A saltatorial study: resilin and power amplification

Sources:

Exoskeleton background: composite material, sclerites, apodemes, tagmata, condyles, levers

The exoskeletal cuticle of a locust is a composite material of chitin (polysaccharide) nanofibres within a protein matrix. (Glass fibres in a resin matrix, as in the hulls of a boat or canoe, is an example of a man-made composite material.) The physical properties of composites differ from the properties of their constituents, and can be a more adaptive compromise of strengths for dealing with forces. For example changing the orientation (‘grain’) of chitin nanofibres layered within their protein matrix is a way to improve how the overall material opposes stresses coming from certain directions.

The most remarkable protein involved in insect cuticle is resilin, sometimes called ‘insect rubber’. It was discovered by Weis-Fogh (1960) in body parts associated with insect flight. Patches of resilin (e.g., prealar arm, see below) within the exoskeleton serve as springs (elastics or shock absorbers); resilin pads near the wingbase distort and store elastic energy, then recoil to direct this energy back into wing oscillation with each change in wingstroke direction. Resilin is involved in many insect skeletal situations, including the jumping and kicking behaviour of the locust of today’s lab.

Arthropod exoskeleton comprises a continuous integument, a kind of flexible shell enclosing the body, but with great variation in thickness and thinness, stiffness and elasticity. Thicker regions make up variously shaped and sized elements called sclerites. Typically these sclerites are separated by external grooves or sutures, and these grooves usually indicate an internal strengthening ridge. The sclerites have obscure names (epimeron, episternum, tergum, sternum, notum etc.). There are sometimes deep pits on the integument surface which indicate an internal cuticular projection for muscle attachment. There are often pits associated with the tentorial arms. Sometimes the sclerites are small distinct and queerly shaped, functioning importantly in articulation to transfer forces, e.g., axillaries at the base of the wing; sometimes they are large convex plates, e.g., covering the dorsal aspect of the abdomen or thorax (notum = tergum). (Sclerite shapes can determine how they direct forces.) Or one sclerite may adjoin another and so articulate and pivot two leg segments, e.g., a cylindrical ‘sclerite’ comprising a segment of a limb: femur, tibia etc. The locust mandible, the locust femur, are also in this sense ‘sclerites’ though not tubular. But no matter sclerite diversity of shape and size, between all sclerites the cuticle continues unbroken, just very thin and ‘membranous’ and so more or less flexible. Sclerites are elements or ‘pieces’ of the exoskeleton. creating an overall result: an animal walking, jumping and flying about in a jointed ‘suit of armor’.

Besides the sclerites of the exoskeleton there are also apodemes. These inflections of cuticle can be the basis of an internal truss as in the case of the tentorium. But more often they are blade shaped offering a surface for muscle insertion and then directing that pull to a very narrow and specific point (e.g., mandibular adductor apodeme). There are also apodemes, one in the locust leg, that are rope-like,
cuticle inflection that has become in effect a piece of string that reaches through the body a very long
distance from the site of its attached muscles. Blade-like apodemes are typically invested with pinnately
arranged muscle fibres (pinnate just means feather-like in their arrangement, angled up from a central
axis). The alternative to a pinnate muscle fibre arrangement is parallel-fibred. Pinnate muscles are
invariably shorter and cannot shorten as far but there can be more muscle packed into a given volume
than for parallel fibres, and so they can be more forceful.

Insects arose from metameric segmented ancestors. There are three tagmata: head, thorax and
abdomen. In the course of evolution segments became grouped to serve different functions. The
segments making up the head tagma house sensory structures and their appendages act to manipulate
food. The thorax arose from three segments as a tagma specialized for locomotion: walk, jump, fly. It is
a relatively rigid box whose rigidity promotes the development of strong forces in associated appendages.
The third tagma is the abdomen, the least departed from the ancestral segment and the simplest in
structure.

Each segment in the abdomen of a locust consists of four ‘megasclerites’: dorsally a **tergite**, below a **sternite** and on each side a **pleuron**: in effect top, sides and bottom. Tergites, sternites and
pleura are subdivided into the smaller sclerites mentioned above. But even the abdomen tagma may be
interpreted as having adapted for certain functions. Each abdominal segment is connected to the next by
a very thin, back-folded membranous cuticle; each is in fact telescoped into the segment anterior to it.
Thus the insect abdomen can greatly change in volume which allows it to act like a bellows, out and in to
serve in breathing (ventilation involving abdominal air sacs) or it can expand greatly to store food
(ingested blood by a mosquito) or eggs.

