

The Spermatophore of the Black Field Cricket *Teleogryllus commodus* (Insecta: Orthoptera: Gryllidae): Size, Structure and Formation

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Abstract. The spermatophore produced by male *Teleogryllus commodus* was investigated in detail. Besides the study of the spermatophore size, structure, and formation, additional information on the number of spermatozoa enclosed in the capsule was obtained. The 3.32–4.37 mm long spermatophore is composed of the bulb-shaped, sperm-containing ampulla, the filamentous tube, and the attachment plate, fixing the device in the genital tract of the female. The ampulla consists of 4 layers: (1) the outer membrane, (2) the liquid-filled middle layer, (3) the internal layer defining the shape of the capsule, and (4) the inner membrane enclosing the sperm package. An almost identical structure was reported for the sperm-encompassing devices of other cricket species. While formation of the main components of the spermatophore takes place in the ampulla of the male accessory glands, definition of the final shape and transfer of the spermatozoa into the sperm sac is realized in the ventral chamber of the phallus and dorsal pouch. Sperm number within one spermatophore was estimated to range from several ten-thousand to few hundred-thousand.

Key words. Spermatophore, spermatozoa, mould, cricket, *Teleogryllus commodus*.

Introduction

Contrary to most vertebrates, which discharge semen as suspension of free spermatozoa in seminal plasma, in numerous invertebrates the spermatozoa are subject to a specific aggregation, before they are transferred to the females. The formed packages are either loosely or tightly wrapped and, in most cases, consist of male accessory secretions, enfolding the spermatozoa and afterwards undergoing a process of hardening and solidification (see overview in MANN 1984). The sperm-encompassing devices are generally termed spermatophores, if they appear as complex, multilayered tunics or capsules. Among invertebrates, shape and size of such spermatophores as well as the number of enclosed spermatozoa are subject to high variation (MANN 1984).

Regarding the Insecta, pre-packaging devices of spermatozoa are observed very frequently, representing a distinguishing characteristic of this class. Spermatophores either occur as naked sperm-drops (i.e., droplet spermatophores) or as typical multilayered capsules, whose building substances (e.g. proteins, glycoproteins, mucopolysaccharides, lipoproteins, phospholipids, etc.) exclusively originate in the male accessory glands (KHALIFA 1949; WIGGLESWORTH 1972; KAULENAS 1976; LEOPOLD 1976). Besides the secretion of spermatophore material, the accessory glands seem to fulfil additional functions such as the production of prostaglandines (PG) or cyclic guanosine 3',5'-monophosphate (cGMP) (DESTEPHANO & BRADY 1977; MANN et al. 1981).

Among 'orthopteroid' insects, specialization in formation, structure, and function of spermatophores has reached a rather high degree. Most comprehensive studies on the structure and formation of the sperm-encompassing devices have previously been carried out for various Blattaria, Mantodea, and Phasmatodea, and the orthopteran families Tettigoniidae, Gryllidae, and Acrididae (MANN, 1984). Concerning the gryllids, respective investigations are reported for *Acheta domesticus* Linnaeus, 1758 (KHALIFA

1949), *Gryllus assimilis* Fabricius, 1753 (SPANN 1934), *Gryllus campestris* Linnaeus, 1758 (REGEN 1924), *Nemobius sylvestris* Bosc, 1792 (GERHARDT 1921; GABBUTT 1954), *Oecanthus pellucens* Scopoli, 1763 (HOHORST 1936), and, more recently, for *Gryllus bimaculatus* DeGeer, 1773 (HALL et al. 2000). Among all of these species, the spermatophore is composed of three main parts, which have already been defined in the early work of LESPÈS (1855): (1) a bulb-shaped, sperm-containing capsule (ampulla; 'vésicule' sensu LESPÈS), (2) a thin, filamentous spermatophore tube ('filet' sensu LESPÈS), and (3) a hook-like attachment plate (handle; 'lamelle' sensu LESPÈS), which serves for anchoring the spermatophore in the female genital tract. As outlined in detail for *G. assimilis*, *G. bimaculatus*, and *A. domesticus*, the wall of the ampulla mainly consists of an outer membrane, a layer of evacuating fluid underneath, a thick internal layer determining the form of the capsule, and an inner membrane enveloping the sperm mass and the pressure bodies (SPANN 1934; KHALIFA 1949; HALL et al. 2000). The pressure bodies themselves are responsible for emptying the spermatophore after its transfer to the female. Differences between the cricket species are noticeable regarding the size of single spermatophores, the time necessary for their formation, and the number of such devices produced within a specified time interval. Until now, nothing has been reported about the number of spermatozoa enclosed in one spermatophore and its dependency on diverse physiological parameters.

