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Size of corpora allata, haemolymph juvenile hormone-III titre, and growth of terminal and subterminal oocytes throughout the reproductive period of the oedipodine grasshopper *Aiolopus thalassinus* under laboratory conditions (Orthopteroidea Caelifera Acrididae)

Abstract - In a population of *Aiolopus thalassinus* (Fabricius, 1781), originated from East Africa, (Kenya), and reared successfully under controlled laboratory conditions at Hannover, the effects of factors controlling reproduction were investigated. The reproductive parameters, like the number of ovariols, period of preoviposition, number of egg pods per female, as well as the changes in the volume of corpora allata (CA), the juvenile hormone (JH) titre of the haemolymph, and the growth of the terminal (t) and subterminal oocytes were studied throughout the egg-laying period. For the data of each reproductive parameter, 58-103 females were used. Up to 22 consecutive gonadotropic cycles per female were evaluated. Neither a correlation was found between the volume of CA and the age of the adult females, nor between these parameters and the size of the t-oocytes. The JH-III titre of the haemolymph was low (< 0.2 pmol/10 µl) during previtellogenesis, increasing after that to a maximum of 2.5 pmol/10 µl and declining drastically with the maturation of the oocytes and the onset of oviposition. After the first egg deposition, the JH-III titre of the haemolymph increased again with the growth of the oocytes in the gonadotropic cycles, but did not start from that low level found during previtellogenesis, and did not show the high values recorded before the first oviposition, when the volume of t-oocytes increased. The growth of the subterminated oocytes often started before deposition of the t-oocytes. The JH-III titre of these individuals did not drastically decline. The simultaneous ripening of two generations of oocytes might account for the short interval of only one or two days passing between two ovipositions. Before the termination of the 2nd gonadotropic cycle, the JH-III titre declined again, but no clear cyclisation of the JH-III titre was found in the haemolyph.

Under laboratory conditions, the East African *A. thalassinus* showed a high fecundity with an average of 36 ovarioles and the possibility to deposite more than 20 egg pods per female. The CA volume varied independently of the JH-III titre of the haemolymph and cannot be considered as a reliable indicator for the latter. The increase of the CA volume, accompanied by a simultaneous rise of the JH titre of the haemolyph, could only be observed during the first 15 days after emergence. In some females, a growth of oocytes was found neither before nor after

the first oviposition, even though some of them had a very high JH-III titre in the haemolymph.

Zusammenfassung - *Größe der Corpora allata, Juvenilhormon-III-Titer in der Hämolymphe und Wachstum der terminalen und subterminalen Oocyten während der Reproduktionzeit des oedipodinen Grashüpfers* Aiolopus thalassinus *unter Laboratoriumsbedingungen (Orthopteroidea Caelifera Acrididae).-*

Bei einer ostafrikanischen Population von *Aiolopus thalassinus* (Fabricius, 1781) aus Kenya wurden verschiedene, die Reproduktion beeinflussende Faktoren untersucht. Die Versuchstiere wurden in Hannover unter kontrollierten Laboratoriumsbedingungen über viele Generationen erfolgreich vermehrt. Außer den reproduktiven Parametern, wie Anzahl der Ovariolen, Präovipositionszeit, Anzahl der Eigelege pro Weibchen, als auch der Wechsel im Volumen der Corpora allata (CA) des Juvenilhormon (JH) -Titers in der Hämolymphe und das Wachstum der terminalen (t) sowie subterminalen Oozyten wurden während der Eilegeperiode registriert. Die Reproduktionsparameter wurden bei 58-103 Weibchen untersucht. Bis zu 22 nacheinander durchlaufene Reproduktionszyklen pro Weibchen wurden bewertet.

Eine Korrelation wurde weder zwischen dem Volumen der CA und dem Alter der Weibchen, noch dem CA-Volumen und der Grösse der t-Oozyten gefunden. Während der Prävitellogenese war der JH-III-Titer in der Hamolymphe niedrig (< 0.2 pmol / 10 µl) erreichte danach ein Maximum von 2.5 pmol / 10 µl, um dann mit Reifung der Oozyten und Eiablage stark abzufallen. Nach Ablage der ersten Oothek stieg der JH-III-Titer in der Hämolymphe mit dem Wachstum der Oozyten in jedem gonadotropen Zyklus wieder an. Der Anstieg begann stets bei höheren Werten als während der Präovipositionszeit und erreichte nicht so hohe Titer wie vor der ersten Eiablage, als das Volumen der t-Oozyten anstieg. Das Volumen der subterminalen Oozyten vergrößerte sich häufig schon vor der Ablage der t-Oozyten. In diesen Individuen sank der JH-III-Titer in der Hämolymphe nicht drastisch ab. Eine simultane Reifung von zwei Oozytengenerationen führte zu Intervallen zwischen zwei Eiablagen von nur 1-2 Tagen. Vor Beendigung des zweiten gonadotropen Zyklus fiel der JH-III-Titer in der Hämolymphe wieder ab. Es konnte jedoch keine eindeutige Zyklisierung des JH-III-Titers in der Hämolymphe festgestellt werden.

Unter Laboratoriumsbedingungen zeigte die ostafrikanische *A. thalassinus*-Population mit durchschnittlich 36 Ovariolen und der möglichen Ablage von mehr als 20 Ootheken pro Weibchen eine hohe Fekundität. Da das CA-Volumen unabhängig vom JH-III-Titer der Hämolymphe beträchtlich variierte, kann das CA-Volumen nicht als Maßstab für den JH-Titer der Hämolymphe verwendet werden. Ein Anstieg des CA-Volumens, begleitet von einer simultanen Erhöhung des JH-Titers, konnte nur während der ersten 15 Tage des adulten Lebens der Weibchen beobachtet werden. Bei mehreren Weibchen wurde ein Wachstum der Oozyten weder vor, noch nach der ersten Oviposition gefunden, obgleich einzelne von ihnen einen sehr hohen JH-III-Titer in der Hämolymphe aufwiesen.

Riassunto - *Dimensioni dei corpora allata, titolo dell'ormone giovanile III, sviluppo degli oociti terminali e subterminali nel corso del periodo riproduttivo di* Aiolopus thalassinus *(Orthopteroidea Caelifera Acrididae).*

E' stato studiato l'effetto dei fattori che controllano la riproduzione in una popolazione di *Aiolopus thalassinus* (Fabricius, 1781), nativo dell'Africa orientale e allevato in condizioni di laboratorio controllate, ad Hannover. I parametri riproduttivi, quali numero degli ovarioli, periodo di preovideposizione, numero di ooteche per femmina, modifiche nel volume dei corpora allata (CA), titolo dell'ormone giovanile (JH) nell'emolinfa, sviluppo degli oociti terminali e subterminali, sono stati studiati nel corso del periodo di ovideposizione. Per i dati di ogni parametro riproduttivo sono state utilizzate da 58 a 103 femmine e sono stati valutati più di 22 cicli gonadotrofici per ogni femmina. Nessuna correlazione è stata trovata tra il volume dei CA e l'età delle femmine adulte, così come tra questi parametri e le dimensioni degli oociti. Il titolo JH-III nell'emolinfa è stato basso (< 0.2 pmol/10 µl) durante la previtellogenesi, aumenta successivamente fino ad un valore massimo di 2,5 pmol/10 µl, declinando drasticamente con la maturazione degli oociti e al momento dell'ovideposizione. Dopo la prima ovideposizione, il titolo dell'JH-III nell'emolinfa aumenta nuovamente con lo sviluppo degli oociti nel corso del ciclo gonadotrofico, ma non ha inizio dal basso livello trovato durante la previtellogenesi e non raggiunge gli alti valori registrati precedentemente alla prima ovideposizione, quando il volume dei t-oociti aumenta. Lo sviluppo degli oociti subterminali spesso inizia prima dell'ovideposizione dei t-oociti. Il titolo di JH-III di questi individui non declina drasticamente. La simultanea maturazione di due generazioni di oociti può determinare un intervallo di soli 1-2 giorni tra due ovideposizioni. Prima del completamento di due cicli gonadotrofici, il titolo JH-III declina nuovamente, ma non si è trovata una chiara ciclizzazione del titolo JH-III nell'emolinfa. In condizioni di laboratorio *A. thalassinus* risulta molto fecondo, con una media di 36 ovarioli e la possibilità di depositare più di 20 ooteche per femmina. Il volume dei CA varia indipendentemente dal titolo del IJ-III dell'emolionfa e non può essere considerato un sicuro indice di riferimento per quest'ultimo. L'incremento di volume dei CA, accompagnato da un simultaneo innalzamento del titolo dell'JH dell'emolinfa, può essere osservato solo nei primi 15 giorni dopo lo sfarfallamento. In alcune femmine non si è notato sviluppo degli oociti né prima, né dopo la prima ovideposizione, anche se alcune di questa hanno evidenziato un titolo di JH-III nell'emolinfa molto elevato.

Key words: *Aiolopus thalassinus*, lifespan of females after emergence, reproduction period, preoviposition period, size of corpora allata, juvenile hormone III titre in the haemolymph, volume of terminal oocytes, influence of subterminated oocytes, number of ovariols, egg pod production.

INTRODUCTION

Growth and reproduction of insects are regulated by hormones. One of these, the juvenile hormone (JH] is synthetizsed in the corpora allata (CA). The neurosecretory cells (NSC) of the brain produce candidate neuropeptides, termed allatotropins and allatostatins, which stimulate and inhibit JH production, respectively (Kataoka *et al.*, 1989; Woodhead *et al.*, 1989; Kramer *et al.*, 1991; Lorenz *et al.*, 1997) reaching the corpora cardiaca (CC) (Wyatt *et al.*, 1984; Feyereisen, 1985; Kempa-Tomm *et al.*, 1989; Neuhäuser *et al.*, 1994). After being released into the haemolymph, the allatotropic hormone activates the CA to synthetise JH (Strong, 1965; Ulrich *et al.*, 1985). For Acrididae, the relationships are shown in Fig. 1.

