

## SELF-HEALING PROPERTIES OF INSECT CUTICLE

### Authors

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### Summary:

Insects are all around us. They live in almost every environment on earth. Every part of every insect and arthropod is made from a material called cuticle [1]. This versatile material makes up the insect's exoskeleton, its tendons, wings and even its eyes, performing a wide variety of functions and exhibiting a very wide range of material properties [2]. Despite being the second-most abundant natural material on this planet [3], very little research has been done into its mechanical properties, and less still on its long term mechanical properties such as recovery and repair.

This paper investigates the recovery properties of the living cuticle tissue of the adult desert locust, *Schistocerca gregaria* (Forsskål 1775). To replicate forces experienced by the locust when jumping (30-60MPa [4]) a cyclic load was applied to a living locust hind leg (tibia) until softening or deformation was seen to occur. The locust was allowed a period of time to recover, and the experiment was repeated in order to gauge whether or not the material had been permanently weakened. The entire procedure was then repeated on tibiae that were first *removed* from the locust. Our experiments show that the living cuticle recovers its original strength and stiffness after such cyclic tests. Also shown is the absence of this recovery in the "dead" cuticle, suggesting that this recovery is not entirely a passive material phenomenon. The insect may play an active part in regulating its material properties.

### Introduction:

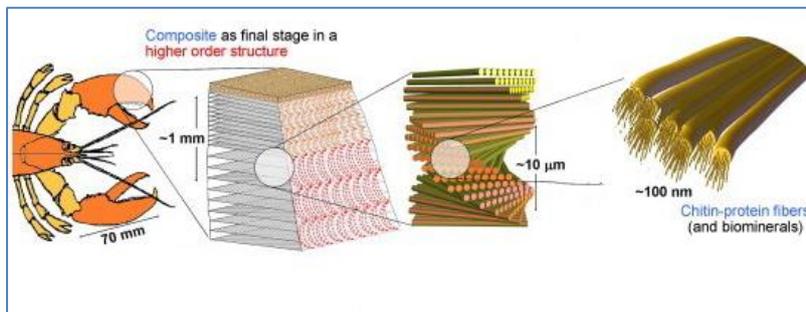


Figure 1 [5] showing the arrangement of chitin fibres in arthropod cuticle

Insect cuticle has a unique structure: fibres of a material called chitin embedded in a matrix of proteins are arranged in layers, oriented in a helical fashion from one layer to the next (Figure 1 [5]). Research has been done into various material properties of insect cuticle, such as stiffness and strength [2], fracture toughness [6], and fatigue [3], all of which used the tibia from the hind leg of the locust, which had been removed from the insect before testing. Bayley [7] conducted tests on live insects into the properties of the buckling region found in the proximal hind-tibia (Figure 2(B)) where the highest forces are encountered when jumping / kicking. This is a region which allows the leg to buckle without breaking (during jumps when the hind leg slips). The area acts like a shock absorber by deforming in such a way that the energy is dissipated back to the tibia upon release. The aim of this paper is to

analyse the behaviour of the living material under cyclic loading, excluding this buckling zone.

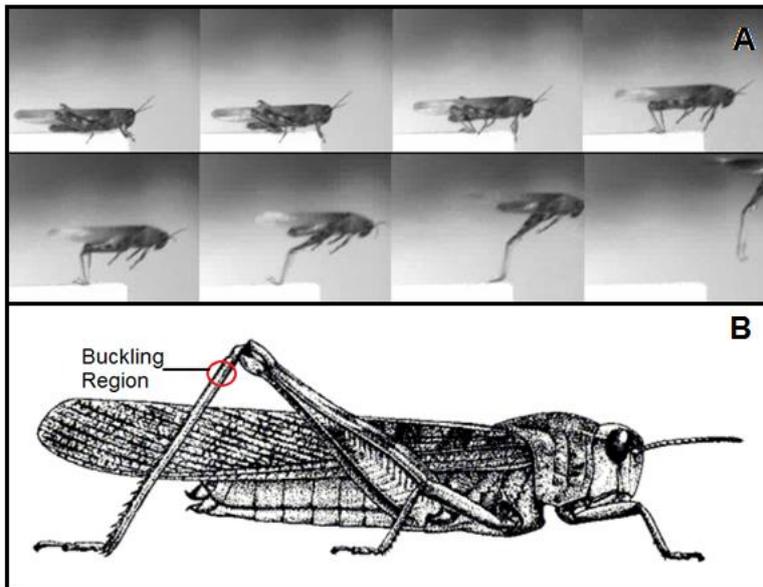


Figure 2: (A) Video micrograph slides animating the insect jump – (Heitler, 2007) [8], and (B) Illustrating the buckling region on the locust hind tibia [9]