In the study presented here, the knowledge on spermatophores is extended by describing in detail the respective sperm-encompassing device of the black field cricket *Teleogryllus commodus* Walker, 1869. Besides an investigation of the spermatophore size and structure, the number of enclosed spermatozoa is crudely estimated from electron micrographs of oriented sections of the ampulla. Results obtained from the study are finally compared with respective literature data of other crickets.

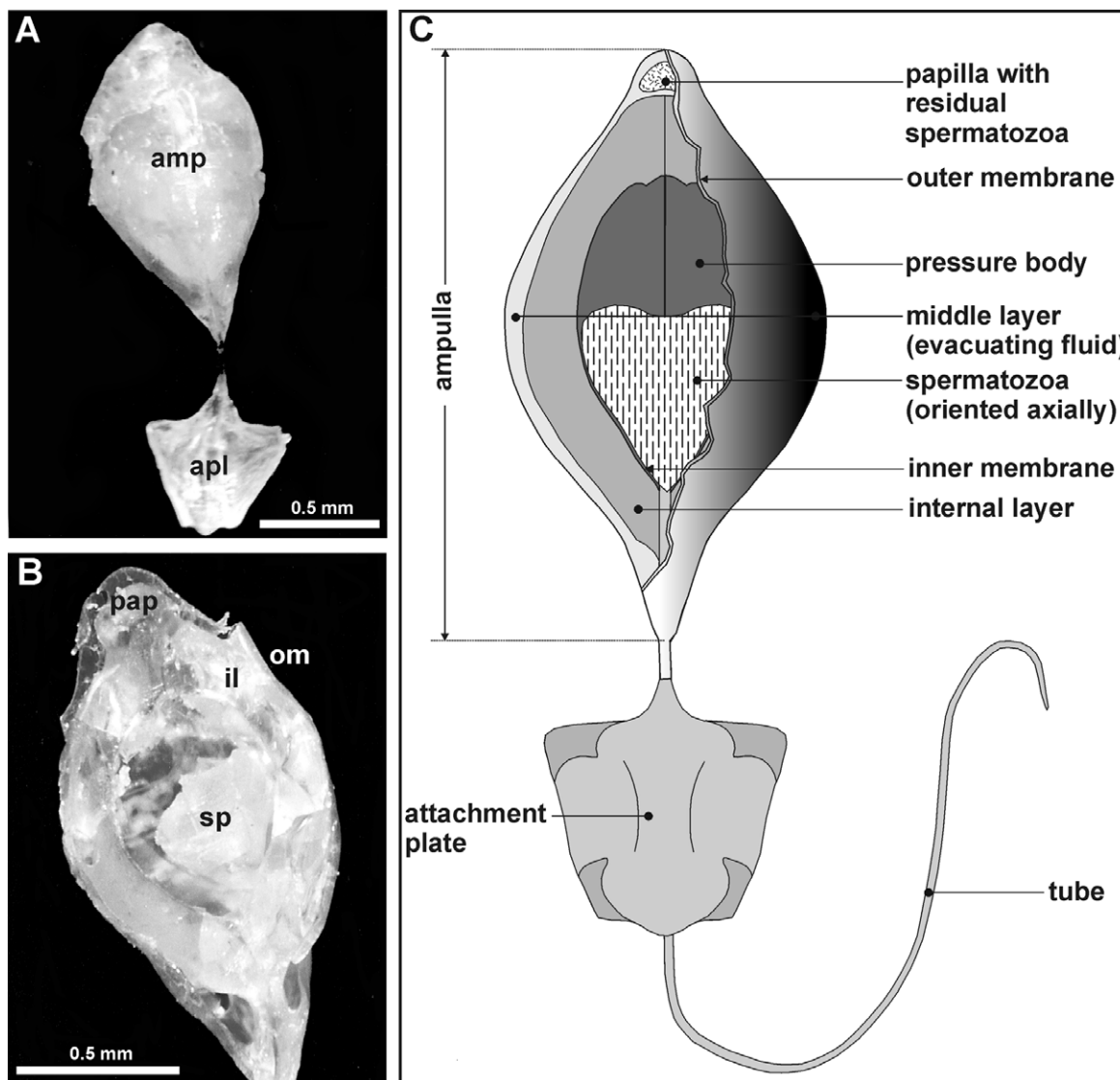


Fig. 1. Spermatophore of *Teleogryllus commodus*. **A–B:** Light-microscopic photographs of spermatophore. Abbreviations: amp = ampulla; apl = attachment plate; pap = papilla; om = outer membrane; il = internal layer; sp = sperm mass. **C:** Scheme illustrating the main parts of the spermatophore and the morphology of the ampulla.

Materials and methods

Animals. Crickets were reared in a climate chamber at the Institute of Zoology, University of Salzburg. During the study, the following setup was used: temperature: 25°C, relative humidity: 60 %, photoperiod: 12 h. While nymphal instars and subadults were kept in specific plastic boxes (LxWxH = 45x30x25 cm) filled with a 3 cm thick layer of peat soil, adults were separated by gender and transferred into glass vessels with a volume of 5 L.

Structure of spermatophores. In order to answer the open questions noted above, it was necessary to remove fully developed spermatophores from sexually mature males of *T. commodus*. This was best realized by securing the cricket under the microscope, lowering the subgenital plate with a preparation needle, and carefully working the spermatophore back and forth until it came loose. Sizes of single components of the evacuated sperm-encompassing devices were measured under the light microscope. The internal structure of the capsule was decoded in two ways: (1) by fixing the spermatophore in ethanol (70 %) for 3 h, producing a longitudinal section of the ampulla, and studying its composition under the light microscope; (2) by preparing the object according to a procedure outlined in

