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# Morphological and functional maturation of a skeletal muscle regulated by juvenile hormone

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**Summary**

**Reproductive behaviour of animals requires a well-adapted muscular system. This study examines the structural and functional development of ovipositor muscle properties in female locusts during reproductive development. A possible regulation by juvenile hormone (JH) was assessed by comparing muscle properties in immature and mature females and with those whose JH production was inhibited by allatectomy early in adult life. The results are related to the reproductive behaviour of locusts.**

**Histological and ultrastructural comparison of muscle fibres and their associated cuticular structures (apodemes) revealed dramatic growth during the first 2 weeks of reproductive development. The cross-sectional area of muscle fibres increased sevenfold, and their mass-per-length 5.3-fold. Ultrastructural examination showed growth of mitochondria, development of sarcoplasmic reticulum and increasing levels of structural organisation of myofibrils. Muscles of mature females displayed pronounced fatigue resistance, contracted more powerfully (twitch, 33.22±10.8 mN; 50 Hz, 623.66±115.77 mN) and had almost two times faster kinetics than those of immature females (twitch, 6.5±2.6 mN; 50 Hz, 14.19±2.58 mN). Together with muscular maturation, cuticular apodemes, which serve as attachment sides for ovipositor muscles, grow considerably in length and width and assume a complex surface structure. Most of the described changes were suppressed in females deprived of JH (allatectomised). The results demonstrate an adaptation of muscle properties to the requirements of reproductive behaviour that is largely regulated by juvenile hormone.**

Key words: insect, locust, *Locusta migratoria migratorioides*, juvenile hormone, reproduction, muscle development, contraction property.

## Introduction

The reproductive development of animals is characterised by the production of gametes, the maturation of various tissues and the expression of behaviour related to mating and birth or oviposition. Morphological and functional changes of the neuro-muscular system are considered a prerequisite for animals to show adequate sexual behaviour, which in turn is necessary for successful reproduction. Steroids have been shown to be intimately involved in the timing and coordination of these processes in vertebrates (Venable, 1966; Bass, 1986; Rand and Breedlove, 1995; Forger et al., 1996; Schlinger, 1997). Among the best-known systems are the androgen-regulated sexual differentiation and maturation of perineal muscles and their innervating motoneurons in mammals (Breedlove, 1984, 1986; Forger et al., 1996; Peroulakis et al., 2002) and of larynx in *Xenopus* (reviewed in Kelley, 1996).

Although evolutionarily remote, similar processes occur in the adult insect, albeit regulated by a different hormone named juvenile hormone (JH). JH, first discovered by Wigglesworth (1934, 1936), regulates several aspects of insect development and reproduction. In cooperation with 20-hydroxyecdysone (20-HE), JH governs metamorphosis by inhibiting the development of adult characters (Riddiford, 1985). In the adult insect, by contrast, JH function has clearly been associated with reproductive development (reviewed by Wyatt, 1997).

Early experimental data suggesting a regulatory function of JH during reproductive development came from behavioural studies on grasshoppers (Strong and Amerasinghe, 1977; Hartmann, 1978) and showed that female sexual behaviour strongly depends on corpora allata activity, the gland producing and releasing JH. Although this effect can differ even within a single insect family (Truman and Riddiford, 1974; Barth and Lester, 1973), the importance of JH became apparent.

By focusing on the nervous and muscular system, recent studies have revealed a variety of developmental changes mediated by JH. Changes in the phonotactic response of female house crickets are mediated by JH *via* gene regulation (Stout et al., 1992, 1993). In the same species, neurogenesis of mushroom body neurons is stimulated by JH (Cayre et al., 1994). Both changes are likely to contribute to the expression of adequate sexual behaviour (reviewed by Strambi et al., 1997). In the male moth *Agrotis ipsilon*, the sensitivity of

olfactory interneurons to sex pheromones is increased by JH (Anton and Gadenne, 1999), thereby enhancing mate recognition and reproduction. Muscles are the output elements of the information provided by the nervous system, and several studies have demonstrated hormonal regulation of degeneration/regeneration and structural muscle properties during metamorphosis and reproductive development.

The remodelling of musculature during metamorphosis of holometabolous insects involves degeneration of existing muscles (Finlayson, 1975; Rheuben, 1992) and the differentiation of new muscles (Stocker and Nüesch, 1975; Bate et al., 1991; Consoulas et al., 1997). These events are mainly regulated by 20-HE in the absence of JH (Weeks and Truman, 1985; Schwarz and Truman, 1983; Luedemann and Levine, 1996; Hegstrom and Truman, 1996; reviewed in Weeks and Truman, 1986). The importance of metamorphosis for flight muscle differentiation and development has been shown by manipulating the levels of JH. Treatment of larval stages with the JH analog methoprene, or implantation of corpora allata, which shortened the length of the larval stadium, caused slowing of muscle growth and inhibited the development of mitochondria and tracheolation in locust (Poels and Beenakkers, 1969; Cotton and Anstee, 1990) and cricket (Novicki, 1989). Chemical allatectomy, however, enhanced flight muscle development and resulted in normal flight muscles of the adultiform (Wang et al., 1993). By contrast, parts of a flight steering muscle in locust (M114c) degenerate shortly after adult emergence while the JH titer is low (Meuser and Pflüger, 1998). Experimentally elevated JH titers prevent muscle degeneration. These studies indicate the importance of low JH levels during the last larval stage for normal development of flight muscles.

In the adult insect, degeneration and regeneration of flight muscles are clearly regulated by JH in different insect species (Tanaka, 1994; Borden and Slater, 1968; de Kort, 1990; reviewed by Finlayson, 1975; Wyatt and Davey, 1996). The close correlation between reproduction and flight muscle degeneration and regeneration has been suggested to serve for the liberation of nutrients when functional muscles are no longer needed. However, little is known about structural and functional changes that might adapt muscle performance to the requirements of reproductive behaviour. A recent study on the longitudinal muscles of female locusts provided the first evidence for a functional adaption of muscle properties controlled by JH (Rose et al., 2001). Changes were shown to be segment- and gender-specific and important for oviposition behaviour.

Oviposition behaviour in insects is not expressed before the female is sexually mature and ensures adequate deposition of eggs. This raises the question of whether the underlying neuromuscular system changes its properties at the time of sexual maturation to adapt for the species-specific requirements of oviposition. In locusts, considerable knowledge has accumulated about various aspects of oviposition (reviewed by Staufer and Whitman, 1997). The female lays its eggs deep down into the soil by rhythmical digging movements of a pair of sclerotized appendages (ovipositor valves) located at the tip of the abdomen (Vincent, 1975; Thompson, 1986; Rose et al., 2000). The digging movements of the ovipositor are thus essential for a successful oviposition. However, whether the muscles associated with the ovipositor undergo structural and functional changes during reproductive development is unknown. Among the ten pairs of ovipositor muscles (Snodgrass, 1935), the dorsal and ventral ovipositor opener muscles are the largest. Their contractions open and close the valves during oviposition digging. The patterned neural input to these muscles, which can be elicited in embryos and larvae (Thompson and Roosevelt, 1998), is provided by motoneurons located in the terminal abdominal ganglion (Thompson et al., 1999).

The present study addresses questions about possible structural and functional maturation of ovipositor muscles during reproductive development. I compared morphological characteristics and functional properties of the dorsal ovipositor opener muscles from immature and mature females. One of the primary goals of this work was to reveal the functional consequences of structural changes and to relate them to the behavioural requirements. A possible regulatory function of JH was assessed by manipulating JH production through allatectomy in combination with JH-analog replacement injections.

The results presented here show a pronounced growth and ultrastructural maturation of ovipositor muscle fibres during reproductive development, together with multiple changes of their contraction characteristics. Inhibition of JH production by allatectomy largely suppressed normal changes, whereas replacement injections with the JH analog methoprene almost restored the normal development. The results thus suggest a regulatory control by JH.

## Materials and methods

### *Animals and preparations*

All experiments were performed on adult *Locusta migratoria migratorioides* (R&F). Animals were obtained from a crowded colony reared at the University of Ulm in laboratory cages at 32±2°C. Colonies were fed daily on wheat seedlings, bran or fresh grass. Prior to experiments, animals were cold-anaesthetised at 4°C for 0.5 h. To expose the dorsal ovipositor opener muscles (M271), animals were mounted with their dorsal side up in a Sylgard™-coated Petri dish. An incision was made along the dorsal midline and the abdominal walls were pinned down laterally. After removing the gut, gonads and adhering tissue, the abdominal cavity was immediately filled with locust saline buffered at pH 6.8 (in mmol $l^{-1}$ NaCl, 140; KCl, 10; $CaCl_2$, 2; $MgCl_2$, 3; 3-[*N*-morpholino]propanesulfonic acid (MOPS), 10; sucrose, 90). For staging, animals were isolated at the day of adult emergence. In our colony, females reached maturity after approximately 14 days. Thus, animals older than 14 days were considered mature and those used for the experiments were between 18 and 25 days old. Females between 1–3 days old