External stimuli coupled with evolution can often determine material properties in the natural world (e.g. mammalian bone structures, properties and geometries). While jumping (Figure 2(A)), the locust tibia experiences bending forces greater than twenty times the locust's body mass [4] over a period of 20-30ms [7]. While kicking, the full extension of the leg can be reached in 5ms [7]. The hind tibia of the locust must undoubtedly undergo several thousand such cycles over the course of its life. Dirks [3] showed that the tibia only suffers from fatigue failure after some 100,000 cycles when cycled at 76% of the material's ultimate strength. Bennet-Clarke [4] illustrated that a locust could only jump 10-20 times consecutively before muscle fatigue (tiredness) sets in – i.e. a rest period is required between every few jumps. We hypothesised that the mechanical properties of cuticle might be different when the material was still part of the living insect. In particular, we considered whether the insect could control the responses of its leg material to cyclic loading, such as that which occurs during walking and jumping.

### **Materials and Methods:**

#### Insects:

Adult desert locusts, obtained locally from Reptile Haven pet store (27 Fishamble Street, Temple Bar West, Dublin 8), were kept in a controlled 12h (35°C) / 12h (20°C) day / night cycle. Insects were fed with fresh plants and dried cereals *ad libitum*.

#### Experimental Setup:

The experimental rig was designed to keep the locust as stationary and comfortable as possible, and to immobilise and secure the tibia for testing in order for consecutive tests on the same sample to be comparable.

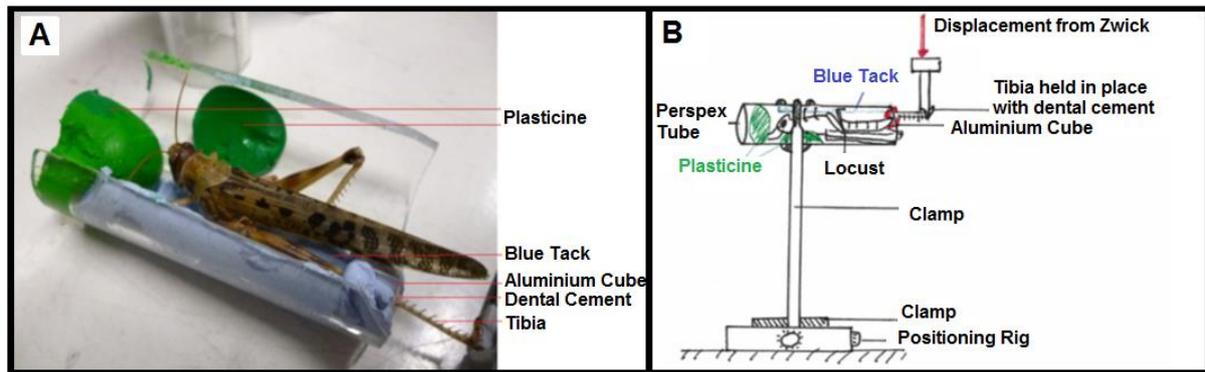


Figure 3: Experimental Rigs – (A) showing rig for housing the locust for duration of the experiment, (B) showing how cyclic load was applied

The locusts were housed in some Perspex tubing for the duration of the experiment as shown in Figure 3(A). During handling, locusts tend to remain stationary when handled by a pincer grip to the pronotum (dark area of hardened cuticle on their back/neck). Plasticine was used to simulate this grip, while the bottom of the tube was lined with blue tack to keep the locust stationary. The tibia was inserted through a hole in an aluminium cube, and the region of the tibia below the femoral-tibia joint was secured in place using fast hardening cold-cure acrylic dental cement (Simplex ACR308, Kemdent, Swindon, UK). This effectively eliminated the “buckling region” analysed by Bayley [7], making the sample being tested more uniform. Precise positioning of this entire setup was possible using the positioning rig shown in Figure 3(B).

#### Loading:

Figure 2(A) shows how the hind leg is loaded during jumping, which is almost exclusively in bending [10]. Our tests – designed to model this – involved fixing the tibia at one end and applying a load to the other (Figure 3(B)). According to Bennet-Clark [4] and Bayley [7] – stresses of 30-60 MPa are applied to the tibia during jumping and kicking. These tests can therefore be said to be an accurate model for the stresses applied *in vivo* for the insect.