previous publications (e.g., MUSIOL et al. 1990; STURM & POHLHAMMER 2000; STURM 2002) and investigating respective sections of the ampulla in the scanning electron-microscope (Cambridge EM-250). To estimate the number of enclosed spermatozoa, cross sections of the ampulla were prepared and the area of the cut sperm mass was measured. Assuming that the spermatozoa are perfectly oriented parallel to each other, their number was simply calculated by dividing the cross-sectional area of the whole sperm mass by the mean cross-sectional area of a sperm tail.

Formation of the spermatophore. For crudely following the production of a spermatophore in males of *T. commodus*, at pre-defined time spans after the beginning of copulation (1, 5, 10, 20 minutes) animals were transferred into liquid nitrogen. Dissection of the abdomen was carried out after thawing at room temperature for 5 minutes. Alternatively to the nitrogen procedure, males were anesthetized in a stream of carbon dioxide, decapitated, and fixed in ethanol (70 %) for several hours. After fixation, cranio-caudal sections were produced with a razor blade and studied under the light microscope. The investigations were additionally supported by the use of histological sections oriented in the same way and stained according to a procedure described by ADAM & CZIHAK (1964).

Tab. 1. Morphometric results for the spermatophore of *Teleogryllus commodus*; stdev = standard deviation.

N = 20	mean [mm]	stdev [mm]
total length	3.85	0.520
ampulla		
length	1.56	0.270
width	0.88	0.140
tube		
length	2.29	0.420
diameter	0.04	0.003
attachment plate		
length	0.76	0.082
width	0.52	0.035
layers of ampulla		
outer membrane	0.02	0.001
middle layer	0.19	0.005
inner membrane	0.01	0.001

Tab. 2. Number of spermatozoa per spermatophore, amount of spermatophores formed per day, and time needed for the production of one spermatophore; stdev = standard deviation.

