Motoric activity of the receptaculum seminis and ductus receptaculi in females of the house cricket
Acheta domesticus (L., 1758) (Insecta: Orthoptera)

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A b s t r a c t : With reference to an earlier study motoric activity of the receptacular complex of female *Acheta domesticus* was investigated. For this purpose, organs of animals belonging to four different reproductive stages (virgin, mated, ovipositing, after oviposition) were isolated and transferred to standard Ringer's solution. For an environmental temperature of 20°C contraction frequencies commonly ranged from 16 to 28 pulses per minute. Thereby, highest values were measured for ovipositing females, whereas lowest values were obtained for mated females. In a second experimental line receptacular complexes were isolated from virgins and studied with regard to the dependence of contraction frequency on the chemical composition of Ringer's solution. Therefor, concentrations of the cations Ca^{++}, Mg^{++} and K^+ were both doubled and halved. Increase of the Mg^{++} concentration by 100% resulted in a significant intensification of motoric activity, whereas doubled concentration of K^+ caused a considerable decline of peristalsis. All other changes of the chemical composition did not produce any noticeable effects. Differences of motoric activity of the receptacular complex among the various reproductive stages are submitted to an intense debate.

K e y   w o r d s : Receptacular complex, myogenic peristalsis, motoric activity, Ringer's solution, *Acheta domesticus*, cricket, Orthoptera.

Introduction

In the reproductive tract of numerous female insects a variable number of receptacula seminis or spermathecae can be observed. These organs serve for the storage of spermatozoa between the processes of copulation and egg fertilization. In Ectognatha an unpaired receptaculum seminis is formed from the ventromedian invagination at the hind margin of the eighth abdominal segment (SNODGRASS 1937, KLASS 2003, STURM 2005, 2008, 2009). The position of the organ chiefly depends on the ontogenetic development of the gonoduct. With regard to the Ensifera the receptaculum is connected with the genital chamber over the so-called ductus receptaculi or spermathecal duct. Through this tubular structure spermatozoa are transported on two occasions: (1) when the female is impregnated; (2) when the eggs become fertilized (SNODGRASS 1937). In some insects the receptacular complex is completed by a receptacular gland, whose secretions are considered to act as nutritious substances for the sperm (CHAPMAN 1998).

Concerning the morphology of the receptacular complex considerable variations can be documented among the insects and even among the Orthoptera (HEBERDEY 1931, MATSUDA 1976). Within the family of the Gryllidae the receptaculum adopts spherical to elliptical shape ($\varnothing \sim 1$ mm), whereas the ductus receptaculi represents an up to 25 mm

long and strongly convoluted tube (⌀: 0.1-0.2 mm). Since parts of the ductal epithelium are glandular themselves, a receptacular gland does not exist (Fig. 1; DALLAI & MELIS 1966, POHLHAMMER et al. 1975, ESSLER et al. 1992, STURM 2005, 2008). Regarding its histology the receptaculum and its duct generally consist of an inner cuticular layer, one layer of epithelial cells, a basement membrane and an outer muscle layer with variable thickness (GILLOTT 1988). Glandular cells show a central cavity which serves for the temporary storage of the secretion and is connected with the lumen by an efferent ductule (GILLOTT 1998). In crickets such as *Teleogryllus commodus* WALKER, 1869 and *Acheta domesticus* (LINNAEUS, 1758) the ductus receptaculi can be subdivided into three regions with great differences concerning epithelial organization and diameter of the lumen (Fig. 1; ESSLER et al. 1992, STURM 2005, 2008, 2009).