The legs of an arthropod are a series of chitinous tubes usually linked by dicondylic joints. The
weight of an insect acting down its leg segments is supported upon these projecting condyles, but still,
between each limb segment, is a relatively thin intersegmental membrane. Not all joints involve two
condyles: the coxa, the most proximal of the leg segments attaches to the thorax by a monocondylic joint.
One large knob, a single pivot point on the thorax wall fits into a depression (socket) in the wall of the
coxa. The intersegmental membrane holds this knob in its socket and the coxa can rotate about a very
wide range of axes relative to the thorax: This is a kind of ‘universal’ joint (like a trailer hitch) and it is no
accident that it is immediately adjacent to the body.

Two condyles fix the plane in which a leg segment can move: i.e., it can only pivot about a single
axis. The dicondylic joints of an arthropod leg each establish a single axis, about which each joint can
pivot. A line between the two condyles reveals this axis (recall pins and crayfish limb). The orientation of
the axes of successive joints changes as one progresses along the limb. They shift each time by about
45º, and this, together with the extensive mobility of the monocondylic basal joint, gives the limb a great
range of capability for movement. There are two types of dicondylic joint. In one type the knobs lie in the
plane of the long dimension of the leg, i.e. the knobs point along the limb. In the other type (the locust
femorotibial joint is an example) the knobs are angled inward. This second type of joint is much stronger.

A muscle which bends a jointed limb is a flexor, moving one part on another and reducing the
reach of the limb; an extensor muscle straightens out the limb and makes it reach farther away from the
body. If the limb movement achieved by a muscle is described in relation to the ground, the muscle may
be called an elevator or a depressor; a muscle is an elevator if it is used to raise a moveable part and a
depressor if it lowers such a part.

**The Locust Jump** *(Heitler 1974)*

The 1974 paper by Heitler was discussed in lecture and presents a paradox: “The extensor
muscle is massive and occupies the greater part of the femoral volume. Its fibres are short, and occur in
chevron blocks; an arrangement which enables the muscle to develop a very large force at its tendon
[apodeme]. The flexor muscle, by contrast, is composed of long, thin parallel fibres, and is of
comparatively small cross-sectional area. This weak muscle must hold the tibia flexed against the full
force of the powerful extensor muscle. The structural specialisations of the femoraltibial joint which
enable this apparent paradox to be resolved are described in this paper. (Heitler 1974).

The resolution of the paradox is leverage. When skeletons translate forces generated by muscles they act as levers, changing the applied force of the muscle in both magnitude and direction. The line of action of the forces developed in a skeleton is typically at some distance from a joint or axis of rotation. The muscles produce rotation of the skeletal parts, one on another. At greater distances (e.g., the shortest distance between the axis of rotation and the line of action of the muscle) the force applied becomes greater. As you increase the effort arm (= lever arm or force arm) the force applied increases. Conversely, the shorter the effort arm, the more muscle force you will need to achieve the same movement. Effort multiplied by effort-arm length = moment of force.

An example of a simple first class lever, one in which the sequence is effort-fulcrum-load, is the prying of a rock out of the ground with a pole. Supposing a pole to be 11 feet long and we wedge one end of it under a 100-lb rock using a smaller rock as a fulcrum. Say the distance along the pole between where we apply effort and the fulcrum is exactly 9 feet. If we push down on the remote end of the pole with 10 lb of force, then because of the leverage of this machine we will exert 90 lb of upward force at the base of the rock. A moment of force is developed which is the product of the effort (10) and the shortest distance between its line of action and the axis of rotation, i.e., the 9-foot effort arm. Force advantage (mechanical advantage) is the ratio of the load to the effort. In the example above, the force advantage of this first class lever is 9: for a force of 1 lb applied we lifted 9 lb of rock.

Another aspect of levers is distance advantage. (This is the reciprocal of the force advantage and the same thing as 'speed advantage'.) Distance advantage is the ratio of the distance moved by the load to that moved by the effort. In the pole and rock example the load might move a half foot up at best, while perhaps the point of application of the effort moved 3 feet down. (We should be using metres of course; to be more scientific.) So the distance advantage would be 0.5/3 = 0.17.

Lever systems of animals commonly involve (if anyone can think of an exception to this I'd like to know) very short effort arms and very long load arms. Animal muscles seem always to work with a bad force advantage but a good distance (or speed) advantage. Muscles shorten over small distances to produce a very large movement of the load. Commonly antagonistic animal muscles combine a 3rd class lever with a first. This is the case with the extensor and flexor muscles of the metathoracic tibia of the locust. The extensor is a first class lever. The flexor is a third class lever.