N = 20	mean	stdev
number of spermatozoa per spermatophore	152,000	57,000
number of spermatophores formed per day (at 25°C)	4.2	2.6
time for emptying one spermatophore [min]	24.6	5.9

Results

Structure and size of the spermatophore

The structure of the spermatophore produced by male *T. commodus* is represented schematically in Fig. 1C. Additional information can be obtained from the micrographs of Figs. 1A,B and 2A–D. In general, the studied spermatophore consists of three components: (1) the bulb-shaped, sperm-containing ampulla, (2) the filamentous tube, and (3) the attachment plate, acting as a device for fixing the spermatophore in the genital tract of the female. The ampulla itself is composed of an outer membrane, a liquid-filled middle layer, an internal layer defining the shape of the capsule and an inner membrane enclosing the sperm package (Figs. 1A–C, 2A). Within the cavity of a functioning ampulla, pressure bodies surround the aggregation of spermatozoa, serving for their discharge out of the spermatophore after copulation. As crudely indicated in Figs. 2B–D, spermatozoa seem to be well arranged within the ampulla with tails oriented parallel to each other and heads lying in pre-defined layers adjacent to the outlet. The attachment plate of the spermatophore is separated from the ampulla by a short tube segment, has a more or less trape-

zoid shape, and is marked by spiny hooks posited at each corner (Fig. 1C). The spermatophore tube has a uniform diameter from start to end. During mating its tip remains closed, preventing a premature discharge of sperm.

Results of spermatophore morphometry are summarized in Tab. 1. Main components of the device are subject to considerable variation in size, expressed by high standard deviations (stdev). For instance, total length of the spermatophore ranges from 3.32 mm to 4.37 mm. Size of the sperm-encompassing devices positively correlates with the size of the investigated males.

Estimation of the sperm number

Sperm number was estimated in the way described in the methods section. Results of this rather simple quantification technique are summarized in Tab. 2. The respective data of the table clearly indicate that the number of spermatozoa is in the order of tens to hundreds of thousands. The high standard deviation further shows that this amount is subject to meaningful fluctuations, probably depending on the physiological state of the male. For the environmental conditions used in this study, the mean number of spermatophores formed per male per day was 4.2 ± 2.6 . Emptying of one spermatophore needed 24.6 ± 5.9 minutes (Tab. 2).

Formation of the spermatophore

Production of the spermatophore in male *T. commodus* has to be evaluated as a highly complex process. A meaningful contribution for solving this question may be given by the photographs of Fig. 3 as well as the schematic illustrations of Fig. 4. Formation of the main components of a new sperm-encompassing device follows directly after copulation and takes place in the ampulla of the accessory glands (Fig. 3). At early stages of the production process (i.e., after c. 1 minute), single layers of the spermatophore head as well as most parts of the tube have already been generated (Fig. 4A). The sperm mass is posited at the apical end of the head (papilla), and the inner layer represents a soft and elastic mass. The attachment plate material is also produced in the accessory glands. Immediately after its formation, the vehicle is transferred downward the ejaculatory duct. While the ampulla of the spermatophore is stored between the ventral valve-like flaps (SNODGRASS 1935: 567) of the male genitalia for finishing its development, the tube and attachment plate material enters the dorsal pouch (Fig. 3A,B) by rhythmic movement of the subgenital plate. Further development of the ampulla is marked by the penetration of sperm mass into the sperm sac and axial orientation of single spermatozoa (Fig. 4B). Within the dorsal pouch, the final shape of the attachment plate develops at the same time (Fig. 4C).

Discussion and conclusions

The present study could clearly demonstrate that the spermatophore produced by male *Teleogryllus commodus* is very similar to respective devices of other cricket species. The investigated spermatophore falls into three structural main parts (i.e., capsule or ampulla, attachment plate, and tube) and therefore unequivocally follows the scheme that was previously described for other gryllids (e.g. REGEN 1924; SPANN 1934; KHALIFA 1949). Correspondence can also be reported concerning the structure of the ampulla, which is essentially composed of four layers (i.e., outer membrane, middle layer, internal layer, and inner membrane). As a uniform quality of all spermatophores of the Gryllidae, the inner membrane directly envelops the sperm package, while the internal layer defines the shape of the capsule due to its remarkable hardness and thickness, and the middle layer contains a so-called evacuating fluid.

