

Motoric activity of the receptacular complex in the cricket *Teleogryllus commodus* (Insecta: Orthoptera: Gryllidae)

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Abstract. The motoric activity of the receptacular complex was investigated in standard Ringer's solution after having isolated the organ from females of the cricket *Teleogryllus commodus* characterized by different reproductive stages (virgin, mated, ovipositing, after oviposition). At 20°C mean myogenic contraction frequencies ranged from 15 to 25 pulses per minute, with highest values obtained for ovipositing females (22.8 ± 7.5) and lowest values for mated females (13.8 ± 6.5). In a second experimental line, the dependence of contraction frequency of receptacular complexes isolated from virgins on the composition of Ringer's solution was studied; concentrations of the cations Ca, Mg, and K were both doubled and halved. Changes of the mean pulse frequency with respect to the standard situation could be only observed for a doubled concentration of Mg, leading to an increase of the motoric activity, and for a doubled concentration of K, causing a significant decline of peristalsis. In-vivo investigations were carried out by cutting a 4 mm² window into the 7th sternite of females and observing the spermathecal activity at 20°C for at least 5 days. Females of the same reproductive stages as described for the in-vitro studies were used. Contractions of the receptacular complex were observed for 70 % of all females, with pulse frequencies being about 20/minute. Differences of receptacular activity among the reproductive stages are discussed, and the effect of various cations on the contraction of muscle fibres is tried to be decoded.

Key words. Receptaculum seminis, ductus receptaculi, motoric activity, myogenic peristalsis, *Teleogryllus commodus*.

Introduction

In most female insects, a variable number of receptacula seminis (spermathecae) serves for the storage of spermatozoa from the time of copulation until the fertilization of the egg. The basic condition in Ectognatha is that an unpaired receptaculum seminis is formed from the ventromedian invagination at the hind margin of segment VIII (SNODGRASS 1937; KLASS 2003). Its position in the adult depends upon the ontogenetic development of the gonoduct. Concerning the Ensifera, the single receptaculum seminis is connected with the genital chamber by a specific ductus receptaculi (spermathecal duct), through which spermatozoa are transported, when (1) the female is impregnated and (2) the eggs are fertilized (SNODGRASS 1937). Besides the receptaculum/receptacula seminis and the ductus receptaculi, the receptacular complex may additionally include a receptacular gland, whose secretions are thought to act as nutrients for the sperm (CHAPMAN 1998: 295 ff). The morphology of the complex is subject to significant variations within the insects and even within the Orthoptera (HEBERDEY 1931; MATSUDA 1976). Regarding the Gryllidae, a spherical to elliptical receptaculum (diameter: ~ 1 mm) is connected with the genital chamber by a long (up to 25 mm), strongly convoluted ductus with a diameter of 0.1–0.2 mm (SPANN 1934; DALLAI & MELIS 1966; POHLHAMMER et al. 1975); since parts of the ductal epithelium are glandular themselves, a receptacular gland does not exist in female crickets. Contrary to the form and arrangement of the receptacular components, their histology is rather uniform among the insects. The receptaculum and its duct generally consist of an inner cuticular intima, one layer of epithelial cells, a basement membrane, and an outer muscle layer with variable thickness (GILLOTT 1988). Glandular cells, as far as present, are usually characterized by a cup-shaped central cavity due to the invagination of the apical cell membrane. The cavity is surrounded by numerous microvilli, and secretion temporarily stored in this extracellular space is depleted into the lumen by an efferent ductule (GILLOTT

1988). As briefly described in the following section, this basic histology could also be found in the Australian field cricket *Teleogryllus commodus* Walker 1869 (ESSLER et al. 1992).

Regarding *T. commodus*, a question that is still not answered sufficiently concerns the mechanisms of sperm release from the receptaculum and its transport through the ductus. According to POHLHAMMER (1978), transport of the spermatozoa is mainly induced by peristaltic contractions of the ductus-associated muscle coat, whereas active motion of single germ cells along a chemogradient may be excluded so far. In general, the receptacular complex of *T. commodus* is innervated by the fourth segmental nerve of the terminal abdominal ganglion (SUGAWARA 1993; Fig. 1A), thus recommending a neural control of sperm release. In accordance with POHLHAMMER (1978), OKELO (1979), who examined *Schistocerca gregaria* Forskal 1775 (Orthoptera: Caelifera), and SUGAWARA (1993) hypothesize that movement of the spermatozoa is exclusively caused by the motoric activity of the receptacular complex, distinguishing between spontaneous myogenic peristalsis of the duct, which produces a slow continuous flow of sperm, and strong twitches evoked by the burst of efferent impulses, which squeeze the spermatozoa into the genital chamber in order to fertilize the eggs. The rest phase between two nervous impulses is assumed to depend upon the physiological state of the female, being about 15 times longer in virgins than in mated females.