Each rig was clamped in place, and samples were tested with a tensile / compression testing machine (Zwick Roell, Ulm, Germany) using displacement control for 3-5 cycles. Readings for the displacement and applied force were recorded. The process was repeated 22 hours after the first test, and again 4 hours later. Only the left tibia of each locust was tested.

Identical experiments were carried out on tibiae which had been removed from the locust and soaked in water to prevent desiccation. These tibiae were cycled in the same manner, left for a rest period of roughly 20 hours (in water), and tested again.

In order to introduce another level of control, both the left (cycled) and the right (idle) tibiae were removed from the locust and tested to failure in order to gauge if the cyclic loading had any effect on the ultimate strength of the material.

#### Formulae Used:

The morphology of the tibia is such that it can be assumed to be a hollow circular cylinder. The actual cross section is more elliptical, and the distal side is reinforced with two rows of spines.

Dimensions of the tibiae were assumed from previous studies ([6], [8]), namely:

- The radius of the tibia = 594 $\mu$ m
- The thickness of the tibia = 50 $\mu$ m
- $I$ , the moment of inertia of the tibia = 0.038mm<sup>4</sup>

Maximum induced stress (force per unit area) was calculated using the following formula:

$$\sigma = \frac{Flr}{I}$$

Where  $F$  = applied force,  $l$  = cantilever length from load application to rigid cement,  $r$  = radius of tibia,  $I$  = moment of inertia of the tibia

Induced strain (normalised distortion of the tibia) was calculated using the following:

$$\varepsilon = \frac{3dr}{l^2}$$

Where  $d$  = recorded displacement ( $r$  and  $l$  as above)

Stiffness (Young's Modulus) of the cuticle for each cycle was measured from the linear slope of the loading portion of the stress-strain curve.

Fitting a trendline to the "loading" portion of this curve, and integrating to give the area under this line yields the energy (MJ/m<sup>3</sup>) required in deforming the cuticle. The area under the "unloading" portion gives the elastic energy recovered during unloading. The difference between the two indicates the amount of energy absorbed by the material during loading.

## Results:

Cyclic softening of the tibia material was observed during all cyclic load tests (Figure 4). On average, 10.3% less stress is required to reach the same displacement for cycle 2 compared to cycle 1. A further decrease in stress of 7.7% is required for cycle 3 (a total reduction of 17.3% of the *original* stress required).

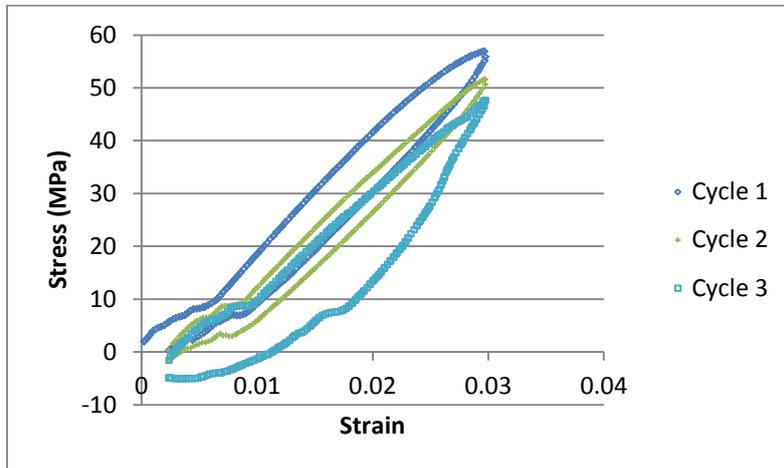


Figure 4: A typical test graph plotting stress (force per unit area) against strain (deflection) The slope of the linear portion of the loading curve in graphs such as this is used to estimate the material's stiffness (Young's Modulus,  $E$  [GPa]). The areas under these curves are used in the energy calculations. The graph also shows that less stress (force) is needed for each subsequent cycle to obtain the same strain (displacement). This shows that each cyclic load is causing the material to soften. No stress is observed in cycles 2 and 3 until a strain of 0.0025 is reached because the tibia has lost contact with the machine due to plastic deformation.

The energy required in loading the tibia was seen to fall by an average of 24.4% from the first to the second cycle, and by a further 5.1% from cycle 2 to cycle 3. Subsequent tests on the same tibia showed almost identical results, indicating the material had recovered from any damage inflicted during previous tests.