According to our current knowledge transport of spermatozoa through the ductus of crickets is mainly accomplished by peristaltic contractions of the ductus-associated muscle coat, whereas active motion of single sperm cells along a chemogradient may be regarded as insignificant so far (POHLHAMMER 1978, ESSLER et al. 1992, STURM 2005). As documented for the exemplary case of *T. commodus*, the receptacular complex is innervated by the fourth segmental nerve of the terminal abdominal ganglion (SUGAWARA 1993) which recommends a neural control of sperm release. It is hypothesized that movement of the spermatozoa is exclusively caused by motoric activity of the receptacular complex, whereby spontaneous myogenic peristalsis can be distinguished from strong twitches evoked by the burst of efferent pulses. Whilst the first process produces a slow continuous flow of sperm, the second one causes the squeezing of spermatozoa into the genital chamber for egg fertilization. Restriction between two nervous pulses depends on the physiological state of the animal and is about 15 times longer in virgins than in mated females (POHLHAMMER 1978, OKELO 1979, SUGAWARA 1993).

In the present contribution myogenic peristalsis of the receptacular complex is measured in vitro, thereby pursuing two main goals: (1) detection of possible correlations between contraction frequency and the physiological state of the females; (2) studying the effects of different cation concentrations of the Ringer's solution on myogenic peristalsis.

Materials and Methods

Animals

A. domesticus was reared in a specific climate chamber at the former Institute of Zoology (now Department of Organismic Biology) in the University of Salzburg. Nymphs and subadults were kept in plastic boxes (L x W x H: 45 x 30 x 25 cm) which were filled with a 3 cm thick layer of peat soil, egg cartons serving as shelter and diverse nutritive substances including fresh lettuce, dry food (Altromin 1222) and water. After imaginal molt the adults were separated by gender and transferred into glass vessels with a volume of 5 liters. The vessels were filled with crumpled sheets of white paper in order to offer some shelter to the animals. In addition, the crickets were supplied with the same food already used for the nymphs and subadults. Rearing of the animals took place under the following standard conditions: constant temperature of 25°C, atmospheric humidity of 60%, photoperiod of 12 h (POHLHAMMER et al. 1975, POHLHAMMER 1978, ESSLER et al. 1992, STURM 1999, 2000, 2002a, 2002b, 2003a, 2003b, 2005, 2008, 2009, 2010, 2011a, 2011b, 2012, 2013, 2014, 2016a, 2016b, STURM & POHLHAMMER 2000).
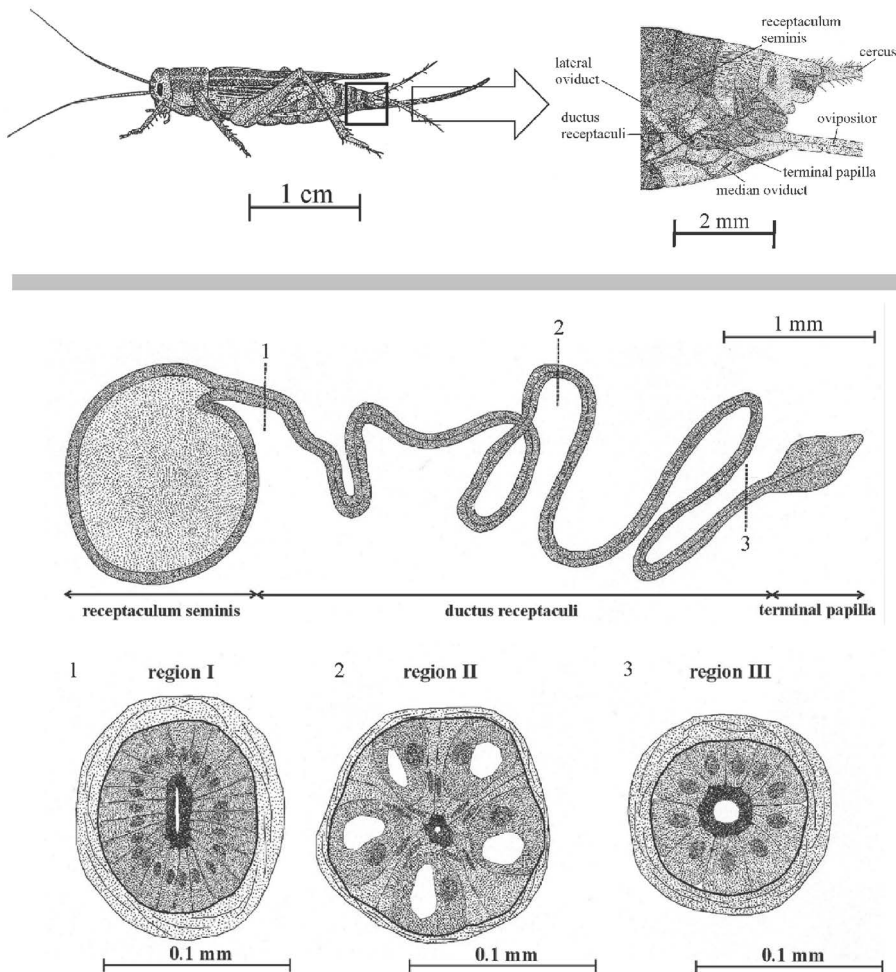
Fig. 1: Position, morphology and histology of the receptacular complex in crickets. Whilst the receptaculum seminis is characterized by uniform histological organization, the ductus receptaculi can be subdivided in at least three regions with different cellular structures and arrangements (ESSLER et al 1992, STURM 2005, 2007, 2008).

