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A DESCRIPTION OF MALE AND FEMALE GENITALIA AND A RECONSTRUCTION OF COPULATORY AND FERTILISATION EVENTS IN *CALOPTERYX HAEMORRHOIDALIS* (VANDER LINDEN) (ODONATA: CALOPTERYGIDAE)

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The genitalia have a "design" remarkably similar to those of other representatives of the family. The main ♀ structures are the bursa copulatrix, a T-shaped spermatheca, a pair of vaginal plates bearing a variable number of mechanoreceptive sensilla, and a ganglion located at the VIII abdominal segment. The ♂ intromittent organ is a curved, sclerotised aedeagus that ends in a distal penis head. This latter structure bears 2 lateral appendages which are covered by recurved spines. A construction of the fertilisation and copulatory events is proposed based on descriptive and experimental evidence in other zygopterans as well as in this species. The female genital anatomy suggests fertilisation occurs in the manner proposed for other odonates. Experimental evidence shown in this work suggests that, during fertilisation, the egg stimulates the mechanoreceptive sensilla and elicits contractile activity of the muscles that surround the sperm storage organs (SSOs). The contractile activity is likely to be mediated by the VIII abdominal segment ganglion. As a consequence of the muscular contractions, the SSOs eject sperm which arrive to the site where the egg is and fertilise it. During copulation, the aedeagus "imitates" the presence of an egg in the vaginal plates and stimulates the mechanoreceptive sensilla inducing spermathecal sperm ejection. It is likely that spermathecal sperm is ejected to the bursa copulatrix where it is removed by the penis head and lateral appendages. After this sperm displacement process, the copulating male's sperm, stored in the seminal vesicle, is transferred, through a canal-like passage, by the aedeagus to the SSOs. Since ♀♀ exhibit a considerable intra- and inter-individual variation in sensillum distribution and number on the plates, it is discussed whether this may have an adaptive significance in terms of retaining more control over stored sperm for ♀♀ during ♂ stimulation.

INTRODUCTION

Insect genitalia are highly diverse and complex (EBERHARD, 1985). The knowledge we have of their morphology and related function varies depending on the insect order. The morphology and function of the male and female genitalia in Odonata are, compared to other insect orders, well documented, in part due to the simplicity of the genitalic apparatus as well as the opportunity that this group offers for observing copulatory events.

In Zygoptera, the major female structures, in terms of copulation and fertilisation, are the vagina, the mechanoreceptive sensilla embedded in the vaginal tract, and the sperm storage organs (SSOs; generally, the bursa copulatrix and the spermatheca; e.g. MILLER, 1987; ANDREW & THEMBARE, 1996; LINDEBOOM, 1998). These structures are located in the VIII and IX abdominal segments and are involved in copulation as well as fertilisation (MILLER, 1987). The male intromittent organ consists of an aedeagus which is a sclerotised and curved structure with a distinct head attached to its distal end (e.g. SRIVASTAVA & SRIVASTAVA, 1986, 1987; ADAMS & HERMAN, 1991; LINDEBOOM, 1998).

Some aspects of the copulatory function of the genitalia of *Calopteryx haemorrhoidalis*, previously known as *C. haemorrhoidalis asturica* by OCHARAN (1983) but later corroborated as *C. haemorrhoidalis* by MAIBACH (1985, 1986) from northern Spain are relatively well known (CORDOBA-AGUILAR, 1999a, 1999b). In this species, and similar to other Calopterygidae (e.g. WAAGE, 1979), males displace both bursal and spermathecal sperm which is followed by sperm transfer (CÓRDOBA-AGUILAR, 1999a). The mechanism of spermathecal sperm displacement is based on the stimulation of the female sensory physiology: during copulation, the aedeagus stimulates the vaginal mechanoreceptive sensilla which control spermathecal sperm ejection (CÓRDOBA-AGUILAR, 1999a, 1999b). Since the male stimulatory mechanism relies on the female structures utilised for fertilisation (to induce sperm ejection, the egg also stimulates the mechanoreceptive sensilla; MILLER, 1987), it has been postulated that the male stimulatory ability has "exploited" a female sensory "bias" (CÓRDOBA-AGUILAR, 1999a). However, it has been an assumption that the fertilisation process in *C. haemorrhoidalis* takes place in the manner proposed for other zygopterans (e.g. MILLER, 1987). My aim in this study of *C. haemorrhoidalis* is twofold: (a) to describe the anatomy of male and female genitalia; and, (b) to provide a more complete view of the function of the genitalia during fertilisation and copulation in relation to the knowledge that has been gathered in this as well as other studies in Zygoptera.

