

**REPRODUCTIVE BEHAVIOR OF
HYPOLESTES TRINITATIS (GUNDLACH) IN CUBA
(ZYGOPTERA: MEGAPODAGRIONIDAE)**

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Received December 4, 2008 / Revised and Accepted May 18, 2009

The study was conducted at the Dos Bocas stream nr Santiago de Cuba, during Aug.-Sept. 2007 (30 d) and in May 2008 (5 d). ♂♂ are territorial, defending perching sites and returning to the same place for several days. Sperm translocation lasted about 9 s and copulation about 7.3 min. It consisted of 2 stages, recognized from the position and movements of ♂'s abdomen. Stage I took ca 6.6 min and is probably associated with sperm removal. Stage II lasted about 41.3 s, involving probably sperm transfer. Oviposition lasted for just over 1 h; the male guarding his mate during ca 30 min, whereafter he returned to his perch.

INTRODUCTION

Compared with other insects, the mechanisms of mating in Odonata have been relatively well studied but the knowledge is confined to a small number of taxa. Research on poorly studied families is needed if we are to make generalizations on the evolution of reproductive behavior within the order (CÓRDOBA-AGUILAR et al., 2003).

The family Megapodagrionidae is widespread in the tropics, but only the biology of *Heteragrion alienum* (GONZALEZ-SORIANO & VERDUGO-GARZA, 1984) and *Paraphlebia quinta* (GONZÁLEZ-SORIANO & CÓRDOBA-AGUILAR, 2003), both from Mexico, has been described. The genus *Hypolestes* is the unique representative of the family in the West Indies. It has two species: *H. clara* from Jamaica and Haiti, and *H. trinitatis* from Cuba and Hispaniola, which differ in penis morphology (WESTFALL & MAY, 1996). According to DUNKLE (1991) most *Hypolestes* collected in Hispaniola have penises different from those

of Cuba (*H. trinitatis*) and Jamaica (*H. clara*); consequently, a third taxon might in the future be recognized as either a third species or a subspecies of *H. trinitatis*. DUNKLE (1991) suggests that behavioral and molecular studies are needed to solve these taxonomical problems. The work described here is a first step in this direction.

H. trinitatis inhabits rivers and streams at the mountains of the central and eastern Cuba (ALAYO, 1968; TRAPERO-QUINTANA & NARANJO, 2003). Little is known on the biology of this genus. The aim of this paper is to describe the reproductive behavior of *H. trinitatis* in Cuba.

STUDY SITE AND METHODS

The study was carried out in a stream at Dos Bocas, 12 km N of Santiago de Cuba, at approximately 200 m a.s.l. The stream bed width varies from 5 to 10 m, with abundant stones and sand. The riparian vegetation consists on trees about 15 m high, grasses of the genus *Cyperus* L. and bushes of *Citrus sinensis* (L.) and *C. nobilis* Andrews, as well as coffee trees (*Coffea arabica* L.). Other Odonata species observed were *Protoneura capillaris* (Rambur), *Neoneura maria* (Scudder), *Enallagma coecum* (Hagen), *Telebasis dominicana* (Selys), *Scapanea frontalis* (Burmeister), *Dythemis rufinervis* (Burmeister), *Macrothemis celeno* (Selys in Sagra), *Progomphus integer* (Hagen) and *Aphylla caraiba* (Selys).

The area was visited daily from 8 August until 1 September 2007 (except days 11; 16; 17; 19; 29 and 30), and every two days from 1 to 26 September (except days 2 and 16). A transect of 355 m along the stream edge was selected for the study, and divided in 71 5-m sections. Behavioral observations were carried out from 09:00 to 15:00 h. (summer time), totalling 180 hours. During this period, the transect was surveyed twice and the types of activity (perching, copulation, oviposition), as well as the position of the insects occupied in the transect when first spotted in the day were recorded. Additionally, in May and June 2008 the reproductive behavior was recorded with a video camera.

We marked 146 *H. trinitatis* individuals, 44 females and 102 males, using a black ink waterproof marker (Staedtler Lumocolor F). Each damselfly was marked with a unique number on its right hindwing and then released at the site of capture. Data collected in 2007 and 2008 were used to determine the mean duration of pre-copulatory, copulatory and post-copulatory events.

RESULTS

MALE TERRITORIALITY

The males perch on marginal vegetation, on stones, or on dry branches overhanging the stream and always in areas with rapids. Confrontations were observed among *H. trinitatis* males, and between *H. trinitatis*, *Scapanea frontalis*, *Macrothemis celeno* and *Enallagma coecum* males. The aggressive behavior took place whenever the intruder flew in front of, or above, the male's perch, and consisted of chasing them a maximum of five meters away, and returning immediately to the same perch.

Perching site fidelity was high: 60% of males were observed at least two days in the same section of the habitat (Fig. 1). The most faithful male was observed at the same perch on each of the 20 observations between 12 August and 18 September 2007.