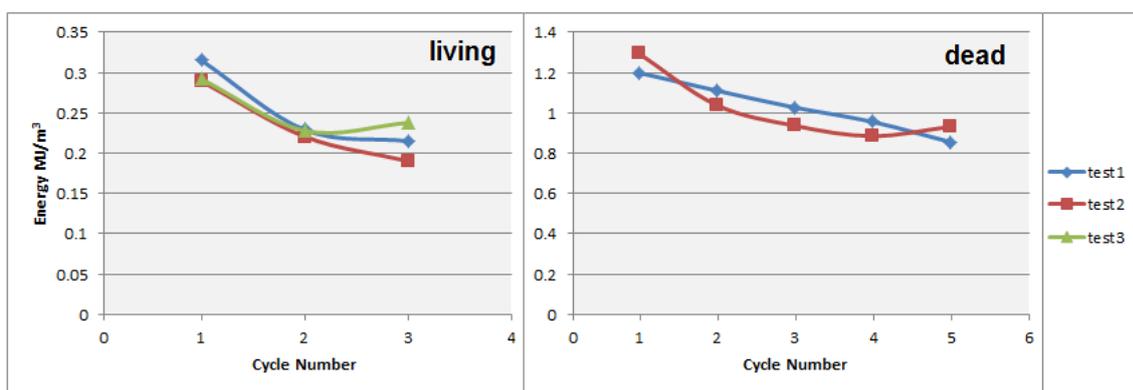
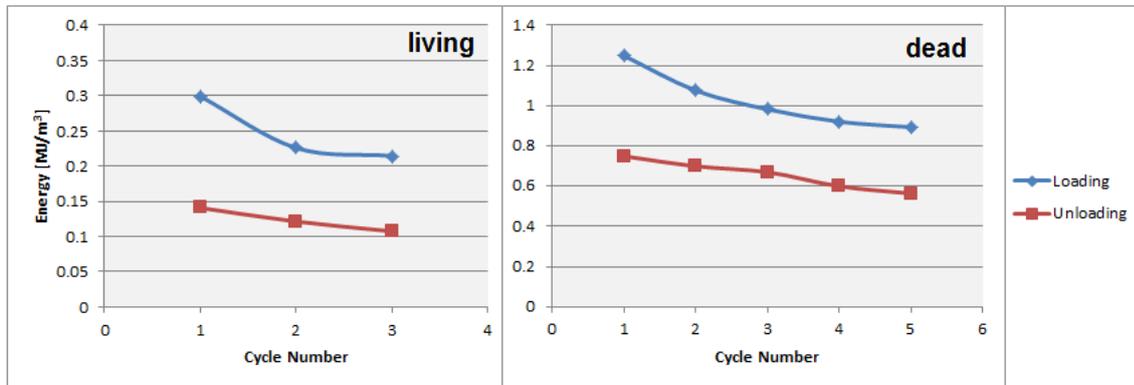


Figure 5: Loading Energies – plotted is the energy required to deform a tibia by 1mm at a rate of 5mm/min for each cycle in three consecutive tests. Both living and dead cuticles show very little variation from one test to the next – the energy required to achieve the same displacement falls during each test, indicating some deformation and / or damage.

The energy for unloading was always significantly lower than that for loading, indicating that some of the applied energy had been absorbed. The difference between loading and unloading energies averaged at 49.5% for the living material, and 35.6% for tibia which were tested *post removal*.



**Figure 6: Plotting the average energies required for loading and unloading all tibiae for each cycle of the tests. Although the batch of locusts used for the “dead” tests were much stiffer to begin with, they absorbed much less energy.**

Stiffness values for individual locusts were seen to vary slightly from test to test (Figure 7). Some showed a decrease in stiffness for subsequent tests (Locust K, M), while some showed an increase (Locust L, N). On average (Figure 9(A)), there was a drop in stiffness of 9.8% from test 1 to test 2. Also notable was that 83% of specimens showed an increase in stiffness from cycle 1 to cycle 2 – the average increase being 12.5% of the original stiffness.