The objective of the study presented here is two-fold: Firstly, myogenic peristalsis of the ductus receptaculi and receptaculum seminis is measured in vitro and also in vivo by applying the transparent window preparation method of SUGAWARA & LOHER (1986). Possible correlations between the frequency of contractions and the reproductive stage of the females are discussed. Secondly, in-vitro motoric activity of the receptacular complex is studied by changing cation concentrations of the Ringer's solution.

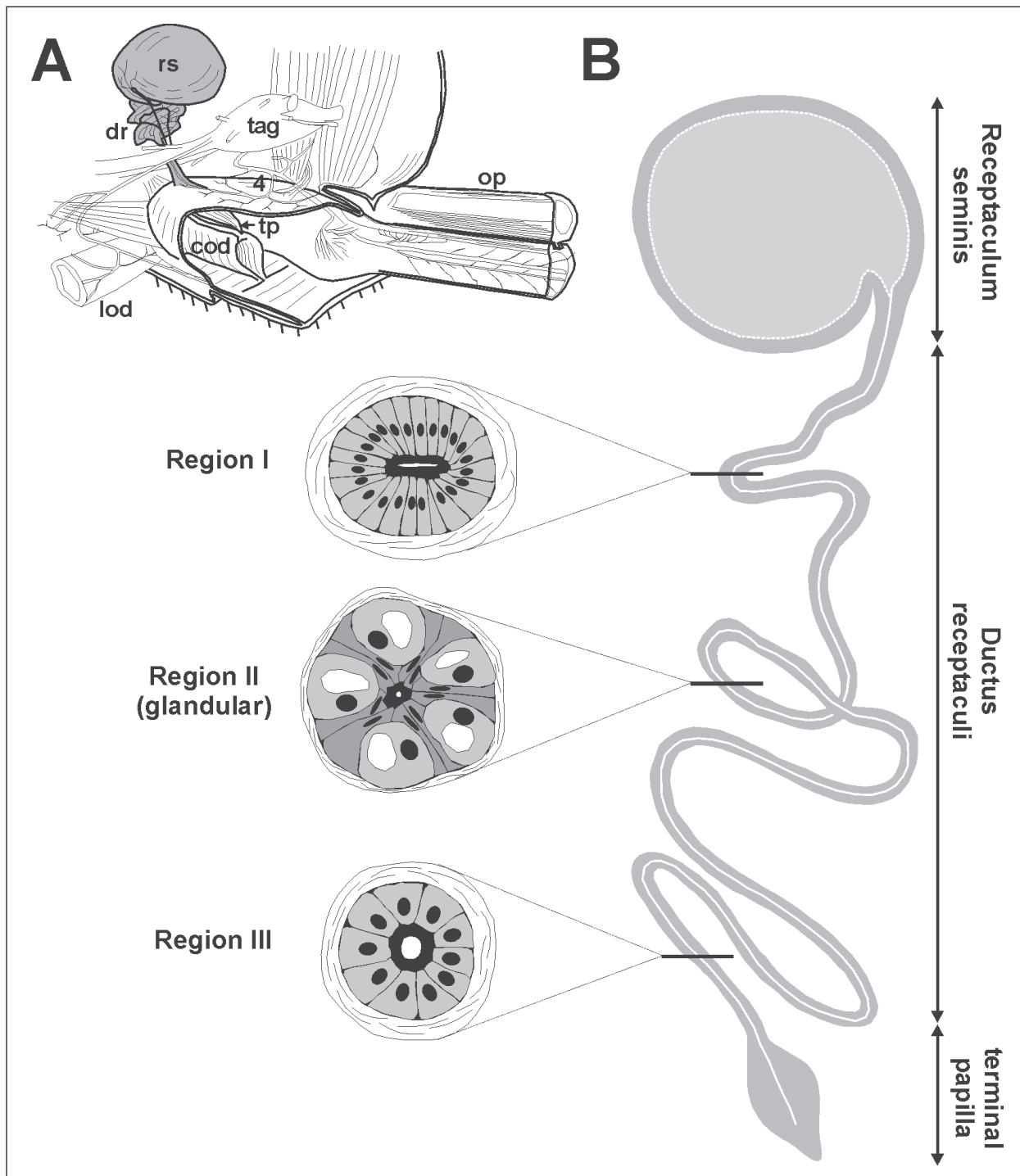


Fig. 1. **A:** Major anatomical components of the genital chamber in female *T. commodus* and their innervation by nerves arising from the terminal abdominal ganglion (tag; modified after SUGAWARA 1993). Abbreviations: cod = common oviduct, dr = ductus receptaculi, lod = lateral oviduct, op = ovipositor, rs = receptaculum seminis, tp = terminal papilla. **B:** Receptacular complex of *Teleogryllus* with its different parts and morphology of the three regions of the ductus receptaculi shown by transverse sections.

