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RESEARCH ARTICLE

Development and deposition of resilin in energy stores for locust jumping

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ABSTRACT

Locusts jump by using a catapult mechanism in which energy produced by slow contractions of the extensor tibiae muscles of the hind legs is stored in distortions of the exoskeleton, most notably (1) the two semi-lunar processes at each knee joint and (2) the tendons of the extensor muscles themselves. The energy is then suddenly released from these stores to power the rapid, propulsive movements of the hind legs. The reliance on the mechanical storage of energy is likely to impact on jumping because growth occurs by a series of five moults, at each of which the exoskeleton is replaced by a new one. All developmental stages (instars) nevertheless jump as a means of forward locomotion, or as an escape movement. Here, I show that in each instar, resilin is added to the semi-lunar processes and to the core of the extensor tendons so that their thickness increases. As the next moult approaches, a new exoskeleton forms within the old one, with resilin already present in the new semi-lunar processes. The old exoskeleton, the tendons and their resilin are discarded at moulting. The resilin of the semi-lunar processes and tendons of the new instar is initially thin, but a similar pattern of deposition results in an increase of their thickness. In adults, resilin continues to be deposited so that at 4 weeks old the thickness in the semi-lunar processes has increased fourfold. These changes in the energy stores accompany changes in jumping ability and performance during each moulting cycle.

KEY WORDS: Locomotion, Kicking, Moulting, *Schistocerca*

INTRODUCTION

Insects use two basic mechanisms to propel jumping with their legs. In the first, direct contractions of muscles can generate sufficient power to move the legs, which act as levers. In bush crickets, which use this mechanism, the propulsive legs are very long relative to body length (Burrows and Morris, 2003) and this increases their effective leverage. In the second mechanism, used by fleas (Bennet-Clark and Lucey, 1967; Sutton and Burrows, 2011), locusts (Bennet-Clark, 1975) and froghoppers (Burrows, 2003, 2006), a catapult amplifies the power of the muscle contractions. This catapult mechanism requires that the energy generated by the slow contraction of the muscles is first stored before being released suddenly to power the rapid movements of the propulsive hind legs. In all insects using this mechanism that have been analysed to date, the energy is stored in distortions of the cuticle so that jumping ability and performance are dependent on the mechanical properties of the skeleton. The hard exoskeleton of insects, however, constrains growth to occur in a series of steps, or moults, which

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means that it must periodically undergo fluctuations in its rigidity and elasticity. The process of moulting also involves discarding the cuticle, which then has to be replaced by a new one. As a consequence, the energy stores will also be subject to these changes, while at the same they must meet increasing demands to store more energy for jumping as the mass of the insect increases.

In insects that use femoral muscles to propel rapid movements of the hind tibiae in jumping, the energy stores are also found in the femora. For example, in locusts, the structures estimated to store 57% of the energy for jumping (Bennet-Clark, 1975) are the paired semi-lunar processes in the distal femur, which are a composite of hard cuticle and resilin (Burrows and Sutton, 2012). These structures are bent by contractions of the extensor tibiae muscles when they are activated with other muscles in the complex motor pattern for jumping and kicking (Burrows, 1995; Godden, 1975; Heitler and Burrows, 1977). The recoil of these structures leads to the rapid extension of the tibiae that propel jumping. A second structure that is suggested to store the remaining 43% of the requisite energy is the tendon of the extensor muscle (Bennet-Clark, 1975), the materials of which are described here. Only the propulsive hind legs have semi-lunar processes and enlarged extensor tibiae muscles.

Locusts and grasshoppers are exopterygote (hemi-metabolous) insects, which develop through a series of nymphal instars that have a similar shape to the adults. At each moult the exoskeleton is shed and is replaced by a new one, which is initially soft so that it can expand to allow growth. Once the old exoskeleton is discarded, the new one, which has already formed inside the old one, must then harden to provide rigidity and protection. Both energy stores, which are part of the exoskeleton, would also be expected to be discarded at each moult and will thus have to be rebuilt. The nymphs are adept at jumping (Katz and Gosline, 1993) as a means of rapidly evading predators and as their main form of locomotion, because wings only develop to enable flying in adults. Prowess at jumping is, nevertheless, also retained by the adults as an escape mechanism, as a way of moving rapidly from one place to another, and as a launch into flight. Any disturbance to such an important locomotory behaviour in both nymphs and adults is thus likely to impose life-threatening challenges. This paper therefore demonstrates the presence of resilin in one store (the extensor tendon) and analyses the development of resilin in both this and the second energy store (the semi-lunar processes) during the repeated moult cycles as a new exoskeleton forms within the old one and then expands and hardens. The correlation between the changes in the resilin of these two stores and the observed difference in the frequency of jumping and its performance (Gabriel, 1985a; Norman, 1995, 1996, 1997; Queathem, 1991) is then discussed.

MATERIALS AND METHODS

Gregarious phase locusts, *Schistocerca gregaria* (Forskål 1775), were raised after hatching from egg pods as first instars through five moults to adults in crowded cages. Each cage contained one

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particular instar and was maintained on a 12 h light:12 h dark cycle, with the temperature held at 37°C during the light period and 25°C during the dark. Under these conditions, these gregarious locusts spent 10.7±0.9 days (mean±s.e.m., N=10 cages followed through all instars) as first instars, 5.6±0.6 days as second instars, 6.3±0.4 days as third instars, 14.0±1.7 days as fourth instars and 11.8±0.5 days as fifth instars. Adults were examined after the final moult from fifth instars and during the following 4 weeks.

The possible presence of the elastic protein resilin in the two energy stores – the semi-lunar processes and the tendon of the extensor tibiae muscle within a hind femur – was analysed in the following way. Dissections of the femur, or transverse sections cut with a thin razor blade at the same region of the distal femur in different locusts (see Fig. 1Ai) were immersed in locust saline (Usherwood and Grundfest, 1965) and examined on an Olympus BX51WI compound microscope (Olympus, London, UK) under ultraviolet (UV) or white epi-illumination. The microscope was fitted with Olympus MPlan ×5/0.1 NA, MPlan ×10/0.25 NA and LUCPlanFLN ×20/0.45 NA objective lenses. The UV light was provided by an X-Cite series 120 metal halide light source (EXFO, Chandlers Ford, Hants, UK) and was conditioned by a Semrock DAPI-5060B Brightline series UV filter set (Semrock, Rochester, NY, USA) with a sharp-edged (1% transmission limits) band from 350 to 407 nm. The resulting blue fluorescence emission was collected in a similarly sharp-edged band at wavelengths from 413 to 483 nm through a dichroic beam splitter. Images were captured with a Micropublisher 5.0 digital camera (QImaging, Marlow, Bucks, UK) and those at the same focal plane under UV and white light were superimposed in Canvas 14 (ACD Systems International, Seattle, WA, USA). Measurements of the thickness of resilin in a semi-lunar process (lines in Fig. 1Aii indicate where these measurements were made), and of the dorsal femoral cuticle were made from these images within Canvas. Once images were superimposed, the structures containing resilin could be identified, allowing the measurements then to be made solely from the non-fluorescing images viewed under white light. This avoided a possible exaggeration of the thickness of resilin caused by any spread of the fluorescence into surrounding tissue that did not contain resilin. This potential error would have increased in older stages as more resilin was deposited.