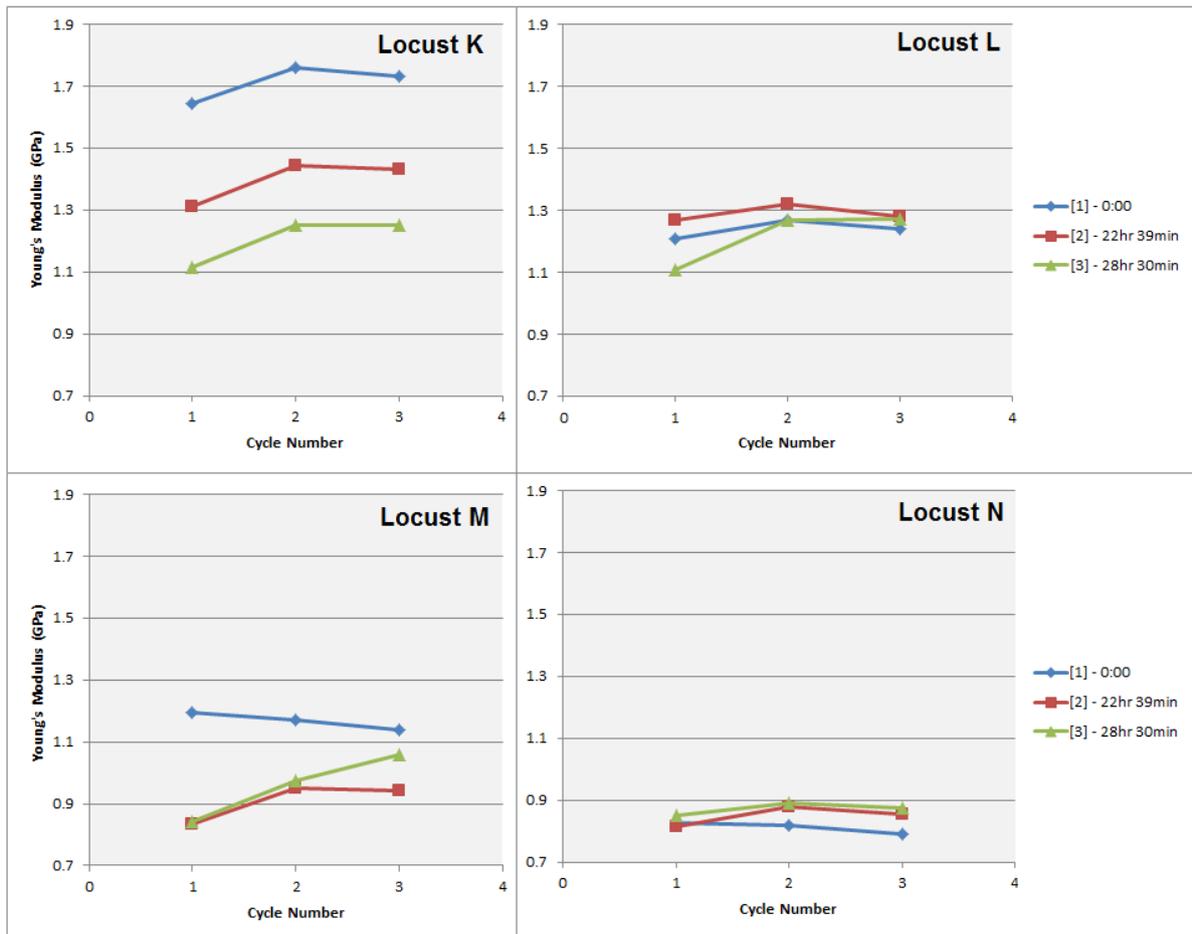


Figure 7: Sample of individual Young's Modulus values for living cuticle. Data points show stiffness values for each cycle for three consecutive tests - the second test being carried out 22 hours 39 minutes after the first, and the third being carried out 28 hours 30 minutes after the first. Stiffness values were calculated from the slope of the linear portion of the loading curve for each cycle.

Stiffness results for the “dead” cuticle (Figure 8) showed a more noticeable trend, with over 80% of samples showing a significant drop in stiffness from test 1 to test 2 (averaging 24.5% - Figure 9(B)).