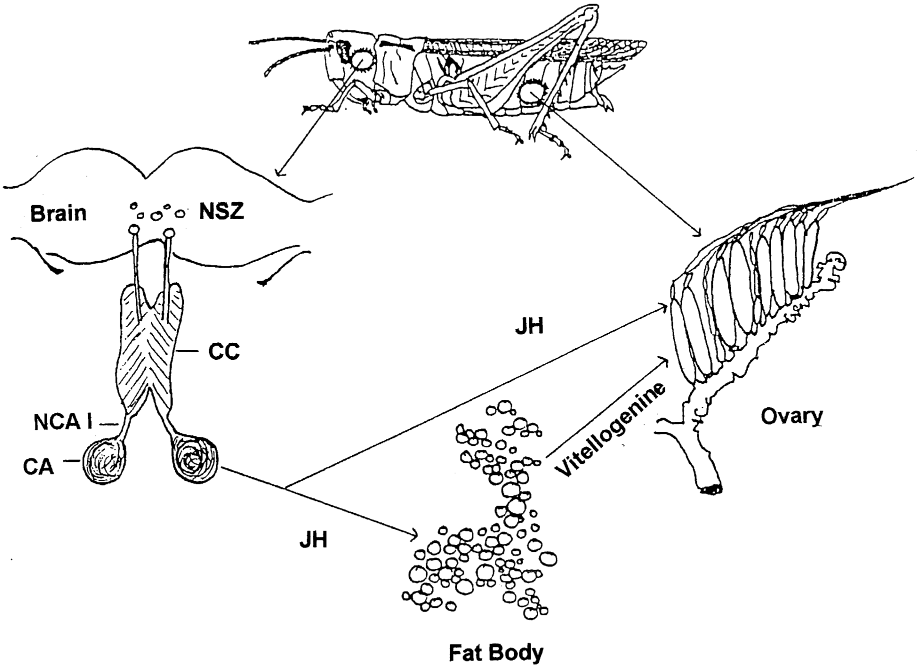

Fig. 1 - Scheme showing the relationship between JH-III and the reproduction organs in acridids;.CA: corpora allata, CC: corpora cardiaca, NCAI: nervus corporis allatum I; NSZ: neurosecretory cells; JH: juvenile hormone.

In *Locusta migratoria migratorioides* (Reiche & Fairmaire, 1850), Horseman *et al.* (1994) reported that in each pars lateralis (PL) of the brain, 13 cells innervate the ipsilateral CA, while four cells (two in each PL) innervate both glands. A radiochemical assay was used to measure hourly JH production by a CA with intact nerve connections to the brain. Then, changes in hormone production due to selective nerve stimulation or transection were assessed. In control preparations, JH production per hour remained approximately constant for at least nine hours. Simultaneous electrical stimulation of all neurons innervating one CA (*i.e.* 13 ipsilateral plus 4 bilateral cells), always inhibited JH production in the African migratory locust, while their transection led to a rapid progressive increase in JH biosynthesis in CA from females with oocytes longer than 4.5 mm showing a strong, neurally mediated inhibition of the CA at certain phases of the vitellogenic cycle as found in *A. thalassinus* (Schmidt &

Othman, 1993). Selective stimulation of the four neurons innervating both CA suggested that they do modulate JH biosynthesis, but the effect varies qualitatively depending on the phase of the vitellogenic cycle.

Studies in Acrididae showed that JH acts in adults as gonadotropine and JH III is the; only juvenile hormone present (Huibregste-Minderhoud *et al.*, 1980; Rembold, 1981; Dale & Tobe, 1986; Osman & Schmidt, 1988; Renucci *et al.*, 1990; Schmidt & Othman, 1994). JH stimulates the vitellogenesis in the fat body and the incorporation of yolk material into the developing oocytes by affecting the follicle cells (Loher, 1965; Couble *et al.*, 1979; Davey *et al.*, 1993). The vitellogenins are synthetized in the fat body and released into the haemolymph to be taken up by the oocytes (Roussel, 1978; Ferenz & Kaufner, 1981; Wyatt, 1988; Glinka & Triseleva, 1989; Ferenz & Aden, 1993). The latter authors showed an ovarian control of JH biosynthesis and identified the active peptide 'allatinhibin'.

Experiments were carried out with *Locusta migratoria* (Linnaeus, 1758) to obtain correlations between neuronal tissues, JH III titre and activity of the ovaries. For that allatectomy, CA implantation, ultrastructural investigations, measurements of the CA volume and determination of the JH III titre in the haemolymph were performed. For the first time, the JH biosynthesis could be measured by in vitro determination of the CA activity (Couillaud & Girardie, 1985; Dale & Tobe, 1986; Renucci *et al.*, 1990). Only few observations were reported on the relation of the JH III titre and egg production in vivo. The investigations in *L. migratoria* (Rembold, 1981) and *A thalassisnus* (Schmidt & Othman, 1994) were restricted to the first gonadotropic cycle, during which the changes of the CA volume, the concentration of JH III in the haemolymph, and the protein content of the terminal (t) oocytes were studied. These parameters were found to be closer related to the volume of t-oocytes, than to the age of fenales after emergence (Schmidt *et al.*, 1990). The acridid *Eyprepocnemis plorans* (Charpentier, 1825) was used to compare the JH III situation in three consecutive gonadotropic cycles (Schmidt *et al.*, 1996). In the latter species no clear cycle of the JH III titre was found in the haemolymph during egg-laying.

The present work aims to study the relations between size of CA, JH-III titre and egg production throughout several gonadotropic cycles in a population of paired adult **FF** of *A. thalassinus,* regarding the influence of the subterminated oocytes.

MATERIAL AND METHODS

Test insects and rearing conditions

Aiolopus thalassinus (Fabricius, 1781) is widely distributed on damp and grazy places throughout the Palaearctic region (Europe, Africa and India) (Hollis,1968; Schmidt & Lilge, 1997). The population used here was sampled at Nairobi,Kenya in 1986. Several generations were bred in the laboratory at Hannover without diapause. The species can be easily reared (Schmidt & Feierabend, 2000). About 4-5 generations were produced per year.

The experiments were mainly performed in 1990-1992. The insects were cultured in a CT room at 25±2°C in plastic cages of 34 x 19 x 20 cm, topped by a 38 x 21 x 28 cm woden frame, one side closed with a door of plexiglass, the others and the roof being of wire gauze for ventilation (Schmidt 1981, 1986). On the bottom of the cage, a box was placed, containing an hydroponic wheat culture, as main green food. The box was replaced every 8-10 days. The cage floor was partly covered with a 1-cm-layer of coarse-grained dry sand.

The cage was heated and lit by a 40 W Osram reflector bulb placed on the ceiling of the cage, illuminating one third of the area. It was carefully placed, thus, the seedlings were protected by a narrow angle of radiation. The temperature raised up to 38°C on the sand underneath during the daily light period from 7-19 h [L/D 12 h], and the temperature gradient consistently decreased to 28°C in all directions during this period.

The humidity varied inside the cage between 15% closer to the bulb, to more than 90% between the seedlings; in the morning, 60 to 75% RH were registered in the middle of the cage at about 24°C. Every morning the humidity was increased by placing a 1.5 cm high rim petri dish (9 cm diam) filled with wet sand or a moist cotton pad inside the cage, to offer the insects drinking water. As additional food, the grasshoppers received a mixture of wheat bran and protein-rich dog food, ad libitum.

After a preoviposition period of a week, a plastic container [6,5 x 6,5 x 5 cm], filled with washed and sterilized sand (250°C for 4 h) of 0.35-0.60 mm grain size and 12% water, was placed for egg deposition, near the hydroponic wheat culture, directly under the lamp. A depth of 5 cm was needed for laying normal egg pods [length up to 3 cm]. The surface temperature measured about 32°C at light period. The water content of the egg-laying substrate was controlled daily by weighing; weight loss was compensated by adding demineralized water. In this way, desiccation of the substrate was avoided. The access to the container was facilitated by a piece of cardboard obliquely arranged. The egg-laying container was almost daily replaced, until oviposition ceased. Almost all egg pods were laid in the container. The eggs deposited were incubated in situ at 30Í1°C. The hatching of the nymphs was checked and recorded daily for three weeks. Each container was kept in a plastic bag to protect the substrate from drying out, and some cellucotton was used to reduce the condensed water on the inner walls of the plastic bag. This method protected the hatchlings from sticking to the precipitated water and dieing.

In a cage, about 80 nymphs were grown to adults under similar conditions, passing five instars during 4-5 weeks. Several generations were reared and compared.

Recording of individual oviposition

For the experiments, from either breeding cage 10 females first emerged (body weight 300-450 mg) were differently colour-marked on pronotum or wings and sexed with 10 males of the same generation. The date of emergence and beginning of the oviposition period were individually registered. The females were daily weighed, and the number of egg pods laid counted for either individuum. The oviposited **F** could be determinated by a remarkable loss of weight (30-90 mg). Prepared insects were not used for calculation of life parameters.

Collection and preparation of haemolymph

Females of known age and body length of 21-29 mm were used for the determination of JH-III titres. Haemolymph (10-40 Bl) was collected after transection of one of the hindlegs near the coxa and by using a calibrated 5 Bl micropipette. The collected haemolymph was immediately transferred into a glass tube containing 1 ml methanol and 5 pmol ethyl JH-III-oate as internal standard (E), and was vigorously shaken in a Vortex homogenizer. The samples were stored in the dark at -15‡C for further processing.

Quantification of haemolymph JH-III

For preparation of the 10-dimethyl (nonafluorohexyl) silyloxy-11-methoxy analyte of JH-III and of the internal ethyl-JH-III-oate and their quantification by combined capillary gas chromatography - selected ion monitoring mass spectrometry (MIS), the method of Rembold & Lackner (1985) was used. The analytical fragment used for the analysis was m/z 376 (for the other JH homologs it is m/z 390) (Fig. 2). According to this method, the limit of the detectability of JH-III was 0.05 pmol per injected sample. Due to a total yield of this micro-analytical method of 65-70%, the zero hormone level is less than 10^{-9} M JH-III concentration in the haemolymph.

