Energy storage and synchronisation of hind leg movements during jumping in planthopper insects (Hemiptera, Issidae)

M. Burrows

Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK

mb135@hermes.cam.ac.uk

SUMMARY

The hind legs of *Issus* (Hemiptera, Issidae) move in the same plane underneath the body, an arrangement that means they must also move synchronously to power jumping. Moreover, they move so quickly that energy must be stored before a jump and then released suddenly. High speed imaging and analysis of the mechanics of the proximal joints of the hind legs show that mechanical mechanisms ensure both synchrony of movements and energy storage. The hind trochantera move first in jumping and are synchronised to within 30 μs. Synchrony is achieved by mechanical interactions between small protrusions from each trochantera which fluoresce bright blue under specific wavelengths of ultra-violet light and which touch at the midline when the legs are cocked before a jump. In dead *Issus*, a depression force applied to a cocked hind leg, or to the tendon of its trochanteral depressor muscle causes a simultaneous depression of both hind legs. The protrusion of the hind leg that moves first nudges the other hind leg so that both move synchronously. Contractions of the trochanteral depressor muscles that precede a jump bend the metathoracic pleural arches of the internal skeleton. Large areas of these bow-shaped structures fluoresce bright blue in ultraviolet light, and the intensity of this fluorescence depends on the pH of the bathing saline. These are key signatures of the rubber-like protein resilin. The remainder of a pleural arch consists of stiff cuticle. Bending these composite structures stores energy and their recoil powers jumping.

INTRODUCTION

The powerful and rapid movements of the legs that propel jumping in insects require an intricate interplay between the mechanics of their joints and the controlling signals from the nervous system. The potential for mechanics to simplify an otherwise complex control problem is enormous. The small size of its body and hence the short length of its legs, means that an insect has little time available to accelerate its body to take-off by extending its legs, and little time for sensory feedback to exert any motor control. Short acceleration times and high energy requirements, mean that insect jumping usually has to be powered by catapult mechanisms and not by direct muscular contractions (Alexander, 1995; Bennet-Clark, 1975; Bennet-Clark and Lucey, 1967). Such mechanisms require the storage of energy during slow contractions of the power-producing muscles and then its sudden release, both of which in turn require complex mechanical designs of the relevant leg joints, and detailed motor patterns.

These problems, and their solutions, are exemplified by the champion jumping insects, froghoppers (Hemiptera, Cercopidae) and some planthoppers (Hemiptera, Issidae). The froghopper *Philaenus* accelerates in less than 1 ms to a take-off velocity of $4.7 \, \text{m s}^{-1}$, experiencing a force of some $550g$ (Burrows, 2003; Burrows, 2006). In the best jumps by males of the planthopper *Issus*, the body is again accelerated in less than 1 ms to a take-off velocity of $5.5 \, \text{m s}^{-1}$, is subjected to a force of $719g$ and is displaced an horizontal distance of $1.1 \, \text{m}$ (Burrows, 2009). To explain these outstanding performances two basic questions about the interplay between mechanics and neural control must be tackled. First, how are the movements of the two hind legs synchronised to propel an effective jump? Second, how is energy stored and then delivered quickly to power a jump when using a catapult mechanism?

Jumping insects have two basic arrangements of the hind legs relative to the body. First, in fleas and orthopteran jumping insects such as locusts, the hind legs are arranged at the side of the body so that they each move in independent but parallel planes. In fleas, a jump is powered by contractions of trochanteral depressor muscles in the thorax (Bennet-Clark and Lucey, 1967; Rothschild and Schlein, 1975; Rothschild et al., 1975; Rothschild et al., 1972), but in locusts, a jump is propelled by rapid extension of the hind tibiae powered by the contractions of the extensor tibiae muscles (Bennet-Clark, 1975; Brown, 1967; Burrows, 1995; Heitler and Burrows, 1977). In locusts, the movements of the two hind legs in jumping are usually synchronised to within a few milliseconds of each other, but a consequence of their arrangement is that successful jumps can be produced when there are larger timing differences, and oriented jumps are even possible when powered by a single hind leg (Sutton and Burrows, 2008). The second arrangement, found in many families of jumping hemipteran bugs, particularly those in the Auchenorrhyncha, is that the two power-producing hind legs are slung beneath the body and move in the same plane as each other close to the under surface of the body (Sutton and Burrows, 2010). Huge trochanteral depressor muscles located in the thorax of froghoppers (Hemiptera, Cercopidae) generate closely synchronised movements of both hind legs. The different force vectors of each hind leg mean that if there are differences in the time at which the forces of the two hind legs are applied to the ground, the body will spin with the consequent loss of energy and reduction in jumping performance (Sutton and Burrows, 2010). Froghoppers that have

lost one hind leg are rare in nature. These same constraints are likely to apply to the planthoppers; the hind legs move in the same plane as each other beneath the body, and the same muscles generate the forces for jumping by a similar motor pattern (Burrows and Bräunig, 2010). The different anatomical arrangement of the proximal joints of the hind legs may, however, allow insights into whether mechanics play a role in synchronising the movements of the hind legs.

Energy storage for jumping is achieved by a number of different mechanical mechanisms in different insects. For example, in fleas it is attributed to a pad of the rubber-like protein resilin between the stiff, cuticular struts of the metathorax (Bennet-Clark and Lucey, 1967). In locusts, paired semi-lunar processes at the femoro-tibial joints of the hind legs are bent by the contractions of the large extensor tibia muscles and can store about half of the energy needs of jumping (Bennet-Clark, 1975). In froghoppers, the contractions of the trochanteral depressor muscles bend bilaterally arranged and bow-shaped pleural arches that are part of the internal skeleton of the metathorax. These structures are composite structures built of stiff chitinous cuticle and resilin (Burrows et al., 2008). In four families of planthoppers (Fulgoroidea), pleural structures (Ursprungsplatte) have been implicated in jumping (Heilig and Sander, 1986; Sander, 1957), but their composition, and their action in jumping is unknown.

This investigation analyses the mechanics of the proximal joints of the hind legs of the planthopper *Issus* (Hemiptera, Issidae) during natural jumping and experimentally imposed movements, to address two questions. First, what are the mechanisms that deliver synchronous movements of the hind legs to power jumping? It is shown that protrusions from each trochanter that contain resilin-like protein provide a mechanical linkage between the two hind legs that can synchronise the jumping movements. Second, how is energy stored during the prolonged contractions of the main power producing muscles that precede a jump? It is shown that these contractions distort and bend the paired pleural arches which are composite structures containing resilin-like protein.

MATERIALS AND METHODS

Adult planthoppers *Issus coleoptratus* (Fabricius 1781) of either sex were collected on ivy (*Hedera*) during August and September in Aachen, Germany (50.77672°N, 6.07023°E and 50.77925°N, 6.06325°E). *Issus* belongs to the order Hemiptera, suborder Auchenorrhyncha, superfamily Fulgoroidea and family Issidae.

To determine the degree of synchrony between movements of the proximal joints of the two hind legs, live *Issus* were restrained ventral surface uppermost in Plasticine, but with the hind legs free to move. They were encouraged to produce movements of both hind legs that had the characteristics of those that occur during natural jumping movements (Burrows and Bräunig, 2010) by touching the body with a fine paint brush. When restrained in the same way, both froghoppers (Hemiptera, Auchenorrhyncha, Cercopidae) (Burrows, 2007b) and leafhoppers (Hemiptera, Auchenorrhyncha, Cicadellidae) (Burrows, 2007a) show a similar sequence of movements, that also appear to underlie natural jumping. The movements were captured at rates of 30,000 images s^{-1} and exposure times of 30 µs (supplementary material Movie 1) with a Photron Fastcam 1024 PCI camera [Photron (Europe) Ltd, Marlow, Bucks., UK] mounted on either a Leica MZ16 or a Zeiss SV6 stereo microscope, and viewing the *Issus* ventrally. The images captured in this way were then fed directly to a computer.