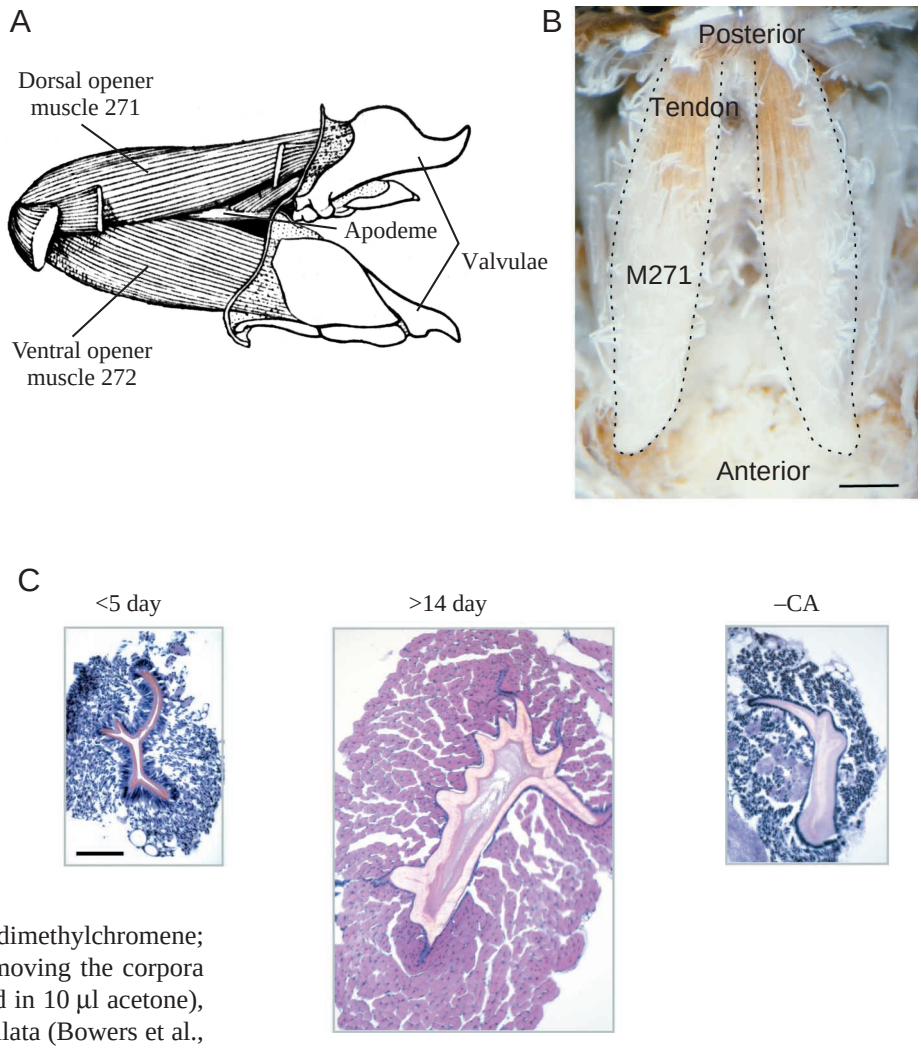


Fig. 1. The ovipositor and its muscles in female grasshoppers. (A) Lateral view showing a diagram of dorsal and ventral muscles, apodeme and valvulae. When muscles 271 and 272 contract, the dorsal and ventral valvulae open. (B) Photograph of a dissection showing a dorsal view on the ovipositor opener muscle M271 from a mature female. The tendon at the posterior insertion site appears magenta-coloured. (C) Transverse sections through the apodeme-muscle complex of an immature (<5 day), mature (>14 day) and allatectomised (–CA) female. Note increased size of muscle fibre bundles and apodeme complexity in mature females. A is adapted from Snodgrass (1935) with permission. Scale bars, 1 mm (B); 0.5 mm (C).

were considered immature. Muscles were numbered after Snodgrass (1935) and named after Thomson (1986).

### *Hormonal manipulation*

To determine the influence of JH on the development of muscle fibres, female locusts were allatectomised by one of two means. Experimental animals were either chemically allatectomised by using precocene (7-ethoxy-6-methoxy-2,2-dimethylchromene; Sigma) or surgically allatectomised by removing the corpora allata. Precocene (500 μg/animal, dissolved in 10 μl acetone), which chemically inactivates the corpora allata (Bowers et al., 1976; Pener et al., 1978) was topically applied onto the dorsal neck fold once at the day of adult emergence. Control animals were treated with acetone only. The technique for surgical allatectomy followed the method published by Strong (1963). In brief, animals were anaesthetised with carbon dioxide and mounted in a dish with their dorsal side up. The head was stretched to expose the dorsal neck membrane and the dish was filled with ice-cold saline until the neck membrane was covered. A tear was made in the midline of the neck membrane and, with two fine forceps, the cephalic air sacs were displaced on one side and the corpora allata from the other side was removed. After both corpora allata had been removed, the animal was dismounted, blotted on filter paper and the neck membrane sealed with histoacryl (B. Braun, Tuttlingen, Germany). The same procedure was applied to sham-operated females, except that in this case the corpora allata were gently pulled but not destroyed or removed. Allatectomised and sham-operated females were then allowed to develop to an age of 18–25 days. In all experiments of this study, sham-operated and non-operated females were indistinguishable in their anatomy and physiology and considered as mature females. However, to be clear on this point, they were named >14 day (non-operated) and >14 day, sham-op. (sham-operated). The survival rate of animals was about 70% for those surgically allatectomised and about 90% for precocene-treated animals. During the experiments no differences were apparent between surgically and chemically allatectomised females. Once the allatectomised animals reached the appropriate age (18–25 days) they usually showed clear signs of inhibited JH production (undeveloped oocytes, cuticle lightly coloured, considerable fat body). Those females showing no signs of inhibited JH production were excluded from the study.

At the seventh day following adult emergence, some of the surgically allatectomised females were either injected with the JH analog methoprene (860 μg in 5 μl acetone, injected once in the abdomen) or an active corpora allata was implanted through an incision in the ventral tergo-sternal membrane of the third abdominal segment. All experimental females were individually marked and held separately from other groups.

### *Histology*

Cuttings of fixed tissue were performed on a cryostat or ultramicrotome. For cryostat sections, the entire valve-muscle complex (Fig. 1A) was fixed overnight in 2.5 mol $l^{-1}$ glutaraldehyde, rapidly frozen in liquid nitrogen and

subsequently cut (12 μm) on a cryomicrotom (Microm, HM500 OM; Walldorf, Germany). Material was transferred to slides, stained with Hematoxylin-Eosin, dehydrated and mounted in Entellan (Merck, Darmstadt, Germany).

Electron microscopy studies were performed on dorsal ovipositor muscle M271 taken from immature, mature or hormonally manipulated females. Muscles were fixed *in situ* using 2% glutaraldehyde for 30 min, post-fixed with 2% osmium tetroxide and subsequently dehydrated and embedded in Epon 812 (Fluka, Seelze, Germany). Thin sections were cut (80–90 μm) and double stained with uranyl acetate and lead citrate. Sections were examined and photographed using a Zeiss EM10 electron microscope.

Semi-thin sections (1 μm) from the middle region of fixed muscles were cut, mounted on slides and stained with Methylene Blue. Sections were examined under a bright field microscope. To determine the cross-sectional area of muscle fibres, a digital picture was taken with a CCD camera (Sony ICX038AK, resolution 752×582). The mean cross-sectional area of a single fibre was determined by calculating the sum of cross-sectional areas of all measured fibres (Scion Image, 4.0.2, Scion Corporation, Frederick, USA) divided by the number of fibres.

To meaningfully compare the performance of muscle M271 in different experimental groups, the mass-per-length of the muscle was estimated. Muscles were fixed *in situ* for 1 h in 2.5% glutaraldehyde and carefully separated from their attachment sites with fine forceps. After measuring the length under a dissection microscope, muscles were blotted on filter paper and weighed (Sartorius, MC210P, Göttingen, Germany).

For scanning electron microscopy studies, valve apodemes were fixed in 2% glutaraldehyde, freed from adhering tissue and separated from the abdomen. After rinsing, specimens were critical-point dried, coated with gold–palladium (20 nm) and examined and photographed in a scanning electron microscope. To quantify their length and width, apodemes were measured under a dissection microscope. The length was measured between the anterior tip and their posterior attachment to the valvulae. The width was measured at the middle of the apodeme. As an overall measurement for apodemal growth the values for length and width were multiplied.

### *Tension recordings*

Isometric tension recordings were performed on dorsal ovipositor muscle M271 (Fig. 1A). Experimental animals were mounted in a Sylgard-coated dish and opened dorsally. The dorsal ovipositor muscle was fixed anteriorly with an U-shaped insect pin where it attaches to the apodeme. The tendon at the posterior side was cut and clamped to the lever arm of an isometric force transducer. Muscle contractions were evoked by stimulating the motor nerve *via* a suction electrode. Stimulus intensity and length of the muscle were individually adjusted to elicit maximal muscle contraction. Between contractions, the preparation was continuously superfused with aerated saline. The response of the transducer was linear over the range used in the experiments and calibrated after each experiment. Proctolin was freshly prepared from a stock solution ($10^{-3}$ mol $l^{-1}$) and bath-applied by means of a pipette. Between different experimental trials the muscle was left unexcited for at least 5 min to recover from previous contractions.

### *Statistical evaluation*

Data are expressed as means ± standard deviation (S.D.). Statistical significance was determined by parametric tests (*t*-test or paired *t*-test where appropriate). When criteria for parametric tests were not met the non-parametric Mann–Whitney rank sum test was applied.

## Results

Visual observation revealed dramatic changes of ovipositor muscle size and appearance during the first 2 weeks of adult life. For a detailed examination, the dorsal opener muscle 271 (Fig. 1A,B) was taken as a representative. Muscle 271 and the valve apodeme, which gives the anterior attachment to the muscle, grow considerably from the 5th to the 15th day of adult life ($N$=10). Muscles from immature females were tiny, almost white in appearance and interspersed with numerous small tracheae. Cross-sections of the entire apodeme-muscle complex revealed relatively flat, small apodemes (Fig. 1C). By contrast, mature animals had well-developed, pink coloured muscle fibres (>14 day; Fig. 1C). Their central apodeme was much larger in size and had a complex three-dimensional structure. Females that were allatectomised (–CA; Fig. 1C) failed to show a comparable growth of muscle fibres or development of complex apodeme structure. The appearance of muscle fibres almost resembled those of immature females.