The results are based on analysis of the following numbers of locusts: first energy store, the semi-lunar processes: 12 fourth instars, 30 fifth instars and 26 adults; second energy store, the muscle tendons: 20 fourth instars, 27 fifth instars and 24 adults; cuticle as a control from the dorsal distal femur: 14 fourth instars, 28 fifth instars and 23 adults. Data are presented as means±s.e.m.

RESULTS

First energy store: the semi-lunar processes

To reveal development changes in the two energy stores for jumping in fourth and fifth instars, and in adults, sections were cut through the distal femora of the hind legs at the level of the paired semi-lunar processes (see Fig. 1Ai).

In sections of fourth instar nymphs on the day that they had moulted from third instars, the semi-lunar processes were visible as paired indentations of the medial and lateral side walls of the distal femur. Both of these structures showed intense blue fluorescence under UV light, indicative of the presence of resilin (Fig. 1A) and distributed in a similar pattern to that in adults (Burrows and Sutton, 2012). In 1 day old fourth instars, the thickness of the resilin in a semi-lunar process was 25±1.5 μm (N=4 locusts, both sides measured; Fig. 1A). Resilin continued to be deposited at a semi-
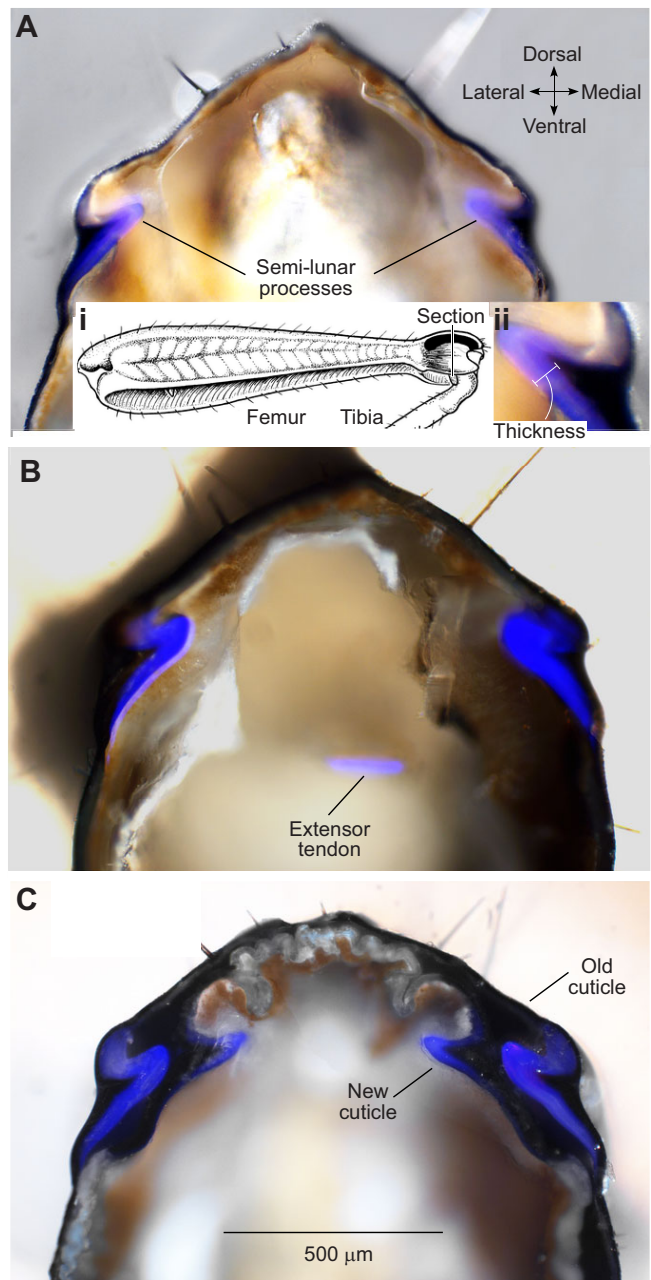

Fig. 1. Semi-lunar processes in fourth instars. (A–C) Transverse sections through the distal hind femur containing the semi-lunar processes. Images of the same section taken under brightfield and ultraviolet (UV) light are superimposed. (A) One day old fourth instar recently moulted from the third instar. (Ai) Drawing of a hind femur to show the plane of sections in this and Figs 2 and 3. (Aii) The region of a semi-lunar process where the thickness of the resilin was measured. (B) Six day old fourth instar. The tendon of the extensor tibiae muscle showing bright blue fluorescence is also visible at the midline. (C) Fourteen day old fourth instar in which the new cuticle of the fifth instar has already begun to form within the old one and is folded repeatedly, particularly below the dorsal surface of the femur. The semi-lunar processes of the new cuticle already fluoresce blue.

lunar process so that after 7 days, or midway through this instar, its thickness had increased to 53.5±1.5 μm (N=4 locusts; Fig. 1B). The tendon of the extensor tibiae muscle was also visible in some sections (Fig. 1B), and showed the same blue fluorescence as in the semi-lunar processes, indicating that it also contained resilin. During the remaining development of this instar, resilin continued
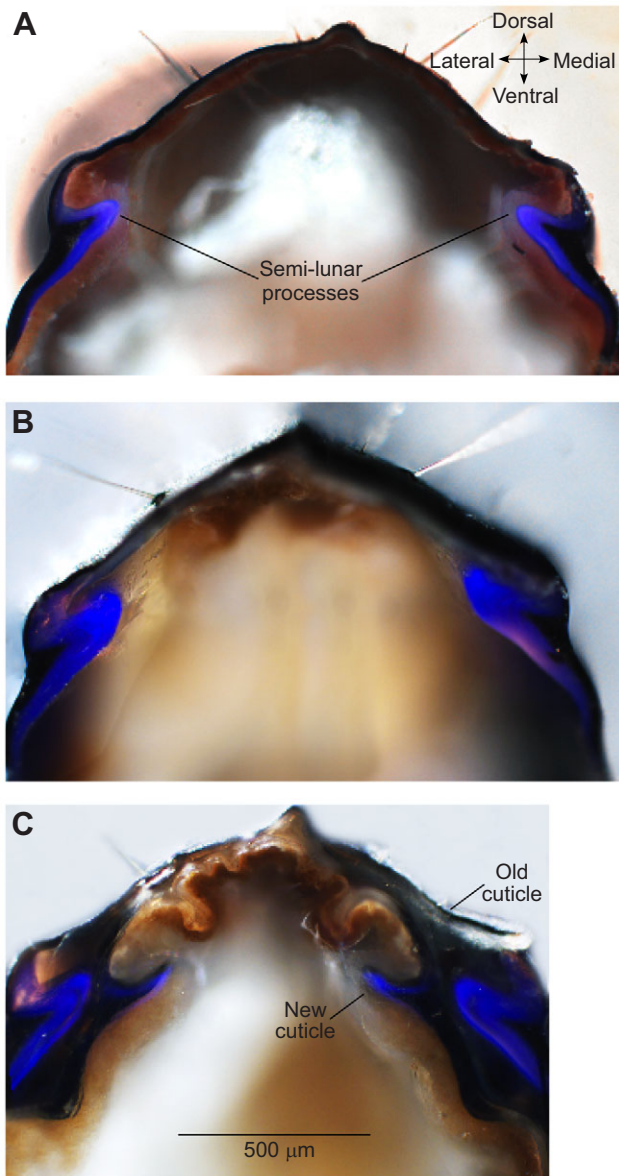
Fig. 2. Semi-lunar processes in fifth instars. (A–C) Superimposed images of transverse sections through the distal hind femur containing the semi-lunar processes viewed under brightfield and UV light. (A) One day old fifth instar, recently moulted from a fourth instar. (B) Eight day old fifth instar. (C) Twelve day old fifth instar in which the new cuticle of the adult has already formed and its paired semi-lunar processes fluoresce blue.