METHODS

Adults of both sexes were collected from a population located in Pontevedra, SW Spain (for a detailed description of this site see CÓRDOBA-AGUILAR, 2000) in summer 1996, 1997 and 1998.

HISTOLOGICAL AND GROSS ANATOMICAL STUDY. – The VIII and IX abdominal segments of females were placed into Bouin's fixative (BANCROFT & STEVENS, 1990) overnight. Samples were then dehydrated by placing them in increasing alcohol concentrations (70, 95 and 100%) over 30-60 min periods, and then left in xylene for one hour. After this, samples were embedded in molten wax (Paraplast Plus, melting point 56°C). Transversal and longitudinal sections (10m thickness) were obtained using a rotary microtome. These were stained using haemotoxylin and eosin as described by HOOPER (1994). Scanning electron micrographs of the female vagina were obtained by preserving samples in 70% alcohol, air-dried, sputtered in gold, mounted on holders and examined under a JEOL JSM-35 scanning electron microscope (SEM).

For the gross anatomical work, male and female structures that were preserved in 70% ethanol were rehydrated, dissected and examined under a stereo microscope (Wild Heerbrugg).

OBSERVATION OF DISSECTED *IN VIVO* ANIMALS. – This was carried out to gain some knowledge of the nature of the fertilisation process. During odonate fertilisation, the egg passes in between the vaginal plates where fertilisation takes place (MILLER, 1987; SIVA-JOTHY, 1987). In *Ischnura elegans*, MILLER (1987) showed that the egg stimulates the vaginal mechanoreceptive sensilla to induce muscular contractions of the SSOs. It was investigated whether a similar mechanism might occur in *C. haemorrhoidalis*. I measured the contractile activity of the SSOs during a simulated passing of the egg from the vagina to the outside. Under a dissecting microscope, an incission was made in the 8th and 9th tergites to uncover the SSOs of 10 females that were in saline water. With this procedure, I was able to see the contractile activity of the SSOs as well as the manipulated egg. The manipulation consisted of introducing a "clean" egg (obtained from the ovary of another female), using a pair of fine scissors, from the outside through the ovipositor blades. This was produced via gentle in-and-out movements of the egg at the level of the vaginal plates. The contractile activity of the spermatheca and the bursa copulatrix was measured as the number of undulating movements per minute at two stages: (a) before the introduction of the egg; and, (b) during the egg manipulation. Since the spermatheca is a T-shaped structure (see below) and each duct of this organ seems to be under control of its corresponding vaginal plate (CÓRDOBA-AGUILAR, 1999b), the contractile activity was divided for each duct. At the end of the manipulation, the egg was inspected to look for attached sperm on its surface. Finally, and using these females, it was also investigated whether there was a relation between the number of sensilla for each vaginal plate and the contractile activity of each spermathecal duct.

Volume (in mm^3) of sperm was measured as indicated by CÓRDOBA-AGUILAR (1999b). Dimensional measurements were made with Optimas® image analysis software (version 6.1) via a CCD camera attached to a compound microscope (Leitz Diaplan). Dimensions are given in mm. Means ± standard deviations are provided.