### *Apodeme growth*

A scanning electron microscopy study was performed to quantify possible changes in size and surface structure of isolated apodemes in greater detail. The study revealed considerable growth of apodemes during the first two weeks of adult development (Fig. 2A,B). Apodemes grew in length and width and assumed a complex three-dimensional structure that is characterised by a surface contour with pronounced edges and corrugations (Fig. 2, compare <5 day and >14 day). Apodemal area increased from 2.06±0.31 $mm^2$ to 7.08±0.72 $mm^2$ for immature and mature females, respectively. Allatectomised females had apodemes that were significantly smaller than those from mature females (3.60±0.67 $mm^2$; –CA in Fig. 2B). In addition, the surface structure assumed a level of complexity that was intermediate between that of immature and mature females. Replacement injections with methoprene were able to significantly reverse the effect of allatectomy (5.25±0.42 $mm^2$; –CA+met in Fig. 2B). However, the values still differed considerably from those measured in mature females.

### *Muscle fibre growth*

As mentioned before, ovipositor opener muscles undergo

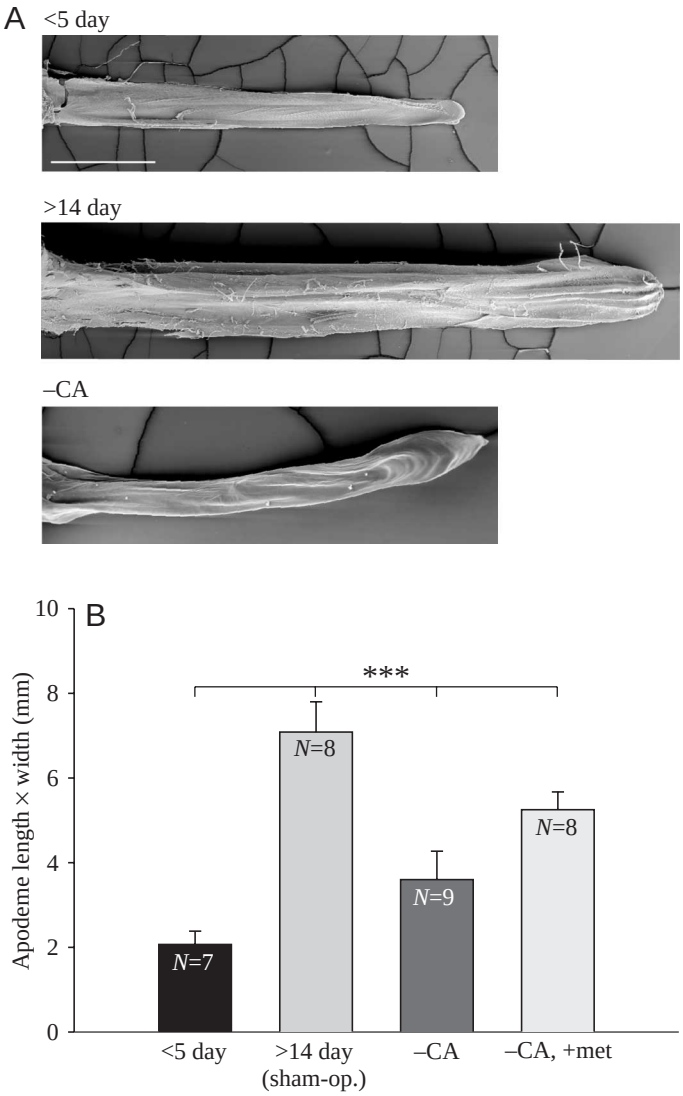


Fig. 2. Growth of valve apodeme during maturation is largely regulated by JH. (A) Scanning electron micrographs of the valve apodeme of females from different experimental groups. Apodemes of mature animals show increased length, width and a more complex surface structure than those of immature and allatectomised females (see also Fig. 1). By contrast, apodemes from immature or precocene-treated females are rather flat with a straight and smooth appearance. (B) Size-index values (length×width) of apodemes from immature (<5 day), mature (>14 day), allatectomised (–CA) and those females injected with the JH analog methoprene (–CA, +met). Apodemal size increases considerably between the 5th and the 14th day after adult emergence. Allatectomy markedly inhibits the increase, but replacement injections with methoprene are able to significantly reverse allatectomy effects. Data are means ± S.D.; ***$P$<0.001. Scale bar in A, 1 mm; anterior is to the right.

hypertrophy during the first 2 weeks of adult life. Their mass-per-length (mass/length) increased from 0.28 to 1.5 mg mm$^{-1}$ (Table 1). Allatectomy significantly reduced this increase to 0.42 mg mm$^{-1}$, whereas additional injection of the JH analog methoprene partially restored the higher mass/length values

Table 1. *Mass and length of ovipositor opener muscle 271*

| | Muscle mass (mg) | Muscle length (mm) | Mass/length (mg mm$^{-1}$) |
|---|---|---|---|
| <5 day | 1.54±0.47 (*N*=10) | 5.5±0.38 (*N*=7) | 0.28 |
| >14 day (sham-op.) | 11.0±2.0 (*N*=10) | 7.3±0.21 (*N*=8) | 1.5 |
| –CA, | 2.76±0.73 (*N*=10) | 6.5±0.31 (*N*=9) | 0.42 |
| –CA, +Met | 6.14±1.26 (*N*=9) | 7.17±0.22 (*N*=8) | 0.85 |

(0.85 mg mm$^{-1}$) seen in mature females. To examine muscle histology and physical dimensions in greater detail, semi-thin sections of M271 were made and evaluated. Transverse sections obtained from muscles of immature females revealed loosely distributed fibres with a high degree of tracheolation and numerous nuclei (Fig. 3A, <5 day). The mean cross-sectional area of muscle fibres was 53.75±15.65 μm$^2$ (Fig. 3B, <5 day). By contrast, muscles from mature females had a much larger cross-sectional area of 377.87±73.54 μm$^2$ (Fig. 3B, >14 day). Their tracheolation appeared not as pronounced as in immature females, possibly because of the large and prominent muscle fibres (Fig. 3A, >14 day). The appearance of muscle fibres from allatectomised females, however, was comparable to immature females with a cross-sectional area of 67.25±21.68 μm$^2$ (Fig. 3B, –CA). As a result of allatectomy, fatty tissue was frequently present in cross-sections of these muscles (ft in Fig. 3A, –CA), but never observed in sections from immature or mature females. Additional injections of methoprene partially reversed the effects of allatectomy on the growth and appearance of muscle fibres (cross-sectional area: 214.25±31.63 μm$^2$, Fig. 3B, –CA+, met).

From these experiments it became apparent that methoprene was not able to completely reverse the effect of allatectomy and therefore additional experiments were performed in which a pair of active corpora allata was implanted, instead of injecting methoprene. The intention behind this experiment was to determine whether there is a difference between methoprene (JH analog) and the natural JH (released from the corpora allata) in their action to restore the normal development. The experiments revealed muscle fibres with an appearance indistinguishable from mature females. Their mean cross-sectional area was 361.0±103.47 μm$^2$, $N$=9 (data not shown) and were thus not significantly different from mature females ($P$>0.05, Mann–Whitney rank sum test).

A possible increase in the number of muscle fibres during reproductive development was accessed by relating the increase in cross-sectional area to the increased in muscle mass. Both the cross-sectional area and the mass of the muscle increased by a factor of 7.1, which suggests that the number of muscle fibres remains constant throughout maturation.

### *Ultrastructure*

To gain further insight into possible maturational changes of muscle fibre structure and organisation, the ultrastructure of muscles was compared. Muscle fibres from immature females

were in close contact with multiple tracheoles and tracheae (Figs 4A, 5A). The cytoplasm associated with the tracheoles contained numerous microtubules oriented parallel with the cuticular tubes. Interfibrillar tracheoles were not seen. In transverse sections, T-tubule openings and dyads were frequently present as well as multiple, small mitochondria (Fig. 4A,C). The myofibrils had an irregular appearance and were not well-defined. The sarcoplasm between the myofibrils covered a relatively large area and contained multiple microtubules, elements of the sarcoplasmic reticulum and T-tubules. Longitudinal sections showed small, elongated mitochondria located at the level of I-band (Fig. 4B). Although Z-lines were clearly visible as patches of electron dense material, their alignment was poor.

The ultrastructure of muscle fibres from mature females was clearly different from immature females. Myofibrils showed a regular appearance and were, at the level of the A-band, clearly defined by surrounding sarcoplasmic reticulum and T-system (Fig. 4D). Well-developed dyads were regularly encountered at the A-band (Fig. 4D, arrow), whereas T-tubule invaginations were restricted to the I-band (T; Fig. 4F). Compared to immature females, mitochondria were much larger and clustered along the I-band (M; Fig. 4E,F). Tracheoles and tracheae associated with single fibres lacked the relatively large cytoplasmatic area seen in fibres from immature females, but their number remained rather constant throughout reproductive development (approx. 8–10 tracheoles per muscle fibre, data not shown).

The muscle fibre appearance from allatectomised females was similar to those of immature females and shared some common characteristics. Their myofibrils remained relatively small, with numerous microtubules apparent (Fig. 5B,C). The mitochondria were clustered at the level of I-band and their size was somewhat larger than those of muscle fibres from immature females (compare Figs 5B,D and 4B,C). The same was found for dyads and elements of the sarcoplasmic reticulum that appeared slightly more developed and better organised than in immature females (compare Figs 4C and 5B).