Morphometric investigations

After haemolymph was taken, the females were deeply cooled (-18°C) for 7 min, then the heads were dissected in Ringer's solution (1:4). After dissecting the cuticle and removing the muscles and fat tissues near the brain, the CA-CC complex became visible by adding a solution of 0.5% methylene blue. The round or ellipsoidal CA were measured by means of an ocular micrometer at magnification of 16 x, and the volume of each gland was calculated as a rotation ellipsoid by the formula

$$V_1(\mathrm{nl}) = \frac{4}{3}\pi \cdot [\frac{(r_1 + r_2)}{2}]^3,$$

in which r_1 means the larger and r_2 the smaller radius.

For reaching the ovaries, the abdomen of either female was dorsally dissected in Ringer's solution (1:4), and 10 t-oocytes were measured starting at the nearest distance to the oviduct, after removal of the ovaries and transferring them to clean Ringer's solution. For better handling and taken the measurements, the ovaries were spred on a glass plate. Females were used only if the t-oocytes were developed more or less similarly. The length and width of the t-oocytes were determined using a binocular microscope, with an micrometer ocular of a suitable magnification. according their different shape, the volume(V_2) was calculated by using the formula

$$V_2(\mathrm{nl}) = \frac{4}{3}\pi \cdot a \cdot b^2,$$

in which a indicates the larger, and b the smaller radius.

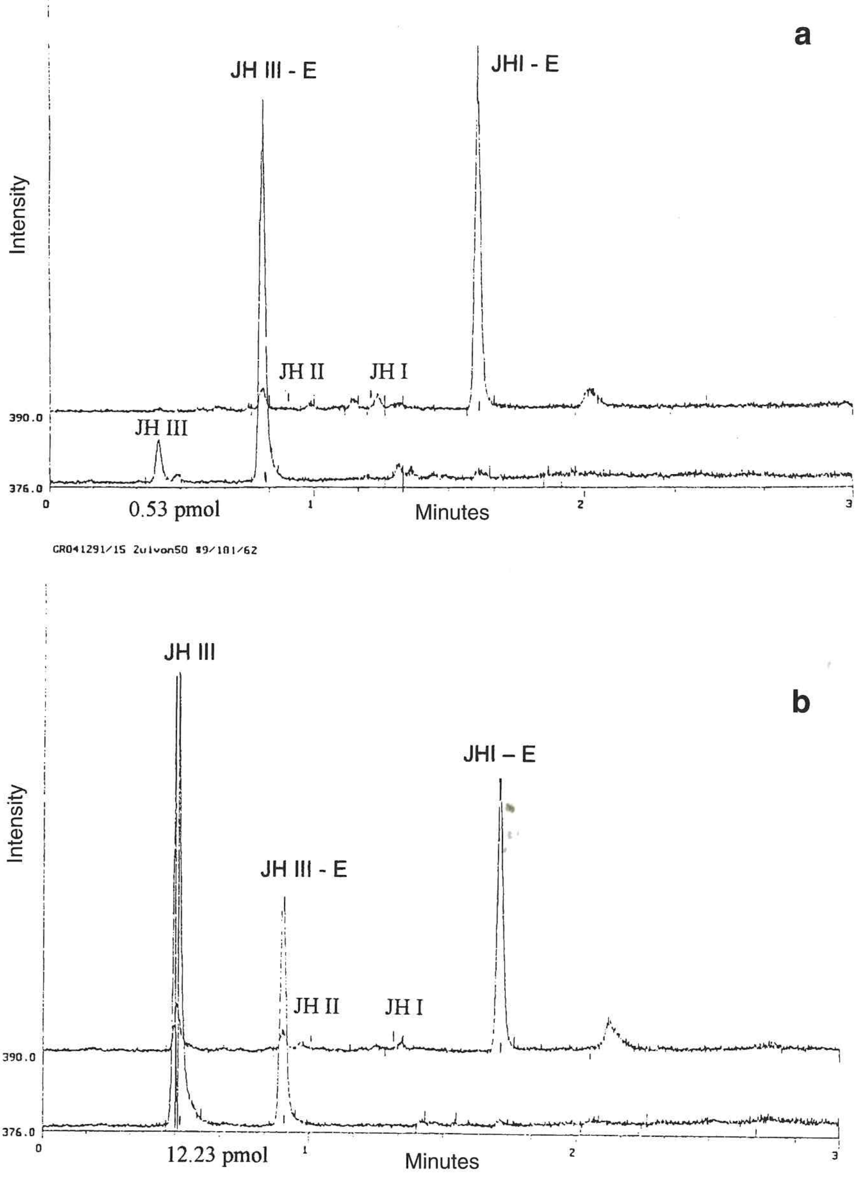

Fig. 2 - MIS signals of JH-III and the ethyl esters JH-III-E and JH-I-E as standard; a: 0.53 and b: 12.23 pmol JH-III/10 µl haemolymph samples of females of *A. thalassinus*; JH-I and JH-II were not detectable in the haemolymph.

The variation of the mean volumes was calculated throughout the different gonadotropic cycles. Individuals containing ovariols in which no resorption [R] bodies (corpora lutea) became visible, were considered having not produced eggs (Singh, 1958; Schmidt & Othman, 1993; Schmidt *et al.*, 1996).

RESULTS

Behaviour parameters and lifespan

In *A. thalassinus*, copulation can take place directly after oviposition. Often one or two males mounted the egg-laying female or waited nearby for copulation. Pairings lasted 10-15 min. When the female was much disturbed, the oviposition process was broken off. The length of egg pods measured 2.2-3 cm. The eggs were placed in the lower half of the pod, the upper part consisting of a foamy secretion.

Egg laying occurred at day time as well as at night, and was specially high at the beginning and termination of the photoperiod (7-19 h). In the morning and at evening, the deposition of eggs was significantly ($p < 0.05$) higher than around midday, which may be depended on the influence of disturbance factors, like daily weighing of the **FF**, cage control, working in the breeding room, etc.

For uptake of food, no part of the light period was preferred. The insects fed at day time as well as at night. Shortly after oviposition, the females showed an increasing intake of food, which may be connected with a compensation of the loss of weight by the eggs deposited. Weight loss was calculated for 10-20% of 300-450 mg body weight per female.

The adult lifespan of females was 44.7±18.7 days (n=94) (Fig. 3). Only marked females paired with males 1:1 during the experiments and died inside the cages were counted.

Reproduction parameters

The number of ovariols varied between 30-42 per female (Fig. 4), the mean number was recorded to 36±3.7 (n=58), for both ovaries. However, counting of the number did not occur in females, when the ovaries were pasted up or torn in pieces by preparation.

Great variability was observed in the period of preoviposition. The first oviposition of the females tested varied between day 6-15. More than 60% of them deposited the first egg pod on day 8-10 (Fig. 5). After preoviposition of about one week; egg deposition increased for six weeks, and declined afterwards (Fig. 6). In the cages, several **FF** were lost by dying and the preparation of inner organs for control during the experimental period, reducing the comparable number.

The number of egg pods was individually counted sometimes for >20 per female. One female produced 22 egg pods, and then it was prepared for control, showing fully

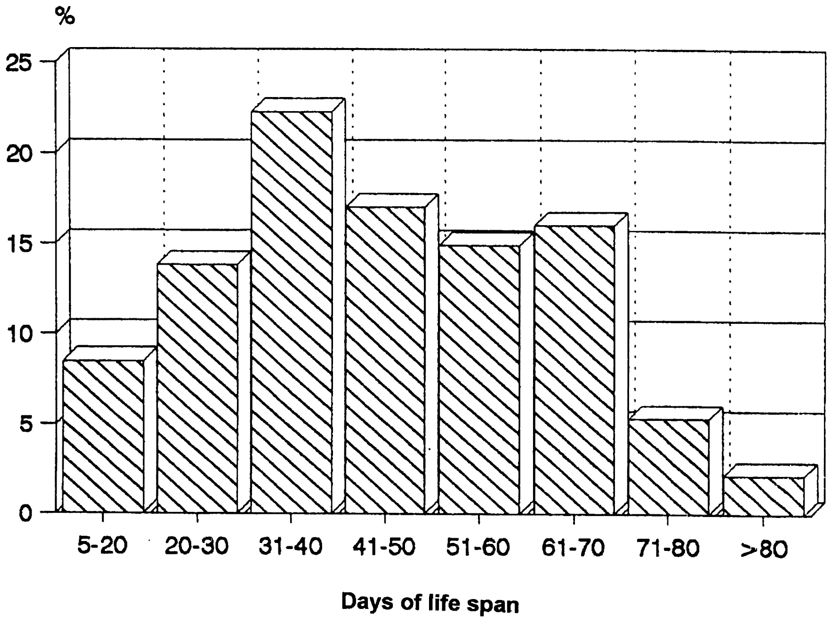

Fig. 3 - Variability of lifespan in 94 females of *A. thalassinus* tested.

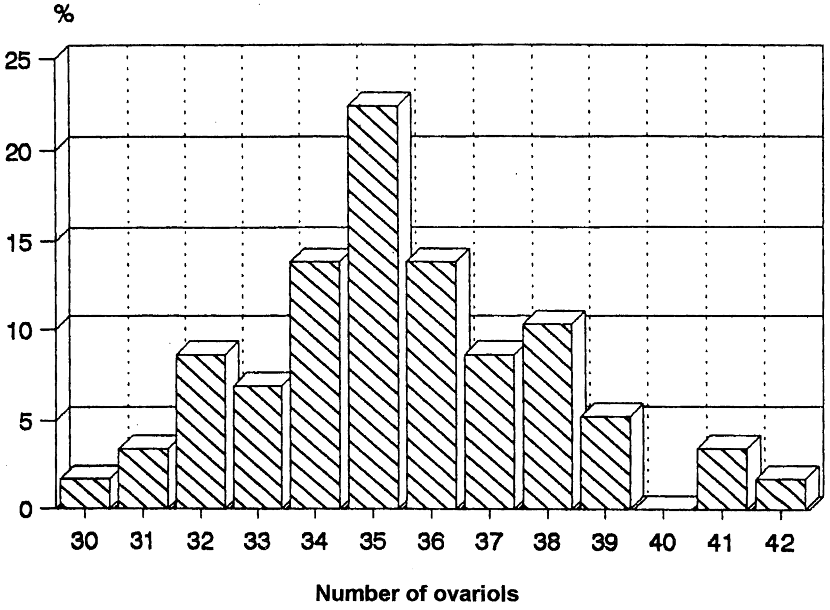

Fig. 4 - Number of ovarioals (both ovaries) from 58 females of *A. thalassinus*.

developed ovaries for producing eggs. The egg-laying intervals often lasted only 1-2 days, because not only the t-oocytes enlarged during the gonadotropic cycles, but also the subterminated oocytes started growing, influenced by the JH-III titre of the haemolymph. The developmental stage of the ovaries could not be assessed. When the female had already laid, further egg depositions followed, independent of the duration of the period of preoviposition.

