Reproductive Behavior of *Eidmanacris corumbatai* Garcia (Orthoptera: Phalangopsidae)

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Comportamento Reprodutivo de *Eidmanacris corumbatai* Garcia (Orthoptera: Phalangopsidae)

RESUMO - Em *Eidmanacris corumbatai* Garcia, os machos adultos apresentam tegminas reduzidas e sem aparelho estridulatório e, portanto, tiveram que desenvolver outros modos de comunicação intra-específica. Durante a corte, os machos usam uma combinação de batimentos com as pernas dianteiras e ondulações das antenas, além da sinalização química através de feromônios, uma vez que as fêmeas se tornam receptivas à cópula quando os machos expõem a glândula metanotal. Essa glândula, localizada no metanoto do macho, é também uma fonte de substâncias das quais a fêmea se alimenta antes de receber o espermatóforo. Durante a cópula, a fêmea destrói o ápice da glândula metanotal para ter acesso à secreção liberada por essa estrutura.

PALAVRAS-CHAVE: Glândula metanotal, presente nupcial, grilo

ABSTRACT - Adult males of *Eidmanacris corumbatai* Garcia have reduced tegmina without stridulatory apparatus. For this reason, they developed other means of intra-specific communication. During courtship, the males use a combination of foreleg drumming and waving of the antennae, in addition to chemical signaling through pheromones. The females become receptive to copulation when the males expose their metanotal gland. This gland, located on the male metanotum, is also a source of substances on which females feed before receiving the spermatophore. During copulation, the female destroys the apex of the metanotal gland to gain access to the secretion released by this structure.

KEY WORDS: Metanotal gland, nuptial gift, cricket

In crickets, the most common strategy used to attract females for mating is to emit high frequency sounds by rubbing the stridulatory apparatus present in the forewings (tegmen). In addition to serving as calling songs, the repertoire can vary to indicate aggression, sexual recognition, hierarchy, courtship and copulation (Alexander 1962, Zefa 2000). However, some species of several families, including Phalangopsidae, have lost the stridulatory apparatus and use other strategies to communicate with conspecific females, such as the emission of low frequency vibration produced by different parts of the body (Loher & Dambach 1989, Desutter-Grandcolas 1995a) or chemical attraction through sexual pheromones (Loher & Dambach 1989). Some crickets also use substrate-borne vibration for intra specific communication (Bell 1980, Mello & Reis 1994, Virant-Doberlet & Cokl 2004).

During the reproductive period in many insect group, the female immediately accepts the male, while in others, such as Diptera, Hemiptera and Orthoptera, the female requires further stimulation that can involve an elaborate behavior or the offer of a nuptial gift (Chapman 1971). This gift is generally a source of nutrients to the female and may represent an important paternal investment (Gwynne 1984, 1988a; Simmons & Parker 1989).

There are several types of nuptial gifts among Orthoptera, including spermatophores (Simmons 1988, Gabbutt 1994) spermatophylaxes (Gwynne 1988b, Simmons 1990, Reinhold 1999), parts of forewings (Alexander & Otte 1967a) and secretion released from glandular structures of the tibia (Mays 1971, Bidochka & Snedden 1985, Fedorka & Mousseau 2002) or metanotum (Hancock 1905, Fulton 1915, Brown 1997, Ono et al. 2004).

The metanotal gland is an elaborate structure present in the Oecanthidae, Eneopteridae, Gryllidae and Phalangopsidae (Walker & Gurney 1967) and has been implicated in the reproductive behavior of some species (Walker 1978, Bell 1980, Gabbutt 1994). This gland is also used as a taxonomic character (Leroy 1964, Walker e Gurney 1967, Desutter-Grandcolas 1995b). Prado & Fontanetti (2005) compared five species of *Eidmanacris* using scanning electron microscope and confirmed the morphological importance of this gland as a specific taxonomic character.
In this work, the reproductive behavior of *E. corumbatai* was investigated and the involvement of the metanotal gland as a source of nuptial gift in this behavior was examined. The ultrastructural morphology of virgin and copulated males was also analyzed to determine how the females gain access to the secretion released from this structure.