to be added to a semi-lunar process so that 1–2 days before the moult to the fifth instar its thickness had increased by 140% to 60.5±1.5 µm (N=4 locusts; Fig. 1C). At this stage, a new exoskeleton was beginning to be laid down within the existing old one and resilin with a thickness of 23.1±1.7 µm (N=4 locusts) was already present within its semi-lunar processes. The new exoskeleton was also highly folded, as was particularly apparent underneath the dorsal surface of the femur. The larger surface area of the new cuticle would allow for an increase in the femur size of the next instar when expanded fully.

After the moult to a fifth instar, a similar sequence of changes was seen in the deposition of resilin and the consequent increase in thickness of the semi-lunar processes. Two days after the moult, the resilin in each semi-lunar process had increased in thickness to

45.6±2.3 µm (N=8 locusts), a 97% increase over that present within the new cuticle forming inside the previous fourth instar (Figs 1C and 2A). Eight days after the moult, the thickness of the resilin had increased further to 57.7±1.4 µm (N=10 locusts; Fig. 2B). At the time of the final moult to adulthood, the thickness reached 68.6±2.1 µm (N=12 locusts), a 150% increase since the emergence of the fifth instar (Fig. 2C). A new, folded exoskeleton was again present at this stage within the existing exoskeleton and resilin with a thickness of 22.4±0.9 µm (N=12 locusts) was already present in the new semi-lunar processes.

The overall changes that occurred in a semi-lunar process during the fifth instar can be seen by comparing its appearance and size in a freshly moulted instar with that in the new cuticle at the time of the next moult (Fig. 3). In the first day after the moult from the fourth instar, a semi-lunar process appeared from the outside as a dark shape clearly delineated from the lighter-coloured surrounding cuticle (Fig. 3A). By the twelfth day of this fifth instar, the dark semi-lunar process was barely distinguishable from the surrounding cuticle, which had become progressively darker and harder (Fig. 3B). If, at this stage, the external cuticle was peeled away from the distal femur, a larger semi-lunar process in the new cuticle was revealed, surrounded by lighter and highly folded cuticle (Fig. 3C). The semi-lunar process of this underlying new cuticle (Fig. 3C) was 95% larger than the semi-lunar process of the old external cuticle (Fig. 3D).

In adults, a similar process of resilin deposition was observed, but no further moulting occurred. One to two days after the final moult to adulthood, the resilin in a semi-lunar process had more than doubled in thickness to 53.4±6.3 µm (N=12 locusts), relative to the new cuticle in the preceding fifth instar (Fig. 4A). Five days after the final moult, the thickness of a semi-lunar process had almost doubled again to 93.4±8.3 µm (N=8 locusts), finally reaching 125.1±13.4 µm (N=6 locusts) in adults 4 weeks after the final moult (Figs 4B and 5A).

All of these changes in the semi-lunar processes that were measured from newly moulted fourth instars to mature adults revealed a repeating pattern of deposition of resilin accompanied by the growth of a new exoskeleton until the final moult was achieved (Fig. 5A). In each instar, the thickness of the resilin layer increased with age, indicating a continual process of deposition, and a new cuticle containing resilin in its semi-lunar processes was laid down prior to the next moult. In adults, the thickness of the resilin layer in the semi-lunar processes continued to be augmented with increasing age. The rate of increase in the thickness of the resilin in the semi-lunar processes was higher in each stage than the rate of increase in thickness of nearby femoral cuticle that did not contain resilin and has not been implicated in energy storage (Fig. 1A). For example, in an adult, the dorsal femoral cuticle increased by a factor of two from a thickness of 14.4±1.6 µm (N=6 locusts) to 29.1±4.2 µm (N=5 locusts), compared with the fivefold increase of the resilin in the semi-lunar processes.

Second energy store: the muscle tendons

The tendon of the extensor tibiae muscle in a hind leg has been suggested to be a second structure that could act as an energy store (Bennet-Clark, 1975). The muscle undergoes a prolonged contraction before a jump and the resulting energy is stored before being released suddenly to power the rapid propulsive movements of a hind leg. The action of the flexor tibiae muscle is also essential. It first pulls the tibia into a flexed position, and its continued contraction then holds it there while the opposing extensor muscle contracts slowly to store the necessary energy.
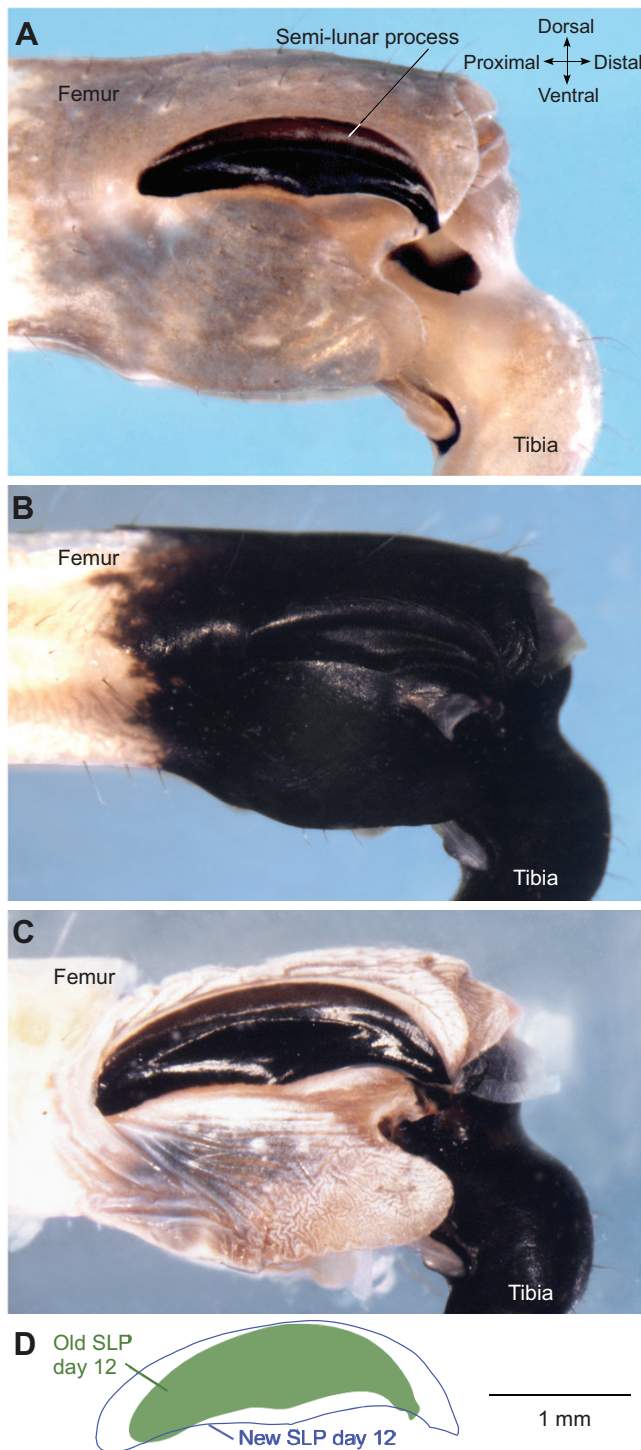
Fig. 4. Semi-lunar processes in adults. Superimposed images of transverse sections through the distal hind femur containing the semi-lunar processes viewed under brightfield and UV light. (A) Adult that had moulted from a fifth instar 1 h before the section was made. (B) Twenty day old adult.