Before measuring the t-oocytes, well prepared ovaries were classified for better comparing the growth of the oocytes. In Table 1 five steps of development were indicated, in relation to the JH-III titre of the haemolymph.

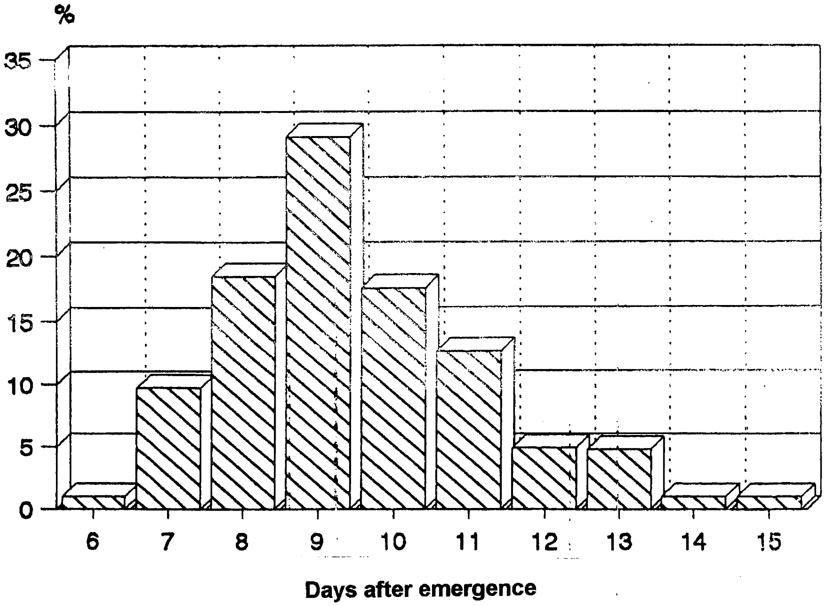

Fig. 5 - Age of 103 adult females of *A. thalassinus* at first oviposition.

Considering the steps of t-oocyte development, it has to be mentioned that the subterminated oocytes have normally reached the steps 3 or 4 before the t-oocytes were deposited. Then, no strong decline of the JH-III titre was observed in the haemolyph.

The t-oocyte volume was barely measurable during the previtellogenesis (on the rule, 3-4 days after emergence). After that the volume increased slowly, reaching maximum shortly before egg deposition. Females dissected often showed a prolonged development of oocytes and, therefore, still a lower volume of t-oocytes, but with a tendency to increase until oviposition.

The volume of the t-oocytes can vary from 1.315 to 2.288 μl, shortly before deposited. The growth of the t-oocytes, in relation to the last oviposition, is shown in Fig. 7

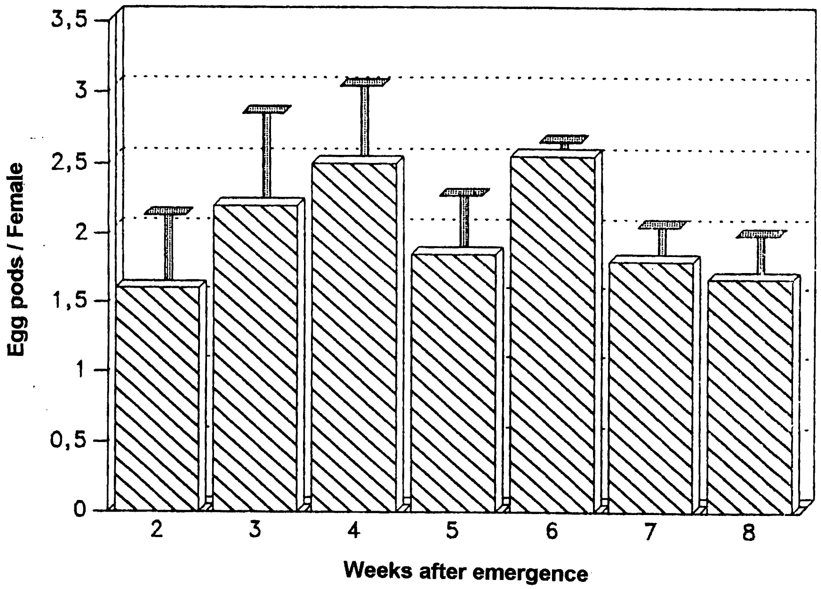

Fig. 6 - Number of egg pods per female and week (± SD) of *A. thalassinus*.

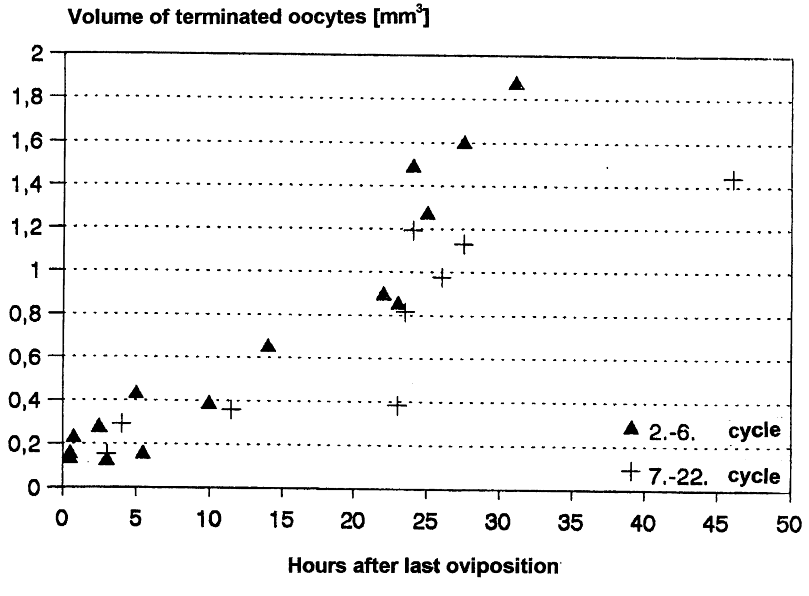

Fig. 7 - Volume of t-oocytes in relation to the period after the last deposition of eggs in *A. thalassinus*, counted in various gonadotropic cycles of oviposition (single values).

during 22 gonadotropic cycles studied, demonstrating that the females began the 7th cycle with some retardation of oocyte growth, mainly in step 4, if compared with the findings during the 2nd-6th gonadotropic cycles.

CA volume and JH-III titre of the haemolymph in relation to the age of females

The same females, in which the t-oocytes were measured, were used for the determination of the CA volume and the JH-III titre of the haemolymph. Neither cyclic activities, nor an initial increase of the CA volume, were observed during the various gonadotropic cycles. However, in 58% of CA measured the right and left gland of the F showed different volumes, and a mean value had to be calculated. For recording the CA volume, it was helpful to differentiate between females before and after the first gonadotropic cycle. After emergence, the CA volume increased parallel with the JH-III titre of the haemolyphe. In Fig. 8, the first value is related to a female of the last nymphal instar, in which no JH was detectable in the haemolymph.

After the first gonadotropic cycle, it was not possible to recognize a relationship between CA volume and JH-III titre of the haemolymph. Neither females with a short interval between two egg depositions, nor individuals with a high JH-III titre in the haemolyph showed significantly larger CA volumes. No increase of the CA volume, higher than during the first gonadotropic cycle, could be established during the period of oviposition.

JH-III titre of the haemolyph and the growth of t-oocytes

In the first gonadotropic cycle, no clear correlation was found between the JH-III titre of the haemolymph and the volume of t-oocytes. On age of 7-9 days after emer-

Table 1 - Differentiation of ovaries basing on the volume of the t-oocytes in relation to the JH-III titre in the haemolymph.

Oocytes	**Growth**	**Volume [µl]**	**Stage**	**JH-III titre**
Step 1	weak	< 0.015	about 3 days emerged	very low
Step 2	small	0.02-0.2	a) eggs even laid b) after about 3 days without oocyte growth c) females have laid without further oocyte growth	variablle
Step 3	strong	0.2-0.36	incorporation of vitellin is starting in oocytes	increased
Step 4	very strong	0.36-1.4-1.7	very strong and fast growth	increased to maximum
Step 5	weak	>1.7 (varied 1.4-2.2)	t-oocyte growth terminated and eggs deposited	strongly declined

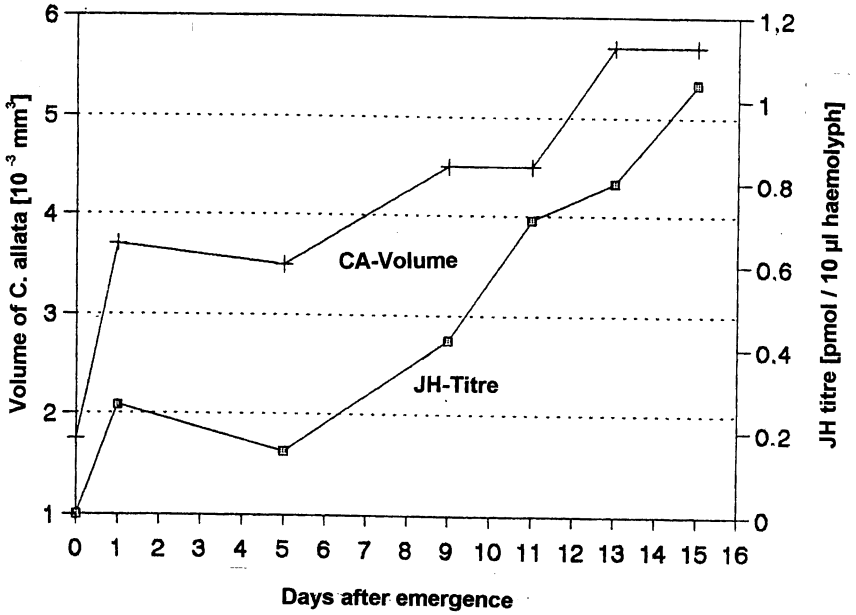

Fig. 8 - Increase of the CA volume and the JH-III titre in the haemolyph of females of *A. thalassinus* after emergence (single values).

gence, four females showed t-oocyte volumes of step 4-5 (Table 1) and JH-III titres of 0.42-0.76 pmol/10 µl haemolyph. On the other hand, in four females, aging 8-15 days after emergence, JH-III titres of 0.23-1.49 pmol/10 µl haemolyph and t-oocyte volumes of step 2 were established. Table 2 represents results of females tested during the first gonadotropic cycle.