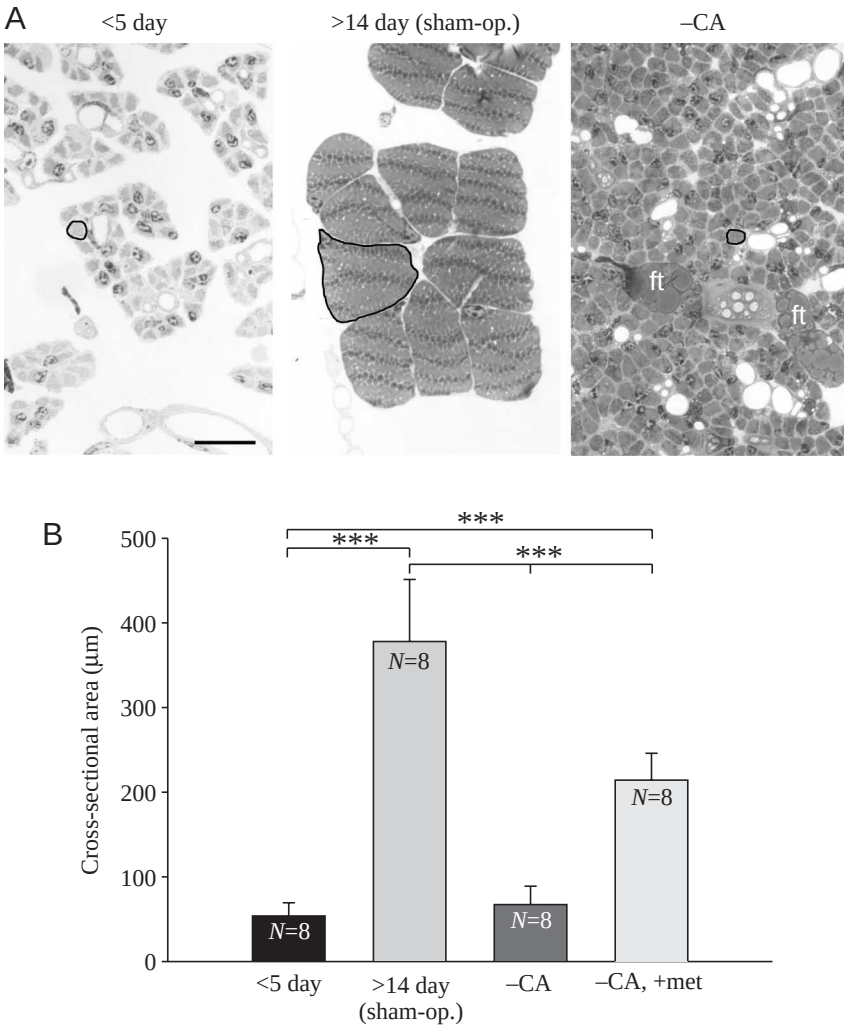


Fig. 3. (A) Cross-sections from dorsal opener muscle 271 reveal dramatic growth of muscles fibres during maturation (a single muscle fibre is outlined in each group for comparison). Muscle fibres from allatectomised females remain almost undeveloped. Fatty tissue (ft) is frequently observed between the fibres of allatectomised females, but not in immature or mature females. (B) Quantification of fibre growth during reproductive development. Cross-sectional area of muscle fibres increase approx. sevenfold during maturation. Allatectomy prevents fibre growth (–CA), whereas methoprene injection reinitiates growth (–CA, +met). Data are means ± S.D.; ***$P$<0.001. Scale bar in A, 20 μm.

*Contractions*

The apparent influence of JH on the morphological development of ovipositor muscle fibres raised questions about their functional properties. Female locusts undergo reproductive development within the first 2 weeks of adult life, then mate and start egg laying. When egg laying becomes necessary the ovipositor is able to perform powerful movements for about 15–25 min while digging the oviposition hole (Rose et al., 2000; Thompson, 1986). To reveal possible functional changes associated with the growth of the ovipositor opener muscle M271, various contraction parameters were measured in immature (<5 day), mature (>14 day, sham operated) and allatectomised (–CA) females.

Generally, contraction measurements of ovipositor opener muscles displayed a rather high degree of individual variation in all experimental groups. This can be due to individual variation of animal size or the current status of muscular modulation by the pentapeptide proctolin, which is known to

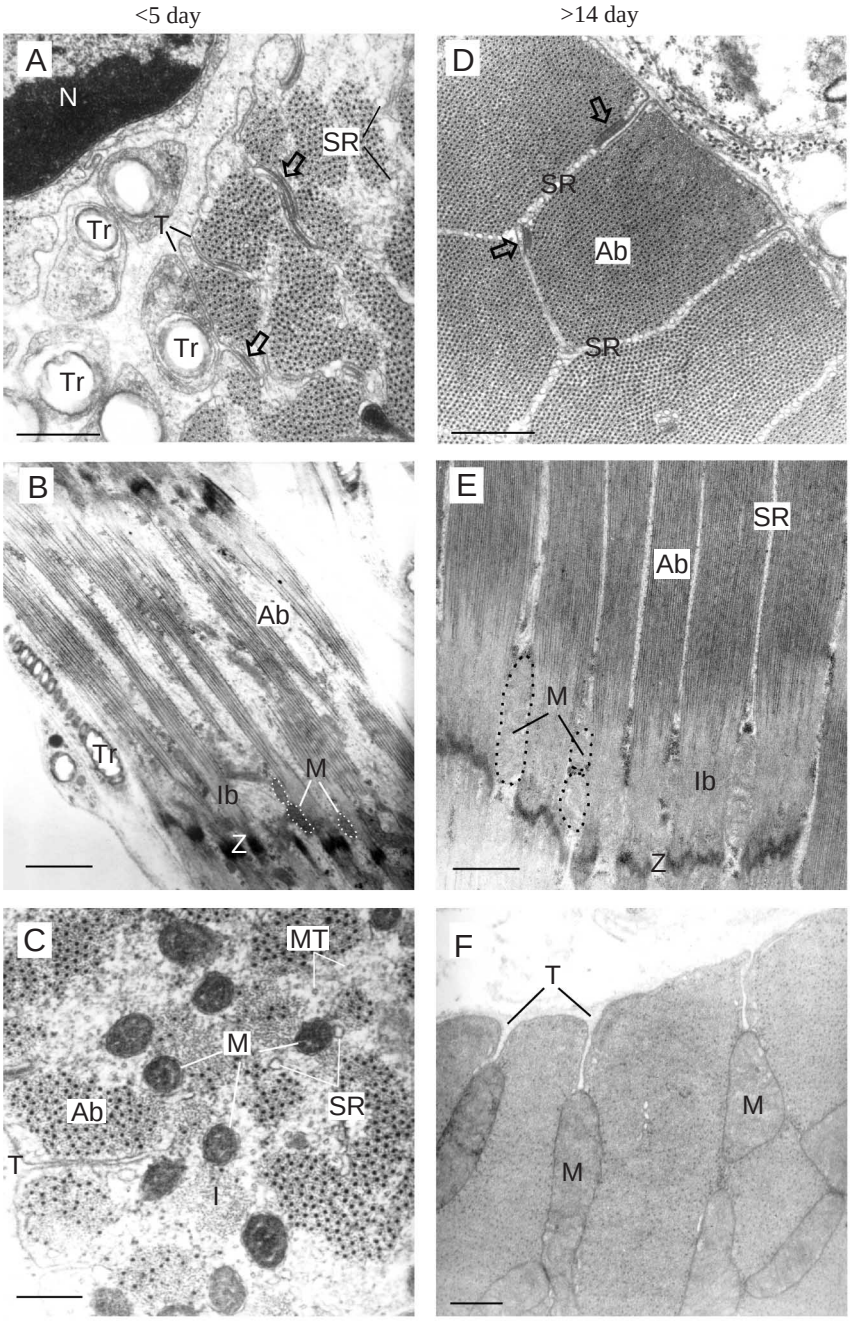


Fig. 4. Electron micrographs of transverse (A,D,C,F) and longitudinal (B,E) sections obtained from dorsal opener muscle 271 of immature (A–C) and mature (D–F) females. In immature females, note the presence of numerous tracheoles (Tr), small irregular myofibrils and mitochondria (M). T-tubules (T) and dyads (open arrow) occur at the level of the A-band (Ab). Multiple microtubules (Mt) are present within the sarcoplasm. Elements of the sarcoplasmic reticulum (SR) are rare and not well developed. By contrast, myofibrils of mature females appear well organised and are divided by regular chains of sarcoplasmic reticulum profiles. Comparatively large mitochondria (M) are present at the level of the I-band (Ib), adjacent to the Z-line (Z). N, nucleus. Scale bars, 0.25 μm (C,F); 0.5 μm (A,B); 1 μm (C,D).

affect muscular contraction (Belanger and Orchard, 1993). However, most differences were robust enough to let a clear picture emerge.

Considering the dramatic increase in cross-sectional area during reproductive development, it was not surprising that muscles from mature females generated considerably more tension during twitch and tetanic contraction than immature females (Fig. 6A,B). Twitch tension increased approx. fivefold (>14 day, sham-op., 33.22±10.8 mN; <5 day, 6.5±2.59 mN) and tetanic tension (50 Hz) approx. 44-fold (>14 day, sham-op., 623.66±115.77 mN; <5 day, 14.19 ±2.58 mN). The twitch/tetanus ratio decreased from 0.45 (<5 day) to 0.05 (>14 day, sham-op.). Removing the corpora allata shortly after adult emergence (Fig. 6B, –CA) largely suppressed the ability of the muscle to exert large tension (twitch, 9.9±2.67 mN; tetanus, 61.56±17.5 mN) and revealed a twitch/tetanus ratio of 0.16. However, these values were still significantly different from immature females (twitch, $P<0.05$; tetanus, $P<0.001$, $N=9$, Mann–Whitney rank sum test). During the experiments it became apparent that contractions of muscles from immature females fused at lower stimulation frequencies (3–5 Hz) than in mature females (>10 Hz, Fig. 6A). A detailed evaluation of the contraction kinetics on the basis of single twitches revealed significantly shorter contraction (time-to-peak) and relaxation (50% relaxation) times for mature females (Fig. 6C). The contraction time declined from a mean value of 309.23±75.69 ms (<5 day) to 140.12±19.84 ms (>14 day, sham-op.). Similar values were measured for the 50% relaxation time (<5 day, 333.67±121.23 ms; >14 day, sham-op., 142.48±27.40 ms). Allatectomy, however, was not able to completely prevent the shift to faster contraction kinetics. The values obtained from allatectomised females were between those measured in immature and mature females (contraction, 178.32±39.15 ms; 50% relaxation, 209.48±45.72 ms). Nevertheless, these results suggest a powerful increase of contraction forces during reproductive development of female locusts that is, at least in part, influenced by JH. Associated with that increase is a shift to faster contraction kinetics.