Histology of the receptacular complex in *Teleogryllus commodus* – a brief overview

For a better understanding of mechanisms standing behind the motility of the receptacular complex and the release of spermatozoa into the genital chamber, a brief description of the receptacular histology is given, which is mainly based on the findings of ESSLER et al. (1992). The ductus receptaculi of female *T. commodus* may be subdivided into three

regions with different histological characteristics (Figs. 1B, 2). Region I near the receptaculum seminis has a cleft-shaped lumen, a cuticular intima with longitudinal grooves and ridges on the surface facing the lumen, and a thick muscle layer (longitudinal and circular muscle fibres) underlying the basis of the epithelium. This short part of the ductus is commonly interpreted as a locking device, preventing the sperm from an uncontrolled flow out of the receptaculum. Within region II (middle region), the epithelium includes

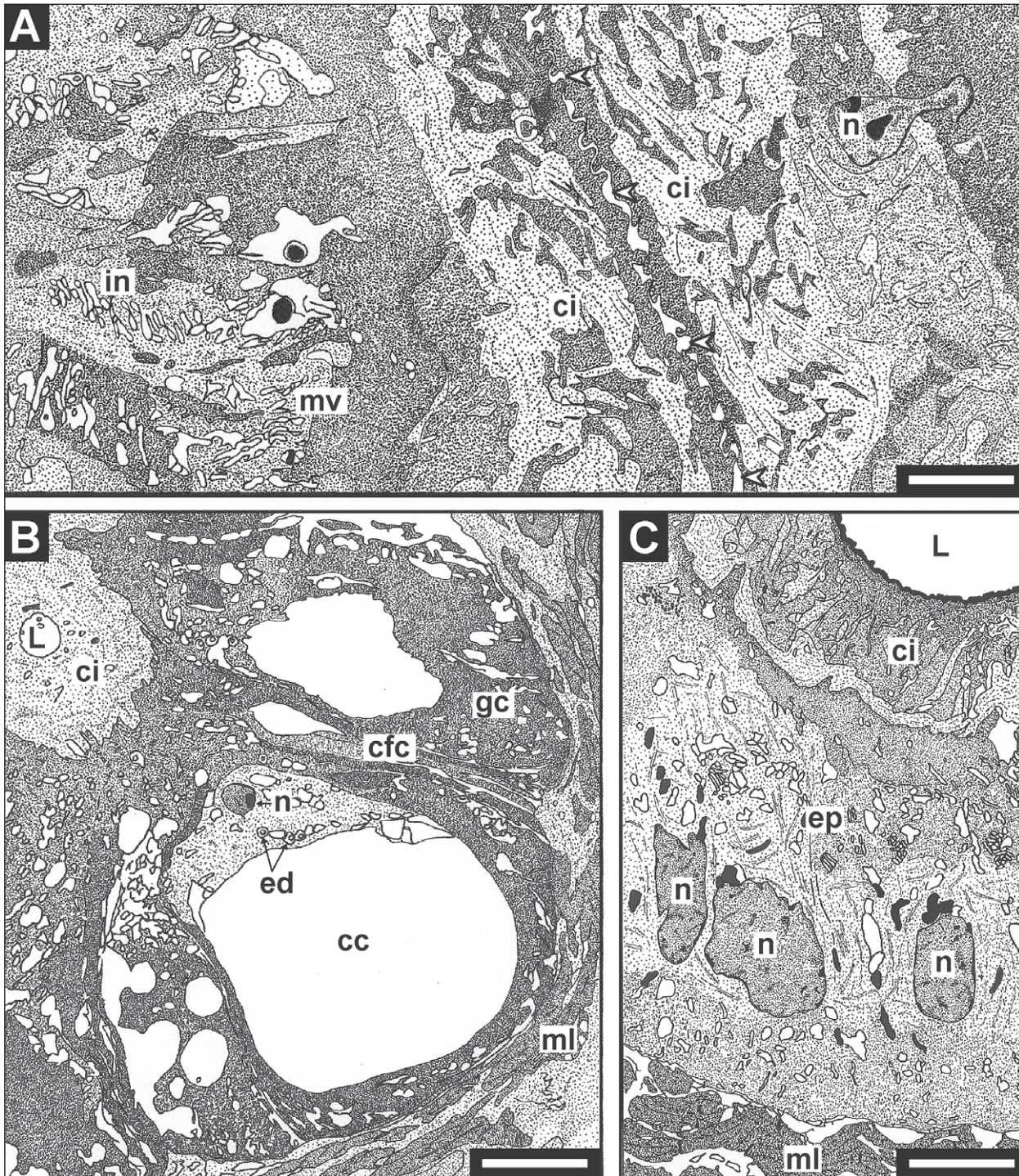


Fig. 2. Histology of the three regions of the ductus receptaculi (modified after ESSLER et al. 1992). **A:** Region I adjacent to the receptaculum seminis (bar: 2 μ m; arrowheads indicate the cleft-like lumen). **B:** Region II representing the middle part of the ductus receptaculi (bar: 10 μ m). **C:** Region III near the opening of the ductus into the genital chamber (bar: 5 μ m). Abbreviations: cc = central cavity, cfc = cuticula-forming cell, ci = cuticular intima, ed = efferent ductule, ep = epithelium, gc = glandular cell, in = interdigitations, L = lumen, ml = muscle layer, mv = microvilli, n = nucleus.

glandular cells (Fig. 2B), which release their secretions into an extracellular storing cavity that is connected with the small ductal lumen by an efferent ductule penetrating the intima. The mighty intima as well as the exocuticular layer lining the ductule are produced by specific cuticula-forming cells, which are interspersed between the glandular cells. In region III of the ductus receptaculi, the lumen is continuously widened except for the opening of the ductus on the terminal papilla, where, again, the diameter of the lumen

reaches a minimum. The cuticular intima of this part is characterized by longitudinal grooves and ridges (Fig. 2C) and the surrounding muscle coat by an enhanced thickness. The cuticular intima of the receptaculum seminis forms spines ('microtrichia') 5–10 μ m in length that are uniformly oriented towards the ductus and are assumed to support the transfer of the spermatozoa.