To determine whether the movement of one hind leg might mechanically influence the movement of the other hind leg, both hind legs of four recently dead *Issus* were forcibly moved into their fully levated and cocked position that they adopt in preparation for a natural jump. They remained in this position until a force was applied to a hind leg. Three series of experiments were undertaken. First, a force was applied to the left femur so that, in turn, it applied a depression force to the left trochanter. Second, the experiment was repeated with a force applied to the right femur and right trochanter. Third, some of the flexible cuticle overlying the ventral surface of the metathorax was removed to allow access to the tendon of the trochanteral depressor muscle of the left hind trochanter. The tendon was then hooked with the bent tip of a minuten pin (size 0) mounted in a holder on a micromanipulator, and pulled in the same direction as it would move were the trochanteral depressor muscle to contract. Images of the induced movements of the hind legs in all three experiments were captured at rates of 2,000 images s^{-1} with the Photron camera (e.g. supplementary material Movie 2). Temperatures in all experiments to determine the kinematics of the hind leg movements ranged from 24 to 30°C.

The anatomy of the proximal joints of the hind legs was examined in live *Issus* and in specimens cleared in 5% potassium hydroxide for 24 h and mounted in distilled water. Anatomical drawings were made with the aid of a drawing tube, and photographs were taken with a Nikon DXM 1200 camera, both attached to the Leica MZ16 microscope. Other dried insects were mounted on specimen holders, sputter coated with gold and then examined in a Philips XL-30 scanning electron microscope.

To indicate whether resilin was present in the mechanical structures associated with the hind legs, fresh *Issus* were placed on the stage of an Olympus BX51WI compound microscope. They were viewed through Olympus MPlan ×5/0.1 NA, Mplan ×10/0.25 NA, and LUCPlanFLN ×20/0.45 NA objective lenses, under ultraviolet (UV) or white epi-illumination. Images were captured with a Nikon DXM 1200 digital camera, as colour (RGB) TIFF files. The UV light was provided by an X-cite series 120 metal halide light source, conditioned by a Semrock DAPI-5060B Brightline series UV filter set (Semrock, Rochester, NY) with a sharp-edged (1% transmission limits) band from 350 nm to 407 nm. The resulting blue fluorescence emission was collected in a similarly sharp-edged band at wavelengths from 413 nm to 483 nm through a dichromatic beam splitter. Images captured at the same focal planes under UV and visible light were superimposed in Canvas X (ACD Systems of America, Miami, FL, USA). To establish whether the fluorescence was sensitive to the pH of the bathing saline, it was changed from its normal value of 7.2 to pH 2 with 2 mol l^{-1} hydrochloric acid, and to pH 12 with 2 mol l^{-1} sodium hydroxide.

RESULTS
Joint movements
Synchrony of movements during jumping

Jumping is powered by huge muscles in the thorax, the tendons of which run through the coxae to insert on the trochantera and cause depression. The coxo-trochanteral joints are therefore the first to move in a jump. Images of natural jumping movements captured at rates of 5000 images s^{-1} (Burrows, 2009) revealed no timing differences between the first depression movements of the two hind trochantera. To determine how closely these initial movements were coupled, eighteen restrained jumps by four adult *Issus* were captured at a frame rate of 30,000 images s^{-1} (Fig. 1 and supplementary material Movie 1). Each *Issus* was restrained on its back so that the jumping movements of the two hind legs did not have to support the mass of the body and were thus completed in 0.3 ms, compared with an average of 1.5 ms in natural jumping. In 16 (89%) of these
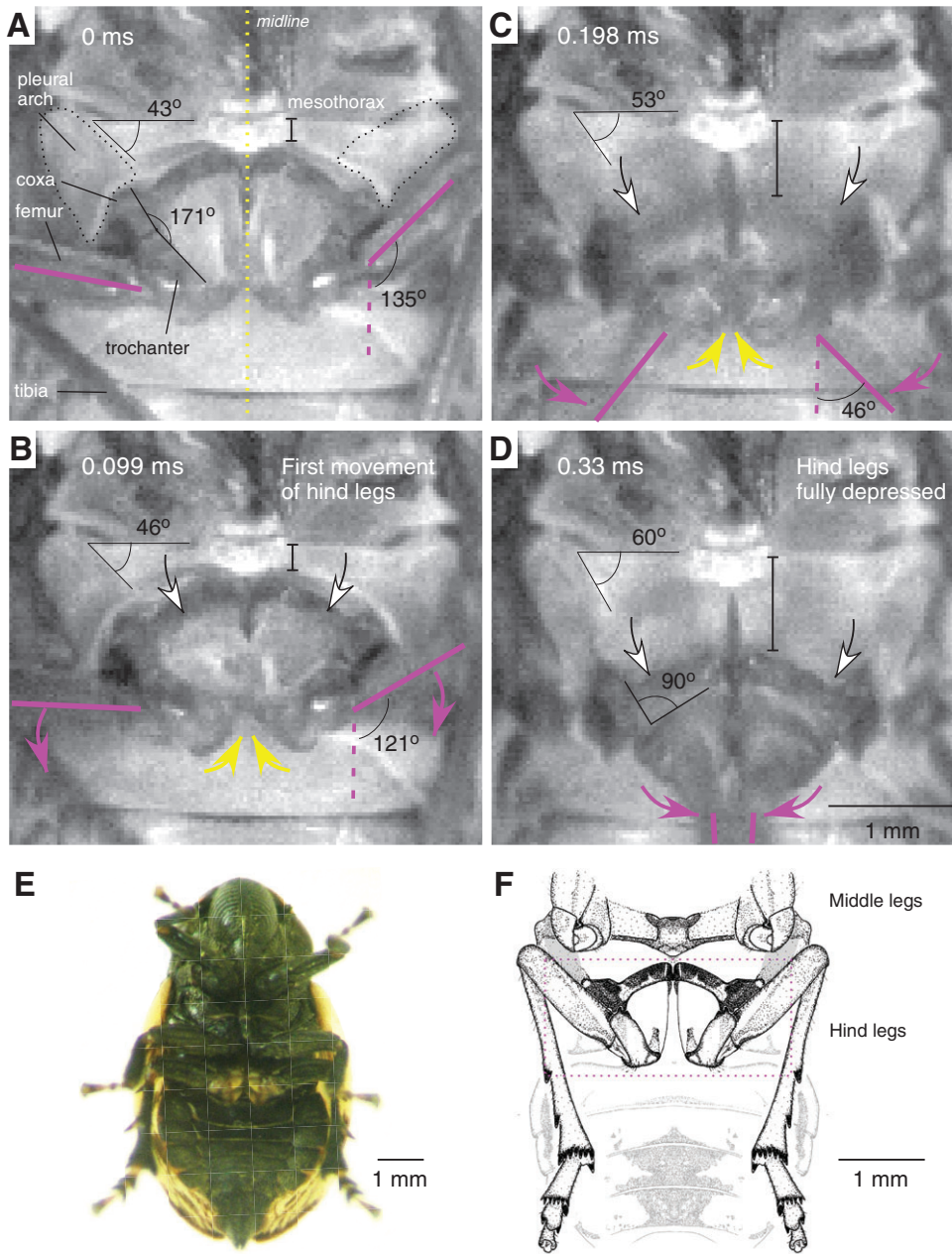
Fig. 1. Movements of the proximal joints of the hind legs during restrained jumping by *Issus*. (A–D) Four images taken from a jump captured at 30,000 images s⁻¹ and each with an exposure time of 0.03 ms. (A) Before a jump, the hind legs were in their cocked position (time 0 ms) with both trochantera fully levated about the coxae. The angle of a femur was measured relative to the longitudinal axis of the body, and the angle of a trochanter relative to its coxa. The vertical black bar indicates the proximity of the anterior edge of the right coxa to the border between the meso- and metathoracic segments. The outlines of the visible parts of the pleural arches are dotted, and their angle is given relative to the transverse axis of the body. The vertical yellow dotted line indicates the midline. (B) 0.099 ms later the first depression movement (yellow arrows) of the hind trochantera began. (C) The depression of the trochantera reached completion. (D) The jump was completed after 0.2 ms, the pleural arches were unfurled, the coxae moved posteriorly, the trochantera were fully depressed, and the femora were parallel to each other at the ventral midline. White arrows indicate the posterior movements of the coxae, and the distance by the vertical black bars; pink arrows and lines indicate the movements of the femora. Bending of the pleural arches is indicated by their angular changes from 43 deg. (A) to 60 deg. (D) relative to a line at right angles to the longitudinal axis of the body at the posterior edge of the mesothorax. (E) Photograph of *Issus* viewed ventrally. (F) Drawing of the middle region of *Issus* viewed ventrally with the dotted pink box indicating the parts of the hind legs shown in the high speed images (A–D).