One muscle is much much larger than the other and they pull at different angles depending on the degree of flexion. The force advantage of the flexor is much greater than that of the extensor when the tibia is maximally flexed (femorotibial angle ~5º is total flexion): note that at this angle the effort arm is greater for the flexor. This is partly the result of the pulley action of the lump. Because the flexor apodeme must run up and over the lump, like a rope over a pulley, the angle of action of the muscle force is changed in a way which increases the length of the effort arm: the shortest distance from the line of action of the force to the axis of rotation. But as the extension of the tibia is carried out and the angle changes from 5 to 150º, the arms are changing for both muscles. And they shift more in favour of the extensor, especially when the flexor moves clear of the lump entirely.

The locust contracts both muscles isometrically and much of the distortion energy is stored in the semilunar processes (see below).

**Today's lab**

We begin with the locomotory tagma of the locust, the thorax, and how its anatomy relates to the coxa and metathoracic legs. (The next lab will address the wings and for that lab we will need to carry over some of the thoracic anatomy studied today-- pleural ridge, pleural and sternal apophyses.) But today we focus on the anatomy of the locust metathoracic leg, especially the femorotibial joint with its important role in power-amplified jumping. A look at KOH cleared material is followed by the same uncleared material with the muscles present.
**Cleared locust thorax**

Some locust thoraxes have been cleared with KOH so that only the exoskeleton remains. Cut one sagittally with microscissors close to the midline and observe its internal surface submerged in 70% alcohol. Staining may be helpful and the demonstrator will explain this procedure. Refer to the diagrams provided and learn to recognize the following: pleural suture (look on the outer surface first, then determine the corresponding internal ridge; the pleural suture is quite twisted in its course and this is confusing but its top end is the fulcrum of the wings, the wing process), pleural apophysis (a projection internally from the pleural ridge, sternum, tergum, monocondylic joint of the coxa, condyle of the metathoracic coxa, sternal apophysis (a projection serving for muscle attachment arising from the floor of the thorax), phragma (pl. phragmata). The phragmata are broad apodemes fore and aft upon which insert the longitudinal wing muscles; associated with the phragma between the pro and mesothorax see if you can find the prealar arm. Make a drawing showing the apophyses viewed from a posterior aspect.

**Preserved locust thorax**

Use a single-edged razor blade (or microscissors) to decapitate a preserved grasshopper. Remove the legs, severing them just distal to the coxae. Then remove the abdomen by making a transverse cut at the first abdominal segment. Bisect the thorax by making a single sagittal cut along the midline. (Put one of the halves aside to use in case you need a second try.) Immerse the half thorax in 70% alcohol, internal surface up, pinning it in a dissecting tray. Use two fine 00 insect pins or minuten pins. With forceps remove obscuring remnants of gut, fat body, Malpighian tubules etc. until you can see the muscles ranged along the inner face of the body wall. Observe all of the structures (if visible) that you identified in the cleared specimen, plus the following features: dorsal longitudinal wing muscles, tergo-sternal (dorso-ventral) flight muscles, pleurosternal muscles, promotors of the coxa, remoters of the coxa. What are the origins and insertions of these various muscles? (The insertion is always taken as the end of the muscle which is displaced significantly.)

The longitudinal wing muscles of this insect, running between the phragma, are termed indirect flight muscles; they run from one phragma to the next. How can they move the wings if they don't insert on them? [To understand this is to understand why they are called indirect.]

**Cleared locust jumping leg**

Obtain a cleared jumping leg and use communal microscissors to remove most of the lateral face of the femur. Then stain this specimen (e.g., chlorazol black) and view the leg under alcohol. Observe: levator apodeme of tibia (also called the extensor apodeme) and the depressor apodeme of tibia (also called the flexor). Note the difference in size between the two apodemes. Observe: dicondylic femorotibial joint, other leg joints including that with the pretarsus. By reference to Fig. 1 of Burrows & Sutton 2012 [obtained on computer or lab screen] determine where the semilunar processes are located in the region of the distal femur; are these semi-lunar regions detectable in the absence of fluorescence, i.e., what can one see of the limits of the paired semilunar processes under white light? In the region of the femorotibial joint locate the structure that Heitler terms a 'lump' and the bifurcate pocket of the flexor apodeme that pulls over this lump. Make a drawing of the leg showing the apodemes that move the tibia.

The paired semilunar processes are a composite of hard cuticle and resilin. The inside surface of a semilunar process consists of a layer of resilin... tightly bonded to the external black cuticule. “The resilin in the semilunar process may be there as a protection against fracture when the semilunar process is excessively loaded. Analogies here would be with plywood in which the laminations prevent fracture and give added strength and with bricks linked by mortar, which prevents cracks from spreading. It may also be there to restore a recoiling semilunar process rapidly to its original shape and thus return all the stored energy and allow a jump or kick to be repeated” (Burrows & Sutton 2012).