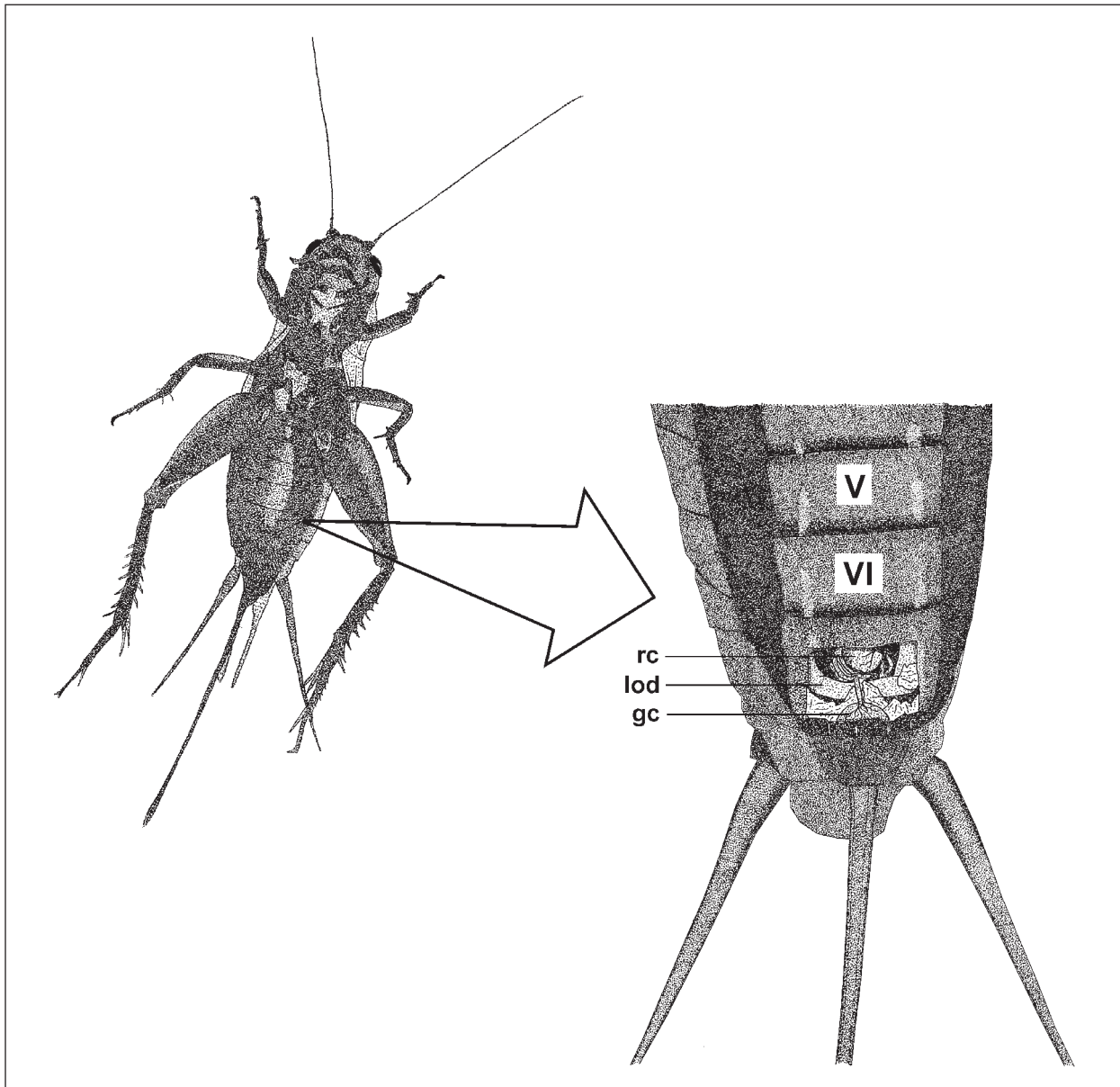


Fig. 3. Transparent window technique introduced by SUGAWARA & LOHER (1986) for in-vivo investigations of the motoric activity of the receptacular complexes in females of different reproductive conditions. Abbreviations: rc = receptacular complex, lod = lateral oviduct, gc = genital chamber.

Material and Methods

Animals. *T. commodus* was reared in a climate chamber at the Institute of Zoology, University of Salzburg. The animals were fed with fresh lettuce, dry food (Altromin 1222), and water, provided by wet cotton wool pads. While nymphs and subadults were kept in specific plastic boxes (L x W x H = 45 x 30 x 25 cm) filled with a 3 cm thick layer of peat soil, adults were separated by gender, keeping them in glass vessels with a volume of 5 litres. In the climate chamber, the following environmental conditions were selected: a constant temperature of 25°C, an atmospheric humidity of 60 %, and a photoperiod of 12 h.

In-vitro experiments. The receptacular complexes were isolated by opening the abdomen of decapitated females ventrally and were afterwards transferred into insect Ringer's solution. Organs of 15 females of each of the following

physiological conditions were studied at 20°C: (1) 10-day old virgins, (2) 10-day old mated females, (3) 11-day old females during oviposition, and (4) 14-day old females after oviposition. The Ringer's solution used for the experiments was composited as follows (LANGE 1993, modified): 150 mM NaCl, 10 mM KCl, 4 mM CaCl₂, 2 mM MgCl₂, 10 mM Hepes, 90 mM saccharose, and 5 mM trehalose. The pH of the solution was