Fig. 3. Growth of the semi-lunar processes. Photographs of the exterior medial surface of the right hind leg of fifth instars. (A) One day old fifth instar. The black semi-lunar process stands out against the pale surrounding cuticle. (B) A different twelve day old fifth instar. The cuticle surrounding the semi-lunar process is now black. (C) The same fifth instar as in B but with the exterior cuticle removed to reveal the new and larger semi-lunar process and folded appearance of the underlying new cuticle. (D) Tracing of the enlarged semi-lunar process (SLP) in the underlying new cuticle in C compared with the smaller one in the overlying old cuticle in B.

The tendons of the extensor and flexor tibiae muscles were therefore examined for the presence of resilin and the time course of its deposition (Figs 5B, 6 and 7). Throughout the greater part of the
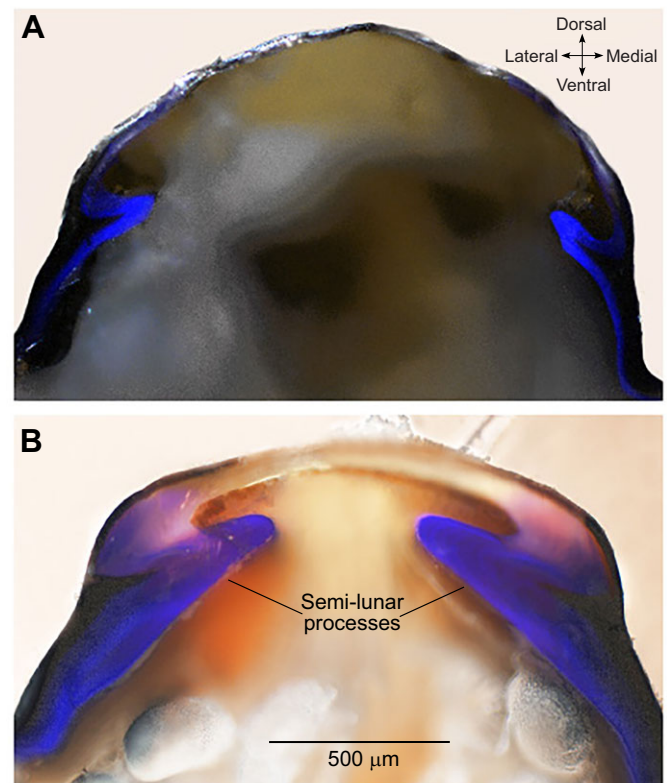
femur, the extensor tendon was flattened laterally to receive the insertions of the muscle fibres that have their origins on the lateral and medial walls of the femur. Only the narrowest edge of the tendon was therefore visible when viewed dorsally. More distally and just proximal to the semi-lunar processes, however, the tendon twisted through 90 deg so that its widest surface, which was devoid of any muscle insertions, was now visible from a dorsal view (Fig. 6A). When this region was illuminated with UV light, the extensor tendon fluoresced with the same bright blue colour as the two semi-lunar processes, thus indicating that resilin is also associated with this second energy store (Fig. 6B). Near to its insertion on the tibia, the tendon transitioned (marked by white arrowheads in Fig. 6A–D) from a hard and stiff structure to a wider, softer and more flexible one. This flexible membrane attached over a large surface area of the dorsal tibia and showed less intense blue fluorescence than the remainder of the tendon (Fig. 6D). The flexor tendon also showed blue fluorescence, again indicating the presence of resilin (Fig. 6E).

Transverse sections taken at different proximo-distal positions along the femur showed resilin in the extensor tendon throughout its changes of orientation, width and thickness (Fig. 7A,B). In all sections, the tendon consisted of a core of blue fluorescence surrounded by harder and in places pigmented material. At the distal end of the femur in 7 day old fifth instars ($N=6$ locusts), the tendon was 312.6±13.2 μm wide, 99.6±6.0 μm thick and had a resilin core 29.5±0.9 μm thick. At the proximal end, it was 550 μm wide, 55 μm thick and with resilin core 15 μm thick. The tendon of the flexor tibiae muscle had a similar cross-sectional structure when viewed with UV light. It had a core of bright blue fluorescence surrounded by harder
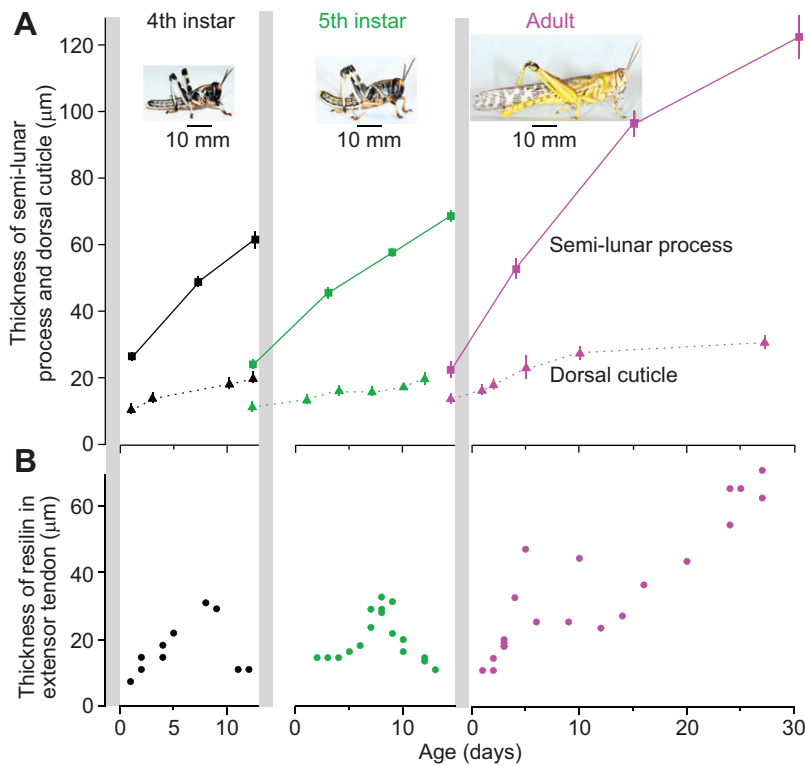
Fig. 5. Cuticular changes in the energy stores during development. (A) Graph of the thickness of the resilin in a semi-lunar process (squares and solid lines) and of a region of dorsal cuticle in the distal femur that contains no resilin (triangles and dotted lines) in fourth, fifth and adult locusts. The insets show photographs of these three stages. The vertical grey bars indicate the occurrence of a moult; the data points represent the means±s.e.m. for a particular age. (B) Changes in the thickness of the resilin core in the tendon of the extensor tibiae muscle. Individual points, each from a different locust, are plotted on the same axes.

material; in the distal femur of a 7 day old fifth instar, it was 180 μm wide, 70 μm thick and had a resilin core 30 μm thick (Fig. 6E).