Females which had deposited eggs showed typical relationships between JH-III titre and t-oocyte volume. In the haemolyph, the JH-III titre strongly increased, starting at steps 2-4, often already reaching the maximum at step 4 (Table 1). The JH-III

Table 2 - Variability between JH-III titre in the haemolyph and t-oocyte volume during the first gonadotropic cycle.

Female no	1	2	3	4	5	6	7	8	9	10
Days after emergence	1	5	7	7	8	8	9	13	15	15
t-oocyte volume [µl]	0.01	0.02	0.93	1.52	0.02	0.74	1.82	0.04	0.03	0.03
JH III titre pmol/10 µl	0.26	0.15	0.76	0.56	0.23	0.71	0.42	0.80	1.04	1.49

titre declined at step 5, when the oocytes were ready for deposition (Figs 9, 10). In females showing t-oocytes already reached the oviduct and being shortly before deposition, a decline of the haemolymphal JH-III titre, typical for step 5, could not be established, if the subterminated oocytes had reached the steps 3-4.

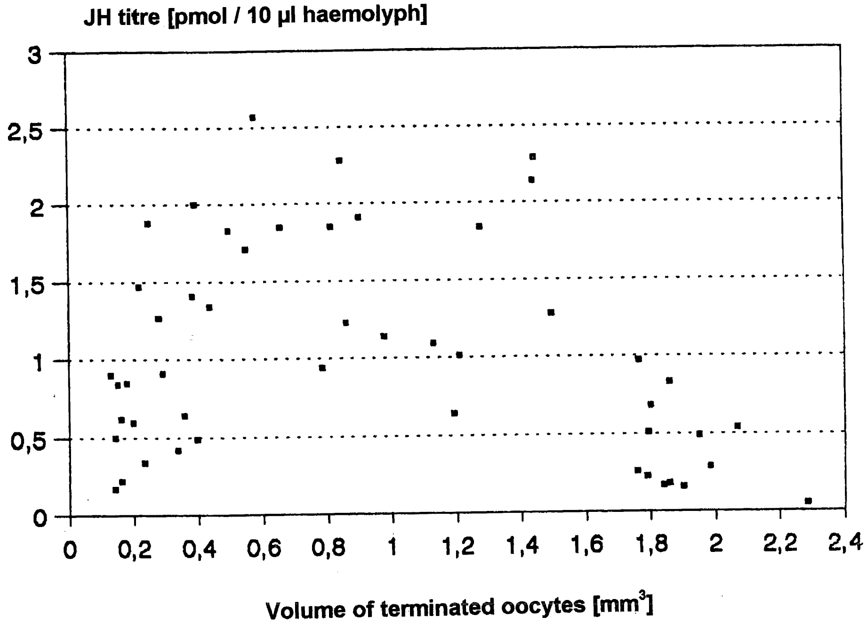

Fig. 9 - Correlation between the JH-III titre in the haemolyph and the volume of t-oocytes in FF of *A. thalassinus* after first oviposition; 2.-22. cycles were combined.

In three females which had deposited eggs for several times, and the last oviposition occurred five days before dissection, the JH-III titre of the haemolymph measured 0.87, 1.75, and 3.71 pmol/10 µl, respectively, although their t-oocyte volumes showed step 2. The last value mentioned was the highest that was found in this study. Also, in **FF** with t-oocytes of step 2, which had not laid eggs, JH-titres were registered of above 1 pmol /10 µl haemolyph (Table 3).

The decline of the JH-III titre in the haemolymph depends on the activity of JH esterases, which could be measured in two individuals. Both females were freezed for 7 min and then haemolymph was again taken to test, whether the JH-III titre had changed during this short period. In both individuals, a lower JH-III titre of about 10% was established than before dieing [0.92¨ 0.81 and 2.57¨ 2.29 pmol / 10 µl haemolymph, respectively], demonstrating that remarkable amounts of JH can be decomposed in a period of 7 min.

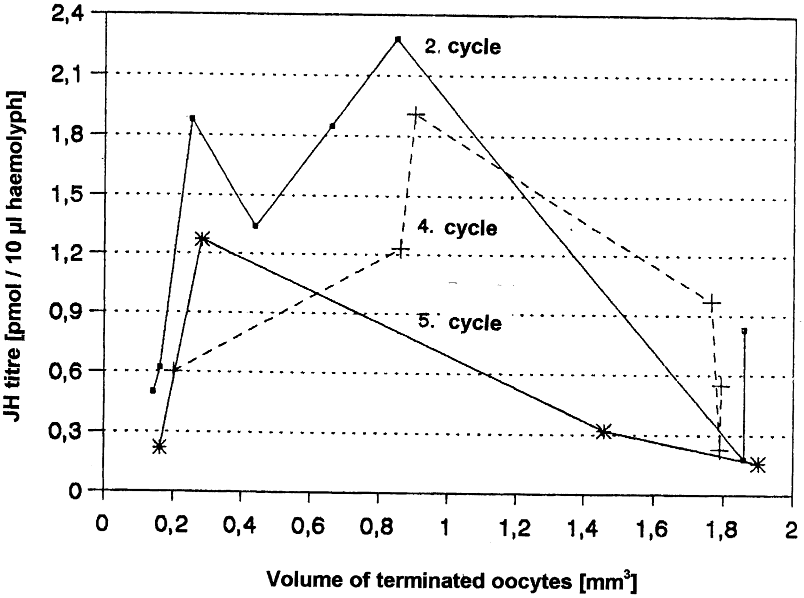

Fig. 10 - JH-III titres in the haemolyph of *A. thalassinus* females at the 2nd, 4th and 5th gonadotropic cycle (single values).

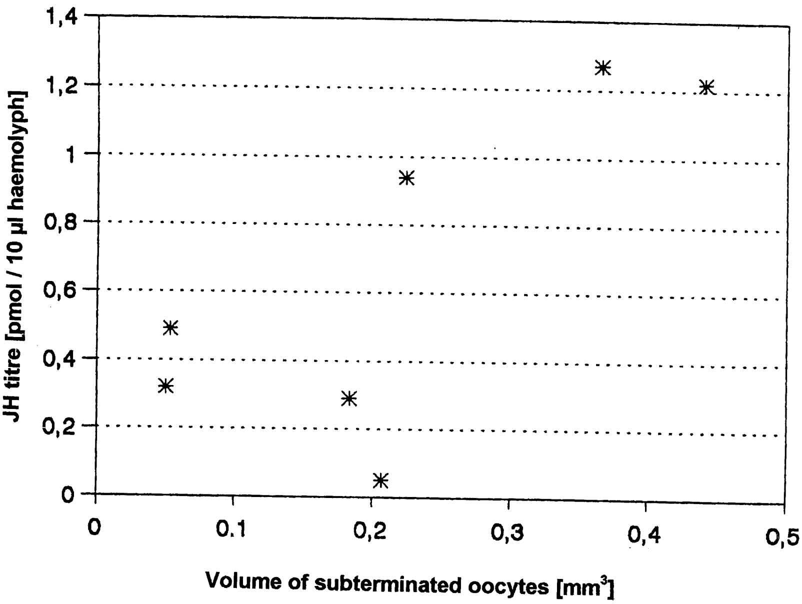

Fig. 11 - Correlation between the volume of subterminated oocytes and JH-III titre, t-oocytes were in the oviduct.

Table 3 - JH-III titres in the haemolymph of egg-laid and not oviposited females with t-oocytes of step 2 (viewed in Table 1).

Female no	**adult age (days)**	**eggs**	**t-oocyte volume (μ^3)**	**JH-III titre / 10 µl haemolyph (pmol)**
1	13	not laid	0.036	0.80
2	15	not laid	0.029	1.04
3	15	not laid	0.030	1.49
4	>40	laid	0.030	1.75
5	>43	laid	0.039	3.71
6	>46	laid	0.029	0.87

DISCUSSION

Fecundity

Regarding the regulation of reproduction, growing conditions of the insects tested should not be under-estimated; suitable food, environmental conditions, group effects, and mating possibilities can influence the reproductive cycle (Tobe & Chapman, 1979; Schmidt, 1980; Injeyan & Tobe, 1981; Dale & Tobe, 1986; Gadot *et al.*, 1989; Schmidt & Othman, 1994; Schmidt *et al.*, 1996). Also, endogenous reproductive parameters have to be considered (Schmidt & Feierabend, 2000).

In Acrididae, egg production and lifespan of females are most important parameters, regarding the fecundity of the species, and the environment, to which the population studied was adapted. Furtheron, the fecundity may depend on the number of ovariols per female, the resorption quotient of the oocytes, preovipotion period, number of egg pods produced per female, the intervals between egg depositions, the hatching rate of nymphs and adult lifespan. However, a long lifespan has not to be connected with a long reproductive period. Species may hibernate without laying eggs in spring (Uvarov, 1977). For *Melanoplus sanguinipes* (Fabricius, 1781), Dean (1981) demonstrated that a higher reproduction rate was followed by a shorter lifespan, showing that the life-history hypothesis cannot be settled for all insects.