Though it has nothing to do with jumping, find the rope apodeme that connects to the pretarsus, the segment bearing the claws. This will illustrate the range of apodeme modifications. From the truss of
a tentorium all the way into a ‘string’, one that acts at an extreme distance from its muscles. In the supplied drawings the tiny rope apodeme muscles are shown as they occur in the tibia (X); there is yet another small muscle in the femur that pulls on this apodeme. When these three tiny muscles contract they pull the rope apodeme within the leg toward the body depressing the pretarsus. So these three tiny muscles are all depressors of the pretarsus. The rope apodeme inserts into the pretarsus above the joint axis and when it is pulled the pretarsus (and the claws) dig into the ground. This movement squeezes an elastic region near the base of the pretarsus; this elastic energy is what returns the pretarsus to the levated state (the claws lifted) when the rope apodeme depressors relax.

**Preserved locust jumping leg**

Obtain a preserved jumping leg, or use one of the legs from the specimen whose thorax you studied above. Use microscissors to expose and observe the depressor and levator muscles of the tibia. Note that the fibres of these muscles have a pinnate arrangement. Locate and observe the origins and insertions of the two antagonistic muscles that move the tibia. Observe the depressor muscle of the tarsus and the levator muscle of the tarsus. In the region of the femorotibial joint locate: condyles of the dicondylic joint, the pocket, the lump, the accessory flexor muscle, accessory extensor muscle, the extensor and flexor ‘tendons’ (= apodemes). Try to find the semilunar processes. There are no muscles in the tarsus, so these segments are not independently moveable on each other.

Make a drawing of the femorotibial joint showing as many of the features above as possible.

**Shock absorbers in the locust metathoracic leg**

Just because a leg is adapted to provide a powerful jump doesn’t mean a locust always makes a powerful jump. Sometimes locusts make mistakes. Their foot slips and instead of the developed force going into the substrate, this energy is let loose within the appendage. In the same way that a bow can be fractured by shooting it without an arrow (Gordon 1978, p. 92) [strain energy in the bent bow cannot escape as kinetic energy via the shot arrow] the metathoracic leg needs protection against damaging itself. There is a special region of rubbery weakness in the cuticle, created by seams of resilin. Manipulate the metathoracic leg: hold the femur and use the index finger of your other hand to bend the the tibia upward beyond maximum normal extension (see Fig. 1B of Bayley et al. 2012). Can you see any external manifestation of the lines of buckling when you examine this area under the microscope? (Demonstration of resilin below).

**Demonstrations**

1. Observe skeleton of a rabbit and a frog to assess the relative development of the hind limb bones.
2. Observe whole-mount of a flea to see disproportionate size of the segments of the hind leg.
3. Resilin as a shock absorber in the proximal tibia of the locust.

From Burrows, Shaw & Sutton 2008: “Resilin is colourless but can be revealed by exploiting ... fluorescent properties accorded to it by its two tyrosine [amino acid] components, of which dityrosine appears to be more important... Dityrosine, trityrosine and resilin ...emit a uniform blue fluorescence when excited by ultraviolet (UV) light. [Body structures besides those containing resilin fluoresce.] To determine if the blue fluorescence is from resilin, it is necessary to use a defining characteristic, which is that the excitation spectra are reversibly pH dependent; the emission spectra are nearly identical and remain pH-invariant, with maxima near 420 nm. With appropriate filters... it is possible to isolate the fluorescence of the high-H form of resilin from its low-pH alternative, so providing an identification signature for the protein.”

From Neff et al. 2000: “To detect autofluorescence, specimens were viewed with a Nikon Microphot microscope... (with a) source of UV illumination. To provide specific filtering of excitation and emission wavelengths we used a Nikon UV-2A filter cube. This cube provides UV excitation at wavelengths of 330-380 nm and allows emitted fluorescence greater than or equal to 420 nm to be detected. The fluorescent intensity of resilin containing structures was high through this filter and its blue
colour was quite distinct from the emissions of other structures, such as the joint membranes or the rigid cuticular segments. This result is in agreement with Weis-Fogh’s finding that resilin emits strongly at 420 nm."

Using a fluorescence microscope the demonstrator will try to show the buckling region of the tibia (compare with Fig. 6 Bayley et al. 2012). Buckling occurs in the cylindrical tibia dorsally and laterally, but not ventrally. Dorsal surface of the leg is that which is directed to the sky when the insect stands on a substrate. The resilin is seen dorsally and laterally along the lines of buckling.