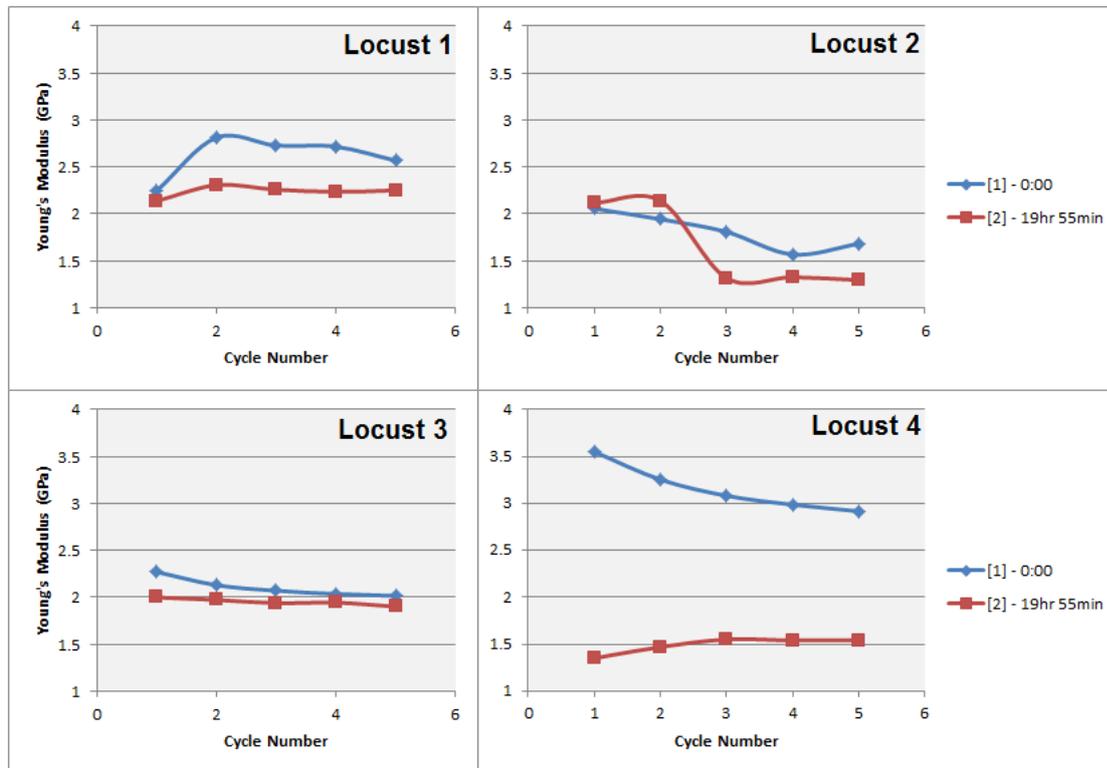


Figure 8: Sample of individual Young’s Modulus values for cuticle *post removal*. Data points show stiffness values for each cycle for two consecutive tests - the second test being carried out 19 hours 55 minutes after the first. Stiffness values were calculated from the slope of the linear portion of the loading curve for each cycle. All specimens show a decrease in stiffness from test 1 to test 2 (the exception being Locust 2 which shows a slight increase for cycle 1 and 2). Only 50% of tests showed an increase in stiffness from cycle 1 to cycle 2.

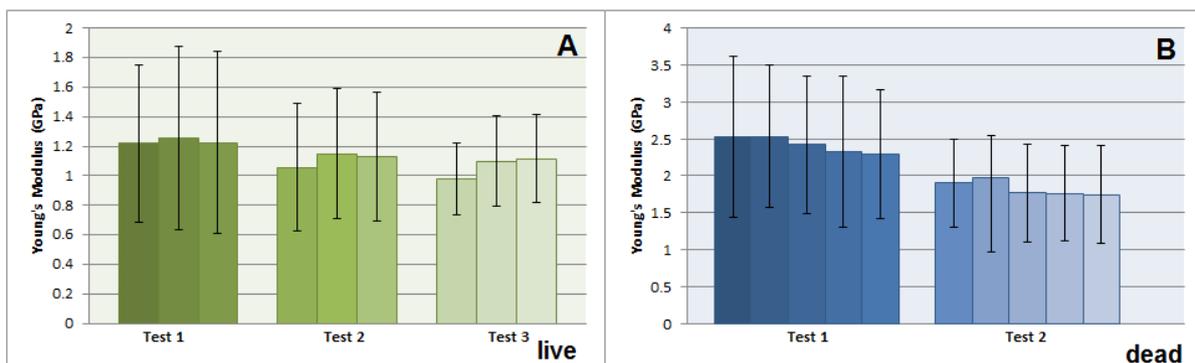


Figure 9: Tests on living insect (A): Average stiffness results for three consecutive tests, with three cycles per test. Tests on removed tibia (B): Average stiffness results for two consecutive tests, with five cycles per test. All values are averages of 5 locusts, error bars calculated using Student t-distribution based on a sample size of 5. The error bars (showing the standard error) are quite large due to the nature of the material itself. Although every effort was made to use similarly aged insects for testing, individual locusts will naturally differ slightly (in terms of size, tibia dimensions etc.). Before any tests were carried out, each tibia will have a slightly different stiffness to begin with. This can be seen in Figure 7. The error bars therefore do not indicate a wide variation of stiffness occurring in any *individual* locust tibia during testing, but rather encapsulate the range of stiffness encountered across *all* samples.