jumps the initial movement of the trochantera of both hind legs occurred within the same frame. In one of the two remaining jumps, the left hind leg moved in the frame before the right leg, and in the other jump the reverse happened. Therefore, even at these high frame rates the greatest detectable timing difference between the movements of the two hind legs was only 30 μs and in most jumps the movements were synchronous.

Coxal movements during jumping
The sequence of jumping movements of the two hind legs began with both moving into their fully cocked position (Fig. 1A), either at the same time or independently, by levation of the trochantera about the coxae. Only a small region of the body and hind legs was captured in these images as indicated by the photograph of the whole *Issus* (Fig. 1E) and the drawing (Fig. 1F). The hind legs then remained in the fully levated (cocked) position for 48.3±3.3 s (mean ± s.e.m.; range 6–134 s in 76 jumps by nine *Issus*) (Burrows and Bräunig, 2010).

During this time, recordings of the electrical activity in the trochanteral depressor muscles showed that they contracted continuously (Burrows and Bräunig, 2010). At the start of a jumping movement, the anterior edge of the hind coxae were about 100 μm behind the transverse posterior edge of the mesothoracic segment (vertical bar in frame designated as time 0 ms, Fig. 1A). The two medial surfaces of the left and the right hind trochantera touched each other at the midline through the flexible arthrodial membrane of the coxo-trochanteral joints. The pleural arches, which could only be seen through the semi-transparent cuticle of the ventral metathorax, were bent toward the midline so that they subtended an angle of 43 deg. to the transverse posterior edge of the mesothorax. The first detectable movements were a simultaneous depression of the left and right trochantera and accompanying movements of the femora (Fig. 1B). The whole jump movement was completed in 0.3 ms (Fig. 1B–D). Both coxae also moved posteriorly by 500–600 μm and the pleural arches unfurled by about 17° (from 43 deg. to 60 deg.).

The same pattern of movements could be induced in dead *Issus*, but they then occurred at slower speeds and with joint movements of lower angular velocities (Figs 2–4). If both hind legs were forcibly moved into their fully cocked positions, they would remain locked with a medial protrusion from the left hind leg touching a similar protrusion from the right hind leg (white arrows in Fig. 2A–C). If a force was then applied to the left femur it would move about the left trochanter, but no other movements occurred. Continued application of force to the left femur, however, resulted in a depression of the left trochanter (Fig. 2B) that, remarkably, also triggered a depression movement of the right trochanter (Fig. 2C). The coxo-trochanteral joints of both hind legs then depressed and these movements were accompanied by extension of both femora. A mechanical interaction between the two sides must have been responsible because no neural coupling acting through sensory and motor pathways was possible in this dead animal. Superimposing tracings of the positions of the mechanical structures before and after the coupled movements of the two legs revealed the mechanical events that followed the imposed movement of the left femur (Fig. 2D). First, moving the hind legs into their cocked position bent the pleural arches inwards at their posterior ends, moved the anterior edges of the coxae forwards and their anterior and medial edges closer together. Second, the force applied to the left femur was transmitted through the left trochanter to the right trochanter causing them both to depress at the same time. Third, both coxae moved posteriorly and their anterior medial edges moved laterally (compare the yellow lines in Fig. 2A,C).

Synchronisation mechanism

The following three experiments were carried in recently dead *Issus* to determine whether the two hind legs were mechanically coupled to each other when they move from their fully cocked position to power a jump (Figs 3, 4).

First, when a depression force was applied to the femur of the left hind leg, the left trochanter suddenly depressed and was always accompanied by a synchronous depression of the right trochanter

and femur (Fig. 3A, Fig. 4B). Both coxae also moved posteriorly indicating that the initial passive levation of the hind legs to their cocked position had bent the pleural arches and moved the coxae anteriorly. The induced depression movements resulting from the force applied to one femur, thus induced both hind legs to move synchronously in the same way as in a natural jump.

Second, a comparable force to that used above was then applied to the femur of the right hind leg (Fig. 3B, Fig. 4C). The result was the same; a synchronous depression of both trochantera and an accompanying movement of both femora. The mechanical linkage between the two hind legs therefore works effectively in both directions and movement of the first leg leads to the depression of the other leg.

Third, the tendon of the large trochanteral depressor muscle was then pulled in the same direction as it would move were the muscle to contract (Fig. 3C, Fig. 4D). This simulation of the action of a single trochanteral muscle again resulted in a simultaneous depression of both trochantera although its direct action was only on the left trochanter. The effect was the same if the left or right tendon were pulled, as it was for the imposed depression movements of either femur.

The movements induced in dead *Issus* were thus similar in the following features to those observed in natural and restrained jumping movements. First, the coxae of both hind legs moved posteriorly as the jump was released. In restrained jumping, the coxae could move by as much as $600\,\mu m$ (Fig. 1, Fig. 4A), but in the induced movements in dead *Issus*, the coxae moved only 48–62% of this distance (Fig. 4B–D). Second, the trochantera of both hind legs moved synchronously in natural and restrained jumps, and in the movements induced in dead *Issus*. In natural jumping, the trochantera rotated at peak angular velocities $66,000\,deg.s^{-1}$ (Fig. 4A) (Burrows and Bräunig, 2010). In restrained jumps, that do not have to support the weight of the body, the peak rotational velocity was more than four times faster at $300,000\,deg.s^{-1}$ (Fig 1, Fig. 4A). In dead *Issus*, forced movements of one trochanter led to peak rotational velocities of $35,250\pm1030\,deg.s^{-1}$ (mean ± s.e.m.;
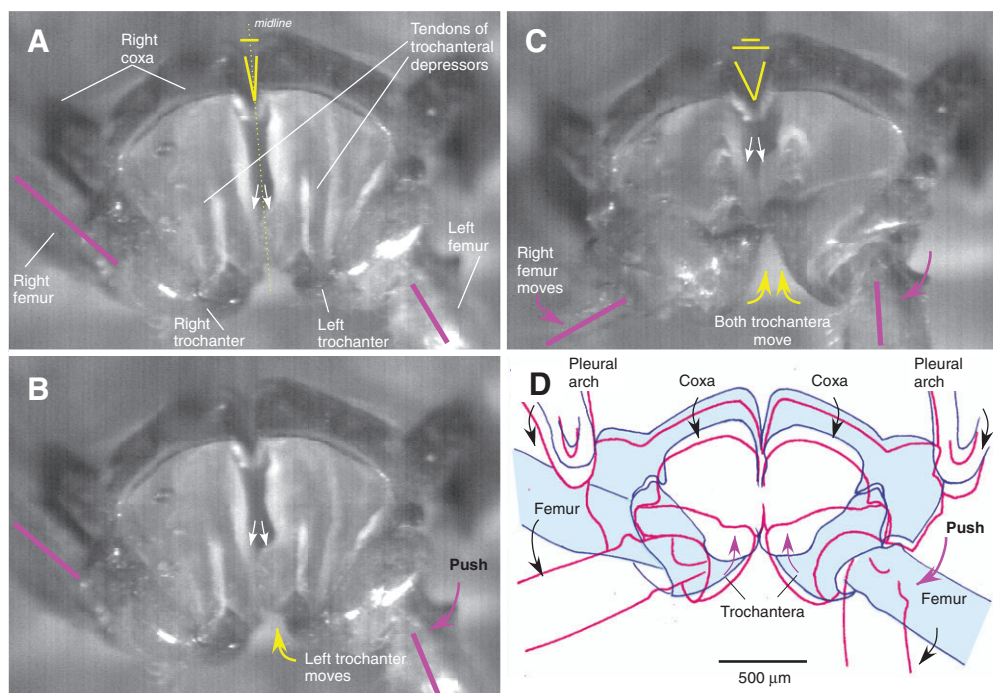