In comparison to our results, Antoniou & Hunter-Jones (1956) observed a very long preoviposition period of 70 days, on the average, in *Eyprepocnemis capitata* Miller, 1929, reared under constant temperature conditions of 28-30°C. Other grasshopper species tested under similar rearing conditions had much shorter preoviposition periods, like *A. thalassinus*. Paired *E. plorans* laid the first egg pod after 23±7 days (Schmidt *et al.*, 1996), crowded *L. migratoria* after 16 days (Rembold, 1981), and crowded gregarious *Schistocerca gregaria* (Forskål, 1775) 18-22 days after emergence (Schmidt & Albütz, 1994, 1999, 2002). The oedipodine *Acrotylus patruelis* (Herrich-Schäffer; 1838), from Italy, produced the first egg pod after 1-2 weeks after emergence and either female laid 230-270 eggs, on the average, containing 21-24-27 ovariols. The oviposition intervals were found between 2.6 and 7 days (Schmidt, 1980).

Under suitable laboratory conditions, lifespan and reproduction rate of females can be much higher than in the field. *Chorthippus brunneus* (Thunberg, 1815) deposited up to 19 egg pods in cages and only up to 8 pods per female under field conditions (Richards & Waloff, 1954). Under this point of view, a number of 22 egg pods per **F**, produced in the population of *A. thalassinus* studied here, may be a top value for the species, which will hardly be reached in the field. Under suitable life conditions, *A. thalassinus* showed a relatively high egg production in the experiments undertaken, due to early egg-laying starting in the 2nd week after emergence, simultaneous growth of two generations of oocytes, and a relatively high number of 36 egg-producing ovariols, on the average.

In Tab 4, the data showed differences which may be due to the various origins of the populations tested. In a study with *A. thalassinus*, originating from Pakistan [Karachi], the number of egg pods and egg production varied from generation to generation and showed no relation to any reproductive parameter tested (Schmidt & Feierabend, 2000).

Table 4 - Fecundity parameters of Aiolopus thalassinus*, reported by various authors under laboratory conditions.*

Preoviposition period	Oviposition interval	egg-pods/female	Origin of population	Authors
7.8 days	2-3 days	9.2	Egypt	Hafez & Ibrahim, 1962
15.1 days	3-6 days	2.1	Turkey	Balogh, 1980
13.8 days	4.5-8.5 days	7.7	Italy	Schmidt & thman, 1994
7-10 days	1-2 days	14	Kenya	this study

Corpora allata volume

Differences in the size and morpho-histology of CA were observed in many orthopteran species during development. Therefore, the CA volume was used as a measure of gland activity. Johnson & Hill (1975) observed an increase and reduction of the CA volume during the first gonadotropic cycle in *L. migratoria*. Schmidt & Othman (1993) described a significant increase of the CA volume at the beginning of the first gonadotropic cycle in *A. thalassinus*. A strong decline took place before oviposition, as shown in the present study. But the largest CA volume did not correspond to the highest JH-III titre of the haemolymph. Schmidt & Othman (1994) demonstrated that the enlargement of the CA volume corresponds to the egg-laying activity.

In *L. migratoria*, Ferenz & Kaufner (1981) could not observe clear cyclic fluctuations, except the strong increase of the CA volume in the first gonadotropic cycle. This agreed with the results of Loher (1965) in *Gomphocerus rufus* (Linnaeus, 1758), in which a general increase of the CA volume was found throughout the adult female lifespan. Also, in *E. plorans* no marked fluctuation of the CA volume could be observed, and thus, no relation to the JH-III titres of the haemolymph was found, except in the first gonadotropic cycle (Schmidt et al., 1996). Similar results were reported for *A. thalassinus* in the present study.

For the blattid *Supella longipalpa* (Fabricius, 1781), Smith *et al.* (1989) reported a decline of the CA size if the oocytes had a volume >0.9 mm^3.

It is remarkable that the volume of both CA differed in most of the females investigated. Such differences were also reported for *L. migratoria* (Ferenz & Kaufner, 1981) and *E. plorans* (Schmidt *et al.*, 1996). In *L. migratoria*, isolated females showed larger CA than grouped ones (Dale & Tobe, 1986).

Growth of oocytes

In Acrididae, oocyte growth is influenced by hormonal factors, as well as by environmental ones, such as regular feeding and contact with males (Tobe & Chapman, 1979; Schmidt & Albütz, 1994; Schmidt & Othman, 1994).

In acridid species, like *A. thalassinus*, there is a period of previtellogenesis, in which vitellogenin is still not incorporated by the t-oocytes. In the African migratory locust, *L. m. migratorioides*, Goltzene & Porte (1978) divided the vitellogenesis of the t-oocytes in two developing phases. In *A. thalassinus*, Schmidt & Othman (1993) considered four growing periods of the t-oocyte. Really five periods could be separated, as shown in the present study (Table 1). Ferenz & Kaufner (1981) reported that yolk deposition did not occur before choriogenesis of the t-oocytes reached a destinct stage. In *M. sanguinipes*, the incorporation of yolk started with an oocyte length of 1 mm (Gillott & Elliott, 1976), and in *G. rufus* with 1.3 mm (LOHER, 1965), after a period of somatic growth. In both acridids, an incorporation of yolk was simultaneously found in the subterminal oocytes, before the t-oocytes were deposited, as observed in *E. plorans* (Schmidt et al., 1996) and *A. thalassinus*, showed in the present study, according to the very short intervals of 1-2 days between two ovipositions.

In the oocyte membrane of *L. migratoria*, receptors were established for a specific uptake of vitellogenin (Röhrkasten & Ferenz, 1986). JH-III seems to interfere with the specific incorporation of vitellogenin from the haemolymph into the oocytes, because JH analoga were able to affect this uptake (Kelly & Davenport, 1976). A similar effect was observed by implantation of CA (Lazarovici & Pener, 1978).

For the cockroach *Periplaneta americana* (Linnaeus, 1758), Weaver & Edwards, (1990) suggested that the ripening of eggs will go on faster, if a high JH-III titre occurs in the haemolymph, and there is a relation between egg production and the JH-III titre of the haemolymph. In *A. thalassinus*, this was not generally observed. In spite of very high JH-III titres of the haemolyph, growth of the oocytes was reduced in some **FF**. In these individuals, other factors may have had an influence, for example, disturbance of vitellogenin synthesis in the fat body or the uptake mechanism of the oocytes.

Ovary ectomy increased the size of the fat body in *M. sanguinipes* (Gillott & Elliott, 1976) and the enreachment of vitellogenin in *Gryllus bimaculatus* De Geer, 1773 (Kempa-Tomm *et al.*, 1990). On the other hand, a strong reduction of JH-III biosynthesis could be demonstrated in *G. bimaculatus* (Wennauer *et al.*, 1989) and

Acheta domestica (Linnaeus, 1758) (Renucci *et al.*, 1987, 1990), if the ovaries were removed.

The stepwise growth of t-oocytes may be responsible for the failed correlation between the volume of CA, the JH-III titre of the haemolymph, and oocyte growth, in connection with innervation processes mentioned in the introduction.

JH-III hormone titre ot the haemolymph and its role in reproduction

In Acrididae, endocrine control of oocyte development is well known, as reported for gryllids (Loher *et al.*, 1988; Orshan & Pener, 1991). Electro-coagulation of mNSC, or cutting the NSC shortly before emergence, inhibited the growth of oocytes and stopped oviposition in *M. sanguinipes* (Gillott & Elliott, 1976) and *L. migratoria* (Lazarovici & Pener, 1978). An early extirpation of the CA had the same effect in *M. sanguinipes* (Dogra & Ewen, 1970; Goltzene & Porte, 1978). Besides allatotropic effects, the mNSC can affect directly the protein synthlesis, as shown in *M. sanguinipes* (Elliott & Gillott, 1978). Couillaud et al. (1989) extracted stimulating and antagonistic factors from NSC of *L. migratoria*. In the latter species (Couble *et al.*, 1979) and *S. gregaria* (Highnam *et al.*, 1963; Pener, 1965), vitellogenin synthesis failed, if CA were removed and JH-III was absent, as shown by treatment with precocene-III in *A. thalassinus* (Osman & Schmidt, 1988). In these cases, vitellogenesis was suppressed, egg maturation and oviposition failed. The effects could be partly suspended by JH injection in *L. migratoria* (Couble *et al.*, 1979), and *A. thalassinus* (Osman & Schmidt, 1988; Schmidt & Othman, 1994).

Haemolymphal JH titres of female grasshoppers were mostly established throughout the first gonadotropic cycle after emergence. Similar to *A. thalassinus*, in *L. migratoria* the JH-III titre was very low until day 5, increased then 10-fold until day 6-9, and declined strongly after day 12 and later. The JH-III titres fluctuated at least by a factor of 7 (Rembold, 1981). In the migratory locust, Johnson & Hill (1975) found a decline of the JH-III titre, when the oocytes were present in the oviduct, before oviposition. Further investigations, in which the JH-III titre of single females was studied until oviposition, showed a similar cyclic trend of the JH-III titre, with values of up to 5 pmol JH-III / 10 Bl haemolymph (Rembold, 1985). In *A. thalassinus*, a better correspondence was found, when the oocyte volume was correlated with the JH-III titre. JH-III titres of 3 pmol / 10 µl haemolymph were maximally measured (Schmidt et al, 1990). In *E. plorans*, no clear cycle of the JH-III titre was found in the haemolymph throughout the first and also the following gonadotropic cycles. In the second and third gonadotropic cycles, the JH-III titre did not reach the same high values as found in the first one. Many negative correlations were observed (Schmidt *et al.*, 1996).