The deposition of resilin in the extensor tendon during the moult cycle was followed in fourth and fifth instars, and in adults, by making transverse sections through the distal part of the femur at the middle of the semi-lunar processes. This plane of section was the same as that used to analyse the resilin in the semi-lunar processes (Fig. 1Ai) and is also shown on the far right of Fig. 7A. Initially, in the fourth instar (N=5 locusts), the central core of resilin was only 10.9±2.1 μm thick (Figs 5B and 8A). During the next few days, more resilin was deposited so that the core of resilin reached a peak thickness of 28.7±1.5 μm (N=5 locusts). Then, in contrast to the semi-lunar processes, deposition apparently stopped and some of the core resilin was resorbed because its thickness just before the next moult had declined to 10.1±0.1 μm (N=5 locusts). At this time, the tendon was surrounded by a loose-fitting stocking of soft cuticle. A similar pattern of change in the resilin of the tendon was repeated in the fifth instars; from an initial thickness of 14.5±0.1 μm, the resilin increased to 29.5±0.9 μm, before declining again to 18.2±1.8 μm (N=6 locusts of each age) just before the moult to adulthood (Figs 5B and 8B). As the final moult approached, the tendon was again wrapped in a loose stocking of soft cuticle. In newly moulted adults, the thickness of the resilin core of the tendon was only 11.7±0.7 μm (N=5 locusts), but again increased progressively with age, reaching a thickness of 76.9±1.9 μm (N=5 locusts) after 4 weeks (Figs 5B and 8C). At this stage, it was particularly apparent that the core of resilin consisted of a continuous, flattened layer encircling a narrow centre that showed no blue fluorescence (see Fig. 8C, bottom image). During each moult, the extensor tendon was discarded still attached to the old cuticle of the tibia (Fig. 9). The constituent resilin was also discarded because when the cast skin was illuminated with UV light, blue fluorescence was revealed within the tendon.

DISCUSSION
Resilin and energy storage mechanisms for jumping

These results show that two cuticular structures in the hind legs that have been implicated in the storage of energy for jumping contain the elastic protein resilin and that the amount of resilin in each varies with the moult cycle. The first of these energy stores, the semi-lunar processes, is distorted by the force generated by the prolonged contraction of the extensor muscle that precedes a jump or kick (Bennet-Clark, 1975; Burrows and Morris, 2001; Burrows and Sutton, 2012). The second, the tendon of the extensor tibiae muscle, which has been proposed as an energy store (Bennet-Clark, 1975), must be stretched by the contractions of the same muscle. In the semi-lunar processes, the resilin forms a composite with hard cuticle (Burrows and Sutton, 2012). Resilin is shown here to form a core along the whole length of the tendon of the extensor tibiae muscle. The resilin in both storage sites was identified by its characteristic blue fluorescence when illuminated by specific wavelengths of UV light (Andersen and Weis-Fogh, 1964; Weis-Fogh, 1960). Another characteristic feature of the fluorescence of resilin is its dependence on the pH of the surrounding fluid (Neff et al., 2001). This dependence has been shown for the resilin in the semi-lunar processes (Burrows and Sutton, 2012) and in the energy stores used by other jumping insects such as froghoppers (Burrows et al., 2008). A third characteristic of resilin that supports its presence in energy stores for jumping is that the distribution of its blue fluorescence is matched by its labelling (Burrows et al., 2011) with an anti-Rec1 resilin polyclonal antibody (Elvin et al., 2005; Lyons et al., 2011). There is thus strong evidence that the fluorescence induced by specific wavelengths of UV light in the two energy stores of locusts analysed here indicates the presence of resilin.

Deposition of resilin

In each instar, resilin in the semi-lunar processes was initially a thin layer but then increased progressively so that by the time of the next
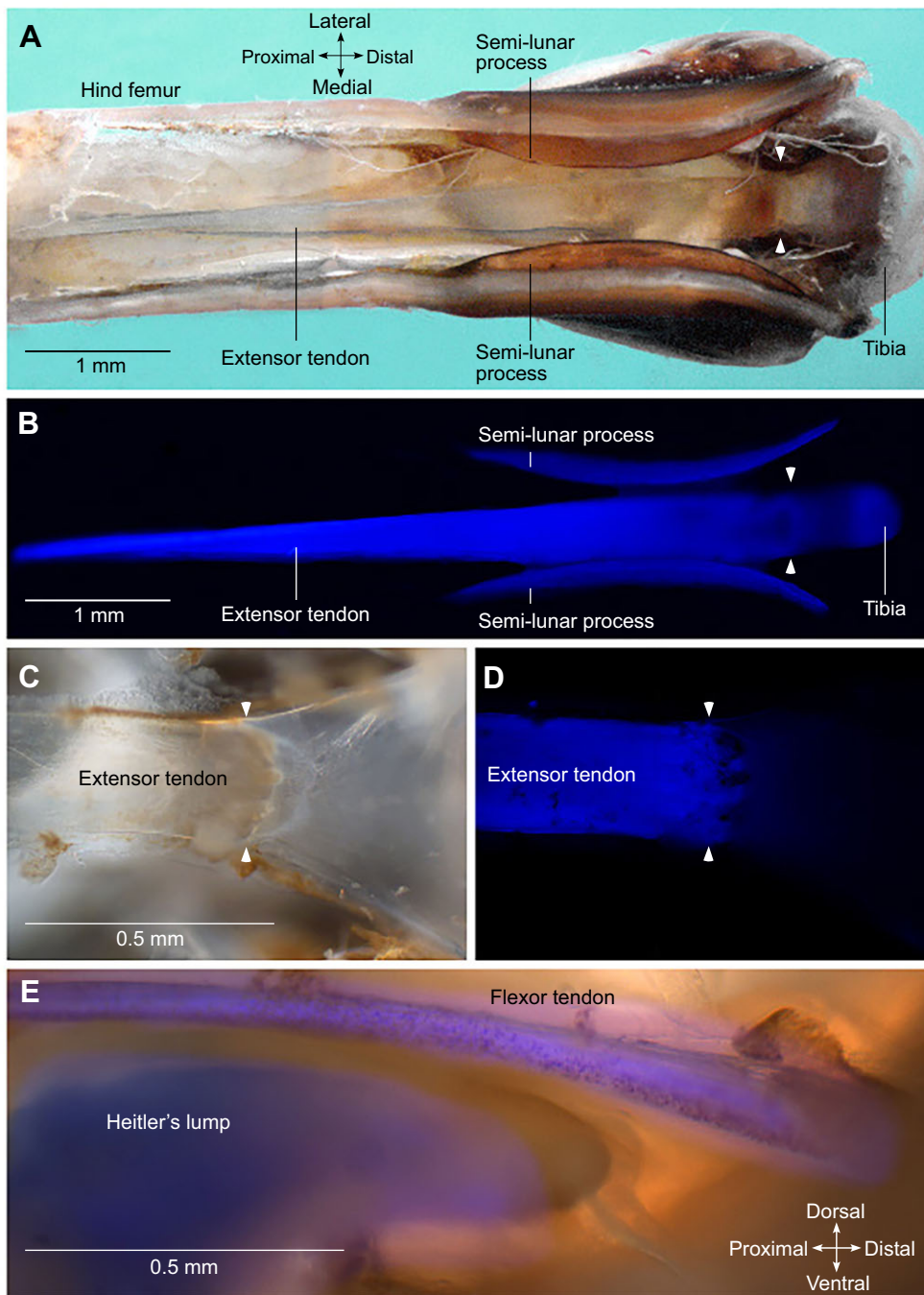
Fig. 6. Tendons of the extensor and flexor tibiae muscles of an adult hind leg. (A) Dorsal view of the distal hind femur of an adult locust under brightfield illumination after removal of part of the dorsal cuticle. The extensor tendon twists from a vertical (proximal on left) to a horizontal orientation as it runs between the two semi-lunar processes to insert on the tibia. (B) Same view under UV illumination reveals intense blue fluorescence of the extensor tendon and the two semi-lunar processes. (C) Brightfield image of the extensor tendon viewed dorsally at its transition (indicated by white arrowheads in A–D) to the flexible membrane that spreads to insert on the tibia. (D) UV illumination of the same area and at the same focal plane shows that the transition is also marked by less intense blue fluorescence in the flexible membrane. (E) Longitudinal section of the flexor tibiae muscle as it passes over an internal protrusion (Heitler's lump) of the ventral, distal femur. Brightfield and UV images are superimposed to show blue fluorescence in both structures.