Fig. 2. Ventral view of both hind legs of a recently dead *Issus*, showing movements of the proximal joints to an applied movement of the left femur. Selected images are shown from a sequence captured at a rate of 2000 images s^{-1} (A) Both hind legs were moved into their fully cocked position where they remained until further experimental intervention. In the absence of any muscle activity, the left femur could be moved through a large angle away from its fully cocked position before it had any impact on the trochanter. (B) A force applied to the left femur (pink arrow) caused a small movement of the left trochanter (yellow arrow), visible as increased wrinkling of the flexible membrane of the left coxo-trochanteral joint. (C) Both left and right trochantera depressed suddenly (yellow arrows) together with the right femur (pink arrow). (D) Tracing of the position of the joints before (cyan tracing and tinting as in A) and after (magenta tracing as in C) the synchronous movements of both hind legs.
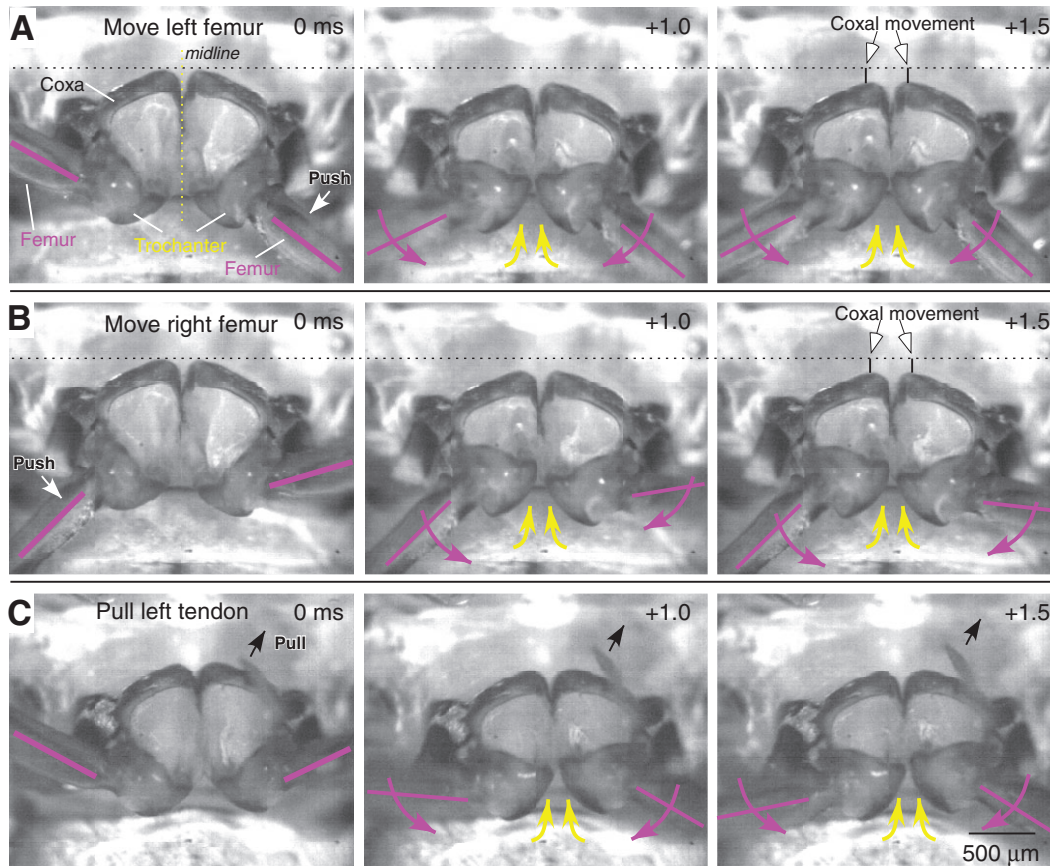
Fig. 3. Ventral view of the hind legs of a recently dead *Issus* showing movements of the hind legs in response to different applied forces. The three sequences of images (in rows) each begin with both hind trochantera in their fully levated positions and were captured at a rate of 2000 images s^{-1}. (A) A force applied to the left hind femur (white arrow) resulted in a synchronous depression of the trochantera of both hind legs and movement of the right hind femur. (B) A force applied to the right hind femur (white arrow) also resulted in a synchronous depression of both hind legs. The horizontal dotted line in A and B indicates the position of the anterior edges of the coxae in the cocked position before the application of any forces. (C) The tendon of the left trochanteral depressor was moved anteriorly (black arrow) to simulate the force that would be applied by this muscle to the left trochanter in jumping. The result was a simultaneous depression of both trochantera. The movements of the proximal joints of the hind legs are indicated in the following ways; coxae by black arrows with white-filled heads and by the horizontal dotted lines; trochantera by curved yellow arrows; femora by curved pink arrows and pink lines.

N=4 *Issus*, five movements in each) in that hind leg and rotational velocities of 26,250±10493 deg. s^{-1} in the other hind leg (Fig. 4B,C). Pulling on the tendon of a trochanteral depressor muscle led to rotational velocities of 23,250±1108 deg. s^{-1} for each hind leg (Fig. 4D).

The mechanical interactions resulted from contact between a medial, cuticular protrusion from each trochanter through the flexible arthrodial membranes of the two coxo-trochanteral joints (Fig. 5). The two protrusions could be seen to touch each other at the midline when both hind legs were viewed posteriorly in their fully levated position that they adopt before a jump (Fig. 5A). Removal of the flexible joint membrane and viewing the joints ventrally, showed that the protrusions arise from the inside surface of the dorsal wall of the each trochanter (Fig. 5B,C). The protrusions are thin and flat, D-shaped structures that each measure 300 μm longitudinally and 100 μm transversely at their widest point. They project ventrally from the inside, dorsal surface of a trochanter. The large tendon of a trochanteral depressor muscle lies ventral to a protrusion and moves relative to it. When viewed under bright-field illumination the protrusions appeared to be made of transparent cuticle (Fig. 5B), but when illuminated with UV light, they fluoresced bright blue (Fig. 5D). When viewed ventrally, the medial

rim of each protrusion showed brighter fluorescence because of the angle the protrusion subtended relative to camera. When viewed posteriorly, the fluorescence was more uniform because more of the flat surface of a protrusion was at right angles to the focal plane of the camera (Fig. 5E).