In-vitro experiments

In a first step the receptacular complexes were removed from females of *A. domesticus* by opening the abdomen of decapitated animals on the ventral side and isolating the organs. The objects were subsequently transferred into insect Ringer's solution (STURM 2000). In total, organs of 25 females belonging to each of the following physiological stages were studied at an environmental temperature of 20°C: (1) virgins with an adult age of 10 days, (2) mated females with an adult age of 10 days, (3) ovipositing females aged 11 days, (4)

females with an adult age of 14 days that had already finished one oviposition cycle. The Ringer's solution used for the experimental lines was composed as follows (LANGE 1993, modified): 150 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Hepes, 90 mM saccharose and 5 mM trehalose. By addition of NaOH or HCl the pH of the produced solution was set to a constant value of 7.2. After its isolation and removal from the female each receptacular complex was observed for 30 minutes. Thereby, every 5 minutes myogenic contractions were counted over a period of 1 minute. Obtained peristalsis data were statistically evaluated using the computer program MS EXCEL$^©$ (version 2010).

In order to decode the possible effect of various cations on the contraction frequency of the receptacular complex, organs of virgins (age: 10 days) were subjected to another experimental line. Concretely speaking, concentrations of KCl, $CaCl_2$ and $MgCl_2$ in the Ringer's solution were both doubled and halved, respectively. Each modification of the solution was tested on 20 receptacular complexes, thereby using the same counting protocol and statistical support as stated above.
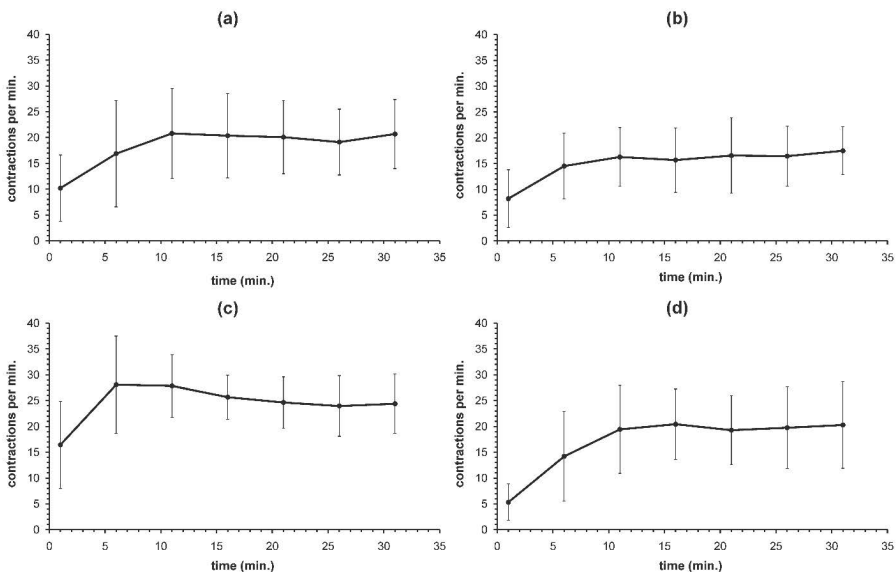
Fig. 2: Graphs depicting the motoric activity of the receptacular complex (mean ± standard deviation) measured in 5-minutes intervals among female house crickets belonging to different physiological states: (**a**) virgins, (**b**) mated females, (**c**) females during oviposition, (**d**) females after oviposition.