FEMALE INTERNAL GENITALIA

Figure 1

THE BURSA COPULATRIX (hereafter bursa). – This organ is a spherical, sac-like structure connected ventrally to the vagina and, posteriorly, to another SSO, the spermathecae. The bursa is the largest organ in the anterior part of the VIII segment. In virgin females, the bursa is transparent and flattened but it is highly visible in mated females because of the sperm it contains (diameter in sperm-carrying individuals, 0.905 ± 0.045 mm, N = 16).

Histological examination of the bursa revealed a simple columnar epithelium overlying the cuticular lining of the organ. Overlying this epithelium are a series of muscle fibres.

THE SPERMATHECA. – This is a T-shaped SSO which is attached ventrally to the bursa via a narrow (diameter, 0.059 ± 0.019 mm; N = 10) duct. The spermathecae appear flattened in virgin individuals. In sperm--containing spermathecae, both ducts are highly visible and have a similar diameter (right duct, 0.056 ± 0.003; left duct, 0.057 ± 0.005 mm; N = 16, paired t-test = -1.31, P = 0.21), length (right duct, 1.1 ± 0.001; left duct, 1.1 ± 0.001 mm; same set of data, paired t-test = -0.39, P = 0.7) and sperm volume (right duct, 0.61 ± 0.08; left duct, 0.62 ± 0.08; same set of data, paired t-test = -0.26, P = 0.8).

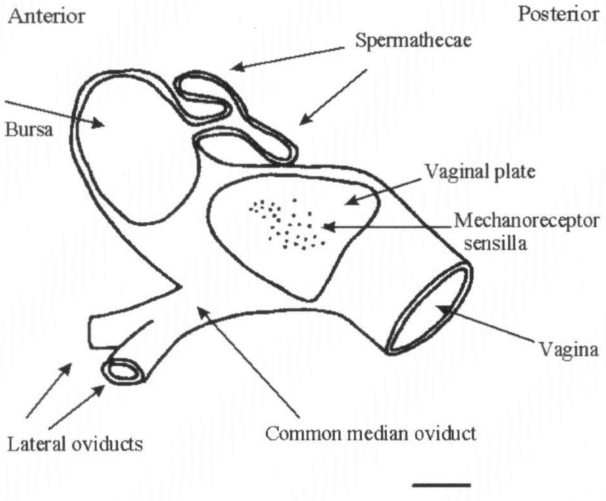
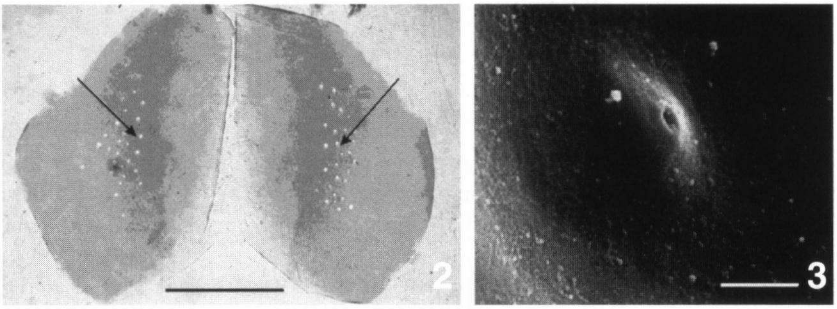


Fig. 1. Lateral stylised view of the female genitalia. [Bar = 0.5 mm].

Histological examination of the spermathecae revealed simple columnar epithelium. A series of longitudinal muscle fibres can be seen over the epithelial layer.

VAGINA. — Ventral to the bursa is the tubular vagina which is a long, cylindrical, cuticle-lined chamber that underlies the gut and runs anterior-posteriorly. Anteriorly, the vagina is connected to the common median oviduct, dorsally to the bursa, and posteriorly it opens into the gonopore, bordered by the ovipositor valves.

