

ASPECTS OF THE REPRODUCTIVE BEHAVIOUR AND PHYSIOLOGY IN THREE NORTH AMERICAN GOMPHIDAE SPECIES (ANISOPTERA)

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Abstract – Field and laboratory observations on the reproduction of *Gomphus externus*, *G. graslinellus* and *Progomphus obscurus* are presented from Illinois, United States. Mating of *G. externus* in the field took 12 min. 2 phases were distinguished, the first consisted of tapping movements of the ♂ hamulus, the second of pumping movements of the ♂ abdomen. The ovary yielded 5100 eggs of which 690 were laid during hand-held oviposition in the laboratory. Only eggs laid by the ♀ but not the ones dissected from the ovary developed a sticky jelly coat around themselves. Eyespots of developing embryos were visible after 13 days. This ♀ had stored approximately 200 thousand sperm (about 160 bundles) in her spermatheca. The sperm were still mobile 3 days after mating. A comparison of the penis horn length and the length of the spermathecal tubes revealed that ♂♂ cannot reach the end of the spermathecal tubes. A *G. graslinellus* ♀ was observed to employ the dipping mode of oviposition. After oviposition she had approximately 1 million sperm remaining. ♀♀ of both spp. showed no muscle contraction response when the cerci were touched with water, but responses to water differed between the 2 ♀♀ on segments 9 and 10. ♂ *P. obscurus* perched on the sandy ground along the bank without territorial behaviour though ♂♂ were observed to follow other ♂♂. The penis morphology of *P. obscurus* was similar to members of the genus *Gomphus*.

Introduction

The most recent and exhaustive summary on gomphid reproductive biology (SUHLING & MÜLLER, 1996) largely relied on anecdotal observations in order to provide a general picture, perhaps with the notable exception of male spacing behaviour, of which experimental studies exist (KAISER, 1974; MILLER & MILLER, 1985; MARTENS, 2001). Basic data are lacking for the majority of the species, as for instance egg numbers

produced per female, mating durations (but see DUNKLE, 1989), female genital morphology and even simple descriptions of the oviposition behaviour (but see DUNKLE, 1989).

The aim of the following account is to note aspects of the reproductive behaviour and physiology of both male and female gomphids. Although the present paper is of anecdotal nature, too, I hope to stimulate further research and to refine testable hypotheses about reproduction of the Gomphidae.

Observations and Results

Field observations were carried out at Timber Creek (40°21'30"N, 89°06'52"W), McLean County, Illinois, USA during June and July 2001. All times reported are local times.

GOMPHUS EXTERNUS

Mating. – On 16 June 2001 a probably just paired mating wheel was blown by the wind from the stream into a tree of the bank vegetation, approximately 8 m above the water. The twig with the mating pair was removed and watched very closely in the shade. Between 15:32 and 15:38 the male was observed to carry out tapping movements with both of the posterior hamuli of his secondary genitalia onto the female subgenital plate. The frequency was about 1 Hz. Between 15:38 and 15:44 the male carried out pumping movements with a frequency of 1 Hz in which only the abdominal segments 2 and 3 were moved. The slightly widened abdominal segments 7 to 9 of the male were firmly pressed to the female frons and fitted neatly along the eye margins of the female. The width of the female frons corresponded well to the width of the abdomen (3.4 mm) of a later caught male. The female seemed to pull the male abdominal segments towards her frons which lead to a very strong bending of the last abdomen segments of the male. At 15:44 the genitals were separated, then the male released the female's head

and flew away. The female sat still until I caught her a few seconds later for hand-held oviposition (STEINER, 1948; McVEY, 1984). As this was unsuccessful the female was collected and kept alive in the laboratory for further examination. In the laboratory, no copulation marks on the head of the female were found when she was examined under a dissecting microscope.