Results

## In-vitro experiments with standard Ringer's solution

Results of the experiments dealing with the contraction frequencies of isolated receptacular complexes originating from female house crickets with different physiological conditions are summarized graphically in Fig. 2. As already outlined in the methods section,

counting procedure was carried out in 5-minutes intervals, and organs were transferred into standard Ringer's solution. Regarding virgins an initial value of 10.2 ± 7.0 contractions per minute was measured which increased to 20.8 ± 7.8 contractions per minute in the 11th minute. This value remained nearly constant during the rest of the counting period. Receptacular complexes isolated from mated females started with 8.2 ± 6.7 contractions per minute, with respective motoric activity reaching its plateau after about ten minutes. Final peristalsis ranged from 15.5 to 17.5 contractions per minute. Most interesting experimental results were obtained for spermathecae dissected from females that were about to oviposit their eggs into the substrate. For this physiological group high contraction frequencies of the organs could be observed already at the beginning of the testing period (16.4 ± 10.1 contractions per minute). This initial value was followed by another strong increase of the motoric activity, whereby maximal frequency could be detected in the 6th minute (28.1 ± 9.3 contractions per minute). During the remaining time span peristalsis was again subject to a slight mitigation. Receptacular complexes of female house crickets, which had already finished one cycle of oviposition, were characterized by an initial motoric activity amounting to 5.3 ± 3.5 contractions per minute. In the further sequel, myogenic peristalsis continuously increased to 20.3 ± 9.6 contractions per minute.

Mean values of the contraction frequencies computed over the entire period of observation are graphically compared in Fig. 3a. As clearly recognizable from the respective diagram, receptacular complexes isolated from virgins averagely contract 18.3 ± 8.4 times per minute, whereas the organs of mated females are distinguished by 15.0 ± 6.5 contractions per minute. This corresponds to a decline of about 20% with respect to the unmated animals. Spermathecae dissected from ovipositing females contract 24.4 ± 8.3 times per minute which represents a highly significant deviation ($p < 0.01$) from the values measured for virgins and mated females. Within the last group (females after oviposition) an average motoric activity of the receptacular complex of 17.0 ± 8.7 contractions per minute was detected which means a highly significant decrease compared to ovipositing crickets.

Effect of modified Ringer's solutions on the motoric activity of the receptacular complex

Influences of modified cation concentrations in the Ringer's solution on the motoric activity of receptacular complexes isolated from virgins are graphically summarized in Fig. 3b. Increase of the $MgCl_2$ concentration by 100% resulted in an enhancement of the standard mean value noted above (18.3 ± 8.4 contractions per minute) to 24.9 ± 8.9 contractions per minute which corresponds to a highly significant deviation ($p < 0.01$). Reduction of the $MgCl_2$ concentration by 50% lead to a motoric activity of the isolated spermathecae being similar to that in the standard solution (19.7 ± 7.4 contractions per minute). By doubling the KCl concentration myogenic peristalsis of the organs was subject to a highly significant decline, with 7.8 ± 2.6 pulses per minute, whereas diminution of the KCl content by 50% produced a contraction frequency of 19.6 ± 7.5 pulses per minute. Modifications of the $CaCl_2$ concentration did not considerably change the motoric activity of the receptacular complexes: Increase of the $CaCl_2$ content in the Ringer's solution by 100% resulted in 17.0 ± 9.6 pulses per minute, whilst reduction of the $CaCl_2$ content by 50% produced 19.5 ± 6.2 pulses per minute.