set to a constant value of 7.2 by adding either NaOH or HCl. After isolation each receptacular complex was observed for 30 minutes. Every 5 minutes myogenic contractions were counted over a period of 1 minute. Frequency data were evaluated statistically using MS Excel[®] (version 2002).

The influence of the various cations on the contraction frequency of the receptacular complex of virgins was tested by doubling and halving the concentrations of KCl, CaCl₂, and MgCl₂ in the Ringer's solution. Each modification of the solution was tested on 20 receptacular complexes to create

Tab. 1. In-vitro contraction frequencies (mean \pm standard deviation; N = 15) of receptacular complexes measured in 5-minute steps. Investigations were carried out at 20°C. Asterisks mark significant differences between adjacent times of measurement (*: p < 0.05; **: p < 0.01). In the rightmost column mean values over the whole time period of analysis were generated and compared between the different reproductive stages (crosses mark significant differences, +: p < 0.05; ++: p < 0.01).

time	1 minute	6 minutes	11 minutes	16 minutes	21 minutes	26 minutes	31 minutes	betw. groups
<i>virgins</i>								
mean	9.6	15.9*	18.6	19.5	19.4	18.2	18.9	17.3
stdev	6.2	10.3	10.8	8.2	7.1	6.4	6.7	8.6
<i>mat. females</i>								
mean	7.3	13.4**	14.9	14.1	15.1	15.4	16.2	13.8
stdev	4.5	6.4	5.7	6.2	7.3	5.8	4.6	6.0
<i>ovipos. females</i>								
mean	14.4	27.3**	26.2	24.1	23.4	22.6	22.2	22.8**
stdev	9.8	9.7	6.0	4.3	5.0	5.9	5.7	7.8
<i>after ovipos.</i>								
mean	4.8	12.9**	17.0	19.2	17.7	18.2	18.8	15.5+
stdev	2.6	8.7	8.5	6.8	6.7	7.9	7.9	8.1