Embedded in the endo-cuticle lining of the vagina are two longitudinally-arranged "vaginal plates" (Fig. 2). This pair of sclerotised structures bears a number of "holes" in its constituent cuticle (Fig. 3). The form of these "holes" corresponds closely to the vaginal mechanoreceptive sensilla described by SIVA-JOTHY (1987) for other



Figs 2-3. Vaginal plates: (2) lateral vaginal plates; the clear "holes" are the mechanoreceptive sensilla. [Bars = 0.5 mm]; 1 (3) terminal surface of a vaginal plate showing the dome of the cuticle over the "holes", typical of campaniform sensilla. [Bars = 1µm].

odonates. When examined under SEM, these "holes" showed the characteristic dome-shaped cap of a campaniform sensillum. There was considerable variation in the number of "holes" (i.e. sensilla) in both plates (52.4 ± 9.9, min-max = 29-69; N = 27). Closer examination revealed four types of "hole" variation: (a) some females had more holes on the left vaginal plate (N = 16); — (b) some females had more on the right vaginal plate (N = 5); — (c) some had a symmetrical distribution (N = 6); these three types of variation differed from a random distribution (chi squared test $\chi^2 = 8.1$, d.f. = 2, $P < 0.05$); – and, (d) although not measured, there was an inter- and intra-individual (right *vs.* left vaginal plate) variation in hole distribution (e.g. some females with a scattered distribution of holes on the plates while others having an aggregated distribution).

THE VIII ABDOMINAL GANGLION. — This is dorsal to the vaginal plates. It is an oval structure which receives nerve fibres from the vaginal plates. Some nerves leave the ganglion and attach to the musculature associated to the outermost layers of the spermathecae and the bursa. These nerves can be clearly distinguished since they look black (in contrast with the white background) in dissected specimens.

MALE INTROMITTENT ORGAN

Figure 4

Two cuticular plates - the anterior lamina - cover the posterior part of the aedeagus. The aedeagus is a curved, sclerotised structure (length from the site of attachment of penis head to the site of attachment to posterior frame 1.423 ± 0.049 mm, N = 70; see Fig. 5). It is connected proximately to a flexible frame - the posterior hamule - and a glandular sac. An inflatable membrane runs ventrally to the aedeagus. Although not measured, the membrane of males interrupted during copulation appeared inflated when compared with that of solitary individuals. Distally, the aedeagus bears the penis head (Fig. 6). The penis head ends in two lateral horn-like appendages of similar size (width of the middle region of the right appendage, 0.073 ± 0.007; width of the left appendage, 0.072 ± 0.007; N = 16, paired t-test = 1.61, P = 0.13) and length (right appendage, 1.198 ± 0.011; left appendage, 1.202 ± 0.007; N = 16, paired t-test = -0.99, P = 0.34) which are covered in recurved spines.

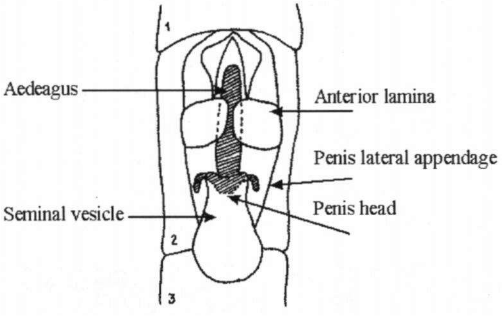


Fig. 4. Ventral plan view of the male genitalia (shadowed is the intromittent organ). Numbers refer to abdominal segments.

Anterior to the intromittent organ, on the III abdominal segment, is the seminal vesicle, a flask-like structure which stores sperm prior to ejaculation.

ACTIVITY OF THE SPERMATHECA PREVIOUS TO AND DURING EGG STIMULATION

Before egg insertion, the spermathecal ducts showed contractile activity (number per minute: right duct, 10.1 ± 10.6; left duct, 12.4 ± 11.4; N = 10 individuals) which significantly increased during egg manipulation (right duct, 60.3 ± 12.2; left duct, 59.1 ± 15.0; paired t-test using log transformed data: for right duct = -6.89, for left duct = -7.87; both $P < 0.0001$). When the inserted eggs were checked, they had a number of spermatozoa attached to their surface.