Oviposition. — The female was kept in a 2 l gauze topped plastic container with a small perch. She was regularly fed by touching her mouthparts with flies, pieces of crickets (thorax, testes, gut) and other small insects. After 3 days, on 19 June 2001, the female was again tested for oviposition by holding her abdomen end into a water filled vial. The female sometimes reacted with a strong muscle contraction and the abdomen was bent between the 4th and the 5th segment. At this time, egg release was observed. After water contact, the female abdomen sometimes showed a pumping movement whereupon the eggs were released. During six such release bouts which were separated by half a minute or more, a total of 690 eggs were laid. This was also

possible when the ventral side of the female was turned upward. When the female abdomen was touched with a drop of water hanging from a pipette only certain parts of the abdomen were responsive to water (Fig. 1). Shortly before the female released eggs she did not react to water on her abdomen tip but this non-responsive time interval was not measured. Such “non-responses” were discarded and not included in Figure 1.

The female died in the evening of that day and was dissected in insect saline. The ovary yielded ca 4400 eggs which were 0.5 mm long and 0.25 mm wide. Although not definitely tested all eggs appeared to be mature (as judged from their similar yellow colour).

Egg development. — Eggs from hand-held oviposition as well as dissected out ones were kept separately at room temperature (ca 23°C) in a

petri dish in which most of the water was replaced every 2 to 3 days. After 1 to 2 days the eggs obtained by hand-held oviposition had formed a thick jelly coat which was sticky as several egg clusters were formed. During the further development individual eggs showed a granulous pattern and the majority showed brown eye spots after 13 days of development. In contrast, eggs dissected from the ovary showed after 5 to 6 days a jelly coat which appeared more irregular and non-sticky. Although some slight patterning was observed the eggs generally retained a milky appearance.

Sperm storage and penis morphology. — The size relationship between the female sperm storage organ and the penis of *G. externus* are shown in Figure 2. The two males investigated differed in the cross section of the low branching process of the penis (Fig. 2). The number

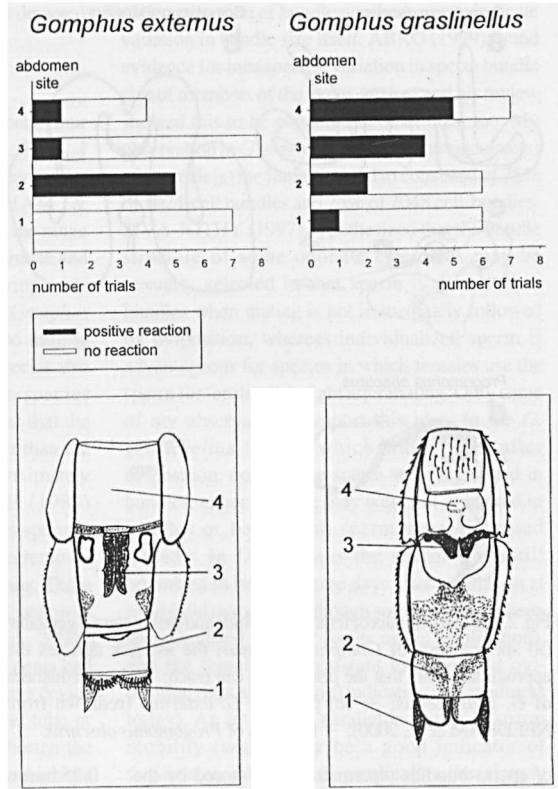


Fig. 1. Reaction by abdomen beating to touch with a drop of water in two gomphid females.

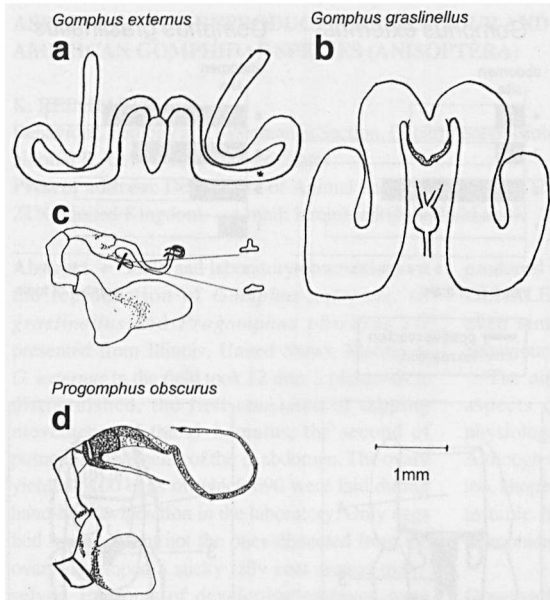


Fig. 2. Internal genitalia of female gomphids and male external genitalia: (a) spermatheca of *Gomphus externus*; the asterisk denotes the approximate point that the penis horns can reach; – (b) spermatheca of *G. grasilinellus*; – (c) penis of *G. externus* (redrawn from NEEDHAM et al., 2000); – (d) penis of *Progomphus obscurus*.