### Materials and Methods

Nymphs of *E. corumbatai* were collected at the Fazenda São Leopoldo Mandique in Descalvado, São Paulo State, Brazil, in September and October 2003. Most of the specimens were late nymphal instars. All of the specimens collected were identified by Dr. Alejo Mesa (Departamento de Biologia, Instituto de Biociências, UNESP, Rio Claro).

Males and females were reared to adulthood in separate glass containers (25 x 30 x 50 cm) containing vermiculite, moistened filter paper, and fish food, and were maintained under 8h light:16h dark photoperiod of artificial illumination at 25°C. After emergence of the adults (about 4 weeks), virgin males and females were placed together to observe their behavior. The combinations: one male + one female, two males + two females, several males + one female, several females + one male, several males + several females, two males + several females, were made immediately before starting the experiment. The observation of each cage was recorded for 4h under red light to minimize any interference and were repeated 10 times for each combination on alternate days.

For scanning electron microscopy, the thoraxes of virgin and copulated males were dissected, fixed in Karnovsky solution (2% glutaraldehyde in 0.025M sodium cacodylate), dehydrated in a graded ethanol series and critical point dried. The samples were subsequently sputter coated with gold and observed with a JEOL scanning electron microscope.

### Results

The results described here were based on the combination of two males + several females since there was no copulation in the other combinations. After placing the crickets together, they remained motionless for about 2h before approaching each other with antennation. After sexual recognition, the males began to wave their antennae in a whipping manner. Initially, the females were unresponsive to the males and continued walking around the container, feeding, and auto-grooming. The waving of antennae was sometimes interrupted for few seconds and, in addition to this movement, the males also jerked their bodies and drummed on the bottom of the container with one of the forelegs. Aggressive behavior was seen when two males met, with both of them waving their antennae. Subsequently, one of the males raised itself from the floor and stretched the distal part of its abdomen to expose a membranous structure between the eighth and ninth tergum (detail in Fig. 1A). This behavior caused the other male to move far from the first male and remain motionless in the corner of the container. About 2h after beginning courtship, the dominant male was approached by a female that used the same antennal movement (Fig. 1B). However, this movement stopped within a few seconds and the female became indifferent to the male’s behavior. The male then turned 180° to direct his genitalia towards the female. The motionless female only showed interest in climbing on the male dorsum when the male completely raised his tegmina at an angle of about 180° to expose the metanotal gland (arrow in Fig. 1C). The female touched his dorsum with her antennae and tried to climb on the male, but the male moved away a few millimeters in a behavior that was interpreted as instigation. The female finally climbed on the male dorsum and immediately began to bite his metanotum. The female remained on the dorsum for about 5 min, which was sufficient for the male to extrude the spermatophore and insert it into the female genitalia (Fig. 1D).

Nuptial gift in *E. corumbatai* was investigated and the involvement of the metanotal gland may also release sex pheromones (Bell 1980). In *E. corumbatai*, the metanotal gland was considered an important source of nuptial gift since the female climbs over the male to reach the spermatophore and insert it into the female genitalia (Fig. 1D). Subsequently the female stopped feeding and dismounted from the male, although both remained connected by their genitalia (end to end) for about 60 min (Fig. 1E). During this time, the male jerked his body quickly. No post-mating behavior was observed.

Comparison of the external ultra-morphology of the thorax of a virgin (Fig. 2A,B) and a copulated male (Fig. 2C,D) showed in the last one that the extremity of the metanotal projections was destroyed by the female.

### Discussion

The findings of this work showed that in *E. corumbatai* the metanotal gland was an important element in stimulating the female to assume a copulatory position (female-above-male). This position is considered plesiomorphic condition and may be related to the evolution of courtship since glands and other female-attracting devices are almost universally present on the dorsal surface of male insects (Alexander & Otte 1967b).