Regarding the present study, the JH-III titre of the haemolymph often varied in relation to the size of the t-oocytes during the gonadotropic cycles of *A. thalassinus*. High JH-III titres of about 2.5 pmol / 10 µl haemolyph were measured during the fastest growth of the t-oocytes and the incorporation period of vitellogenin in steps 3-4 (Table 1). In females, containing almost ripe t-oocytes, or when the latter had reached the oviduct and the eggs were close to being deposited, the JH-III titre declined, as shown by Schmidt *et al.* (1990) for the first gonadotropic cycle. But the decline of the JH-III titre was not significant if the subterminal oocytes had already developed reaching step 3-4.

Different opinions have been expressed upon the influence of various factors on the regulation of the JH-III titre of the haemolymph. The JH-titre is influenced by JH biosynthesis, JH catabolism, JH excretion and JH binding to various proteins of internal organs (Osir & Riddiford, 1988). In *L. migratoria*, Emmerich & Hartmann (1973) showed that JH is bound to a carrier with a MW of 220 KD in the haemolymph. Couillaud & Girardie (1985) supposed that JH-III can bind to body tissues and that binding and excretion are the most important processes for the variability of JH-III concentration in the haemolymph, whereas Ferenz & Kaufner (1981) considered JH biosynthesis as the most important regulating factor of the JH titre of the haemolymph.

For the gryllid *A. domestica*, Renucci *et al.* (1990) registered an inhibition of JH-III production and further development of oocytes, if eggs remained in the ovaries, due to having no possibility for deposition. This may inhibit the CA activity, or affect the brain. The suggestion was supported by oophorectomy, not influencing the JH-III titre in *L. migratoria* (Johnson & Hill, 1975).

Schmidt & Osman (1988) showed in *A. thalassinus* and Schmidt & Albütz (1994) in *S. gregaria* that male pheromones can be involved and accelerate female maturation and oviposition, confirming the findings of Loher (1960) and observation of Highnam & Lusis (1962). Schmidt & Othman (1994) stated that a male pheromone will act on the oogenesis through an activation of the CA, stimulating the biosynthesis of JH-III which interacts with the protein synthesis in the haemolymph and yolk uptake of the oocytes. All external factors may directly stimulate the CA through the nervous and/or neuroendocrine system depending on the physiological state of the insect.

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REFERENCES

Antoniou A., Hunter-Jones P., 1956 - The life history of *Eyprepocnemis capitata* Miller (Orth., Acrididae) in the laboratory. - Entomological Month. Mag. 92: 364-368.

Balogh A.A., 1980 - Some studies on the egg laying behaviour, fecundity and fertility of *Aiolopus thalassinus* (Fab.) (Orth., Acrididae). - Türk Bit Kor Derg 4(1): 29-43.

Couble P., Chen T.T., Wyatt G.R., 1979 - Juvenile hormone controlled vitellogenin synthesis in *Locusta migratoria* fat body: cytological developement. - J. Insect Physiol. 25: 327-337.

COUILLAUD F., GIRARDIE A., 1985 - Control of C-16 juvenile hormone biosynthesis in active corpora allata of female African locusts. - Int. J. Invertebr. Reprod. Dev. 8 (6): 303-315.

COUILLAUD F., GIRARDIE A., GIRARDIE J., 1989 - Identification of gonotropic and antigonotropic factors from the nervous part of the corpora cardiaca in the African locust.. - Int. J. Invertebr. Reprod. Dev. 16 (1-3): 17-22.

DALE J.F., TOBE S.S., 1986 - Biosynthesis and titre of juvenile hormone during the first gonotropic cycle in isolated and crowded *Locusta migratoria* females. - J. Insect Physiol. 32 (9): 763-770.

DAVEY K.G., SEVALA V.L., GORDON D.R.B., 1993 - The action of juvenile hormone and antigonadotropin on the follicle cells of *Locusta migratoria*.- Int. J. Invertebr. Reprod. Dev. 24 (1): 39-46.

DEAN J.M., 1981 - The relationship between lifespan and reproduction in the grasshopper *Melanoplus*. - Oecologia (Berlin) 48: 385-388; Germany.

DOGRA G.S., EWEN A.L.B., 1970 - Egg laying by allatectomized females of the migratory grasshopper, *Melanoplus sanguinipes*. - J. Insect Physiol. 16: 461-469.

ELLIOTT R.H., GILLOTT C., 1978 - The neuro-endocrine control of protein metabolism in the migratory grasshopper, *Melanoplus sanguinipes* (Orth., Acrididae). - J. Insect Physiol. 24 (2): 119-126.

EMMERICH H., HARTMANN R., 1973 - A carrier lipoprotein for juvenile hormone in the haemolymph of *Locusta migratoria*. - J. Insect Physiol. 19: 1663-1675.

FERENZ H.-J., ADEN E., 1993 - Ovarian control of juvenile hormone biosynthesis in *Locusta migratoria* (Orthoptera: Acrididae). - Entomol. Gener. 18 (1-2): 9-17.

FERENZ H.-J., KAUFNER I., 1981 - Juvenile hormone synthesis in relation to oogenesis in *Locusta migratoria,* 135-145. In: PRATT G. E., BROOKS G. T. (Eds), Juvenile Hormone Biochemistry, Elsevier, North Holland Biochemical Press Amsterdam, The Netherlands.

FEYEREISEN R., 1985 - Regulation of juvenile hormone titer: Synthesis. - In: KERKUT G. A., GILBERT L. I. (EDS), Comprehensive Insect Physiology, Biochemistry and Pharmacology, Pergamon Press 7: 391-429; Oxford, New York, Toronto, Sydney, Paris and Frankfurt.

GADOT M., BURNS E., SCHAL C., 1989 - Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica* effects of grouping and mating. - Arch. Ins. Biochem. Physiol. 11 (3): 189-200.

GILLOTT C., ELLIOTT R.H., 1976 - Reproductive growth in normal, allatectomized, median-neurosecretory-cell-cauterized and ovariectomized females of *Melanoplus sanguinipes*. - Can. J. Zool. 54: 162-171.

GLINKA A.V., TRISELEVA T.A., 1989 - Differential regulation of protein biosynthesis at translational level in the fat body cells of adult *Locusta migratoria*. - Mol. Reprod. Dev. 1 (4): 278-282.

GOLTZENE F., PORTE A., 1978 - Endocrine control by neurosecretory cells of the pars intercerebralis and the corpora allata during the earlier phases of vitellogenesis in *Locusta migratoria migratorioides* R. & F. (Orthoptera). - Gen. Comp. Endocrinol. 35 (1): 35-45.

HAFEZ M., IBRAHIM M.M., 1962 -On the ecology and biology of the grasshopper Aiolopus thalassinus (F.) in Egypt (Orthoptera: Acrididae).. - Bull. Soc. Ent. Egypte 46: 189-214.

HIGHNAM K.C., LUSIS O., 1962 - The influence of mature males on the neusecretory control of the ovarian development in the desert locust. - Quart. J. Micr. Sci. 103: 73-83.

HIGHNAM K.C., LUSIS O., HILL L., 1963 - The role of the corpora allata during oocyte growth in the desert locust, *Schistocerca gregaria*. - J. Insect Physiol. 9: 587-596.

HOLLIS D., 1968 - A. revision of the genus *Aiolopus* Fieber (Orthoptera: Acridoidea). - Bull. Brit. Mus. (Nat. Hist.) Entomol. 22 (7):-307-355.

HORSEMAN G., HARTMANN R., VIRANT-DOBERLET M., LOHER W., HUBER F., 1994 - Nervous control of juvenile hormone biosynthesis in *Locusta migratoria*. - Proc. Natl. Acad. Sci. USA 91: 2960-2964.

HUIBREGTSE-MINDERHOUD L., VAN DEN HONDEL-FRANKEN M.A.M., VAN DER KERK-VAN HOOF A.C., BIESSELS H.A.W., SALEMINK C.A., VAN DER HORST D.J., BEENAKKERS M.T., 1980 - Quantitative determination of the juvenile hormones in the haemolymph of *Locusta migratoria* during normal development and after implantation of corpora allata. - J. Insect Physiol. 26: 627-631.

INJEYAN H.S., TOBE S.S., 1981 - Phase polymorphism in *Schistocerca gregaria*: assessment of juvenile hormone synthesis in relation to vitellogenesis. - J. Insect Physiol. 27 (3): 203-210.

JOHNSON R.A., HILL L., 1975 - Activity of the corpora allata in the adult female migratory locust. - J. Insect Physiol. 21: 1517-1519.

KATAOKA H., TOSCHI A., LI J.P., CARNEY R.L., SCHOOLEY D.A., KRAMER S.J., 1989 - Identification of an allatotropin from adult *Manduca sexta*. - Science (Washington) 243: 1481-1483.

KELLY T.J., DAVENPORT R., 1976 - Juvenile hormone induced ovarian uptake of a female specific blood protein in *Oncopeltus fasciatus*. - J. Insect Physiol. 22: 1381-1393.

KEMPA-TOMM S., HOFFMANN K.H., Engelmann F., 1990 - Vitellogenins and vitellins of the mediterranean flied cricket *Gryllus bimaculatus* - isolation, characterization and quantification. - Physiol. Entomol. 15 (2): 167-178.

KEMPA-TOMM S., KASSEL U., HOFFMANN K.H., 1989 - Vitellogenin synthesis and secretion in crickets, *Gryllus bimaculatus*. - Comp. Biochem. Physiol. 94 B: 201-205.

KRAMER S.J., TOSCHI A., MILLER C.A., KATAOKA H., QUISTAD G.B., LI J.P., CARNEY R.L., SCHOOLEY D.A., 1991 - Identification of an allatostatin from the tobacco hornworm *Manduca sexta*. - Proc. Nat. Acad. Sci. USA 88: 9458-9462.

LAZAROVICI P., PENER M.P., 1978 - The relations of the pars intercerebralis, corpora allata and juvenile hormone to oocyte development and oviposition in the African migratory locust. - Gen. Comp. Endorinol. 35 (4): 375-389.

LOHER W., 1960 - The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. - Proc. R. Entomol. Soc. (B) 153: 380-397.