Tab. 2. Development of receptacular contraction frequencies (mean \pm standard deviation; N = 20) with proceeding time in different insect Ringer's solutions. All measurements are based on virgin females and were carried out at 20°C. Asterisks mark significant differences between adjacent times of measurement (*: p < 0.05; **: p < 0.01). In the rightmost column mean values over the whole time period of analysis were generated and compared between the different Ringer's solutions (crosses mark significant differences, +: p < 0.05; ++: p < 0.01).

time	1 minute	6 minutes	11 minutes	16 minutes	21 minutes	26 minutes	31 minutes	betw. groups
<i>standard</i>								
mean	9.6	15.9*	18.6	19.5	19.4	18.2	18.9	17.3
stdev	6.2	10.3	10.8	8.2	7.1	6.4	6.7	8.6
<i>2 x MgCl₂</i>								
mean	14.3	25.3**	26.7	25.7	25.3	25.7	26.1	24.2+
stdev	2.7	3.6	2.1	1.6	2.5	1.8	1.3	3.6
<i>0.5 x MgCl₂</i>								
mean	10.0	20.9**	20.2	20.7	20.7	20.5	20.3	19.0+
stdev	2.1	6.8	3.7	3.5	3.2	3.4	3.3	5.4
<i>2 x KCl</i>								
mean	6.5	6.0	6.4	7.1	8.6	8.6	7.7	7.3**
stdev	3.5	3.7	3.8	3.6	4.6	4.8	4.3	4.6
<i>0.5 x KCl</i>								
mean	12.6	24.3**	22.7	21.4	18.7	17.1	18.1	19.3**
stdev	9.2	8.3	5.6	6.3	6.5	6.1	5.3	7.5
<i>2 x CaCl₂</i>								
mean	4.6	14.9**	17.8	19.5	19.0	18.8	19.3	16.5
stdev	2.8	6.2	4.5	4.2	5.8	6.1	5.5	6.6
<i>0.5 x CaCl₂</i>								
mean	13.1	18.6*	19.7	20.5	21.0	20.4	20.7	19.2
stdev	3.9	6.3	6.5	5.4	4.8	4.9	4.6	6.2

a meaningful basis for a statistical evaluation of the results.

In-vivo experiments. 4 \times 15 females of the same reproductive stages as used for the in-vitro studies were selected. Preparation of the animals was carried out according to SUGAWARA & LOHER (1986), narcotizing the crickets in a CO₂ stream and cutting a 4 mm² window into the 7th sternite (Fig. 3). For obtaining access to the receptacular com-

plex, the organ had to be unwrapped from fat body tissue. Preparation was finished by closing the window with a transparent film sealed with a mixture of wax and paraffine (1:1). Females were observed every day under the stereomicroscope by applying the same counting procedure as in the in-vitro experiments. The results were compared with the other observations.