Contact could be seen between the protrusions of the left and right hind legs in individual images taken from high speed sequences of imposed movements of one hind leg in a dead *Issus* (Fig. 6 and supplementary material Movie 2). The imposed movement of a hind leg and the induced rotation of its trochanter caused its trochanteral protrusion to move against the protrusion of the other stationary leg and in so doing initiated a movement of that leg. The two hind legs then depressed synchronously from their levated positions. The protrusions provided the only observable point of contact between the moving parts of the two hind legs. Their interactions thus provide a mechanical means of transferring information when sensory and other neural mechanisms have been eliminated.

Energy storage

Do mechanical features of the proximal joints of the hind legs play a role in storing and delivering the energy necessary to power a jump? Movements of both coxae and pleural arches occurred during
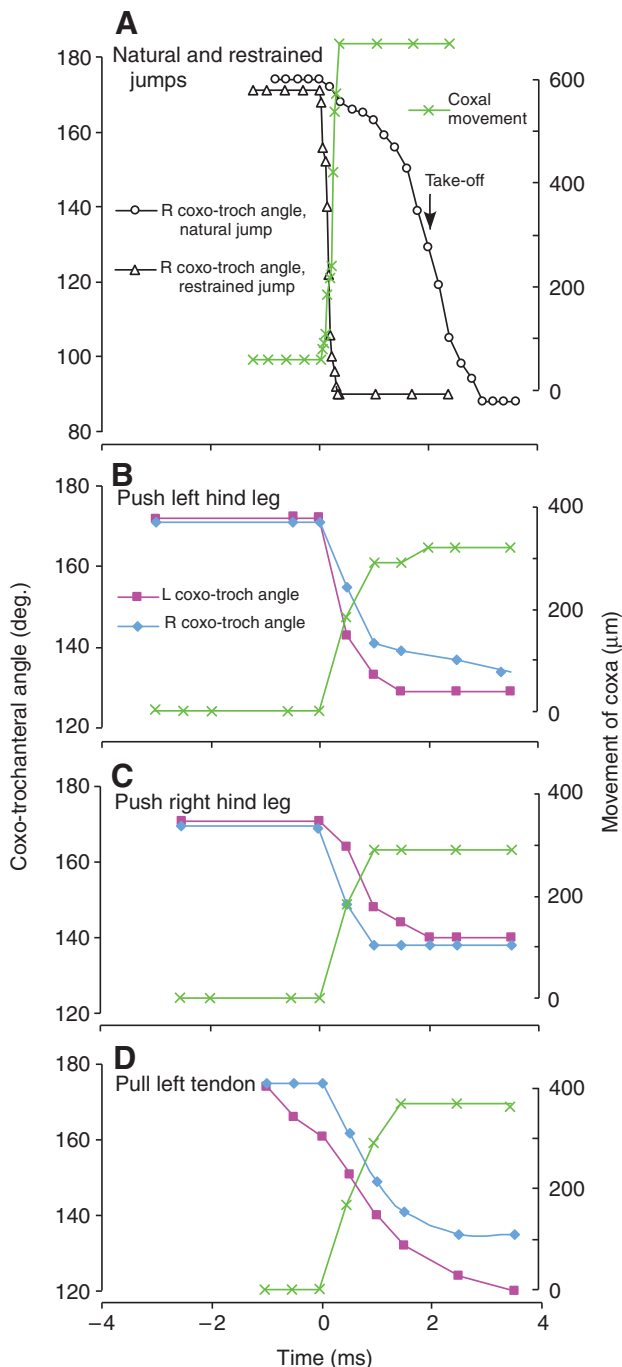
Fig. 4. Graphs of the angular rotations of the coxo-trochanteral joints and movements of the coxae during natural and induced movements of the hind legs. (A) Natural jumping (open circles) in which the body weight has to be supported, and restrained jumping (open triangles and crosses) in which the hind legs kick freely in the air. (B,C) Synchronous movements of the left and right hind legs induced by imposed movements of the left (B) or right (C) femur in dead *Issus*. (D) The tendon of the left trochanteral muscle in a dead *Issus* was moved to simulate a contraction, with the result that both hind legs moved.

both natural jumping (Fig. 1, Fig. 4A) and during imposed movements of the coxo-trochanteral joints (Figs 2, 3, Fig. 4B,C). The structure of the pleural arches, their association with the coxae, and the movements of the coxo-trochanteral joints were therefore investigated. The aim was to see if they could store and then release the energy generated by the prolonged contractions of the huge

trochanteral depressor muscles before a jump (Burrows and Bräunig, 2010).

In cleared specimens from which the thoracic muscles were removed, the lateral part of the hind coxae themselves could be seen to project anteriorly and dorsally to form an integral part of the internal skeletal structures that extended to the hinges of the hind wings (Fig. 7A). Drawings of these pleural arches from both ventral (Fig. 7B) and dorsal aspects (Fig. 7C) and scanning electron microscopy (Fig. 7D) show that they provide a mechanical link from each coxa to the hind wing on the same side of the body. They also emphasise that a pleural arch consists of several different cuticular parts, each with a complex shape. Before a jump was released, these pleural arches were bent so that the two hind coxae were moved forwards by the prolonged contractions of the large trochanteral depressor muscles (Fig. 1). When the pleural arches recoiled to their former shape the energy that they have stored would act in a line that caused the two coxae to move posteriorly as a single unit, and each trochanter to depress. The two hind coxae are normally tightly coupled to each other at the midline. In the cleared specimen in Fig. 7A, they have pulled apart to expose an ovoid area of close apposition between their medial surfaces (drawing in Fig. 7C). In the scanning electron micrograph the two coxae appear fused to each other at the midline (Fig. 7D). No asymmetries were observed between any structures of these proximal joints of the hind legs.

Illuminating the metathorax with UV light after removing the flexible membrane on its ventral surface, revealed bilaterally symmetrical regions of bright blue fluorescence in the pleural arches (Fig. 8A). The fluorescence extended anteriorly from the lateral edge of each coxa before disappearing dorsal to the large depressor muscles of the trochantera. Removing metathoracic muscles revealed that the fluorescence extended from the coxae almost to the hinges of the hind wings (Fig. 8B). Not all of a pleural arch showed this blue fluorescence; a medial region of a coxa and another region at the wing hinge did not fluoresce and appeared to be made from chitinous cuticle. Similarly if a pleural arch was viewed from a medial aspect, the blue fluorescence was seen to start close to a coxa, but a ventral part of this region and a dorsal part at the wing hinge showed no fluorescence (Fig. 8C). The fluorescent regions of a pleural arch were curved in both the anteroposterior and dorsoventral planes, and in the specimen shown in Fig. 8 had dimensions of 1100 μm anteroposteriorly, 250 μm dorsoventrally and 280 μm from left to right. Each pleural arch is thus a composite structure of the fluorescent and non-fluorescent cuticle.

DISCUSSION

The mechanics of the proximal joints of the hind legs determine two key features that enable powerful and rapid jumping movements of *Issus* to be generated. First, mechanical interactions between the hind legs when initially moving from their cocked positions provide a simple and reliable mechanism that ensures the power from both legs is delivered at the same time. Second, to meet the requirement of both power and rapidity of the jumping movement, which could not be met by direct contraction of the muscles, energy is stored in advance. Bow-shaped parts of the internal thoracic skeleton, that are composites of stiff chitinous cuticle and rubbery resilin-like protein, are bent by the slow contractions of large muscles and the energy they store is then delivered quickly to power jumping.