of sperm bundles (spermatodesms) stored by the female differed between the right and the left spermathecal tube. There were about 60 sperm bundles in one tube of the spermatheca but about 100 in the other. In order to count the spermatozoa the spermatheca was removed, placed in 1 ml saline and drawn ten times through a pipette. This destroyed the binding between the spermatozoa within a bundle. A drop of the homogenized solution was then placed onto a hemocytometer and counted under 400 \times magnification. The number counted multiplied by the solution yielded averages of 237600, 207900, and 168300 sperm (mean \pm s.e.: 203500 \pm 14400) giving an average of 1280 sperm per bundle. It is, therefore, likely that one sperm bundle has undergone either ten or eleven cell division cycles during spermatogenesis (expected numbers are 2¹⁰ = 1024 and 2¹¹ = 2048). The sperm were mobile in insect saline.

GOMPHUS GRASLINELLUS

Oviposition. – On 24 June 2001 at 15:09 a female was observed to lay eggs by dipping her

abdomen onto shallow water above a fully mossed stone near the bank. As mentioned for another gomphid *O. uncatus* (SCHÜTTE, 1996) she faced upstream while dipping. After 15 dips the female rested in the adjacent bank vegetation where she was captured. The female was kept alive in the same 2 l jar and fed with the same material as the *G. externus* female mentioned above. Two days after capture I carried out the same muscle contraction tests as with the *G. externus* female. *G. grasilinellus*, however, seemed to move the whole abdomen upon water contact. There were differences in responsiveness to water in the central parts of the segments 9 and 10, respectively (Fig. 1), whereas females of both species did not respond when the water drop touched the end of the cerci.

The female did not have copulation marks on the head. During dissection I observed that many eggs were not fully matured and it was impossible to count them. Mature eggs were 0.6 mm long and

0.25 mm wide.

Sperm storage. – The female spermatheca is depicted in Figure 2. Both sides of the spermatheca were densely packed with sperm but no sperm bundles were visible. The sperm were found to be immobile in insect saline. They were counted as mentioned above and yielded a mean (\pm s.e.) of 968550 (\pm 28400) sperm.

PROGOMPHUS OBSCURUS

Males of this species seemed to become reproductively more active in the afternoon with regard to perch occupation or female pursuing behaviour though this was not quantified. Males perched on the bare sandy ground although many higher perches were available. Males were sitting as close as 5 cm to each other and no sign of site attachment or territorial behaviour could be found. However, neighbours seemed to influence each others decisions: up to three males were seen chasing a (presumably) female. The behaviour of the males thus resembled very much that of *O. forcipatus* as described by KAISER (1975) and

MILLER & MILLER (1985). The penis is depicted in Figure 2.

Discussion

Some aspects of observed reproductive behaviour fit into the general gomphid scheme. Thus, I confirmed that a female *G. externus* may have over 5000 eggs ready to lay (NEEDHAM & HEYWOOD, 1929). The egg size was in the range of European representatives of the Gomphidae and the suspicion that a complete jelly covering coats only the eggs of members of the genus *Gomphus* (SCHUTTE, 1996) was supported. The mating duration of 12 minutes recorded for this species also corresponds well to other *Gomphus* species (DUNKLE, 1989). I found in *G. externus* that the paired appendices of the penis are shorter than the tubes of the spermatheca, reaching approximately to one third of the length. WAAGE (1984) suggested on the basis of a similar penis-spermathecal tube length relationship that sperm removal may not occur in *Arigomphus submedianus*. There was a similarity at the penis structure of *P. obscurus* to other gomphids (see NEEDHAM et al., 2000). The paired structures of the *P. obscurus* penis had an U-shaped cross section which may act as a device to suck out sperm from the spermathecal tube or may enhance sperm transfer and thus shorten the duration of copulation. In *G. externus*, in which the female stored sperm bundles, no such U-shape was found in the penis horns, but a small distal hook instead. In this species I observed a regular tapping prior to the pumping movements. I speculate that this tapping may serve to manipulate the female either by initiating sperm release (see CORDOBA-AGUILAR, 1999 for an example of female sensory exploitation to simulate sperm release) or as a form of intra-pair courtship where the tapping provides some information to the female (see SANTOLAMAZZA CARBONE & CORDERO RIVERA, 1998; EDVARDSSON & ARNQVIST, 2000 for beetle examples of such a cryptic female choice).

