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**Dependence of the sperm number on the adult age of the male
black field cricket *Teleogryllus commodus* WALKER
(Insecta: Orthoptera)**

Robert STURM

A b s t r a c t : The number of spermatozoa stored in the spermatophore of *Teleogryllus commodus* was studied with regard to its dependence on the adult age of the males. For this purpose respective sperm capsules were isolated from animals belonging to four different age groups: 1-10 d, 11-20 d, 21-30 d, 31-40 d. Counting of the spermatozoa was carried out by using stereological methods introduced in previous studies. Thereby, from each age group 20 spermatophores were subjected to the quantitative investigation. In general, the number of filled sperm capsules declines from 94% in the youngest age group to 42% in the oldest one. Concerning the sperm number per spermatophore a reduction from $152,000 \pm 67,000$ germ cells determined for the youngest group to $73,000 \pm 59,000$ cells detected for the oldest group can be observed. The results underline a valuable age-dependence of male fertility which has been already reported for female crickets.

K e y w o r d s : Spermatophore, sperm number, stereology, *Teleogryllus commodus*, cricket, Orthoptera.

Introduction

Within the group of field crickets transfer of spermatozoa from the male to the female is carried out with the help of a spermatophore which is fixed at the female genital aperture during copulation (MANN 1984, STURM 2003a, 2003b, 2011a, 2014). By production of pressure within the sperm capsule the spermatozoa are released from the ampulla and transported through a long tube which had been introduced in the female ductus receptaculi before. Supported by myogenic peristalsis taking place along the duct, the germ cells finally reach the receptaculum seminis or spermatheca, where they are stored for an indeterminate period of time (ESSLER et al. 1992, STURM 2005). During fertilization of the eggs the spermatozoa are transferred back to the terminal papilla (= end piece of the ductus receptaculi), where they get in contact with the oocytes being moved through the median oviduct like on an assembly line (POHLHAMMER 1978, CHAPMAN 1998, STURM 2000, STURM & POHLHAMMER 2000).

Previous studies yielded evidence that the number of spermatozoa per spermatophore is subject to both inter- and intraspecific variations (STURM 2003a, 2003b, 2011a, 2014). Among different cricket families and species the number of sperm cells transmitted during copulation of the female ranges from few thousands to few millions, whereby in some cases a correlation of this amount with female fecundity can be observed (WHITMAN 2008, STURM 2014, 2016). Within a given cricket species size of the ampulla and its capacity for the absorption of sperm cells commonly increases with the body size of the male, so that

larger crickets are able to transfer more spermatozoa than small crickets (STURM 2011a, 2014). It has to be noted in this context that the number of sperm per spermatophore produced in a given size class of males is already subject to high variations which complicates the drawing of definitive conclusions. Current studies could demonstrate that also female crickets exhibit a dependence of their egg production capacity on body size, with larger individuals releasing higher numbers of eggs (WHITMAN 2008, STURM 2016a).

Previous studies on the daily and total fecundity of various female field crickets came to the result that egg-laying activity and development of mature oocytes in the ovaries represent two physiological parameters which strongly depend on the animals' ages (STURM 1999, 2008, 2011b). Therefore, highest daily fecundity can be registered within the first 10 to 15 days of the adult life span. After that phase of increased oviposition activity, fecundity becomes continuously declined towards to female's death. Similar studies for the male are still very sparse, so that they are to move in the center of the present contribution. Concretely speaking, the age-dependent development of sperm number per spermatophore was investigated for the black field cricket *Teleogryllus commodus* WALKER 1869 which had already stood in the focus of numerous previous studies (Fig. 1; STURM 2003a, 2003b, 2011a, 2011b, 2014).