Using only those females with an asymmetrical number of sensillum (9 out of 10), the undulating movements were higher for those ducts whose corresponding vaginal plate had more sensilla (Tab. I; Fisher exact test, $P = 0.01$).

Table I
Relationship between the proportion of contractile movements for each duct and the number of sensillum on each vaginal plate in asymmetrical females (females with biased sensillum distribution)

Activity	Proportion of sensilla on each vaginal plate	
Biased contractile activity on spermathecal ducts	Left-biased	Right-biased
Left duct with more contractions	6	0
Right duct with more contractions	0	3

DISCUSSION

The genitalic anatomy described here is very similar to that of other calopterygid species (see, for example, PFAU, 1971; WAAGE, 1979; SIVA-JOTHY & TSUBAKI, 1989; ADAMS & HERMAN, 1991; LINDEBOOM, 1998). Due to the anatomical similarities with species whose copulatory and fertilisation mechanisms have been documented (e.g. *Ischnura elegans*, MILLER, 1987), I will construct a hypothetical sequence of events that is likely to occur during fertilisation and copulation in *C. haemorrhoidalis*.

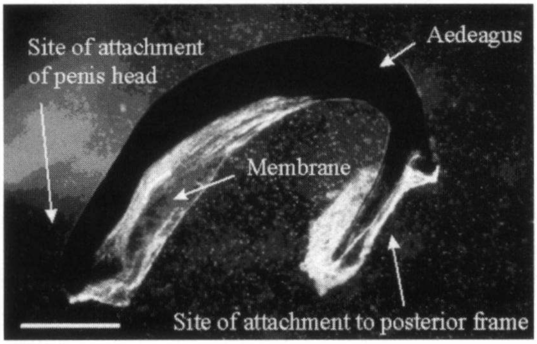


Fig. 5. Lateral view of a dissected aedeagus. [Bar = 0.5 mm].

FERTILISATION

Fertilisation takes place when the sensilla present in the vaginal plates detect an egg in the vagina (MILLER, 1987; for a similar process in Anisoptera see SIVA-JOTHY, 1987). MILLER (1987) suggested that during fertilisation the egg stimulates the campaniform sensilla present in the vaginal plates. Campaniform sensilla detect deformations of the cuticle in which they are imbedded (BARTH, 1981; Mc IVER, 1985). The sensilla would send, via the VIII abdominal ganglion, a stimulus to the muscles that cover the SSOs. These muscles would contract and release the sperm stored in the SSOs (MILLER, 1987). Both, the contractions of the SSOs and the motility of the sperm could enable sperm to reach and fertilise the egg (MILLER, 1987). The sperm penetrates the egg through the micropyle.

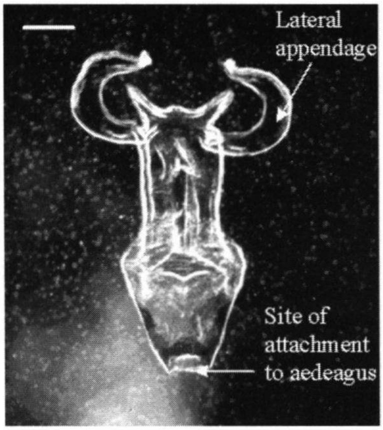


Fig. 6. Plan view of a dissected and extended penis head and its lateral appendages. [Bar = 0.5 mm].

In *C. haemorrhoidalis* females, the components involved in the process of fertilisation postulated by MILLER (1987) are also present: the vaginal plates with their "holes" (sensilla); the VIII abdominal ganglion and the muscles covering the SSOs. Moreover, a nervous connection was detected between the sensilla, the ganglion and the SSO muscles. The egg manipulation results suggest that the egg induces the contractile movements of the spermatheca. This was supported by the observation of attached sperm on the egg's surface. Therefore, according to the information provided here, there is no reason to suppose that the mechanism of fertilisation proposed by MILLER (1987) should not operate in *C. haemorrhoidalis*.