### *Proctolin modulation*

It has been reported that the pentapeptide proctolin is required for a normal function of the locust ovipositor opener muscle (Belanger and Orchard, 1993). During activation of motoneurons a constant realease of proctolin was shown to maintain contractability of ovipositor

muscle fibers, whereas depletion of proctolin stores after prolonged activation led to declined muscular contractions (Belanger and Orchard, 1993). To determine if the effect of proctolin on the contraction of ovipositor muscle changes with the maturational and hormonal status of the female, stimulus evoked contractions were measured in the absence and presence of $10^{-9}$ mol $l^{-1}$ proctolin (Fig. 7A). Ovipositor muscles of all three experimental groups responded to the application of $10^{-9}$ mol proctolin with a significant increase of tension (Proctolin $10^{-9}$ mol $l^{-1}$; Table 2). To test for the existence of endogenous proctolin, 10 single test stimuli (0.3 Hz) were applied before and after a high frequency, conditioning stimulation (50 Hz). Conditioning stimulus lasted for 5 s and was intended to release endogenous proctolin from motor terminals on muscle 271 (Fig. 7B). These experiments revealed significantly increased forces (42%) in ovipositor muscles of mature females only. By contrast, contraction forces obtained from immature females showed a 21% decrease, whereas allatectomised females showed a 14% increase, but both were not statistically different [Pre-stimulation (50 Hz), saline; Fig. 7B, Table 2]. However, in immature females, a pronounced recovery from decreased contraction forces was observed within the following 5 min (not shown). These experiments suggest that muscle ability to respond to exogenous proctolin is independent of maturational status. Pronounced modulation of muscle contractions by endogenous proctolin, however, occurred in mature females only. To test if these results did indeed depend on the lack of proctolin release from motor units of immature and allatectomised females as suggested by the result, the experiment was repeated with proctolin ($10^{-9}$ mol $l^{-1}$) added to the bath shortly after the control stimulation. If the existence of proctolin was the limiting factor of contraction strength in immature and allatectomised females, additional application of proctolin would be expected to increase contractions markedly. The results however, show that proctolin was not effective in changing the values dramatically compared to those obtained without proctolin [Pre-stimulation (50 Hz), proctolin $10^{-9}$ mol $l^{-1}$; Table 2]. Immature females again displayed decreased tension production which was now statistically significant. Forces measured in mature and allatectomised females were in the range of those measured without proctolin, but a statistically significant difference was detected for allatectomised females only.

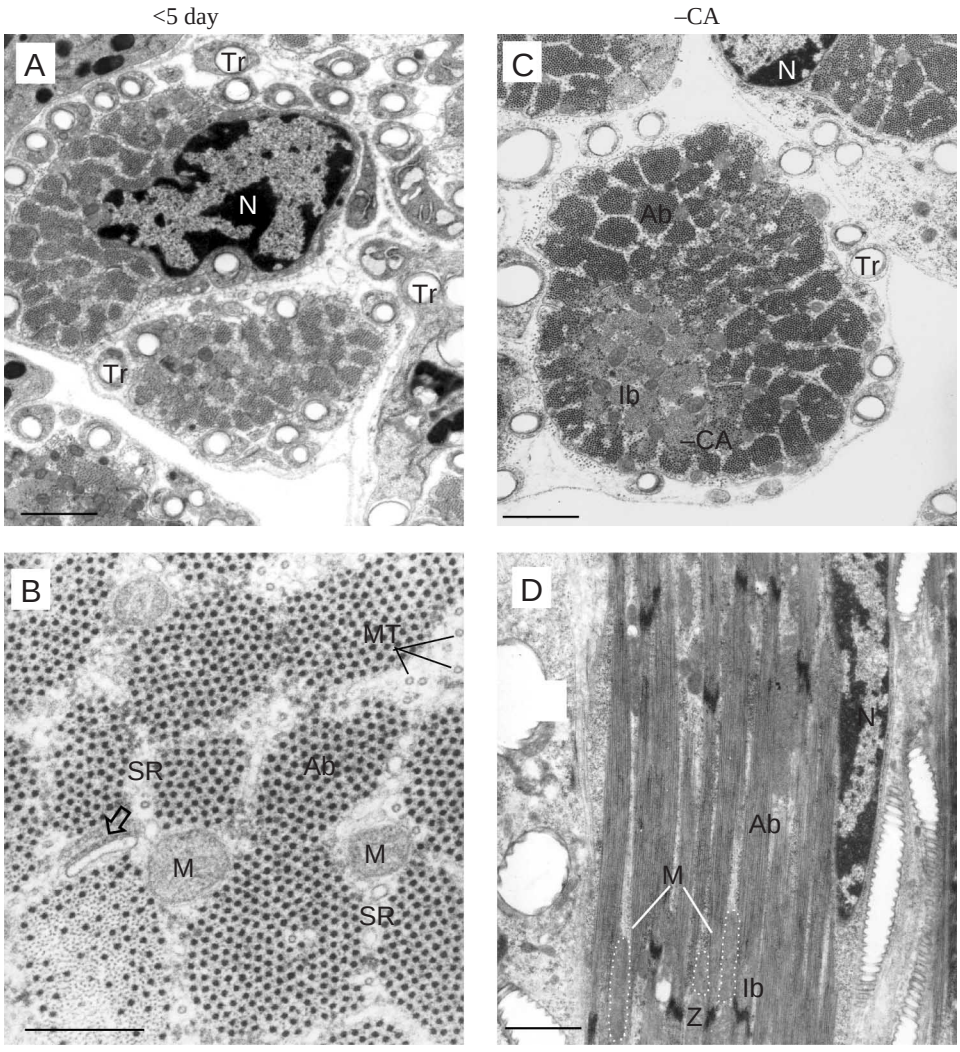


Fig. 5. Electron micrographs of opener muscle from immature (A) and allatectomised (B,C,D) females. (A,C) Overview of myofibril structure. Note the abundance of tracheoles (Tr) surrounding the myofibrils and the similarity of basic organisation and structure of muscle elements in immature and allatectomised females. (B,D) Transverse (B) and longitudinal (D) sections of muscle fibres obtained from allatectomised females reveal the presence of dyads (open arrow in B) and numerous microtubules (MT in B), almost comparable to those seen in immature female fibres (compare to Fig. 4C). However, elements of the sarcoplasmic reticulum (SR) surrounding the myofibrils and mitochondria (M) appear somewhat better organised and larger when compared to fibres of immature females. Ab, A-band; Ib, I-band; N, nucleus; Z, Z-line. Scale bars, 1 μm (A,C,D); 0.25 μm (B).

## Discussion

The present study investigated the maturation of ovipositor muscle properties and their regulation by juvenile hormone. The ovipositor opener muscles

provide, in cooperation with other muscles, the driving force for digging the oviposition hole. Adequate and flexible oviposition is important in the locust for protection and survival of their offspring. The results presented here indicate that ovipositor muscles undergo dramatic morphological and functional maturation during reproductive development. Changes comprise growth and structural maturation of muscle fibre elements and associated apodeme, dramatically increased contraction forces, and altered contraction kinetics. Modulation of muscle contractions by the pentapeptide proctolin was not altered during reproductive development, whereas release of endogenous substances seems to increase contraction forces in mature females only. Maturational changes could partially be blocked by allatectomy and reappeared after replacement injections with the JH analog methoprene, indicating the important role of JH in regulating these processes.

### *JH regulation of muscle development*

The influence of JH on muscle development seems to be a common phenomenon in adult insects. Flight muscles of different insect species undergo degeneration and/or regeneration regulated by JH (Stegwee et al., 1963; Borden and Slater, 1968; Tanaka, 1994). In the colorado potato beetle, flight muscle regeneration, which is initiated by rising JH titers, involves growth of muscle fibres and mitochondria and thus bears some resemblance to the findings of the present study (Stegwee, 1964; de Kort, 1990). The involvement of JH in regulating structural differentiation of muscle fibres has been shown in studies on larval locust. Here, flight muscles undergo accelerated structural differentiation after application of the JH analogue methoprene (Cotton and Anstee, 1990). When the last instar larvae moults to the early adult, however, their normal flight muscle maturation is compromised (e.g. reduction of the normal increase in the cross-sectional area of muscle fibres).