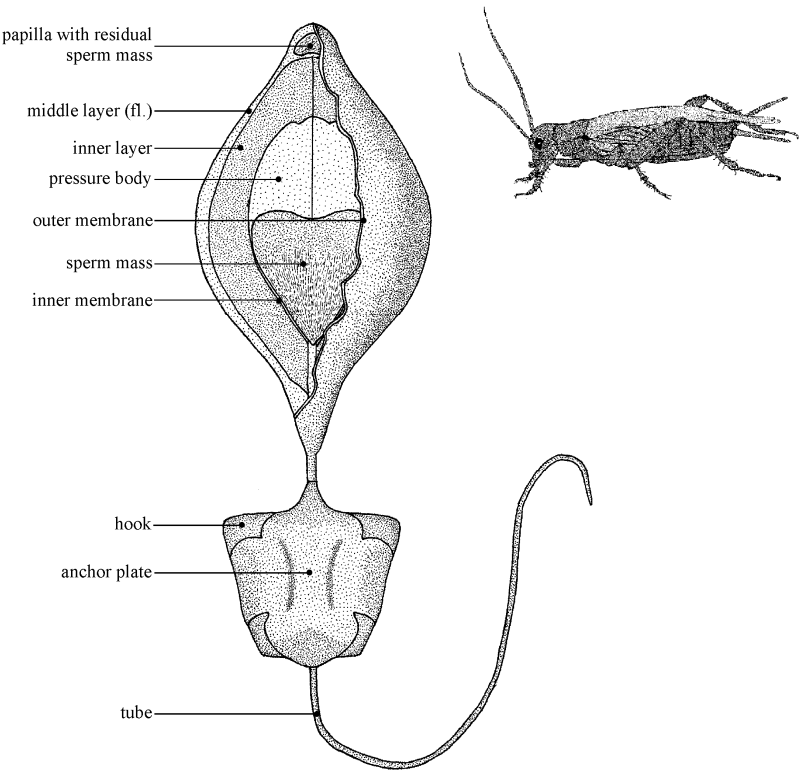


Fig. 1: Shape and morphology of the spermatophore (total length: ca. 5 mm) separated from males of the black field cricket *Teleogryllus commodus*.

Materials and Methods

Animals

The black field cricket was reared in a climate chamber situated at the former Institute of Zoology (= Department of Organismic Biology), University of Salzburg. Farming of the animals took place under constant thermal conditions (25°C), whereby a photoperiod of 12 h and a relative humidity of ca. 60% were adjusted. Immediately after the hatching process nymphs were kept in so-called baby boxes (20 x 10 x 5 cm), where they had to remain until reaching the fifth to sixth nymphal instar. The animals were fed on fresh lettuce, fine-grained oat flakes and water. Older nymphs were kept in larger plastic boxes (50 x 30 x 20 cm) which were filled with a 3 cm thick layer of peat soil. Additionally, the boxes were equipped with egg cartons serving as shelter for the animals. The young crickets were fed on fresh lettuce, standard diet for laboratory animals (Altromin 1222) and water supplied in form of moistened cotton pads. Immediately after their imaginal molt the animals were separated by gender and transferred to glass vessels with a uniform volume of 5 L, respectively. The vessels were equipped with crumpled sheets of paper serving as shelter and food (lettuce, standard diet, water) which was renewed each day (STURM 1999, 2000, 2002a, 2002b, 2003a, 2003b, 2011b, 2016b). Age of the male individuals was documented precisely, whereby four different adult age groups were defined: 1-10 d, 11-20 d, 21-30 d, 31-40 d. Males of each age category were kept in separate glass vessels in order to minimize intraspecific competition.

Isolation of the spermatophore and sperm counting

For spermatophore production males of each age category were brought into the direct vicinity of females. Immediately before starting the copulation process male crickets were separated from the females and anesthetized in a stream of CO₂. Sperm capsules were carefully removed from the genital chamber with the help of special feather tweezers. Afterwards the structures underwent a process of fixation in 70% ethanol. The ampulla of the fixed spermatophores was subjected to a transversal section directly above the sperm mass. Quantification of the spermatozoa being situated in the ampulla was conducted in the scanning electron microscope under adjustment of standard conditions (3-4 nA, 15-20 kV). Sperm number was estimated by application of stereological methods introduced by STURM (2003b, 2011a, 2014). Thereby, the diameter of a single sperm cell is compared with the average diameter of the sperm mass. From each age category 20 spermatophores were investigated in the way noted above. Results were evaluated statistically by using standard calculation software (Microsoft EXCEL®).