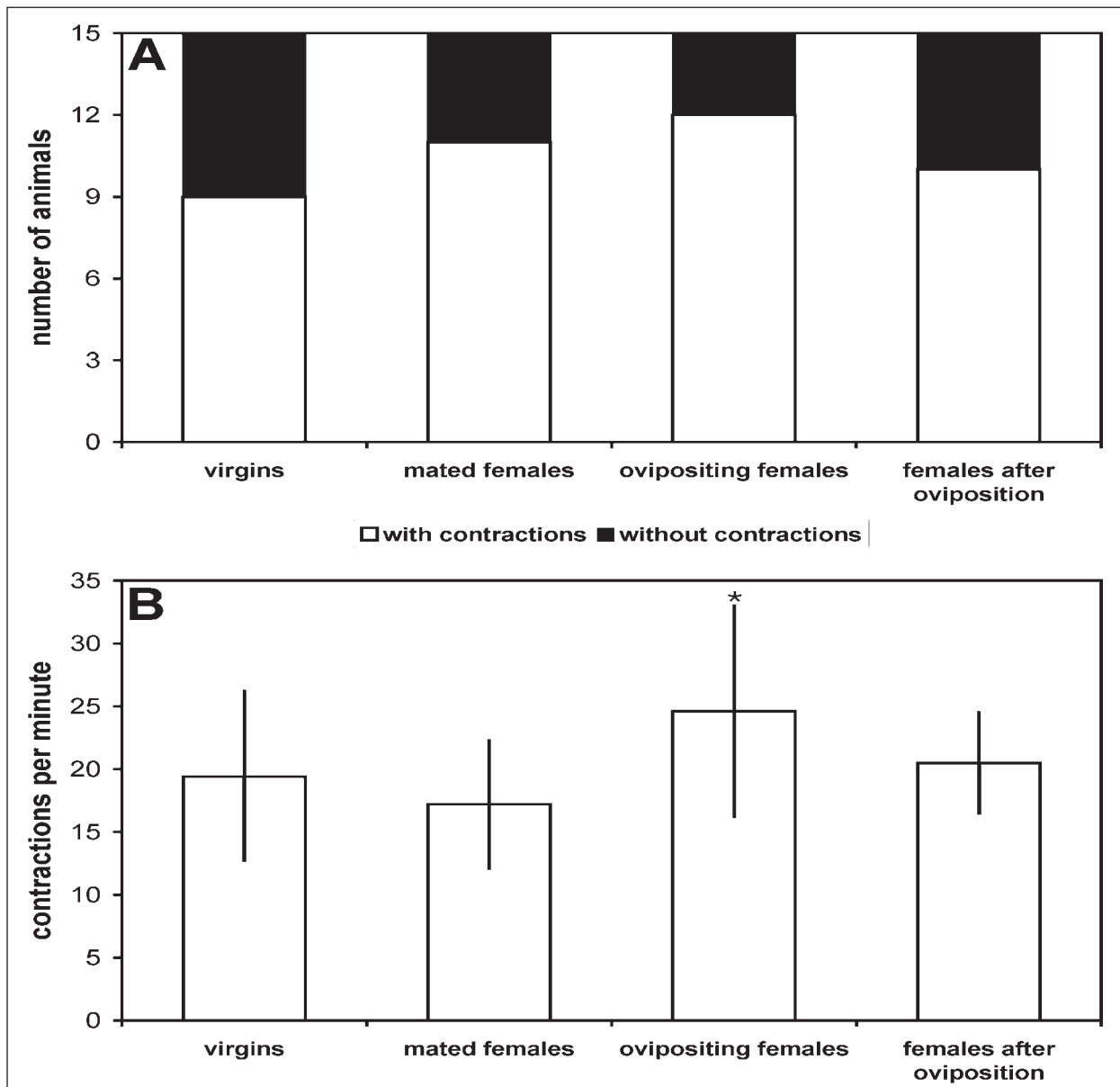


Fig. 4. Results of the in-vivo experiments carried out at 20°C according to the method outlined by SUGAWARA & LOHER (1986). **A:** Ratio between the number of active (white) and inactive (black) receptacular complexes in females of different reproductive stages. **B:** Contraction frequencies (mean \pm standard deviation) computed over the whole experimental time range (asterisk marks significant change, $p < 0.05$).

Results

In-vitro studies. Results on the contraction frequency of isolated receptacular complexes in insect Ringer's solution are summarized in Tab. 1. For virgins, 9.6 ± 6.2 contractions were counted in the first minute, and frequency increased within the first 10 minutes. In the 11th minute 18.6 ± 10.8 contractions were counted. This value was constant over the remaining period of investigation. The receptacular complexes from mated females made 7.3 ± 4.5 contractions per minute at the beginning, again reaching a plateau after about 10 minutes with mean frequency varying between 14 and 16. Spermathecae isolated from females during oviposition showed higher contraction frequencies already at the beginning of the testing period (14.4 ± 9.8), followed by a more rapid increase of the parameter than in the other groups. Maximum frequency was measured in the 6th minute (27.3 ± 9.7), but values

slightly decreased in the remaining time span. Receptacular complexes of females having finished oviposition initially exhibited a low contraction frequency (4.8 ± 2.6) and then permanently increased the frequency to 18.8 ± 7.9 contractions per minute.

Mean values of the contraction frequencies computed over the whole period of observation are compared in the right-most column of Tab. 1. While in virgins 17.3 ± 8.6 contractions per minute were measured, this frequency is decreased to 13.8 ± 6.0 in mated females, then grows significantly in ovipositing females with 22.8 ± 7.8 contractions per minute, and eventually drops to 15.5 ± 8.1 contractions per minute in females that had finished their oviposition activity.

Effects of modified Ringer's solution on the motoric activity of isolated receptacular complexes from virgin females are summarized in Tab. 2. Doubling of the $MgCl_2$ concentration resulted in an increase of the contraction frequency

with respect to the standard situation; maximum contraction activity was measured in the 11th minute with 25–27 contractions per minute. In Ringer's solution with the halved concentration of $MgCl_2$, behaviour of the isolated spermathecae was very similar to that in the standard solution, with mean frequencies of 20–21 contractions per minute. After doubling of the KCl concentration, average contraction frequency exhibited a significant decline, with 6.0–8.6 pulses per minute. Sigmoidal increase of the frequency and generation of a plateau were not observed in this specific case. In Ringer's solution with halved concentration of KCl, contraction frequency increased rather fast at the beginning, reaching a peak at minute 6 (24.3 ± 8.3 pulses per minute), and then continuously retarded. Changes of the $CaCl_2$ concentration did not significantly modify the motoric activity.