These studies clearly show that JH is involved in the regulation of different aspects of flight muscle function and differentiation. The results from the present study suggest in addition a pronounced structural and functional maturation of non-flight muscles leading to fibres well adapted for a stage-specific behaviour (e.g. oviposition). A recent study on locust abdominal longitudinal muscles showed that this type of hormonal regulation of muscle maturation is not an exception. Longitudinal muscles were shown to undergo hypertrophy along with changes in their contractile properties during reproductive development (Rose et al., 2001). These results agree well with the data from the present study and thus provide a further example where JH regulates morphological and functional

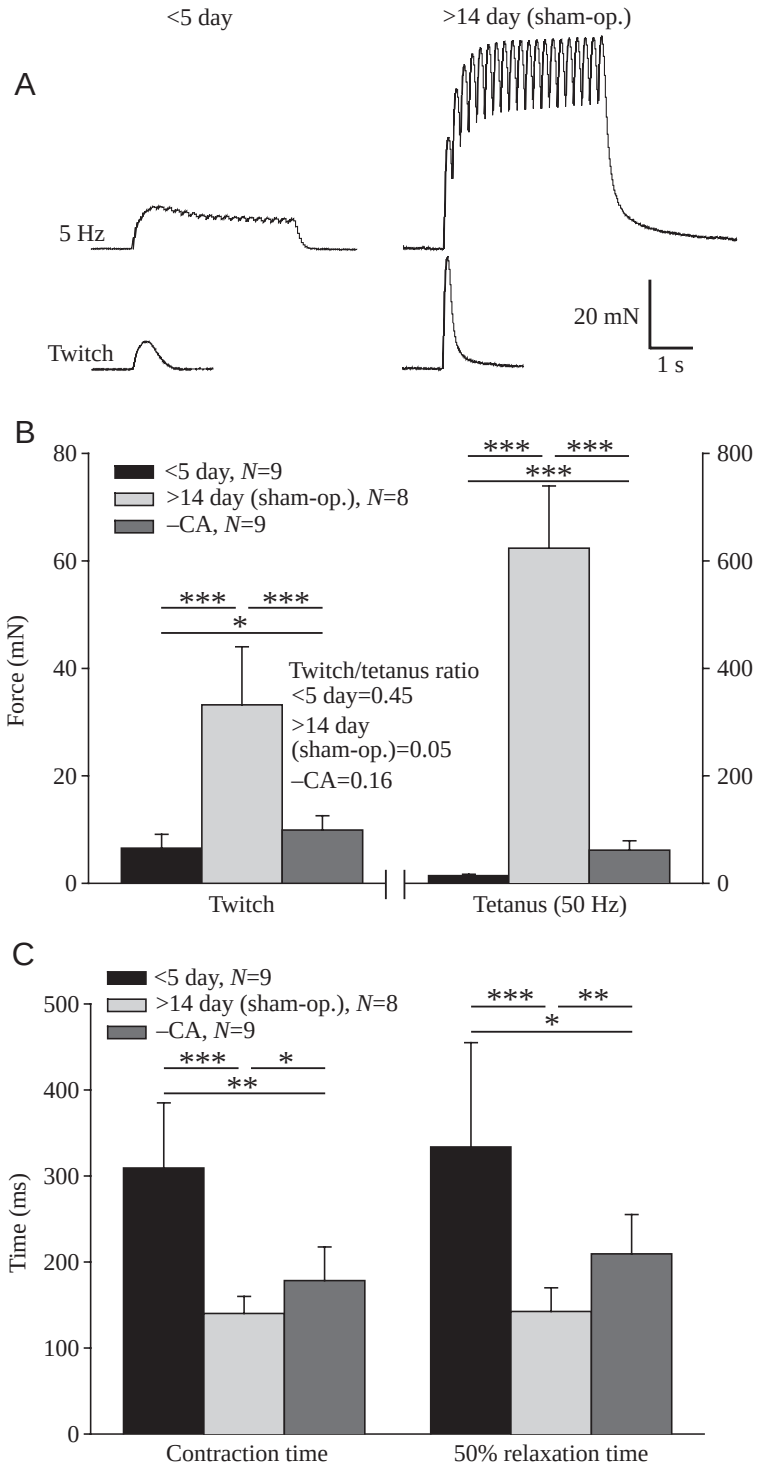


Fig. 6. Contraction force and kinetics of ovipositor muscle 271 obtained from immature (<5 day), mature (>14 day, sham-op.) and allatectomised females (–CA). (A) Examples of muscle contractions from immature (left) and mature females (right), showing considerably increased forces and altered kinetics. (B) Quantification of forces exerted during twitch and tetanus (50 Hz) contractions. The twitch/tetanus ratios for all groups are shown. (C) Contraction and relaxation kinetics of single twitches. Muscles from mature females contract and relax significantly faster than those of immature and allatectomised females. Data are means ± S.D.; **P*<0.05; ***P*<0.01;****P*<0.001.

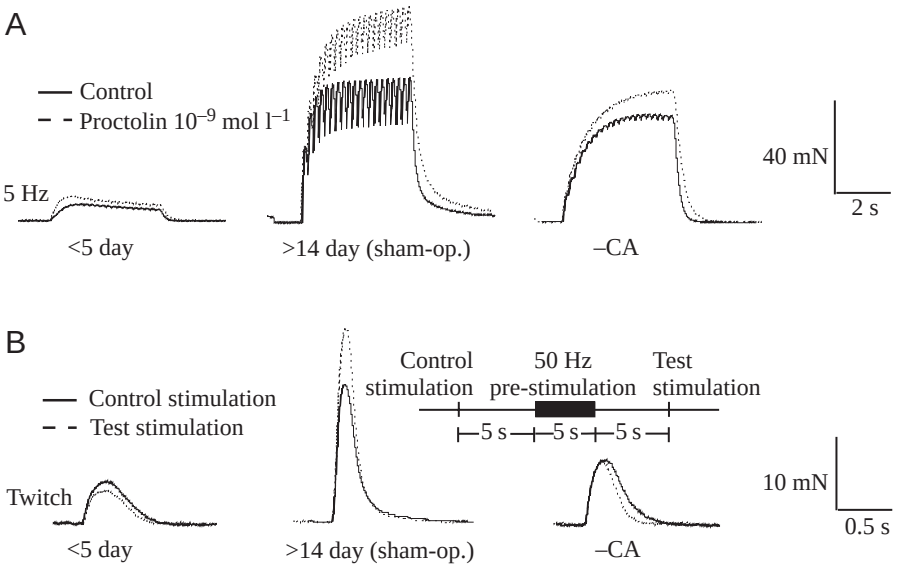


Fig. 7. Modulation of ovipositor muscle contractions by proctolin or high frequency pre-stimulation. (A) Application of $10^{-9}$ mol $l^{-1}$ proctolin increases contraction force in all experimental groups. (B) To test for endogenous release of proctolin, contractions evoked by control stimulations are compared with those evoked by test stimulations. Shortly before the test stimulation, a high frequency pre-stimulation (50 Hz) was applied, as shown in the inset. Pre-stimulation was intended to release endogenous proctolin from neuronal elements innervating the muscle. Significant increase of contraction forces is observed in mature females only.

changes of muscles required in certain life stages (e.g. reproduction, diapause, dispersal flight).

Many aspects of juvenile hormone action on insect tissue during development and adult life are mysterious. In the present study allatectomy was carried out in an attempt to remove the natural source of JH and to examine whether this treatment inhibits the observed changes on muscle structure and function. Most of these changes, however, were only partially inhibited by allatectomy, except for the cross-sectional area and some aspects of the ultrastructural maturation. This is interesting, because it indicates some kind of development that is independent of a functional corpora allata and could be explained by the existence of other internal sources of JH or of substances with similar actions in insects. An alternative source of JH was located in the ovary of *Aedes*, which are able to synthesise JH from farnesoic acid (Borovsky et al., 1994). Substances with JH activity are the thyroid hormones [especially 3′,3,5-triiodo thyronine (T3)]. T3 was shown to mimic some aspects of JH action on follicle cells of *Locusta migratoria* (Davey, 2000). The food of locusts provides a potential source of T3 and the author suggests that thyroid hormones are ingested by locusts. However, evidence for additional mechanisms influencing maturation in adult insects are provided by studies investigating the influence of metamorphosis on the subsequent maturation of insect flight muscles in locust and crickets. Wang et al. (1993) reported inhibited growth of muscles in overaged or supernumerary *Schistocerca gregaria* nymphs, whereas precocious adults developed almost normal muscles. Studies on crickets underline the importance of metamorphosis for subsequent flight muscle maturation. Injection of the JH analog methoprene into last-instar nymphs of the cricket *Teleogryllus oceanicus* slowed subsequent flight muscle growth and blocked ultrastructural maturation (Novicki, 1989). It seems reasonable to suppose therefore that parts of the structural maturation of insect muscles are influenced by their hormonal history during metamorphosis, although it must be pointed out that flight behaviour occurs shortly after moulting, while oviposition is delayed until at least 2 weeks later. Therefore, in the adult insect elevated JH titers apparently regulate additional maturation of specific muscles to adapt them to the demands of reproduction.

Table 2. *Quantified data from the experiments shown in Fig. 7, together with a set of control experiments using bath-applied proctolin*

| | | Pre-stimulation (50 Hz) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Proctolin ($10^{-9}$ mol $l^{-1}$) | | Saline | | Proctolin ($10^{-9}$ mol $l^{-1}$) | |
| | *N* | Force change (%) | *P* value | Force change (%) | *P* value | Force change (%) | *P* value |
| <5 day | 6 | 25±20 | 0.006** | –21±19.6 | 0.08 | –30.5±23.8 | 0.006** |
| >14 day (sham-op.) | 8 | 31±30 | 0.04* | 42±24 | 0.002** | 33.3±10.3 | 0.004** |
| –CA | 9 | 11±9 | 0.013* | 14±24 | 0.11 | 16.7±14 | 0.004** |

Proctolin was applied at $10^{-9}$ mol $l^{-1}$; see text for details.
Values are means ± S.D. *P* values were obtained from paired *t*-tests.

The growth described in the present study was not only restricted to the muscles but also the apodeme, which functions as an insertion element of muscle fibres. Smith (1964) speculated that muscles and their integumental attachment co-develop during ontogenesis. In the adult flesh-fly, *Sarcophyga falculata*, Schlein (1972) reported post-emergence enlargement of an apodeme by the deposition of endocuticle and growth of associated muscle fibres influenced by endocrine factors. The results of the present study support the notion that the form and size of cuticular attachment sites functionally fit their attaching muscles. In addition, hormonal synchronisation of muscular and apodemal growth makes sense in terms of functionality: large and powerful muscles develop considerable tension that need to be tolerated by their cuticular attachment sites.

Although there is no doubt that JH has a pivotal role in regulating reproductive development, the mechanisms by which JH reveals its action on the tissue are not understood. This study was not designed to examine the mechanisms of JH action, but it can be speculated that one, if not the primary, role of JH is to provide muscle and apodeme with sufficient amounts of protein needed for their growth. Studies on adult locusts showed increased levels of persistent storage protein (PSP) during their reproductive development (Wyatt, 1990; Wyatt et al., 1992). In chemically allatectomised animals, by contrast, PSP maintained a low rate of synthesis, whereas application of JH or an analogue elevated PSP synthesis. The function of PSP is to provide amino acids for the construction of the adult integument and other organs in metamorphosis (Kanost et al., 1990). In adult insects, storage proteins may serve a similar function and thus limit organ growth when their synthesis is depressed by low JH levels.