Results

The first aspect covered by the study dealt with the filling state of the spermatophore. Thereby, a simple differentiation between 'filled' and 'unfilled' sperm capsules was carried out. As illustrated in Fig. 2a, the proportion between filled and unfilled spermatophores is subject to a continuous change with increasing age of the males. Concretely speaking, crickets belonging to the first age group (1-10 d) produce 94% filled and 6% unfilled sperm capsules. Within the following age category (11-20 d) 78% of the spermatophores contain germ cells, whereas males aging 21-30 d are characterized by 56% of filled sperm capsules.

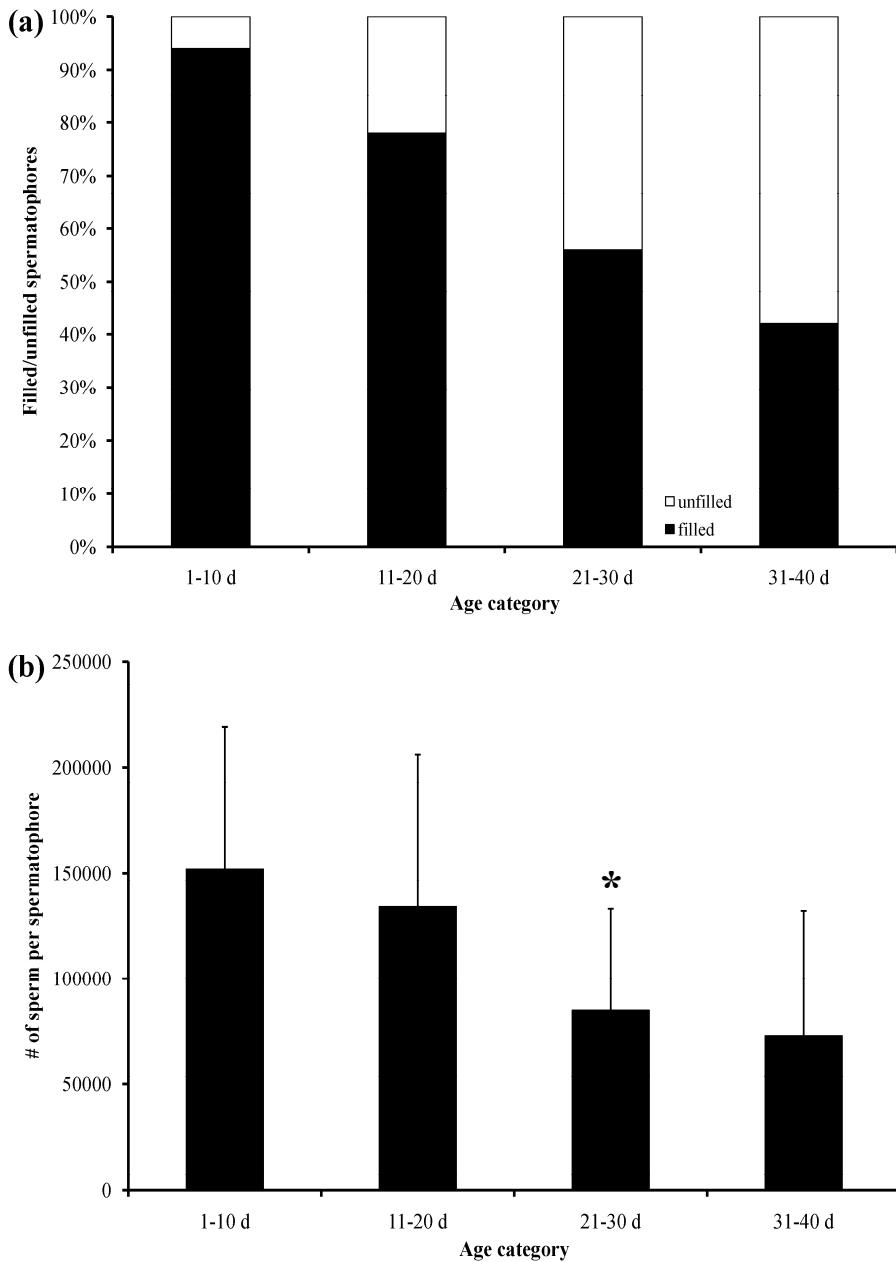


Fig. 2: Results of sperm quantification of male crickets belonging to different age categories: (a) Age-dependence of the percentage of filled/unfilled spermatophores (N = 80); (b) Age-dependence of the number of sperm per spermatophore (N = 80). The asterisk indicates a significant difference ($p < 0.05$) between adjacent mean values.