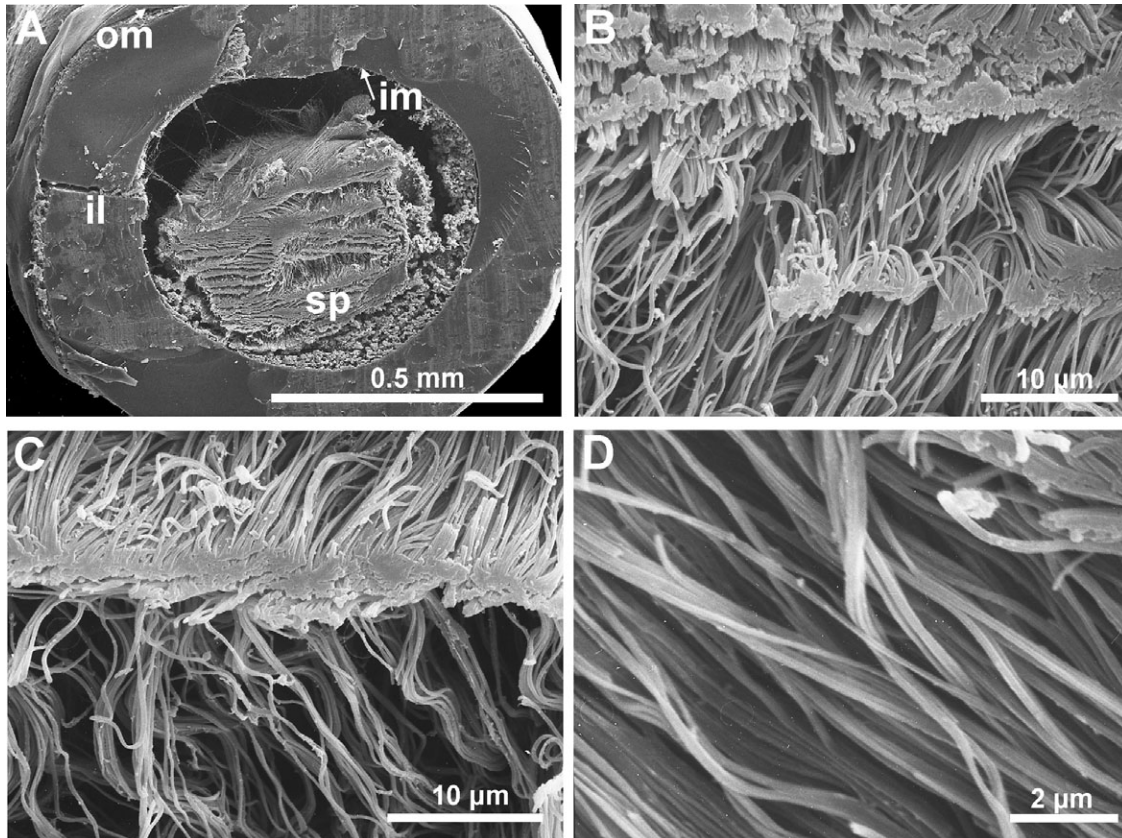


Fig. 2. Spermatophore ampulla of *Teleogryllus commodus*. **A:** Electron micrograph of a cross-sectioned ampulla, with its multi-layered structure (abbreviations: see Fig. 1). **B–D:** Detailed photographs of the sperm mass with single, axially oriented spermatozoa.