### *Functional maturation*

Structure and performance of muscle fibres are tightly bound. Thus, the present study aimed at revealing possible functional alterations of ovipositor opener muscle fibres concomitant with their growth and structural maturation during reproductive development. The most dramatic functional change was the increase in contraction force, which was not surprising, because the maximum force produced by a muscle should be proportional to its cross-sectional area (Josephson, 1975). For twitch tension, this increase can thus be largely assigned to the growth of the muscle, since its mass-per-length values increased accordingly (mass/length, 5.6-fold; twitch tension, 5.1-fold). By contrast, tetanic tension increased 43-fold and cannot be explained solely by the growth of the ovipositor muscle. Other factors must be responsible, of which the organisation of myofibrils is of primary importance. The results from the present study show a poor alignment and some kind of disorganisation of myofibrils in immature females that will result in a lower amount of filament overlap. Since it has been shown for invertebrate and vertebrate muscle fibres that the maximum tension depends critically on the amount of filament overlap (Weis-Fogh, 1956; Gordon at al., 1966), this structural feature may limit the tension produced.

The relatively high maximum tetanic tension of the ovipositor opener muscle from mature females results in a low twitch/tetanus ratio, which is indicative of a muscle type where graded force production is necessary (Aidley, 1985). During the course of oviposition, valves open and close repeatedly to dig a cavity in which the abdomen is lowered and the eggs are eventually laid. Once the female has performed a few cycles of digging movements, the tip of the abdomen retracts and the valves open to press against the dug part of the oviposition hole to stabilise the walls of the cavity (Thompson, 1986; Rose et al., 2000). Since egg laying is performed in a substrate of different compactness, one can imagine that the complex digging movements require muscles capable of graded contractions, especially during the sweeping digging movements.

The brevity of muscle contraction depends to a large extent on the development and organisation of the sarcoplasmic reticulum and the transverse tubules (Josephson, 1975). Thus, the increased shortening and relaxing velocities observed for the ovipositor muscle from mature females may be the result of a well-developed sarcoplasmic reticulum. Indeed, the results from the present study suggest a higher degree of sarcoplasmic reticulum organisation and development for mature, and to a lesser extent for allatectomised, females, as compared to immature females. By contrast, a slowing of contractions during reproductive development has been shown for abdominal longitudinal muscles of female locusts (Rose et al., 2001). Here, muscles from mature females contract significantly slower than those from immature or allatectomised females. This may be explained by the specific functional requirements of longitudinal muscles during oviposition where they must tolerate dramatic lengthening, which is accompanied by the fragmentation of their Z-lines (Jorgensen and Rice, 1983). As a consequence, longitudinal muscles display elastic properties that give rise to slow contraction kinetics (Rose et al., 2001). The ovipositor opener muscles, by contrast, representing a more conventional muscle type, provide the driving force for rhythmic digging movements.

### *Proctolin*

The normal function of locust ovipositor muscles has been shown to depend critically on the pentapeptide proctolin (Belanger and Orchard, 1993). Endogenous proctolin is released from motoneurons and affects the tension produced by the muscle. The experiments presented here show that exogenously applied proctolin significantly increased force production in immature, mature and allatectomised females. This implies the presence of a functional modulatory receptor system, even in the immature stage. However, when the muscular system was tested for endogenous proctolin through high-frequency stimulation of the motor nerve prior to a test pulse, only mature females responded with a significant increase of tension. Although this result suggests that the structures releasing proctolin somehow improve their ability to produce, store and/or release proctolin during reproductive

development, it seems likely that other mechanisms such as fatigue are also involved. This is also suggested by the finding that additional proctolin, applied shortly before the conditioning stimulus, did not reverse the effects obtained without proctolin (compare Pre-stimulation values in Table 2). These results would therefore be interpreted as mainly affected by the fatigue of the muscle fibres after the conditioning stimulus. Fatigue-resistant muscles, capable of sustained activity, generally contain mitochondria comprising a large volume of the fibre (Hoyle and Mc-Neill, 1968; Strokes et al., 1975; reviewed in Josephson, 1975). A look at the sizes of mitochondria of muscles from immature, mature and allatectomised females and their fatigue-resistance revealed a clear correlation. The ovipositor opener muscle from immature females (small mitochondria, Fig. 4C) are non-resistant against fatigue as indicated by the fact that, after the conditioning, stimulus twitch contraction forces declined, but slowly recovered thereafter. Even the presumed release of endogenous proctolin by the conditioning stimulus, or exogenously applied proctolin, had no significant effects since fatigue seems to be the limiting factor for contraction strength. By contrast, the ovipositor opener muscle from mature females, which contain relatively large mitochondria (Fig. 4F), was fatigue-resistant and proctolin effects were clearly visible (increased tension). The same is true for allatectomised females, although their mitochondria were not as large as those from mature females.

The ovipositor muscles of locusts are exclusively important for reproductive behaviour in the adult female. The results of the present study suggest a pivotal role for JH in the regulation of ovipositor muscle structure and function. Although some aspects of muscle maturation appeared to be regulated independently of a functional corpora allata, the importance of JH is apparent. JH obviously synchronises muscle and apodeme development with the maturation of reproductive organs. This development changes muscle performance and adapts their properties to enable powerful, graded contractions as well as endurance. The evolutionary advantage of a retarded muscle development may lie in the savings of metabolic energy during the early developmental stages.

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## References

**Aidley, D. J.** (1985). Muscular contraction. In *Comprehensive Insect Biochemistry and Physiology*, vol. 5 (ed. G. A. Kerkut and L. I. Gilbert), pp. 407-437. Oxford: Pergamon Press.

**Anton, S. and Gadenne, C.** (1999). Effect of juvenile hormone on the central nervous processing of sex pheromone in an insect. *Proc. Natl. Acad. Sci. USA* **96**, 5764-5767.

**Barth, R. H. and Lester, L. J.** (1973). Neuro-hormonal control of sexual behaviour in insects. *Annu. Rev. Entomol.* **18**, 445-472.

**Bass, A. H.** (1986). A hormone-sensitive communication system in an electric fish. *J. Neurobiol.* **17**, 131-156.

**Bate, M., Rushton, E. and Currie, D.** (1991). Cells with persistent twist expression are the embryonic precursors of adult muscles in *Drosophila*. *Development* **113**, 79-89.

**Belanger, J. H. and Orchard, I.** (1993). The locust ovipositor opener muscle: proctolinergic central and peripheral neuromodulation in a centrally driven motor system. *J. Exp. Biol.* **174**, 343-362.

**Borden, J. H. and Slater, C. E.** (1968). Induction of flight muscle degeneration by synthetic juvenile hormone in *Ips confusus* (Coleoptera: Scolytidae). *Z. Vergl. Physiol.* **61**, 366-368.

**Borovsky, D., Carlson, D. A., Ujvary, I. and Prestwich, G. D.** (1994). Biosynthesis of (10R)-juvenile hormone III from farnesoic acid by *Aedes aegypti* ovary. *Arch. Insect. Biochem. Physiol.* **2**, 75-90.

**Bowers, W. S., Ohta, T., Cleere, J. S. and Marsella, P. A.** (1976). Discovery of insect anti-juvenile hormone in plants. *Science* **193**, 542-574.

**Breedlove, S. M.** (1984). Steroid influences on the development and function of a neuromuscular system. *Prog. Brain. Res.* **61**, 147-170.

**Breedlove, S. M.** (1986). Cellular analyses of hormone influence on motoneuronal development and function. *J. Neurobiol.* **17**, 157-176.

**Cayre, M., Strambi, C. and Strambi, A.** (1994). Neurogenesis in an adult insect brain and its hormonal control. *Nature* **368**, 57-59.

**Consoulas, C., Anezaki, M. and Levine, R. B.** (1997). Development of adult thoracic leg muscles during metamorphosis of the hawk moth *Manduca sexta*. *Cell Tissue Res.* **287**, 393-412.

**Cotton, G. and Anstee, J. H.** (1990). A structural and biochemical study on the effects of methoprene on flight muscle development in *Locusta migratoria* L. *J. Insect Physiol.* **36**, 959-969.

**Davey, K. G.** (2000). Do thyroid hormones function in insects? *Insect Biochem. Mol. Biol.* **30**, 877-884.

**de Kort, C. A. D.** (1990). Thirty-five years of diapause research with the Colorado potato beetle. *Entomol. Exp. Appl.* **56**, 1-13.

**Finlayson, L. H.** (1975). Development and degeneration. In *Insect Muscle* (ed. P. N. R. Usherwood), pp. 75-149. London: Academic Press.

**Forger, N. G., Frank, L. G., Breedlove, S. M. and Glickman, S. E.** (1996). Sexual dimorphism of the perineal muscles and motoneurons in spotted hyenas. *J. Comp. Neurol.* **375**, 333-343.

**Gordon, A. M., Huxley, A. F. and Julian, F. J.** (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.* **184**, 170-192.

**Hartman, R.** (1978). The juvenile hormone-carrier in the haemolymph of the acridine grasshopper *Gomphocerus rufus* L.: Blocking of the juvenile hormone's action by means of repeated injections of an antibody to the carrier. *Wilhelm Roux's Arch.* **184**, 301-324.

**Hegstrom, C. D. and Truman, J. W.** (1996). Steroid control of muscle remodeling during metamorphosis in *Manduca sexta*. *J. Neurobiol.* **29**, 535-550.

**Hoyle, G. and McNeill, P. A.** (1968). Correlated physiological and ultrastructural studies on specialized muscles. Ib. Ultrastructure of white and pink fibers of the levator of the eyestalk of *Podophthalmus vigil* (Weber). *J. Exp. Zool.* **167**, 487-522.