In the rightmost column of Tab. 2, mean pulse frequencies resulting from the various solutions were computed over the whole time span of investigation. Most remarkable are the noticeable increase of contraction frequency with doubled $MgCl_2$ concentration and the highly significant drop of the motoric activity with doubled KCl concentration.

In-vivo experiments. As documented in Fig. 4A, the ratio between active and non-active receptacular complexes varied among the observed groups of females, with a minimum in virgins (9:6) and a maximum in ovipositing crickets (12:3). Considering all groups (60 exemplars), 70 % of the studied females exhibited a valuable motoric activity of the receptaculum seminis and ductus receptaculi, whereas in 30 % such contractions could not be detected unambiguously.

Mean contraction frequencies computed over a maximum time range of 15 days (note: in most females, the window in the 7th sternite already became non-transparent after 5 days) are summarized in Fig. 4B. In each of the reproductive stages the in-vivo pulse frequency of the spermathecae behaved similar as in the in-vitro experiments, with lowest motoric activity being recognizable in mated females (17.3 ± 6.2 contractions per minute) and highest frequency occurring in ovipositing animals (24.6 ± 8.7 contractions per minute).

Discussion

As shown by the detailed studies of ESSLER et al. (1992), the receptacular complex in female *Teleogryllus commodus* includes a muscle coat of variable thickness, which surrounds the epithelium and generates peristaltic contractions running along the ductus receptaculi. In the present contribution, in-vitro experiments with the receptacular complex could demonstrate that the mean frequency of these contractions ranges from 15 to 25 pulses per minute. Furthermore, mean contraction frequencies of spermathecae vary among virgin females, mated females, ovipositing females, and females after oviposition, with highest frequency found in ovipositing crickets and lowest frequency in mated females. Consequently, enhanced myogenic activity of the receptacular complex at the time of egg fertilization and oviposition appears highly plausible. However, this does not seem to be a consequence of increased permeability of the muscle cell membranes for pulse-generating cations, which is underlined by the results obtained from the periodic measurements (Tab. 1). According to the generated data most remarkable differences of peristalsis may be recognized within the first 10 minutes. Thereafter, motoric activity behaves rather uniform among the different reproductive stages, thereby indicating a kind of adaptation of

the organs to the ionic concentration of the Ringer's solution. According to the results an additional value of such experiments could be the development of an improved Ringer's solution by eliminating adaptational effects due to slight changes in the cationic composition.

In-vitro experiments using modified Ringer's solutions could demonstrate that noticeable differences of the pulse frequency with respect to the standard Ringer's solution only occur in two cases. (1) If the KCl concentration is doubled, mean contraction frequency declines significantly to about 30 % of the standard value. (2) If the $MgCl_2$ concentration is doubled, contraction frequency undergoes a distinct increase. In contrast, changes of the concentration of Ca, which among other is responsible for the depolarization of the cell, producing a signal cascade, at the end of which the contraction of the fibre can be observed (RÜEGG 1988: 115 ff), do not seem to influence the motoric activity of the receptacular complex. Increase of the extracellular concentration of K inhibits the motoric activity of muscle cells, because the flow of K ions, necessary for the hyperpolarization of the fibre, is blocked by an osmotic and charge gradient arranged in the opposite direction. Concerning the increasing effect of Mg on the pulse frequency, the capability of this cation to generate small potentials was already investigated by FATT & KATZ (1952). Indeed, in insect muscle cells the ion seems to be characterized by the production of additional pulses.

The results from in-vivo measurements essentially agree with those obtained from the in-vitro investigations. Again, differences of peristalsis could be found among the four reproductive states, with highest contraction frequencies occurring in ovipositing females and lowest frequencies in mated crickets. Computed discrepancies are, however, not highly significant (Fig. 4B), therefore partly underlining the argument of SUGAWARA (1993), according to which changes of the sperm flow are exclusively caused by the strength and frequency of efferent nervous pulses. Otherwise, continuity of the flow can be only guaranteed by a myogenic activity, and changes of this activity by either specific transmitters (modulators) or modifications of local ion concentrations (see in-vitro experiments) should not be fully excluded in future investigations. Forthcoming studies, however, should focus to a greater extent on the neurogenic control of muscle activity and its behaviour in females at different time points of the reproductive cycle.

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