COPULATION

Males seized females without previous courtship as they flew near their perch, and settled on the riparian vegetation. Subsequently, sperm translocation took place, the whole process lasting 9 ± 0.14 s (mean \pm standard error) ($N = 5$).

Copulation lasted 7.3 ± 0.53 min ($N = 17$) and from the position and movements of the male's abdomen, we distinguished two stages. In stage I, the male maintains the first and second segments parallel to the female's ninth and tenth. He then flexes the third segment dorsally in the articulation with the second, while the fourth, fifth and sixth segments remain aligned with the third. The male bends the remaining segments ventrally to restrain the female by her prothorax (Fig. 2a).

In this position, the first and second abdominal segments of the male contact the three distal segments of the female and later separate to a distance of 1.0 to 1.5 mm, due to movements caused by both insects. This sequence was repeated with a frequency of 18.3 ± 0.51 min⁻¹ ($N = 8$), causing in and out movements of the penis in the vagina. This stage had a duration of 6.6 ± 0.69 min ($N = 13$). In the second stage the described movements cease and the male adopts a new position with his abdomen. He forms an obtuse angle between the third and fourth segment with the vertex directed ventrally. The movements in this stage consist of flexing the abdomen dorsally in the articulation between segments two and three to return later to the initial position. This sequence was repeated 6 to 42 times ($N = 5$) and the stage lasted 41.3 ± 6.44 s ($N = 15$) (Figs 2b, 3).

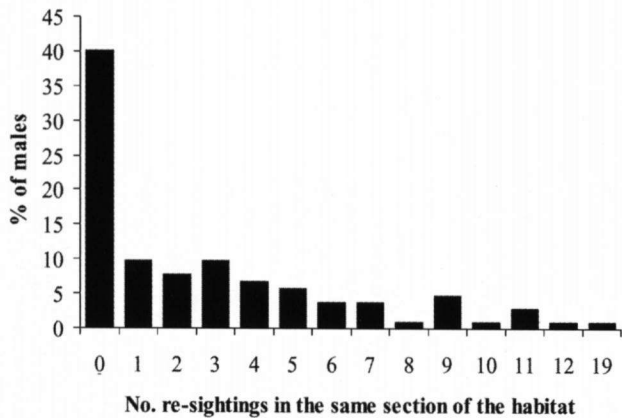


Fig. 1. Frequency of males observed in the same section of the habitat during 30 days (8 Aug.-26 Sept. 2007) at Dos Bocas stream, NE of Santiago de Cuba. The marked five-meter sections along the stream are considered as sections of the habitat. An individual re-sighted i times in the same section of the habitat was $i+1$ times observed in this section.

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OVIPOSITION

Oviposition occurred between one and five meters from the site of mating and always in areas of flowing water. The substrata used by females for oviposition

were submerged petioles and limbs of leaves of *Cyperus* sp., dry branches fallen on the water or roots of terrestrial plants that hang into the water. When the tandem settles on a stone inside the stream, the female touches different places of the surface, several times, with the terminal abdominal segments. This behavior could be assumed as a way of checking whether the substrata is appropriate for laying eggs.

Oviposition lasted 67 ± 3.95 min ($N = 4$), with the male guarding the female for 32.3 ± 3.39 min ($N = 6$), before returning to his perch. The shortest periods of guarding was observed in a male that copulated three times in one hour 45 minutes: 10.9 min, 4.7 min and 3.4 min. The last two matings occurred with females that were ovipositing at a distance less than 50 cm from the perch.

In 2007, 12 couples were detected in copula and 32 ovipositing. A 91% of copulations and 100% of ovipositions detected were observed for the first time between 11:00 and 15:00 h (Fig. 4).



Fig. 2. Position adopted by a couple of *Hypolestes trinitatis* during mating: (a) stage I, the male's abdominal segments 3 to 6 remain aligned; – (b) Stage II, the segments 3 and 4 form an obtuse angle.

DISCUSSION

COMPARISON WITH REPRODUCTIVE BEHAVIOR OF OTHER ZYGOPTERA

Territorial behavior has been described in some other Megapodagrionidae species. For example, *Paraphlebia quinta* from Los Tuxtlas, Mexico has two male morphs, one large with dark wings and territorial behavior, and the other smaller with hialine wings and no territorial behavior (GONZÁLEZ-SORIANO & CÓRDOBA-AGUILAR, 2003). Territorial males are faithful to their perching sites and defend them from all other conspecific males. Territorial behavior has also been reported in *Heteragrion alienum* and *H. albifrons* (GONZÁLEZ-SORIANO & VERDUGO-GARZA, 1984; GONZÁLEZ-SORIANO, 1997).