Sequence of events controlling jumping
The production of a jump involves a sequence of interactions between the mechanical arrangements of the hind legs and the motor commands sent to their muscles.
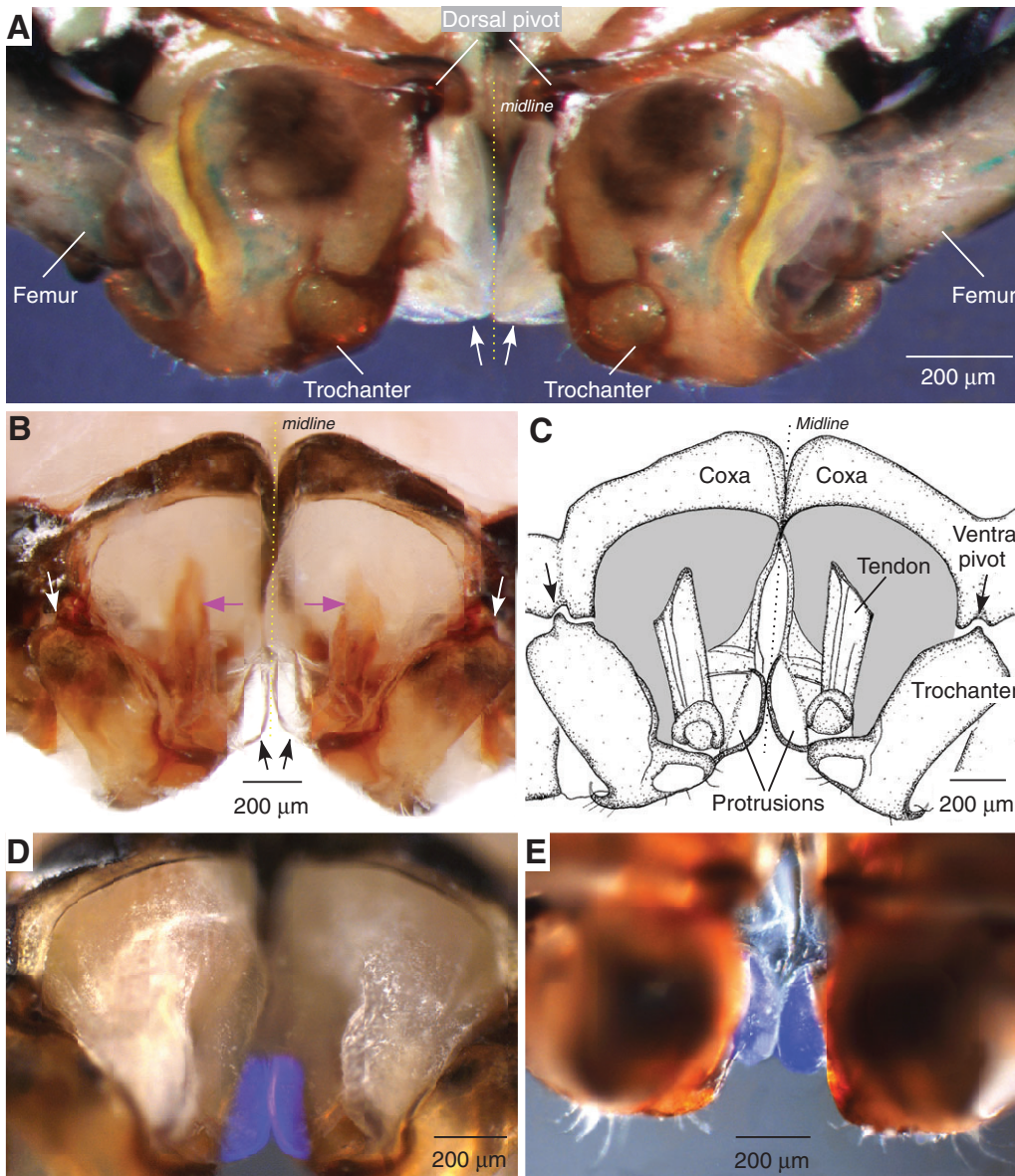
Fig. 5. Structure of medial protrusions from the hind trochantera. (A) Photograph of the proximal joints of the hind legs in their fully cocked position and viewed posteriorly. Medial protrusions (white arrows) from each trochanter touch each other at the midline. (B–D) The proximal joints viewed ventrally. (B) Photograph after removal of the flexible membrane of the coxo-trochanteral joints to show the transparent, medial protrusions of the trochantera (black arrows), the large tendons of the trochanteral depressor muscles (pink arrows), and the ventral pivots of the coxo-trochanteral joints (white arrows). (C) Drawing of a ventral view with the key elements labelled. (D) Ventral view of intact coxo-trochanteral joints illuminated with UV light and with bright-field and UV images superimposed. The protrusions fluoresce bright blue. (E) Posterior view of the same joints under UV and bright-field illumination with the protrusions again showing bright blue fluorescence.

(1) Cocking of the hind legs is brought about by a high frequency burst of spikes supplying trochanteral levator muscles (Burrows and Bräunig, 2010). This leads to the mechanical engagement of a protrusion on each coxa with a specialised patch of cuticle on a dorsal femur (Burrows, 2009).

(2) Prolonged contraction of the trochanteral depressor muscles then occurs in which the motor spikes to the large thoracic part of this muscle are synchronised on both sides (Burrows and Bräunig, 2010). In this state *Issus* is fully prepared for jumping which can then be triggered suddenly by a number of different sensory stimuli.

(3) The mechanical arrangements of the coxo-trochanteral joint ensures that the hind legs do not depress during this period. Instead the prolonged muscle contractions cause the composite pleural arches on both sides of the metathorax to bend and store energy. A further consequence is that both coxae are moved anteriorly because the tendons of the contracting muscles arise in the thorax and run through the coxae to insert on the trochantera.

(4) The jump movement begins with the synchronous depression of the trochantera propelled by the energy released by the unfurling of the pleural arches. The depression of these joints moves both hind legs downwards so that the tarsi are forced firmly against the ground. The continuing depression of both trochantera is accompanied by extension of the tibiae in movements that accelerate the body in less than 1 ms in the best jumps (Burrows, 2009).

(5) Mechanical contact between trochanteral protrusions of the two hind legs ensures that when one hind leg moves, the other hind leg will follow. The power of both hind legs is thus delivered synchronously.

(6) Once airborne, the hind legs are trailed behind and aid steering by acting as rudders. Upon landing the hind legs may be cocked again in preparedness for a further jump and thus may make no contact with the ground during walking.