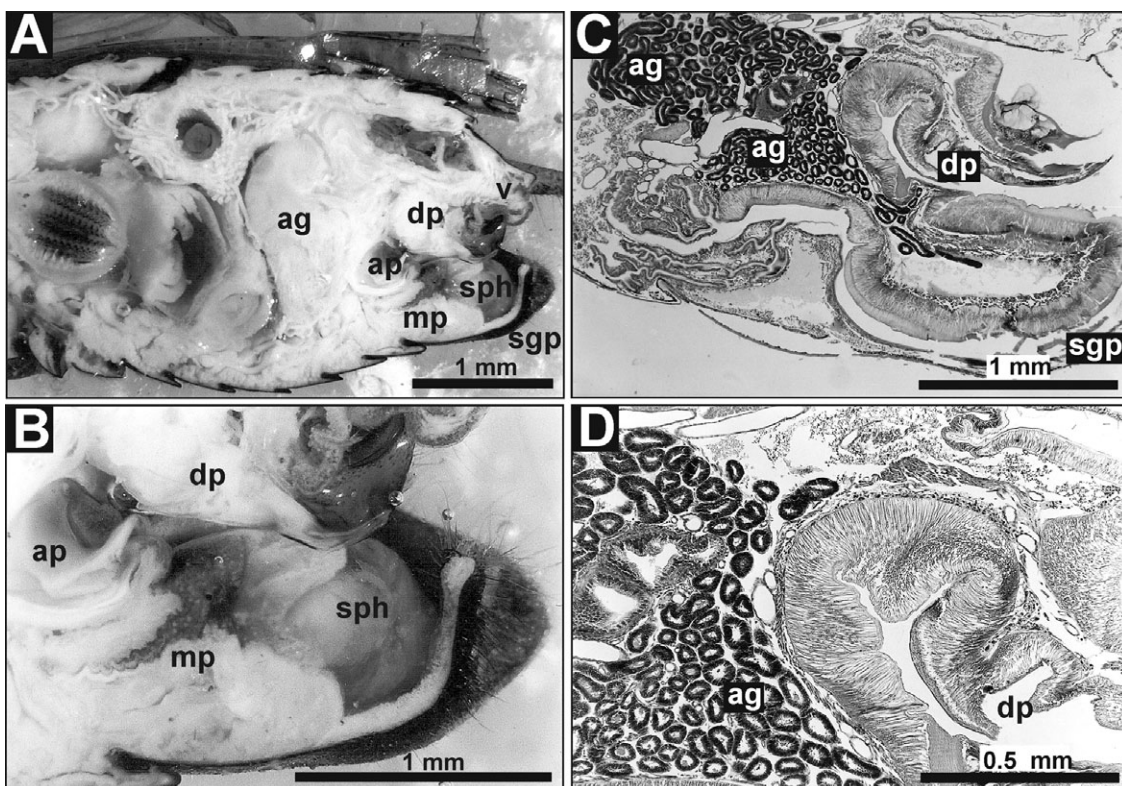


Fig. 3. Male abdomen of *Teleogryllus commodus*. **A–B:** Median longitudinal sections, overview (A) and genital region in detail (B). Abbreviations: ag = accessory glands; ap = ampulla of the accessory glands; dp = dorsal pouch; mp = median pouch; sgp = subgenital plate; sph = spermatophore; v = virga (terminology from SNODGRASS 1935). **C–D:** Histological sections through the male abdomen, overview of genital region (C) and detail (D).