The males of *Hypolestes trinitatis* defend perching sites unlike some species such as *Palaemnema desiderata* (Platystictidae; GONZÁLEZ-SORIANO et al., 1982), *Cora marina* (Polythoridae; GONZÁLEZ-SORIANO & VERDUGO-GARZA, 1984) or *Perithemis mooma* (Libellulidae; DE MARCO & RESENDE, 2004) that defend the oviposition site. Apparently, in *Hypolestes trinitatis*, the territory provides an advantageous position from which males could intercept females for mating. Similar arguments have been used to explain *Heteragrion alienum* territorial behavior (GONZÁLEZ-SORIANO & VERDUGO-GARZA, 1984).

Two stages of the copulatory process have also been distinguished in *P. quinta*, although the abdominal movements are different from those of *H. trinitatis* (GONZÁLEZ-SORIANO & CÓRDOBA-AGUILAR, 2003). During the first stage, *P. quinta* males carry out a series of abdominal elevation movements (pumping movements) rather than the flexing movements described for *H. trinitatis* in this paper.

Like *H. trinitatis*, the males of *Heteragrion alienum* guard their females in tandem during oviposition, while in *P. quinta* the guarding occurs without contact (GONZÁLEZ-SORIANO & VERDUGO-GARZA, 1984; GONZÁLEZ-SORIANO & CÓRDOBA-AGUILAR, 2003).

The ability of the males to remove the sperm stored by the female from previous matings is a widespread phenomenon in Odonata (CÓRDOBA-AGUILAR et al.,

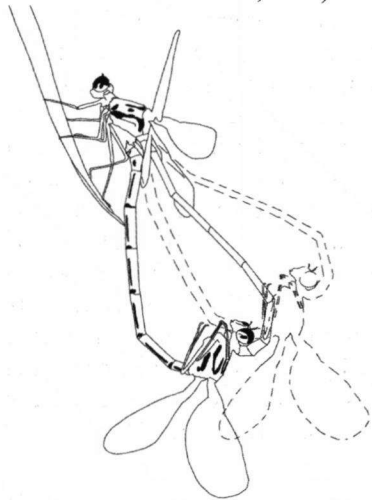


Fig. 3. Stage II in *Hypolestes trinitatis* mating. Movements in this stage consist in flexing the abdomen dorsally in articulation of segments 2 and 3 and subsequently returning to the initial position (with continuous lines). The position adopted after the flexion is represented with dashed lines.

2003). The first stage of mating is associated with sperm removal, and the remaining ones with insemination (MILLER & MILLER, 1981; CORDERO & MILLER, 1992). The kind of abdominal movements that occur during stage I of *H. trinitatis* mating and the presence of four distal projections covered with spines in the penis (unpublished data), suggest that sperm removal takes place in this species.

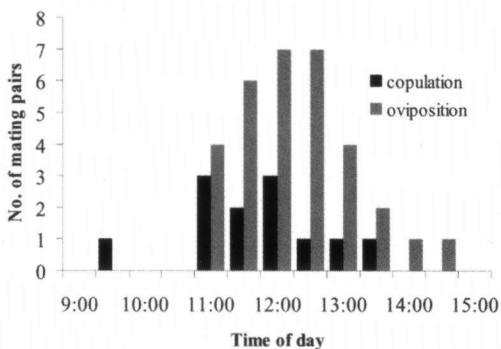


Fig. 4. Daily reproductive activity in *Hypolestes trinitatis* at Dos Bocas stream, Santiago de Cuba. Data were taken over 30 days (8 August - 26 September 2007).

POST-COPULATORY GUARDING

Post-copulatory guarding presumably prevents the females from mating with other males. Thus the last male to copulate with a given female is the one that fertilizes the eggs (FINCKE, 1984; CORDERO & MILLER, 1992). Despite the benefits, the time invested in the post-copulatory guarding limits the male to obtain new matings, and if a male is territorial, compromises his territory defense (ALCOCK, 1982, 1994). Oviposition is prolonged in *H. trinitatis* and involves contact guarding. During this process the male cannot respond to other mating opportunities unless he abandons the female. In theory, leaving a female before she finishes oviposition could be a way of minimizing the costs of guarding. Our results show the male that spent the least time in tandem with his mate was in fact the most efficient in obtaining matings (three matings in 1:45 h, guarded 10.9 min, 4.7 min and 3.4 min). However, this is a unique case and more observations are needed to confirm this hypothesis. Another hypothesis is that the female's receptivity could diminish with the advance of oviposition; once the male has ensured his paternity over some eggs, this would reduce possible loss of fitness should she remate (ALCOCK, 1992).

ACKNOWLEDGEMENTS

We thank ADOLFO CORDERO RIVERA, who revised the first version of the manuscript and provided some literature. NILIA CUELLAR ARAUJO advised us on the English grammar. We thank also RODOLFO NOVELO GUTIERREZ and JOHN A. ALLEN for their valuable comments.

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