The secretion released by this gland can be considered a nuptial gift since the female climbs over the male to reach the nuptial meal (Gwynne 1997). In insects, the nuptial gift is considered to be a sexually selected character, i.e., a character associated with mate choice and reproductive success (Stearns & Hoekstra 2003).

Mate choice by females is based on the size of the nuptial gift, with females choosing males more capable of providing larger quantities of the gift (Forrest 1991, Fedorka & Moussau 2002). As a result, the selected male has a successful copulation (Forrest 1991); a smaller gift would result in premature interruption of the copulation and, consequently, in a low level of oocyte fertilization (Sakaluk 1985).

The ability of the nuptial gift to alter the offspring fitness is controversial (Wickler 1985, Gwynne 1986, Sakaluk 1986), but it is unquestionably a form of reproductive effort. Reproductive effort has two components: “mating effort” (the effort expended in finding a member of the opposite sex in order to mate) and “paternal investment”, in which the fitness of the male’s offspring is increased (Trivers 1972, Low 1978). The nuptial gift in *E. corumbatai* may contribute both to mating effort and paternal investment, thereby increasing the reproductive success and offspring fitness.

In addition to serving as the source of a nuptial gift, the metanotal gland may also release sex pheromones (Bell 1980). In *E. corumbatai*, which has lost its stridulatory apparatus, the
Figure 1. *E. corumbatai* reproductive behavior. A: Agonistic behavior between males fighting for a female. The dominant male stretches the abdomen and exposes a membranous structure between the eighth and ninth tergite (detail). B: Waving movement of the antennae during the courtship. The receptive female with the same movement of the antennae. C: Male with its lifted tegmina exposing the metanotal gland (arrow), in a position that facilitates female climbing on its dorsum. D: Female feeding on the male metanotal gland, while the male extrudes the spermatophore and inserts it in the genital opening of the female. E: Final position of copulation: male and female connected by the genitalia (end to end).
metanotal gland not only serves as the source of a nuptial gift, but also appears to be a source of chemical signaling, as far as the female only showed interest in climbing on the male dorsum after the tegmina had been raised and this gland exposed.

In crickets, odors may serve as attractants and repellents (Otte & Cade 1976). The results suggest that *E. corumbatai* used repellent odors during aggressive behavior, when the male extruded a structure present between the final tergites, and this resulted in agonistic communication, probably mediated through odor emission.

During courtship, *E. corumbatai* males used a combination of vibrations made by waving the antennae and drumming one foreleg. Waving of the antennae may displace air that can be felt by a conspecific. Foreleg drumming is also important and may be used in the intraspecific communication (Bell 1980, Mello & Reis 1994).

Figure 2. Metanotal gland of *E. corumbatai*. A: Glandular projections on the virgin male metanotum. B: Detail showing the intact apex (arrow). C: Glandular projections on the copulated male metanotum. D: Detail showing the destroyed apex (arrow) (Bar = 25μm) (P = projection).
Scanning electron microscopy showed that the female destroyed the distal end of the glandular structure during courtship feeding. A similar event occurs in *Allonemobius socius* (Scudder) in which the female destroys the tibial spur to gain access to male hemolymph during copulation. In this species, the nuptial gift size influences female reproductive fitness (Fedorka & Mousseau 2002).

In *Oecanthus nigricornis* Walker, the metanotal gland improves mating success, but is not essential (Bell 1980). In contrast, in non-acoustical species such as *E. corumbatai*, the metanotal gland must be an essential component of mating behavior, being used both in intra-specific communication and as a nuptial gift source.

**Acknowledgments**

I am grateful to Fernando Varotti for technical assistance and suggestions given throughout all phases of this study. I also thank Dr. Edson Zefa for critical review of the manuscript, Dr. Alejo Mesa for identification of the specimens and Guilherme Galassi for assistance in collecting the specimens in the field.

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Received 09/IX/05. Accepted 17/I/05.