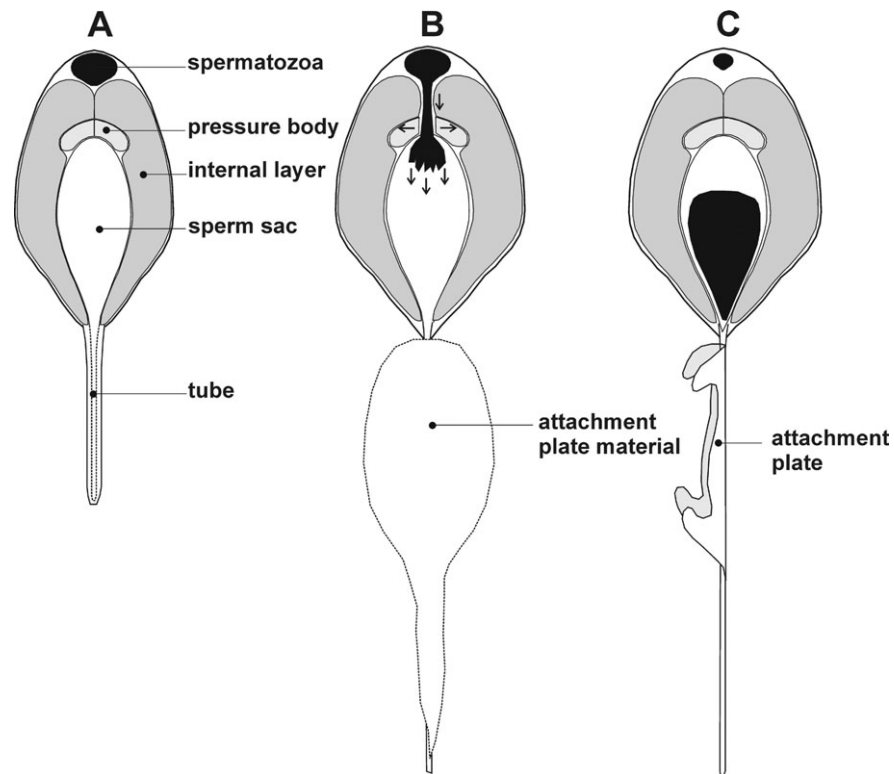


Fig. 4. Illustration describing the development of the spermatophore in male *Teleogryllus commodus*. **A:** After ca. 1 min. **B:** After ca. 10 min. **C:** After ca. 20 min (fully developed spermatophore). Open questions still remain concerning the precise formation of the attachment plate and the transfer of spermatozoa into the sperm sac.

Main criteria for distinguishing the spermatophores of various cricket species are, e.g., the shape of the terminal papilla, ampulla, and attachment plate as well as the size of the device, correlating with the mean size of the male. As listed in Tab. 1, the total length of the spermatophore in *T. commodus* is 3.85 ± 0.52 mm. In males of *Gryllus campestris* and *G. assimilis*, both significantly exceeding the size of the black field cricket, sperm-encompassing devices reach lengths of 5.0 and 5.5 mm, respectively (REGEN 1924; SPANN 1934). Contrary, in the much smaller bush cricket (*Nemobius sylvestris*) spermatophore length rarely exceeds 3.0 mm. As another interesting and unique fact, spermatophores of this species considerably vary in size among each other (GERHARDT 1921; GABBUTT 1954). Concerning the formation of the spermatophore in *T. commodus*, major stages of development could be decoded using the techniques described in the methods section. While production of the spermatophore material takes place in the lumen of the accessory glands, final development is realized in the ventral chamber and dorsal pouch of the external male genitalia after transport of the vehicle through the ductus ejaculatorius. The observed stages correspond well with the respective results for *Gryllus bimaculatus* outlined by HALL et al. (2000). Despite the significant data obtained from the study presented here, two questions could not be clarified: (1) the formation of the attachment plate material and (2) factors responsible for the transfer of spermatozoa from the papilla into the sperm sac. HALL et al. (2000) could distinguish between different types of secretion involved in the attachment plate formation. Until now, such a distinction was not possible for the spermatophore of *T. commodus*. Regarding the movement of spermatozoa into the sperm sac, chemotaxis is assumed to be the driving force but further studies are needed.

In the present contribution, the number of spermatozoa enclosed in one spermatophore was evaluated by a simple quantification method. It has to be noted that a successful application of this technique is only possible if spermatozoa are oriented more or less parallel within the sperm package and spaces between the spermatozoa are negligible. Both demands seem to be confirmed by SEM imaging (Fig. 2). As listed in Tab. 2, sperm number shows significant fluctuations among the investigated males, but, however, is in the order of hundreds of thousands. Assuming that a female of *T. commodus* oviposits 1,000–2,000 fertilized eggs during its adult life-time, about 1–2 % of all spermatozoa stored in the receptaculum seminis finally combine their DNA with the female genotype. This small rate of spermatozoa being successfully involved in reproduction seems to be rather plausible, if a meaningful part of non-functional sperms released from the spermatophore and a penetration of the egg chorion by numerous spermatozoa, from which only one reaches the nucleus, is considered. While numbers of spermatozoa per spermatophore are not available for other cricket species until now, this amount can range from one or two in some crustaceans to 10^8 – 10^{10} in *Octopus dofleini* Wulker, 1910 (Cephalopoda: giant octopus; MANN et al. 1981). The spring-tail *Dicyrtomina ornata* Nicolet, 1842 (Collembola) produces a droplet spermatophore enclosing about 600 spermatozoa. Concerning the number of spermatophores generated in one day, data for different gryllids diverge significantly. While *G. assimilis* produces up to 12 spermatophores per day (SPANN 1934), which is two to three times more than the respective number of *T. commodus* (Tab. 2), *Acheta domesticus* only generates two to three spermatophores within 24 hours (KHALIFA 1949). Better correspondence can be observed regarding the time of sperm evacuation, which for all crickets studied until now varies between 20 and 40 minutes (MANN 1984).

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