**Jorgensen, W. K. and Rice, M. J.** (1983). Superextension and supercontraction in locust ovipositor muscles. *J. Insect. Physiol.* **29**, 437-448.

**Josephson, R. K.** (1975). Extensive and intensive factors determining the performance of striated muscle. *J. Exp. Zool.* **194**, 135-154.

**Kanost, M. R., Kawooya, J. K., Law, J. H., Ryan, R. O., Vanheusden, M. C. and Ziegler, R.** (1990). Insect haemolymph proteins. *Adv. Insect Physiol.* **22**, 299-396.

**Kelley, D. B.** (1996). Sexual differentiation in *Xenopus laevis*. In *The Biology of* Xenopus (ed. R. Tinsley and H. Kobel), pp. 143-176. Oxford: Oxford University Press.

**Luedeman, R. and Levine, R. B.** (1996). Neurons and ecdysteroids promote the proliferation of myogenic cells cultured from the developing adult legs of *Manduca sexta*. *Dev. Biol.* **173**, 51-68.

**Meuser, S. and Pflüger, H. J.** (1998). Programmed cell death specifically eliminates one part of a locust pleuroaxilliary muscle after the imaginal moult. *J. Exp. Biol.* **201**, 2367-2382.

**Novicki, A.** (1989). Control of growth and ultrastructural maturation of a cricket flight muscle. *J. Exp. Zool.* **250**, 263-272.

**Pener, M. P., Orshan, L. and De Wilde, J.** (1978). Precocene II causes atrophy of corpora allata in *Locusta migratoria*. *Nature* **272**, 350-353.

**Peroulakis, M. E., Goldman, B. and Forger, N. G.** (2002). Perineal muscles and motoneurons are sexually monomorphic in the naked mole-rat (*Heterocephalus glaber*). *J. Neurobiol.* **51**, 33-42.

**Poels, C. L. M. and Beenakkers, A. M.** (1969). The effects of corpus allatum

implantation on the development of flight muscle and fat body in *Locusta migratoria*. *Ent. Exp. Appl.* **12**, 312-324.

**Rand, M. N. and Breedlove, S. M.** (1995). Androgen alters the dendritic arbors of SNB motoneurones by acting upon their target muscles. *J. Neurosci.* **15**, 4408-4416.

**Rheuben, M. B.** (1992). Degenerative changes in the muscle fibers of *Manduca sexta* during metamorphosis. *J. Exp. Biol.* **167**, 91-117.

**Riddiford, L. M.** (1985). Hormone action at the cellular level. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, vol. 8 (ed. G. A. Kerkut and L. I. Gilbert), pp. 37-84. Oxford: Pergamon Press.

**Rose, U., Ferber, M. and Hustert, R.** (2001). Maturation of muscle properties and its hormonal control in an adult insect. *J. Exp. Biol.* **204**, 3531-3545.

**Rose, U., Seebohm, G. and Hustert, R.** (2000). The role of internal pressure and muscle activation during locust oviposition. *J. Insect. Physiol.* **46**, 69-80.

**Schlein, Y.** (1972). Factors that influence the post-emergence growth in *Sarcophaga falculata*. *J. Insect. Physiol.* **18**, 199-209.

**Schlinger, B. A.** (1997). Sex steroids and their actions on the birdsong system. *J. Neurobiol.* **33**, 619-631.

**Schwarz, L. M. and Truman, J. W.** (1983). Hormonal control of muscle atrophy and degeneration in the moth *Antheraea polyphemus*. *J. Exp. Biol.* **111**, 13-30.

**Smith, D. S.** (1964). The structure and development of flightless coleoptera: a light and electron microscopic study of the wings, thoracic exoskeleton and rudimentary flight musculature. *J. Morphol.* **114**, 107-184.

**Snodgrass, R. E.** (1935). The abdominal mechanisms of a grasshopper. *Smith. Miscell. Coll.* **94**, 1-87.

**Staufer, T. W. and Whitman, D. W.** (1997). Grasshopper oviposition. In *The Bionomics of Grasshoppers, Katydids and Their Kin* (ed. S. K. Gangwere, M. C. Muralirangan and M. Muralirangan), pp. 231-280. New York: CAB International.

**Stegwee, D.** (1964). Respiratory chain metabolism in the Colorado potato beetle – II. Respiration and oxidative phosphorylation in 'Sarcosomes' from diapausing beetles. *J. Insect. Physiol.* **10**, 97-102.

**Stegwee, D., Kimmel, E. C., DeBoer, J.A. and Henstra, S.** (1963). Hormonal control of reversible degeneration of flight muscle in the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera). *J. Cell. Biol.* **19**, 519-527.

**Stocker, R. F. and Nüesch, H.** (1975). Ultrastructural studies on neuromuscular contacts and the formation of junctions in the flight muscles of *Antheraea polyphemus* (Lep.). I. Normal adult development. *Cell Tissue Res.* **159**, 245-266.

**Stout, J., Hao, H., Atkins, G., Stiedl, O., Ramseier, J., Coburn, P., Hayes, V., Henley, J. and Kim, P.** (1993). JHIII regulates phonotaxis in crickets by controlling expression of nicotinic receptors in auditory interneurons. In *Insect Neurochemistry and Neurophysiology* (ed. M. Loeb), pp. 343-346. Boca Raton: CRC Press.

**Stout, J., Hayes, V., Zacharias, D., Henley, J., Stumpner, A., Hao, J. and Atkins G.** (1992). Juvenile hormone controls phonotactic responsiveness of female crickets by genetic regulation of the response properties of identified auditory interneurons. In *Insect Juvenile Hormone Research: Fundamental and Applied Approaches* (ed. B. Mauchamp, F. Couillaud and J. C. Baehr), pp. 265-283. Paris: IRNA.

**Strambi, A., Strambi, C. and Cayre, M.** (1997). Hormonal control of reproduction and reproductive behavior in crickets. *Arch. Insect. Biochem. Physiol.* **35**, 393-404.

**Strokes, D. R., Josephson, R. K. and Price, R. B.** (1975). Structural and functional heterogeneity in an insect muscle. *J. Exp. Zool.* **194**, 379-408.

**Strong, L.** (1963). A simple apparatus for use in removing corpora allata from locust. *Bull. Ent. Res.* **54**, 19-21.

**Strong, L. and Amerasinghe, F. P.** (1977). Allatectomy and sexual receptivity in females of *Schistocerca gregaria*. *J. Insect. Physiol.* **23**, 131-135.

**Tanaka, S.** (1994). Endocrine control of ovarian development and flight muscle histolysis in a wing dimorphic cricket, *Modicogryllus confirmatus*. *J. Insect. Physiol.* **40**, 483-490.

**Thompson, K. J.** (1986). Oviposition digging in the grasshopper. I. Functional anatomy and the motor program. *J. Exp. Biol.* **122**, 387-411.

**Thompson, K. J. and Roosevelt, J. L.** (1998). Comparison of neural elements in sexually dimorphic segments of the grasshopper, *Schistocerca americana*. *J. Comp. Neurol.* **394**, 14-28.

**Thompson, K. J., Sivanesan, S. P., Campbell, H. R. and Sanders, K. J.** (1999). Efferent neurons and specialisation of abdominal segments in grasshoppers. *J. Comp. Neurol.* **415**, 65-79.

**Truman, J. W. and Riddiford, L. M.** (1974). Hormonal mechanisms underlying insect behaviour. *Adv. Insect. Physiol.* **10**, 297-352.

**Venable, J. H.** (1966). Morphology of the cells of normal, testosterone-deprived and testosterone-stimulated levator ani muscles. *Am. J. Anat.* **119**, 271-302.

**Vincent, J. F. V.** (1975). How does the female locust dig her oviposition hole? *J. Entomol.* **50**, 175-181.

**Wang, Z., Chen, X. and Haunerland, N. H.** (1993). Flight muscle development in juvenile and adult forms of the desert locust, *Schistocerca gregaria*. *J. Insect. Physiol.* **39**, 325-333.

**Weeks, J. C. and Truman, J. W.** (1985). Independent steroid control of the fates of motoneurons and their muscles during insect metamorphosis. *J. Neurosci.* **5**, 2290-2300.

**Weeks, J. C. and Truman, J. W.** (1986). Steroid control of neuron and muscle development during the metamorphosis of an insect. *J. Neurobiol.* **17**, 249-267.

**Weis-Fogh, T.** (1956). Tetanic force and shortening in locust flight muscle. *J. Exp. Biol.* **33**, 668-684.

**Wigglesworth, V. B.** (1934). The physiology of ecdysis in *Rhodnius prolixus*. II. Factors controlling moulting and metamorphosis. *Quart. J. Microsc. Sci.* **77**, 191-222.

**Wigglesworth, V. B.** (1936). The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). *Quart. J. Microsc. Sci.* **79**, 91-121.

**Wyatt, G. R.** (1990). Development and juvenile hormone control of gene expression in the locust fat body. In *Molecular Insect Science* (ed. H. H. Hagedorn, J. G. Hildebrand, M. G. Kidwell and J. H. Law), pp. 163-172. New York: Plenum Press.

**Wyatt, G. R.** (1997). Juvenile hormone in insect reproduction – a paradox? *Eur. J. Entomol.* **94**, 323-333.

**Wyatt, G. R. and Davey, K. G.** (1996). Cellular and molecular actions of juvenile hormone. II. Roles of juvenile hormone in adult insects. *Adv. Insect. Physiol.* **26**, 1-155.

**Wyatt, G. R., Kanost, M. R., Chin, B. C., Cook, K. E., Kawasoe, B. M. and Zhang, J.** (1992). Juvenile hormone analog and injection effects on locust hemolymph protein synthesis. *Arch. Insect. Biochem. Physiol.* **20**, 167-180.