(a)

(b)
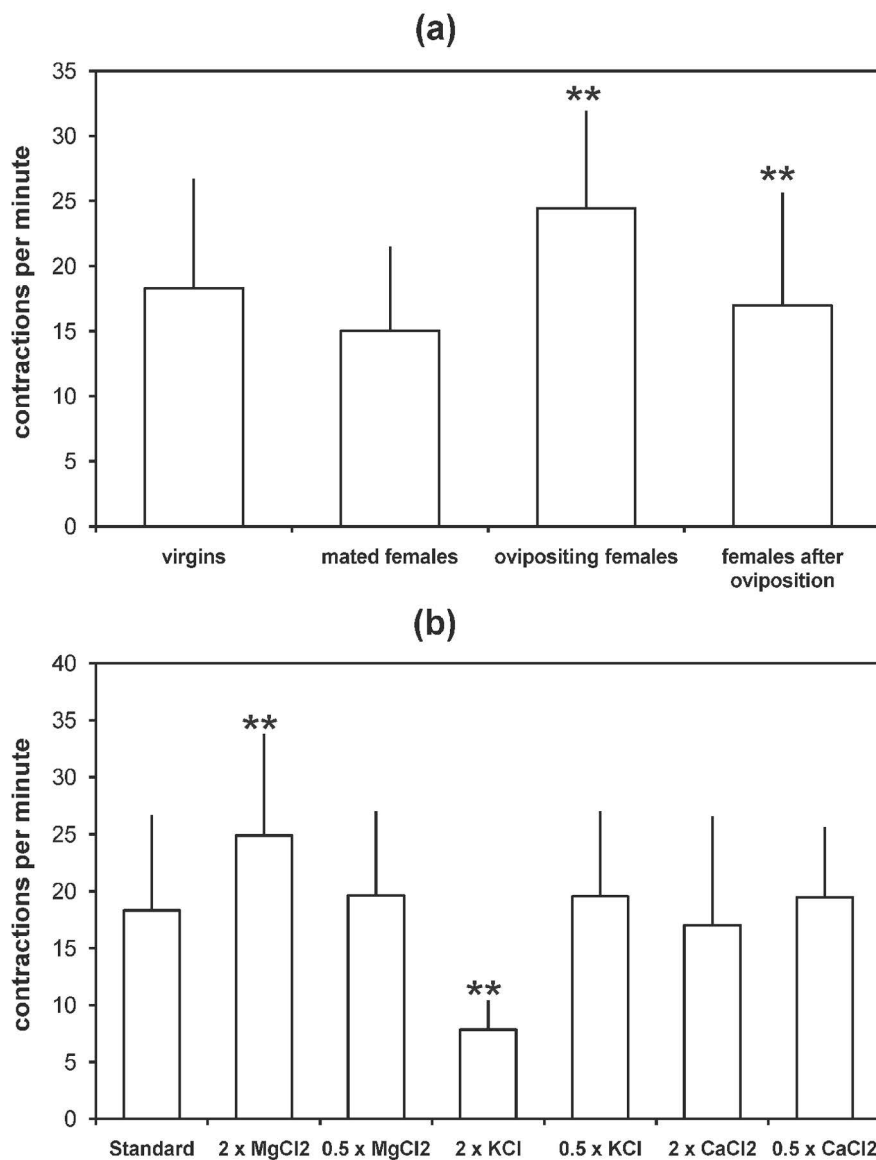
Fig. 3: (**a**) Comparison of mean values of contraction frequencies of the receptacular complexes between the different physiological groups. Double asterisks indicate highly significant differences between neighboring columns ($p < 0.01$). (**b**) Effect of variable cation concentrations in the Ringer's solution on the contraction frequency of the receptacular complexes. Double asterisks mark highly significant deviation from the standard value (left column) measured for virgins.