## Discussion:

### Energy:

Insect cuticle softens and deforms during testing. This is done by absorbing some of the energy applied during loading. When left to rest, the material fully recovers. Cyclic softening of the material can be observed in the stress strain curve (Figure 4). 10.3% less stress is required to reach the same displacement for cycle 2 compared to cycle 1. A further decrease in stress of 7.7% is required for cycle 3 (a total reduction of 17.3% of the *original* stress required). This shows that the material is softening significantly. The loading energy (Figure 5) required in deforming the cuticle also decreases for subsequent cycles – averaging a 24.4% drop from cycle 1 to cycle 2, and a 5.1% drop from cycle 2 to 3. This drop was seen to occur for both the living cuticle and the tibiae removed from the insect (though more pronounced in the living). Almost identical energy values were observed for subsequent tests on each sample (Figure 5), showing that the tibia has recovered fully during the rest period. This data is in agreement with that of Bayley [7] who showed the cuticle's potential for absorption and release of energy when kicking and jumping. Our results show this potential for the entire tibia, not just the specialised buckling region studied by Bayley [7]. The “dead” cuticle is not as efficient at absorbing energy – only absorbing 35.6% of the applied loading energy. It is possible that the dead cuticle has lost some of its elasticity *post mortem*. The exact mechanism at work here is unclear, but this is not an unusual phenomenon for a natural material. Figure 6 shows that the loading and unloading curves differ considerably. The graph reveals that on average, 49.7% less energy is required for unloading compared to loading. This indicates that roughly 50% of the energy applied to the tibia is actually absorbed. This is effectively energy lost that cannot be used for jumping.

Plastic deformation is also noticeable. On cycles 2 and 3, the stress remains at zero (Figure 4) until the strain of 0.0025 is reached, as the tibia has lost contact with the testing machine due to plastic deformation. Plastic strain (deformation) accounts for 8.33% of the total applied strain. Jenson and Weis-Fogh [11] noticed this plastic deformation visually and also its recovery once the material was left to rest. They theorised that as secondary bonds seem to be absent in the material, that deformation that does not break any primary bonds should have no permanent effect. Cuticle is a viscoelastic material, which means the imposition of a stress results in an instantaneous elastic strain (true for all materials) which is followed by a time dependant viscous strain (which is a form of an-elasticity) (Callister [12]). The rate of strain applied determines the response of the material. A higher applied strain rate would result in an apparently stiffer material. The strain rate (5mm/min) used in these experiments is much slower than those experienced when jumping [7], so the plastic deformations observed (due to viscous strain), and the amount of energy absorption observed would be much less *in vivo*.

### Stiffness:

The stiffness of a material is a measure of how easily it deforms or deflects under a given stress. Figure 7 shows a sample of Young's Modulus values for individual locusts during testing. No general pattern of behaviour can be seen in these results. Some individuals exhibit a decrease in stiffness from test 1 to test 2; with others showing an increase (the increase or decrease is usually less significant between test 2 and test 3). Figure 9(A) plots the averages of all these stiffness values. On average, there is a decrease in stiffness of 9.8% between test 1 and test 2, and a further reduction in stiffness of 4.3% between test 2 and test 3. It appears that applying a small number of high load cycles does not stimulate the

insect to make any significant changes to the stiffness of the cuticle material. One would assume that during natural locomotion, the tibiae of the locust could be cycled in a fashion similar to the tests carried out here. Small (<10) numbers of high load cycles can be endured on a regular basis so long as regular rest periods are also taken. Figure 8 and Figure 9(B) show that this recovery is absent for “dead” cuticle. On average, the decrease in stiffness between test 1 and test 2 is 24.5% for dead cuticle - more than double that of the corresponding value for living cuticle. Figure 8 shows that unlike the living cuticle samples, all “dead” samples showed a drop in stiffness between test 1 and test 2 (the exception being a slight increase for cycles 1 and 2 for Locust 2). These results suggest that the recovery of the cuticle is not purely due to the material itself, but may be somehow regulated by the insect.