Synchronisation of hind leg movements

The two hind legs of *Issus* begin their propulsive movements for power jumping within 30 μs of each other, and in most jumps no timing differences could be detected between the two hind legs. The measurement of timing differences was limited by the capture rate of 30,000 images s^{-1} used in this analysis, but the differences revealed are tiny and must be approaching the limits of biological
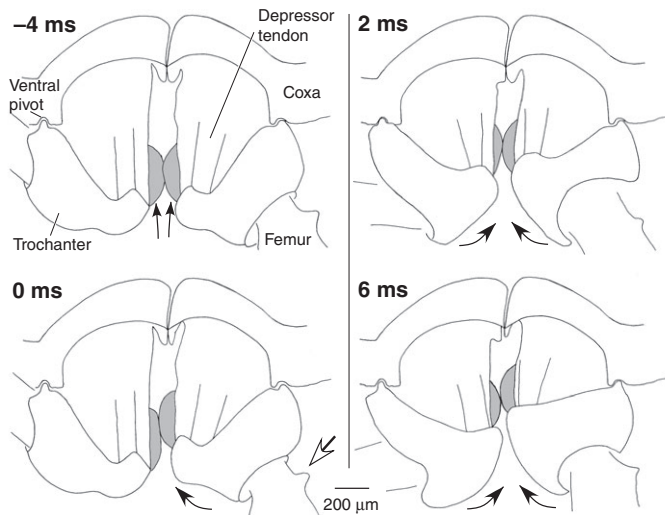
Fig. 6. Drawings of ventral views of the proximal joints of the hind legs (as in Figs 1–3 and Fig. 5B–D) during an imposed movement of the left hind leg captured at 2000 images s^{-1}. The medial protrusions of each hind leg touch each other. They are shaded and indicated by the straight black arrows in the first frame (left column, time=–4 ms), which shows the fully cocked position. The force applied to the left hind leg (open arrow) caused the left trochanter to move (second frame; time=0 ms). In subsequent frames (right column, 2 ms and 6 ms), the movement of the left protrusion caused the right hind leg to move (curved black arrows).
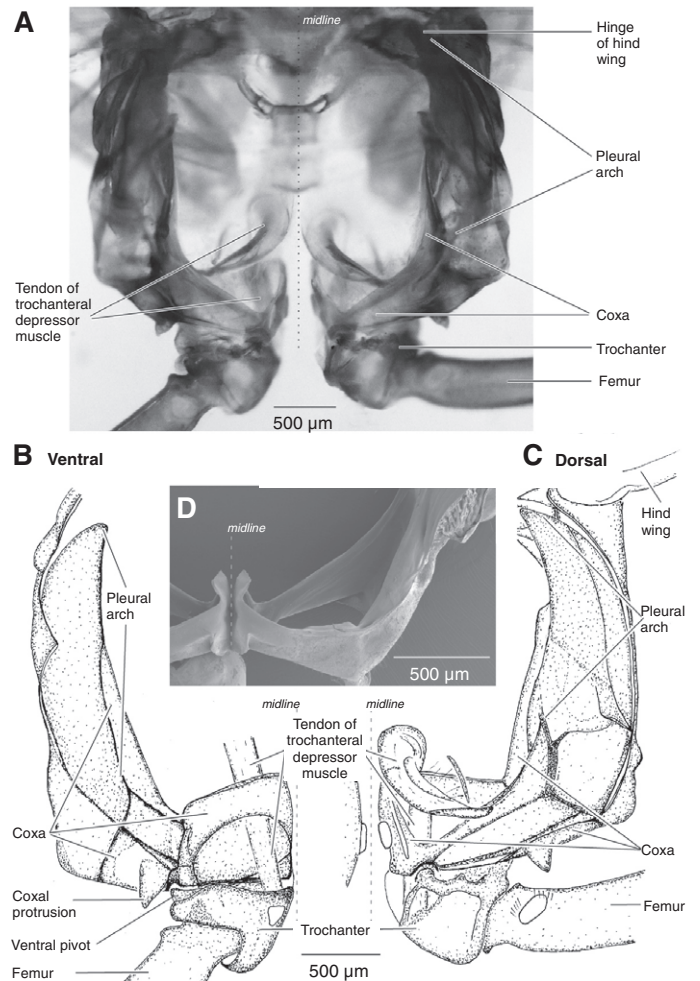

Fig. 7. Internal skeletal structures as energy stores for jumping. (A) Ventral view of a cleared thorax. The two coxae have separated at the midline. (B) Drawing of a ventral view of the right half of the metathorax to show the pleural arch running from the hinge of the hind wing dorsally to the coxa ventrally. (C) Drawing of a dorsal view of the right half of the metathorax. (D) Scanning electron microscope image of the left coxa, viewed ventrally.

relevance and beyond biological control mechanisms. To all intents and purposes, the movements of the hind legs are synchronous and the thrust for jumping is thus applied to the ground by both hind legs at the same time.

The mechanism of this precise synchronisation of natural jumping movements would appear to be mechanical. In dead *Issus*, a force applied to one hind leg, or to the tendon of one trochanteral depressor muscle, when both hind legs are in the cocked position that they would adopt before a natural jump, leads to a sudden depression of that hind leg. Remarkably, this movement of a hind leg is accompanied by a simultaneous depression of the other hind leg. These experiments imply that there must be a mechanical coupling between the two hind legs when they are cocked and dependent on the position, velocity or acceleration of the movement of the first leg. Analysis of the anatomy of the proximal joints revealed a medial protrusion from the trochanter of one leg that touches a similar protrusion of the other leg. These protrusions fluoresce under UV illumination indicating the presence of an elastic resilin-like protein (see Energy storage, below). The protrusions are therefore expected to be elastic, storing and releasing energy when deformed and returning to the their natural shape after any deformation. In experimentally induced movements of the hind legs, the first movement of either hind leg is therefore transferred mechanically to the other hind leg by these protrusions.

In natural jumping, rotation of the trochantera is the first observable movement of the hind legs. It would therefore seem probable that the same mechanical linkage between the two trochantera, as observed experimentally, would also be used in natural jumping. If one hind leg moves first, its trochanteral protrusion would interact mechanically with the protrusion of the other hind leg, thereby causing both to depress within 30 μs of each other. The nervous system does not therefore need to make complex computations to ensure the observed level of synchronisation between the two sides through the co-

ordinated contractions of two bilateral sets of muscles. Indeed, it is hard to envisage neural mechanisms that could achieve these levels of synchrony given that the duration of even a single motor action potential is at least 30 times longer. Moreover, even if a bilaterally symmetrical motor pattern could be generated with this degree of synchrony, it still has to be delivered to the muscles, generate the same forces on the two sides, and then act on the joints to deliver the observed, highly synchronised movements. Instead a mechanical solution means that all the nervous system needs to do is to send signals that bring the muscles of both legs to similar levels of force production to meet the energy requirements of a jump. The complex neural processing of some sensory signals that occurs in the brains of weakly electric fish, barn owls and echo locating bats does allow the computation of microsecond time differences in arrival of those signals (Carr, 1993). By contrast, in motor processing, the delivery of tightly coupled movements will always be at the end of long chains of neural processing and mechanical filters, each with possible timing errors.

Mechanical synchronisation of the two hind legs would be advantageous whatever the mechanism for triggering the release of
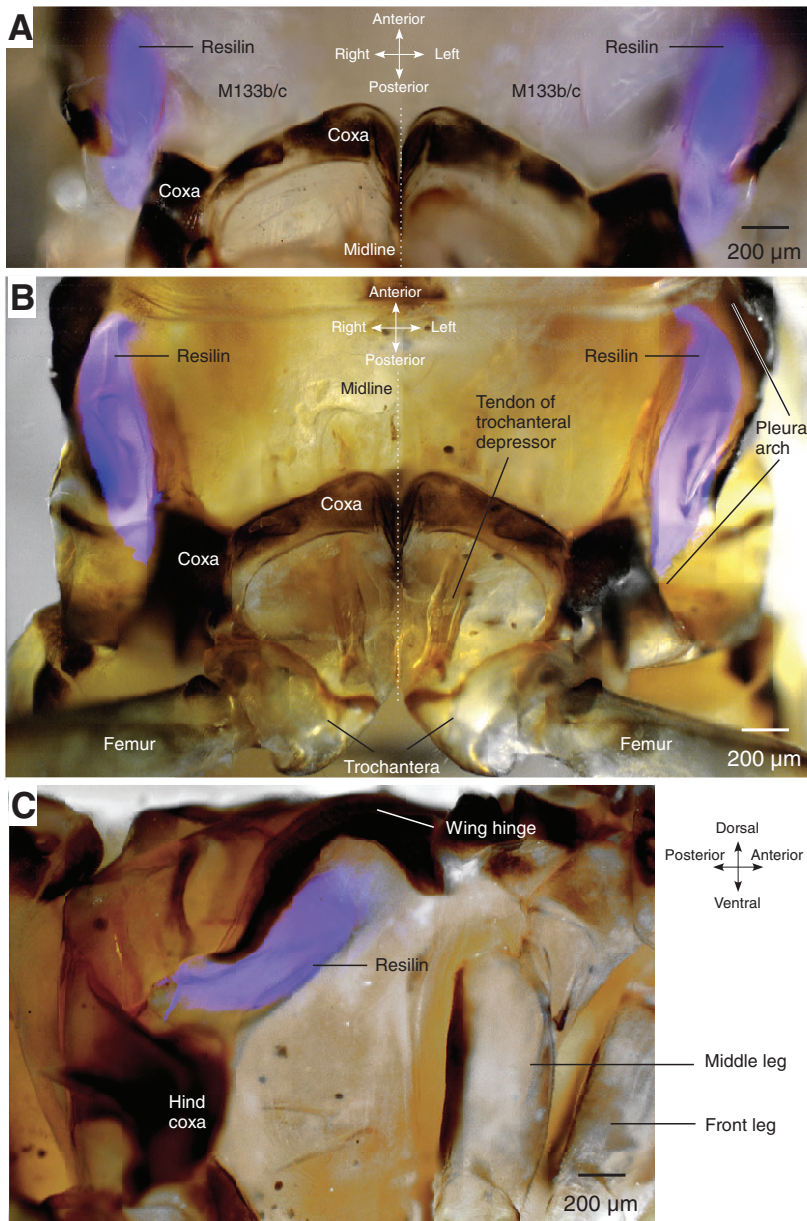
Fig. 8. Energy storage and resilin-like protein. UV and bright-field images are superimposed to show the blue fluorescent and non-fluorescent parts of each pleural arch. (A) Photographs of a ventral view of the posterior metathorax. Blue fluorescence extends anteriorly from the left and right coxae and disappears anteriorly beneath the large trochanteral depressor muscles (M133b/c). (B) Ventral view of the metathorax, after removal of the trochanteral muscles and some lateral, external cuticle. The fluorescence in the pleural arches extends almost to the boundary between the meso- and metathorax. (C) The inside surface of a pleural arch viewed from the midline. The fluorescence curves dorsally from the thoraco-coxal joint but stops short of the wing hinge.