COPULATION

During copulation, two processes take place sequentially in zygopterans: the removal of rival sperm and the transfer of sperm from the copulating male to the female vagina (e.g. WAAGE, 1979; SIVA-JOTHY & TSUBAKI, 1989; LINDEBOOM, 1998).

The male aedeagus and the penis head enter in the vagina and SSOs (e.g. WAAGE, 1979; MILLER, 1987; CORDERO & MILLER, 1992). In Zygoptera, it has been suggested that the aedeagal membrane inflates as the penis head is extended into the SSOs (MILLER, 1987). Once the penis head has extended, the male's abdominal flexions help the male genitalia to move in and out of the female genital tract. These in and out movements manipulate the rival sperm present in the female's SSOs: the stored sperm are trapped on pointing spines of the penis head and are removed (WAAGE, 1979; MILLER & MILLER, 1981; MILLER, 1987; SIVA-JOTHY & TSUBAKI, 1989; CORDERO & MILLER, 1992; CORDERO et al., 1995; SAWADA, 1995; SIVA-JOTHY & HOOPER, 1995; LINDEBOOM, 1998). In *C. haemorrhoidalis*, it has been shown that the penis head and lateral appendages enter the bursa copulatrix and physically displace the sperm stored in this site (CÓRDOBA-AGUILAR, 1999a, 1999b).

Spermathecal sperm is also displaced but, unlike other calopterygids (e.g. *C. maculata*, WAAGE, 1979), the mechanism is not physical: the aedeagus, via the in and out copulatory abdominal flexions that help the penis head to directly remove sperm, also stimulate the mechanoreceptive sensilla embedded in the vaginal plates (CÓRDOBA-AGUILAR, 1999a). Since the sensilla coordinate spermathecal sperm ejection during fertilisation, the aedeagal stimulation results in spermathecal sperm displacement. My egg manipulation results suggest that those spermathecal ducts with a higher number of sensilla on their corresponding plate had more contractile activity. This means that, in asymmetrical females and during aedeagal stimulation, one spermathecal duct might consistently release more sperm. This is in agreement with the observed patterns of unequal volumes of stored sperm in both spermathecal ducts after aedeagal stimulation (CÓRDOBA-AGUILAR, 1999a).

After the aedeagal stimulation, is likely that the sperm moves to the bursa from which is physically removed by the penis head and lateral appendages.

Once the removal of rival sperm has finished, the copulating male's sperm is transferred from the seminal vesicle to the aedeagus (via compression of the vesicle caused by the close contact of the male and female genitalia; WAAGE, 1979; SRIVASTAVA & SRIVASTAVA, 1986; MILLER, 1987). The aedeagal inflatable membrane forms a canal-like passage which allows the copulating male's sperm to travel to the bursa and/or spermatheca where it is stored (MILLER & MILLER, 1981; MILLER, 1987). The filling of sperm is similar in volume for spermathecal ducts as in solitary individuals (this work) and in recently-copulated ones (CÓRDOBA-AGUILAR, 1999a), volumes do not differ.

VARIATION IN THE NUMBER OF SENSILLA

Interestingly, the sensilla in the vaginal plates of *C. haemorrhoidalis* are distributed non-randomly. SIVA-JOTHY (1987a) detected a variation in sensillum size (in cap diameter) in female dragonflies, and proposed that this variation could serve to provide more precise information, through differences in stimulus intensity, about the position of the egg in the vagina during fertilisation. It is unlikely that the asymmetric disposition of sensilla in both vaginal plates serves a similar function in *C. haemorrhoidalis*. Why most females show a bias in sensilla to their left hand vaginal plate or/and, in general, are asymmetrical in distribution is unclear. One possible function is that asymmetrical females may retain more control over stored spermathecal sperm during sperm displacement. The logic of this hypothesis is that those females with fewer sensilla on one vaginal plate would keep more stored sperm in the corresponding spermatheca at the end of the stimulatory process (CÓRDOBA-AGUILAR, 1999b). One way this can be proved is investigating whether asymmetrical females gain any benefit of retaining sperm compared to symmetrical females.

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