#### Control Experiments:

The control experiment of loading both cycled (left) and idle (right) tibiae to failure after the above tests were conducted showed no significant differences in yield strength or stiffness, confirming that the applied cyclic loads had little or no effect on the living cuticle tissue.

#### Applying a load increases the stiffness:

An increase in stiffness between the first and second cycle is apparent in 83% of live tests (Figure 7) and over 50% of dead tests (Figure 8). This increase is statistically significant, averaging 12.5% of the original stiffness. This indicates that the material stiffens somewhat after initial loading. A possible explanation for this lies in the material's laminar fibre-matrix structure. Chitin fibres embedded in a viscoelastic matrix are arranged in a layered fashion [5], and in the tibia, the orientation of these fibres is predominately parallel to the tibia's major axis [13]. A phenomenon experienced when loading such materials is noted by Harris [14]: fibres in off-axis layers, or slightly misaligned fibres in 0° layers can reorient themselves slightly in the viscoelastic matrix when stress is applied. This realignment of fibres to best resist the applied stress can slightly increase the strength and stiffness of the material. When straining the locust tibia, plastic deformation was observed (partially due to fibre and layer realignment), leading to a stronger, stiffer material. When left to rest, these fibres return to their original position in the viscoelastic matrix, releasing any residual strains. The first cycle is the only one that leads to this increase in stiffness (see Figure 7), as this fibre realignment would only occur once and not incrementally for each cycle.

#### Hydration regulation mechanism of the locust is controlled centrally:

Dirks [6] showed in a previous study that desiccation or dehydration of a sample can lead to embrittlement of the material, essentially increasing its strength and stiffness, but lowering its toughness, and making it more vulnerable to crack propagation. When cementing the live tibia samples, it was important not to cement the knee-joint itself, as this was seen to cause a gradual stiffening of the tibia cuticle over time. The harder Exocuticle is absent at joints such as this to allow articulation. The waxy water-resistant layer (Epicuticle) may also be absent here, allowing absorption of fluid (or in this case, dental cement) into the joint. This shows that the water-regulation mechanism which is so important in maintaining homeostasis (constant material properties) for each region of the cuticle is centrally regulated, and can be interrupted if a barrier is introduced. Even a thin film of cement could cause much damage upon hardening. Perhaps other mechanisms than hydration are also centrally regulated, such as those which enable the insect to regulate its material properties. This will require further study.

### Importance of this study:

Viscoelastic materials such as plastics and polymers have been widely studied by materials scientists the world over. Such materials display time dependant strain and recovery, but only biological materials can actively regulate such recovery. Cuticle-based biomaterials have the advantage of being biodegradable and biocompatible, and so could be very useful in the biomedical industry for devices such as bandages, surgical sutures or drug delivery systems. Understanding the ability of other species to repair and heal damage could be useful to humans in the long term. Although the mechanisms at work in these experiments are currently not clear, if the active recovery ability of cuticle could be mimicked, one such use could be in treatment of muscle or bone injury. Hyland [15] believes that understanding such mechanisms as those at work here could prove very useful in the design and engineering of bio-polymer based biomaterials for a variety of biomedical applications, such as regenerative medicine or tissue engineering. It is possible that the cuticle experiences some microdamage (in the form of tiny cracks) when loaded. The insect may be actively repairing this damage using a remodelling process similar to that found in mammalian bone remodelling – which uses bone cells called osteocytes to locate and repair microdamage [16]. This would be one explanation of why the living material shows more pronounced recovery than the dead. If this is true, it would be a very important breakthrough. Investigations involving staining techniques may reveal if this is occurring.

### **Conclusion:**

Living insect cuticle was seen to absorb 49.5% of the applied energy during loading; this caused deformation which, when left to rest, was seen to recover fully. This was expected, as the insect would endure countless such cycles (and rest periods) in a lifetime while jumping. Although residual deformation was observed during the cyclic tests, no significant change in stiffness or strength was observed in subsequent tests, suggesting that any damage caused had been recovered from. Cuticle *removed* from the insect absorbed much less (35.6%) of the energy applied during loading, and the recovery of its original stiffness was absent. The reduction in stiffness between test 1 and test 2 for dead cuticle averaged at 24.5% compared to 9.8% reduction for the living material.

Insect cuticle continues to yield interesting results the more it is studied. A study such as this could be combined with others to assemble a complete picture of this material and its mechanical properties. Such information could prove very useful when considering the mechanical behaviour of a cuticle-inspired bio-mimetic material in the future.

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