a jump. If the release mechanism involves the force generated by the main trochanteral depressors overcoming mechanical locks, then the first leg to unlock will trigger the synchronous movements of the other hind leg. Other possibilities for a release mechanism include the actions of small parts of the trochanteral depressor muscles, or other muscles. Recordings from the coxal parts of trochanteral depressors in froghoppers have not revealed convincing activity that is correlated with release of a jump (Burrows, 2007b). In *Issus*, however, the small coxal part of the trochanteral depressor muscle is innervated by a neuron that has a giant, ovoid axon with axes of $50 \times 30\,\mu m$ and an unusual pattern of branching within the central nervous system (Burrows and Bräunig, 2010). Such an axon would have a conduction velocity of almost $5\,m\,s^{-1}$ and would reduce the journey time of spikes to its muscle by 0.13 to 0.28 ms compared with those of the other motor neurons to the metathoracic muscles. This implies a need for speedy signalling that is yet to be explained. Invoking such mechanisms for releasing a jump, still has to meet the requirement of synchrony of action on both sides and again raises the question of how neural control could achieve this without mechanical linkages playing a major role.

Why are synchronous movements of the hind legs needed?
Synchrony of leg movements is not a universal requirement of jumping animals, but is dependent on the orientation of the power-producing legs and their plane of movement. In locusts, for example, the hind legs can move at times a few milliseconds apart without detrimental effects on the direction or power of a jump. If the synchronisation is worse than this, then the leg to move first will have propelled the locust forward to such an extent that when the second leg extends it will not be able to contact the ground. The second leg will thus over-extend and damage may sometimes occur despite an in-built safety mechanism that allows further bending of the tibia and thus absorbs forces that would otherwise damage the joints. In planthoppers, the movement of the two hind legs in the same plane beneath the body means that any asynchrony in the thrust applied by the hind legs will result in the body spinning

(Burrows, 2009) with the consequent loss of energy and a less effective jump.

Energy storage

The trochanteral depressor muscles of *Issus* that power a jump, like those of froghoppers, contract for seconds before a jump is released (Burrows, 2007b; Burrows and Bräunig, 2010). High-speed images show their contractions bend the pleural arches [Ursprungsplatte (Heilig and Sander, 1986; Sander, 1957)] and move the coxae anteriorly. As the trochantera begin to depress in a jump the pleural arches unfurl and resume the shape and position they normally adopt before jumping.

The fluorescence of both the pleural arches (Fig. 7) and the medial protrusions of the trochantera (Fig. 4D,E) had the same properties as the two key signatures of the elastic protein resilin (Burrows et al., 2008; Neff et al., 2001; Weis-Fogh, 1960). First, it was excited by only a narrow wavelength of UV light. The fluorescence was only captured if an emission filter transmitting wavelengths from 413 nm to 483 nm was used. With other emission filters (e.g. CY3 and GFP) no fluorescence was detected. Second, the fluorescence was reversibly pH dependent, declining in acidic and increasing in alkaline pH. The remaining part of the pleural arch is made from stiff chitinous cuticle. The pleural arches are large structures relative to body size. The whole of the pleural arch is some 1400 μm long anteroposteriorly, or 21% of body length. The region containing resilin-like protein represents 16% of body length and along with that in froghoppers is the largest found in insects.

As in froghoppers, a pleural arch appears to act as a composite energy storage device, with the resilin-like protein acting to ensure that the stiff cuticle does not fracture and through its almost perfect elastic properties, to return the body to its original shape after a jump. The mechanics thus provide a mechanism that overcomes the limitation of muscle that is unable to generate the necessary power and speed for jumping by direct contractions.

ACKNOWLEDGEMENTS

REFERENCES

Alexander, R. M. (1995). Leg design and jumping technique for humans, other vertebrates and insects. *Philos. Trans. Roy. Soc. Lond. B* **347**, 235-248.

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.

Brown, R. H. J. (1967). The mechanism of locust jumping. *Nature* **214**, 939.

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

Burrows, M. (2003). 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. (2007a). Anatomy of hind legs and actions of their muscles during jumping in leafhopper insects. *J. Exp. Biol.* **210**, 3590-3600.

Burrows, M. (2007b). Neural control and co-ordination of jumping in froghopper insects. *J. Neurophysiol.* **97**, 320-330.

Burrows, M. (2009). Jumping performance of planthoppers (Hemiptera, Issidae). *J. Exp. Biol.* **212**, 2844-2855.

Burrows, M. and Bräunig, P. (2010). Actions of motor neurons and leg muscles in jumping by planthoppers (Hemiptera, Issidae). *J. Comp. Neurol.* (in press).

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

Carr, C. E. (1993). Processing of temporal information in the brain. *Annu. Rev. Neurosci.* **16**, 223-243.

Heilig, S. and Sander, K. (1986). Zahnradsektoren zur Koordination der Sprungbeine-eine lavale Synapomorphie der fulgoromorphen Zikaden (Homoptera, Cicadina, Fulgoroidea). *Zool. Jb. Syst.* **113**, 307-317.

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

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.

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

Rothschild, M., Schlein, Y., Parker, K. and Sternberg, S. (1972). Jump of the oriental rat flea *Xenopsylla cheopis* (Roths.). *Nature* **239**, 45-47.

Rothschild, M., Schlein, J., Parker, K., Neville, C. and Sternberg, S. (1975). The jumping mechanism of *Xenopsylla cheopis*. III. Execution of the jump and activity. *Philos. Trans. Roy. Soc. Lond. B* **271**, 499-515.

Sander, K. (1957). Bau und Funktion des Sprungapparates von *Pyrilla perpusilla* WALKER (Homoptera-Fulgoridae*). Zool. Jb. Jena (Anat.)* **75**, 383-388.

Sutton, G. P. and Burrows, M. (2008). The mechanics of elevation control in locust jumping. *J. Comp. Physiol. A* **194**, 557-563.

Sutton, G. P. and Burrows, M. (2010). The mechanics of azimuth control in jumping by froghopper insects. *J. Exp. Biol.* (in press).

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