LOHER W., 1965 - Hormonale Kontrolle der Oocytenentwicklung bei der Heuschrecke *Gomphocerus rufus* L. (Acrididae). - Zool. Jb. Physiol. 71: 677-684.

LOHER W., BAKER F., SCHOOLEY D.A., 1988 - Hormonal regulation of egg production in crickets. - Proc. 18th Intern. Congr. Entomol., Vancouver BC, Canada; Abstracts p. 118.

LORENZ M.-W., Gäde G., HOFFMANN K.H., 1997 - Interspecific actions of allatostatins. - Mitt. Dt. Ges. allg. angew. Ent. 11: 549-553.

NEUHÄUSER T., SORGE D., STAY B., HOFFMANN K.H., 1994 - Responsiveness of the adult cricket (*Gryllus bimaculatus* and *Acheta domesticus*) retrocerebral complex to allatostatin-I from a cockroach, *Diploptera punctata*. - J. Comp. Physiol. B. 164: 23-32.

ORSHAN L., PENER M.P., 1991 - Effects of the corpora allata on sexual receptivity and completion of oocyte development in females of the cricket, *Gryllus bimaculatus*. - Physiol. Entomol. 16: 231-242.

OSIR E.O., RIDDIFORD L.M., 1988 - Nuclear binding sites for juvenile hormone and its analogs in the epidermis of the tobacco hornworm. - J. Biol. Chem. 263 (27): 13812-13818.

OSMAN K.S.A., SCHMIDT G.H., 1988 - Wirkung von Precocen-III auf die hormonale Steuerung der Oogenese und Larvensterblichkeit bei *Aiolopus thalassinus* (Fabr.). (Saltatoria; Acrididae). - Mitt. Dt. Ges. allg. angew.Ent. 6 (4-6): 347-363.

PENER M.P., 1965 - On the influence of the corpora allata on maturation and sexual behaviour of *Schistocerca gregaria*. - J. Zool. 147: 119-136.

REMBOLD H., 1981 - Modulation of JH-III titer during the gonotropic cycle of *Locusta migratoria*, measured by gas chromatography-selected ion monitoring mass spectrometry, 11-20. - In: PRATT G. E., BROOKS G. T. (Eds), Juvenile Hormone Biochemistry, Elsevier, North Holland Biomedical Press, The Netherlands.

REMBOLD H., 1985 - Mikroanalytische Bestimmung von Juvenylhormonen. - Chimia 39 (11): 348-354.

REMBOLD H., LACKNER B., 1985 - Convenient method for the determination of picomol amounts of juvenile hormone. - J. Chromatography 323: 355-361.

RENUCCI M., STRAMBI A., STRAMBI C., 1987 - Ovarian development and endocrine control of the vitellogenesis in the house cricket *Acheta domestica*. - Gen. Endocrinol. (Life Science Advances) 6: 83-92.

RENUCCI M., STRAMBI A., AUGIER R., CHARPIN P., 1990 - Ovaries and regulation of juvenile hormone titer in *Acheta domesticus* L. (Orthoptera). - Gen. Comp. Endocrinol. 78 (1): 137-149.

RICHARDS O.W., WALOFF N., 1954 - Studies on the biology and population dynamics of British grasshoppers. - Anti-Löcust Bull. 17.

RÖHRKATEN A.., FERENZ H.-J., 1986 - Properties of the vitellogenin receptor of isolated locust oocyte membranes. - Int. J. Invertebr. Reprod. Dev. 10: 133-142.

ROUSSEL J.-P., 1978 - Effect of juvenile hormone on blood-vitellogenin in allatectomized females of *Locusta migratoria*. - C. R. Hebd. Seances Acad. Sci., Ser. D, 287 (14): 1309-1312.

SCHMIDT G.H., 1980 - Zur Bionomie von *Acrotylus patruelis* (Herrich-Schäffer) (Insecta; Saltatoria, Acrididae). - Zool. Beitr. N. F. 26: 133-159.

SCHMIDT G.H., 1981 - Growth and behaviour of *Acrotylus patruelis* (H.-S.) larvae in temperature gradients under laboratory conditions. - Zool. Anz- 206 (1/2): 11-25.

SCHMIDT G.H., 1986 - Use of grasshoppers as test animals for the ecotoxicological evaluation of chemicals in the soil. - Agric. Ecosystems Environment 16: 175-188.

SCHMIDT G.H., ALBÜTZ R., 1994 - Laboratory studies on pheromones and reproduction of *Schistocerca gregaria* (Forsk.), the desert locust. - J. Appl. Entomol. 118: 378-391.

SCHMIDT G.H., ALBÜTZ R., 1999 - Identification of solitary and gregarious populations of the Desert Locust *Schistocerca gregaria* (Caelifera: Acrididae) by experimental breeding. - Entomol. Gener. 24 (3): 161-175.

SCHMIDT G.H., ALBÜTZ R., 2002 - Sexual maturation and yellow coloration of adult males in gregarious *Schistocerca gregaria* in relation to volatiles emitted (Caelifera: Acrididae). - Entomol. Gener. 26 (2): 121-141.

SCHMIDT G.H., FEIERABEND H., 2000 - Population dynamics of the grasshopper species Aiolopus thalassinus under contolled laboratory conditions shoiwing the influence of intrinsic factors (Orthopteroidea: Caelifera: Acrididae). - Entomol. Gener. 24 (4): 266-270.

SCHMIDT G.H., FRIEDEL K., REMBOLD H., 1996 - Studies on the size of Corpora allata, the Juvenile hormone III titer in the hemolymph and growth of terminal oocytes throughout three consecutive gonadotropic cycles in *Eyprepocnemis plorans* (Charp.) (Insecta: Saltatoria: Acrididae). - Eur. J. Entomol. 93: 131-144.

SCHMIDT G.H., LILGE R., 1997 - Geographische Verbreitung der Oedipodinae (Orthopteroidea, Caelifera, Acrididae) in Europa und Randgebieten, mit Hinweisen zur Ökologie und Biologie. - Verlag Dr Kovac, Hamburg, xiii+149 pp.

SCHMIDT G.H., OSMAN K.S.A., 1988 - Male pheromones and egg production in Acrididae, 701-706. In: SEHNAL F., ZABZA A., DENLINGER D, L, (EDS), Endocrinological Frontiers in Physiological Insect Ecology, Vol. II, Techn. Univ. Press, Wroclaw, Poland.

SCHMIDT G.H., OSMAN K.S.A., REMBOLD H., 1990 - Hemolymph juvenile hormone titers and growth of terminal oocytes throughout the first gonadotrophic cycle in *Aiolopus thalassinus*. - Naturwissenschaften 77: 194-195.

SCHMIDT G.H., OTHMAN K.S.A., 1993 - Changes in the size of corpora allata, in the juvenile hormone III titer in the hemolymph and the protein content of terminal oocytes throughout the first gonadotropic cycle in *Aiolopus thalassinus* (Insecta: Orthoptera, Acrididae). - Arch. Ins. Biochem. Physiol. 24: 45-54.

SCHMIDT G.H., OTHMAN K.S.A., 1994 - Untersuchungen zur pheromonalen Steuerung der Reproduktion von virginen und verpaarten Weibchen der Feldheuschrecke *Aiolopus thalassinus* (Fabr.) (Insecta, Orthoptera, Acrididae). - Zool. Anz. 233 (3-4): 75-116.

SINGH T., 1958 - Ovulation and corpus luteum formation in *Locusta migratoria migratorioides* (Reiche & Fairmaire) and *Schistocerca gregaria* (Forskål). - Trans. R. Ent. Soc. London 110: 1-20.

SMITH R.F., YAGI K., TOBE S.S., SCHAL C., 1989 - In vitro juvenyle biosynthesis in virgin and mated female of brown-banded cockroaches *Supella longipalpa*. - J. Insect Physiol. 35 (10): 781-786.

STRONG L., 1965 - The relationships between the brain, corpora allata and oocyte growth in the central american locust, *Schistocerca sp.* II. The innervation of the corpora allata, the lateral neurosecretory complex and oocyte growth. - J. Insect Physiol. 11: 271-280.

TOBE S.S., CHAPMAN C.S., 1979 - The effects of starvation and subsequent feeding on juvenile hormone synthesis and oocyte growth in *Schistocerca americana gregaria*. - J. Insect Physiol. 25 (9): 701-708.

ULRICH G.M., SCHLAGINTWEIT B., EDER J., REMBOLD H., 1985 - Elimination of the allatotropic activity in locusts by microsurgical and immunological methods: evidence for humoral control of the corpora allata, hemolymph proteins and ovary developement. - Gen. Comp Endocrinol. 59: 120-129.

UVAROV B.S., 1977 - Grasshoppers and Locusts. Vol 2, Centre for Overseas Pest Research, London.UK.

WEAVER R.J., EDWARDS J.P., 1990 - The role of the corpora allata and associated nerves in the regulation of ovarian cycles of the oviparous cockroach *Pleplaneta americana*. - J. Insect Physiol. 36 (1): 51-59.

WENNAUER R., KASSEL L., HOFFMANN K.H., 1989 - The effects of juvinyle hormone 20 hydroxyecdysone precocene II and ovariectomy on the activity of the corpora allata in-vitro in adult female *Gryllus bimaculatus*. - J. Insect Physiol. 35 (4): 299-304.

WOODHEAD A P, STAY B; SEIDEL S L; KHAN M.A., TOBE S.S., 1989 - Primary structure of four allatostatins: Neuropeptide inhibitors of juvenile hormone synthesis. - Proc. Natl. Acad. Sci. USA 86: 5997-6001.

WYATT G.R., 1988 - Vitellogenin synthesis and the analysis of juvenile hormone action in locust fat body. - Can. J. Zool. 66 (12): 2600-2610.

WYATT G.R., DHADIALLA T.S., ROBERTS P.E., 1984 - Vitellogenin synthesis in locust fat body: Juvenile hormone-stimulated gene expression, 475-484. In: HOFFMANN J. A., PORCHET M. (Eds), Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones. Springer-Verlag, Berlin, Germany.

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