the female.

These behaviors are likely to occur in a number of other species related to the one studied here (see previous Identification section). The flat truncate scraper, strongly ridged file, and distinct brush of medially-directed setae all occur in at least five other species of *T. (Bellardina)*. In addition, in six other species of *T. (Bellardina)*, there is a spine-like "scraper" structure, and there is no "file" structure but instead a roughened or bumpy surface at midlength of the ninth sternite apical lobes, indicating either a lack of stridulation, or possibly a different mechanism. These species also lack a brush of setae on the mesal aspect of the outer gonostylus.

The evidence that favors a stridulation function for the male apical lobe of the ninth

sternite (file) and outer gonostylus (scraper) of the species we studied also constitutes evidence against two other alternative hypotheses that have been proposed to explain the rapid divergent evolution of genitalic structures, species isolation by lock and key (Shapiro & Porter 1989), and sexually antagonistic coevolution (Chapman *et al.* 2003, Arnqvist & Rowe 2005.) There is no female “lock” with which the stridulatory structures mesh; and stridulation by the male exerts no appreciable coercive force on the female.

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RESUMEN

Un macho de *Tipula (Bellardina)* sp. estriduló (produjo vibraciones) por medio de movimientos estereotipados de estructuras de sus órganos genitales durante varios minutos de la cópula. Los machos de algunas especies relacionadas poseen estructuras similares, lo cual sugiere que también estridulan durante la cópula.

Palabras clave: cortejo copulatorio, evolución órganos sexuales, selección sexual, selección críptica en hembras, Costa Rica.

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