Within the oldest age group (31-40 d) only 42% of the produced spermatophores include spermatozoa.

The more important aspect of the study concerns the concrete number of sperm cells stored in the spermatophores of male crickets with different ages (Fig. 2b). Within the youngest age group filled sperm capsules contain $152,000 \pm 67,000$ cells. Males of the second age category produce spermatophores with $134,000 \pm 72,000$ cells in the ampullae. For the third age category $85,000 \pm 48,000$ sperm cells per capsule could be counted which means a significant difference ($p < 0.05$) with respect to the preceding group. Within the oldest age category male crickets produce spermatophores including $73,000 \pm 59,000$ cells which corresponds to 46% of the cell number evaluated for the youngest animals.

Discussion

The results of this contribution largely confirm the hypothesis, according to which fertility (expressed by the number of produced sperm cells) of the male black field cricket becomes reduced with proceeding adult age. Based on a stereological sperm counting procedure (STURM 2003a, 2011a, 2014) it could be found that the amount of spermatozoa contained in the ampulla experiences a reduction of more than 50% from youngest to oldest animals. Therefore, adult males pass through a similar fertility development as adult females, where egg production is subject to a considerable decline from early to late stages of the adult life span (STURM 2008, 2010, 2011b, 2016a).

Concerning possible reasons for the decline of fertility between different age groups several theories can be formulated. Previous studies could demonstrate that food consumption is subject to a continuous decrease with proceeding adult age (HOFFMANN 1985, GEWECKE 1995), so that the energy supplied for reproductive processes (spermatogenesis, copulation, spermatophore production, sperm transfer) also experiences a permanent reduction. During late stages of the adult phase animals are seized by a kind of senescence, with all physiological processes being reduced to a minimum. Another reason for the phenomena reported above might be a decreasing competitiveness of older males with respect to their younger combatants. This again results in a massive drawback of older animals with regard to the utilization of food resources (WHITMAN 2008, STURM 2008, 2011b).

It has to be noted in this context that sperm counting according to the previously introduced microscopic and stereological techniques bears rather high inaccuracies, because almost perfect orientation of the spermatozoa within the ampulla has to be presupposed for a successful application of the methods (KHALIFA 1949, STURM 2003a, 2003b). Although the sperm flagella are characterized by ideal parallel alignment, this arrangement can be partly destroyed by the fixation and cutting process, so that counting work becomes dramatically complicated. In future studies confirmation of the results presented here is tried to be found. Additionally, further cricket species such as the house cricket or the Mediterranean field cricket will be included in the investigation.

Zusammenfassung

Abhängigkeit der Spermienzahl vom Adultalter der männlichen australischen Feldgrille *Teleogryllus commodus* WALKER (Insecta: Orthoptera). Die Anzahl der Spermatozoen, welche in der Spermatophore von *Teleogryllus commodus* gelagert sind, wurde in Bezug auf ihre eventuelle Abhängigkeit

vom Adultalter der Männchen untersucht. Zu diesem Zweck wurden entsprechende Spermienkapseln aus Tieren isoliert, die vier verschiedenen Altersgruppen zugeordnet werden können: 1-10 d, 11-20 d, 21-30 d, 31-40 d. Die Zählung der Spermatozoen erfolgte unter Verwendung stereologischer Methoden, welche bereits in früheren Studien zur Vorstellung gelangten. Dabei wurden insgesamt 20 Spermatophoren jeder Altersgruppe der quantitativen Untersuchung zugeführt. Generell nimmt die Anzahl der mit Keimzellen befüllten Spermienkapseln von 94 % in der jüngsten Altersgruppe auf 42 % in der ältesten ab. Hinsichtlich der Spermienzahl pro Spermatophore kann eine Reduktion von 152.000 ± 67.000 Zellen in der jüngsten Gruppe auf 73.000 ± 59.000 Zellen in der ältesten Gruppe beobachtet werden. Die Resultate unterstreichen eine verwertbare Altersabhängigkeit der männlichen Fertilität, welche schon zuvor für weibliche Grillen festgestellt werden konnte.

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