Discussion

Detailed histological studies of ESSLER et al. (1992) as well as STURM (2005, 2008) yielded evidence that the receptacular complex of field crickets such as *T. commodus* or *A. domesticus* includes a muscle coat of variable thickness. This layer represents the outer-most structural component of the organ and is responsible for peristaltic contractions running along the ductus receptaculi. For the exemplary case of the house cricket the mean frequency of receptacular contractions ranges from 16 to 28 pulses per minute and thus largely corresponds with the value measured for the Australian field cricket (15 to 25 pulses; STURM 2005). Based on comprehensive in-vitro experiments it could be addition-ally demonstrated that mean contraction frequencies of spermathecae differ between virgins, mated females, ovipositing females and females having finished an oviposition cycle. Highest frequency could be measured in ovipositing crickets, whereas lowest frequency was found in mated females. This means that maximal myogenic activity of the receptacular complex occurs at the time point of egg fertilization and oviposition.

As exhibited by the graphs of Fig. 2, most significant differences of myogenic peristalsis may be observed within the first 10 minutes of the experiment. After this initial phase motoric activity is characterized by uniform behavior among the different physiological stages. Hence, a kind of adaptation of the organs to the ionic concentration of the Ringer's solution has occurred (STURM 2005). Based on these experimental results a modified Ringer's solution could be produced, where such adaptational effects are eliminated from the very beginning, so that more appropriate comparisons of single experimental lines can be made.

According to the results obtained from in-vitro experiments with modified Ringer's solu-tions considerable differences of the pulse frequency with respect to the standard Ringer's solution can be only observed in two cases: (1) Doubling of the KCl concentration causes a decline of the contraction frequency to about 30% of the standard value; (2) Doubling of the $MgCl_2$ concentration, on the other hand, results in a significant increase of the contraction frequency. Any changes of the $CaCl_2$ concentration, however, do not seem to perform a valuable effect on the motoric activity of the receptacular complex, although Ca^{++} plays an important role with regard to the depolarization of the cell, the initiation of an intracellular signal cascade and the contraction of the fiber (RÜEGG 1988). If the extracellular concentration of K^+ is remarkably enhanced, motoric activity of the muscle fiber becomes inhibited, because the flow of this cation into the cell is blocked by an osmotic and charge gradient arranged in the opposite direction. If the extracellular concentration of Mg^{++} is considerably increased, the cation generates small electric potentials which may produce additional pulses (FATT & KATZ 1952). Concerning this point future studies should increase our understanding of molecular processes.

Zusammenfassung

Motorische Aktivität von Receptaculum seminis und Ductus receptaculi in Weibchen des Heimchens *Acheta domesticus* (L., 1758) (Insecta: Orthoptera). In Anlehnung an eine frühere Studie wurde die motorische Aktivität des Receptacularkomplexes des weiblichen Heimchens *Acheta domesticus* untersucht. Zu diesem Zweck wurden die betreffenden Organe aus Tieren, welche zu vier verschie-denen Reproduktionsstadien zählen (virgine, verpaarte, Eier legende Tiere, nach der Eiablage), isoliert und in Standard-Ringerlösung transferiert. Bei einer Umgebungstemperatur von 20°C

reichten die Kontraktionsfrequenzen in der Regel von 16 bis 28 Impulse pro Minute. Dabei wurden die höchsten Werte für Weibchen gemessen, welche sich gerade bei der Eiablage befanden, wohingegen die niedrigsten Werte für verpaarte Weibchen erhalten wurden. In einer zweiten Serie von Experimenten wurden die Organe lediglich aus virginen Weibchen entnommen und hinsichtlich der Abhängigkeit der Kontraktionsfrequenz von der chemischen Zusammensetzung der Ringerlösung studiert. Dazu wurden die Konzentrationen der drei Kationen Ca^{++}, Mg^{++} und K^+ verdoppelt und halbiert. Eine 100%-ige Erhöhung der Mg^{++}-Konzentration hatte eine signifikante Steigerung der motorischen Aktivität zur Folge, wohingegen eine entsprechende Erhöhung der K^+-Konzentration zu einer signifikanten Reduktion der Peristalsis führte.Alle anderen Veränderungen der chemischen Zusammensetzung brachten keine nennenswerten Effekte hervor. Unterschiede der motorischen Aktivität des Receptacularkomplexes zwischen den einzelnen Reproduktionsstadien werden einer ausführlichen Debatte zugeführt.

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