moult it had more than doubled in thickness. In the tendon of the extensor muscle, deposition also occurred in each instar and in the adult, resulting in the central core of resilin becoming thicker. However, in contrast to that in the semi-lunar processes, the resilin in the tendon became thinner as the next moult became imminent. In adults, resilin deposition in both energy stores also continued for at least the first 4 weeks of adult life, with its thickness increasing fivefold. From sections of both energy stores taken at different times relative to the moult cycle, it was apparent that there must be a steady increase in the amount of resilin laid down in a repeating pattern during development and well after the final moult to adulthood. Moreover, as the time for the next moult approached, a new exoskeleton was already forming within the old one and resilin was already present in structures that could be recognised as the new

semi-lunar processes. At each moult, the resilin in both the semi-lunar processes and some of the resilin in the tendons was shed with the old exoskeleton. This indicates that the composite cuticular structures that make up the energy stores must be reformed at each moult.

Does the deposition of resilin follow similar patterns in other insects? In locusts, the resilin in the ligament of the forewing hinge and in the pre-alar arm of the moveable plate between the forewings is deposited before the final moult to adulthood and for many days after this moult (Neville, 1963). Cross-linking of the resilin occurs in parallel with this deposition and the elasticity imparted to these structures is due to the presence of resilin (Weis-Fogh, 1960). In fleas (Siphonaptera), resilin in the thoracic energy storage devices for jumping appears to be laid down only late in development
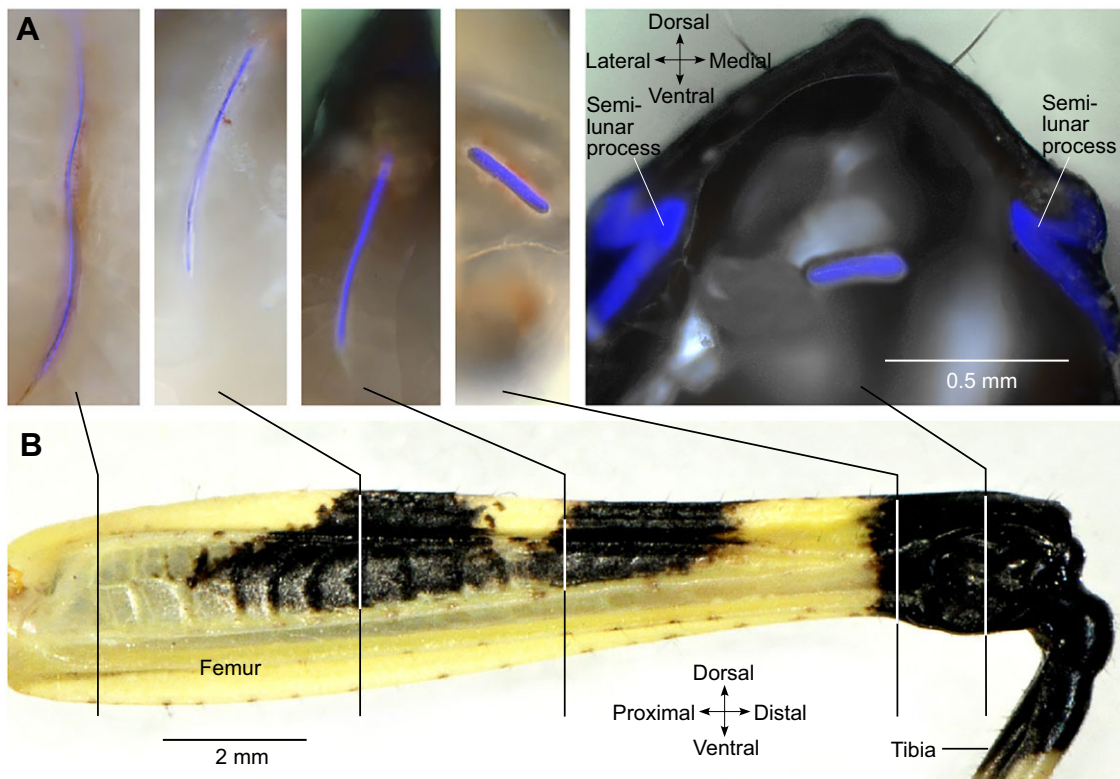
Fig. 7. Shape of the hind extensor tendon. (A) Brightfield and UV images are superimposed to show the blue fluorescence of the tendon of a fifth instar as it changes in orientation and width at different proximo-distal positions along the femur. In the right-hand image, blue fluorescence also occurs in the paired semi-lunar processes. (B) Photograph of the right hind femur viewed medially to indicate the planes of the transverse sections shown in A.