I calculated an average of 1280 sperm per bundle. Because the cell division of spermatogonial clones follows a geometric series of 2^n , expected numbers of spermatozoa are $2^{10} = 1024$ or $2^{11} = 2048$. The deviation from this expected numbers can either reflect a (rather unlikely) unnoticed complete breakdown of some bundles (leading to an

underestimation of bundle number), or can indicate variation in bundle size itself. ÅBRO (1999) found evidence for intraspecific variation in sperm bundle size of members of the genus *Aeshna* and his review showed this to be plausible in other taxa, too. My observation in *G. externus* show that the ejaculate(s) of the male(s) the female mated to consisted of 75% of 1024-cell bundles and 25% of 2048 cell-bundles. SIVA-JOTHY (1997) hypothesized that the bundle structure of some odonate ejaculates may be sexually selected in that sperm is delivered in bundles when mating is not immediately followed by oviposition, whereas individualized sperm is advantageous for species in which females use the sperm for fertilization right after mating. Only some of my observations support this idea. In the *G. graslinellus* female which was caught after oviposition, none of the sperm were organized in bundles, either because they were not delivered in bundles or because all sperm were dispensed already. In *G. externus* the sperm were still organized in bundles three days after copulation at hand-held oviposition (though some must have been individualized in order for its use in fertilization), and the female was resistant to hand-held oviposition right after mating (indicating non-readiness to lay). An interesting parallel is shown in sperm mobility (which may be a good indicator of fertilization ability). In *G. graslinellus*, the (already individualized) sperm was immobile in saline whereas in *G. externus* individual sperm homogenized by me was mobile. An alternative explanation for this difference is that females may manipulate the dispersion of the sperm bundles in relation to the total number of sperm stored. The *G. externus* female had five times less of sperm than *G. graslinellus* which was even caught after oviposition. Thus, the *G. externus* females may have to show some sperm economy.

NEEDHAM & HEYWOOD (1929) and DUNKLE (1989, 2000) mention that certain gomphid males fly with slightly raised abdomens. Thereby, the widened abdomen segments which are found in several gomphid species could perhaps serve as an intra- or intersexual signal. DUMONT (1977) mentions that *Lindenia tetraphylla* may use the abdomen end in territorial threat, a suggestion that could not be confirmed for *Cacoides latro* (MOORE & MACHADO, 1992). I observed in *G. externus* that the end of the abdomen was firmly

pressed upon the female's frons and eyes. Because the female seemed to assist in this connection with her legs I speculate that females may receive information about the male's size during such a connection. However, I have not investigated individual variation in this trait which would be a prerequisite to serve as a marker, e.g. for individual quality or body size. Alternatively, a widened abdomen could have been selectively advantageous for the dipping mode of oviposition in ancestor gomphid females and be favoured by selection. It may then occur in males because of a genetic correlation between the sexes.

The two females also differed in how they responded when water touched their abdomen segments 9 and 10. It may be that the muscle contraction response is linked to the mode of oviposition to either lay eggs in a large batch that is formed while the female sits on a perch and is then released in total, or to the dipping mode of egg laying (SCHÜTTE, 1996). *G. graslinellus* was observed to employ the dipping oviposition mode and in this species the strongest muscle contraction response (abdomen beating) was at or near the subgenital plate. In contrast, *G. externus* had the strongest response when water touched segment 10, the site where eggs accumulate before release. In addition, *G. externus* showed no reaction shortly before an egg release bout.

All aspects of egg development were as previously described, including the duration of coat development as well as differences between laid eggs and those dissected out (*G. s. similimus*, SCHÜTTE, 1996; *Ophiogomphus cecilia*, C. Schütte, pers. comm.).

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