(Rothschild and Schlein, 1975). In froghoppers (Hemiptera, Cercopoidea) the nymphal stages do not jump as many species live in a protective froth. The adults, like locusts, use a catapult mechanism and need energy stores to meet the huge energy demands of jumping. Correlated with these behavioural observations is that little resilin is present in nymphal structures that will develop into the energy stores of the adults. Large amounts

of resilin appear at the final moult to adulthood when the energy stores must be ready for jumps to propel an adult from the nymphal froth (Burrows et al., 2008). By contrast, the nymphal stages of planthoppers (Hemiptera, Fulgoroidea) live a free life in the same habitat as the adults and, like them, jump prodigiously. As in locusts, all nymphal instars and the adults have resilin in functional energy storage devices. Taken together, these observations point to
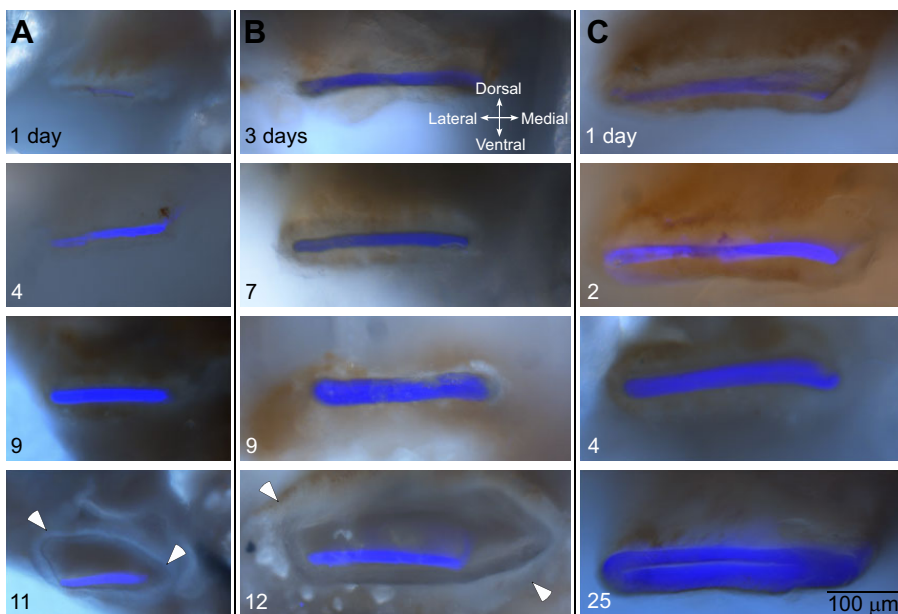

Fig. 8. Developmental changes of resilin in the tendon of the hind extensor tibiae muscle. (A) Fourth instar. (B) Fifth instar. (C) Adult. The numbers indicate the age in days of the locust from which the images were made. The loose-fitting membrane around the tendon in the later stages of each instar is indicated by white arrowheads. Brightfield and UV images are superimposed to show the blue fluorescence in the core of the tendon.
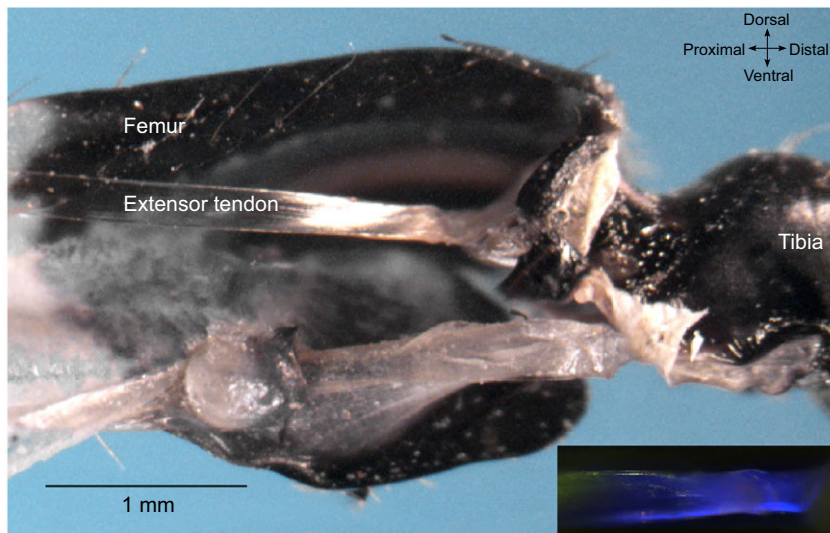
Fig. 9. Cuticle shed by a fifth instar at moulting. Photograph of the cast cuticle of a hind femur of a fifth instar from which the medial wall has been removed to reveal the tendon of the extensor tibiae still attached to the cuticle of the tibia. The inset shows part of this tendon illuminated with UV light and fluorescing bright blue, indicating the presence of resilin.

a clear correlation between the presence of resilin and the mechanism for energy storage in jumping.

Development of jumping ability

If jumping in locusts depends on energy storage, then how is this important behaviour sustained during fluctuations in the rigidity and elasticity of the exoskeleton imposed by moulting? Locusts grow in a series of step changes in size and mass from first instars (mass of a few milligrams) to adult males (mass of 1–1.5 g) or larger females (mass of 2.5–3 g) (Katz and Gosline, 1993). Before a moult, two cuticles are present: the new one is soft and the old one hard. After a moult, the new soft cuticle takes time to harden once more. The enormous muscular forces needed to generate jumping are close to the tolerances of the cuticle even in 3–4 week old adults where the exoskeleton is fully hardened, so that there is the ever-present danger that structural damage may occur (Bennet-Clark, 1975). During a moulting period, the softer skeleton is less able to withstand the forces that would normally be generated in jumping. Should jumping performance be degraded, however, the dangers of predation will rise steeply. In each instar, jumping distance gradually increases and then falls with the imminence of the next moult (Gabriel, 1985a; Queathem, 1991). This measure of jumping performance thus follows the moult cycles. Similarly, in adults, jump distance also increases in the first 3 weeks after the final moult so that mature adults can jump three times further than young nymphs (Gabriel, 1985a). Adults have thicker and stiffer semi-lunar processes than young nymphs and thicker cuticle (Gabriel, 1985b). What mechanisms enable jumping performance to change?

Contractions of the extensor muscle on the day of the moult in all locusts result in deformations of the femoral cuticle (Norman, 1995). If the forces generated are too high then cuticular deformations may be irreversible. After a moult, when the new exoskeleton is still soft, contractions of the extensor muscle that deform the walls of the femur transmit less force to the tibia. Within limits, this can be seen as a mechanism for protecting other weak parts of the leg from irreparable damage. A day or two after the moult, the most common damage is a rupture of the insertion of the extensor tendon on the proximal tibia. This is because the cuticle of the proximal femur has become harder with the result that larger forces are directly transmitted to the distal structures, which may still be weak. Five days after the final moult to adulthood, the incidence of damage falls, but now the most common damage is breakage of

the extensor tendon. Fourteen days after the final moult, the incidence of damage declines still further with only rare breakages of the extensor tendon now occurring.

At the time of a moult, a locust lessens the probability of inflicting these damaging effects on its hind legs by adopting two strategies (Norman, 1995). First, the frequency with which kicks or jumps can be elicited by the same stimulus falls in the days before a moult to reach zero on the day of the moult, and only recovers to its original level some 3 days after the moult. This means that the locust will only attempt to kick or jump in the most exceptional circumstances. An unwillingness or inability to jump has been recognised as a behavioural indicator of the imminence of a moult (Hughes, 1980). Second, the characteristic motor pattern that generates a normal kick or a jump (Burrows, 1995; Heitler and Burrows, 1977) is not produced by newly moulted locusts (Norman, 1995); the extensor and flexor muscles do not co-contract in such a way that enables energy to be generated slowly and then stored. The resulting movements are thus not powered by a catapult-like release of stored energy. Instead, the extensor and flexor tibiae muscles contract separately to produce movements that are much slower and much less powerful than a normal jump. Two neural changes are associated with the lack of expression of the normal motor pattern at a moult. First, the effectiveness of positive feedback from sensory neurons to the motor neurons that participate in the co-contraction is reduced. In newly moulted locusts, a particular sensory neuron, for example, produces fewer spikes in response to the same stimulus than it does in an older locust (Norman, 1996). As a result, its direct synapses with the motor neurons evoke fewer motor spikes, which in turn generate lower muscular forces (Norman, 1996). Second, repetitive spikes in the single fast motor neuron innervating the extensor tibiae muscle decline in amplitude to a greater extent in locusts at the time of the moult compared with mature locusts. This in turn reduces the amplitude of the excitatory potentials evoked in flexor tibiae motor neurons at their direct, central synaptic connections and thus reduces the effectiveness of the co-contraction of the extensor and flexor muscles (Norman, 1997).

To these properties which alter jumping performance, we can now add the changes that occur in the two energy storage devices. The present study has shown that the thickness of resilin in the semi-lunar processes is low at the start of each new instar but then progressively increases. A similar increase also occurs in the resilin at the core of the extensor muscle tendon, but as the moult

approaches the resilin then becomes thinner. These changes in the mechanical properties of the energy stores thus parallel the observed changes in jumping performance.

References

Andersen, S. O. and Weis-Fogh, T. (1964). Resilin. A rubberlike protein in arthropod cuticle. *Adv. Insect Physiol.* **2**, 1-65.

Bennet-Clark, H. C. (1975). The energetics of the jump of the locust *Schistocerca gregaria*. *J. Exp. Biol.* **63**, 53-83.

Bennet-Clark, H. C. and Lucey, E. C. A. (1967). The jump of the flea: a study of the energetics and a model of the mechanism. *J. Exp. Biol.* **47**, 59-76.

Burrows, M. (1995). Motor patterns during kicking movements in the locust. *J. Comp. Physiol. A* **176**, 289-305.

Burrows, M. (2003). Biomechanics: froghopper insects leap to new heights. *Nature* **424**, 509.

Burrows, M. (2006). Jumping performance of froghopper insects. *J. Exp. Biol.* **209**, 4607-4621.

Burrows, M. and Morris, G. (2001). The kinematics and neural control of high speed kicking movements in the locust. *J. Exp. Biol.* **204**, 3471-3481.

Burrows, M. and Morris, O. (2003). Jumping and kicking in bush crickets. *J. Exp. Biol.* **206**, 1035-1049.

Burrows, M. and Sutton, G. P. (2012). Locusts use a composite of resilin and hard cuticle as an energy store for jumping and kicking. *J. Exp. Biol.* **215**, 3501-3512.

Burrows, M., Shaw, S. R. and Sutton, G. P. (2008). Resilin and chitinous cuticle form a composite structure for energy storage in jumping by froghopper insects. *BMC Biol.* **6**, 41.

Burrows, M., Borycz, J. A., Shaw, S. R., Elvin, C. M. and Meinertzhagen, I. A. (2011). Antibody labelling of resilin in energy stores for jumping in plant sucking insects. *PLoS ONE* **6**, e28456.

Elvin, C. M., Carr, A. G., Huson, M. G., Maxwell, J. M., Pearson, R. D., Vuocolo, T., Liyou, N. E., Wong, D. C. C., Merritt, D. J. and Dixon, N. E. (2005). Synthesis and properties of crosslinked recombinant pro-resilin. *Nature* **437**, 999-1002.

Gabriel, J. M. (1985a). The development of the locust jump mechanism. I. Allometric growth and its effect on the jumping performance. *J. Exp. Biol.* **118**, 313-326.

Gabriel, J. M. (1985b). The development of the locust jump mechanism. II. Energy storage and muscle mechanics. *J. Exp. Biol.* **118**, 327-340.

Godden, D. H. (1975). The neural basis for locust jumping. *Comp. Biochem. Physiol. A Physiol.* **51**, 351-360.

Heitler, W. J. and Burrows, M. (1977). The locust jump. I. The motor programme. *J. Exp. Biol.* **66**, 203-219.

Hughes, T. D. (1980). The imaginal ecdysis of the desert locust, *Schistocerca gregaria*. I. A description of the behaviour. *Physiol. Entomol.* **5**, 47-54.

Katz, S. L. and Gosline, J. M. (1993). Ontogenetic scaling of jump performance in the African desert locust (*Schistocerca gregaria*). *J. Exp. Biol.* **177**, 81-111.

Lyons, R. E., Wong, D. C. C., Kim, M., Lekieffre, N., Huson, M. G., Vuocolo, T., Merritt, D. J., Nairn, K. M., Dudek, D. M., Colgrave, M. L. et al. (2011). Molecular and functional characterisation of resilin across three insect orders. *Insect Biochem. Mol. Biol.* **41**, 881-890.

Neff, D., Frazier, S. F., Quimby, L., Wang, R.-T. and Zill, S. (2001). Identification of resilin in the leg of cockroach, *Periplaneta americana:* confirmation by a simple method using pH dependence of UV fluorescence. *Arthropod Struct. Dev.* **29**, 75-83.

Neville, A. C. (1963). Growth and deposition of resilin and chitin in locust rubber-like cuticle. *J. Insect Physiol.* **9**, 265-278.

Norman, A. P. (1995). Adaptive changes in locust kicking and jumping behaviour during development. *J. Exp. Biol.* **198**, 1341-1350.

Norman, A. P. (1996). Proprioceptive feedback in locust kicking and jumping during maturation. *J. Comp. Physiol. A* **179**, 195-205.

Norman, A. P. (1997). Excitatory interactions between antagonistic motor neurones underlying locust kicking and jumping during maturation after the adult moult. *J. Comp. Physiol. A Sens. Neural. Behav. Physiol.* **181**, 231-237.

Queathem, E. (1991). The ontogeny of grasshopper jumping performance. *J. Insect Physiol.* **37**, 129-138.

Rothschild, M. and Schlein, J. (1975). The jumping mechanism of *Xenopsylla cheopis*. I. Exoskeletal structures and musculature. *Philos. Trans. R. Soc. B Biol. Sci.* **271**, 457-490.

Sutton, G. P. and Burrows, M. (2011). Biomechanics of jumping in the flea. *J. Exp. Biol.* **214**, 836-847.

Usherwood, P. N. R. and Grundfest, H. (1965). Peripheral inhibition in skeletal muscle of insects. *J. Neurophysiol.* **28**, 497-518.

Weis-Fogh, T. (1960). A rubber-like protein in insect cuticle. *J. Exp. Biol.* **37**, 889-907.