

## RESEARCH ARTICLE

### Fracture toughness of locust cuticle

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#### SUMMARY

**Insect cuticle is one of the most common biological materials, yet very little is known about its mechanical properties. Many parts of the insect exoskeleton, such as the jumping legs of locusts, have to withstand high and repeated loading without failure. This paper presents the first measurements of fracture toughness for insect cuticle using a standard engineering approach. Our results show that the fracture toughness of cuticle in locust hind legs is  $4.12 \text{ MPa m}^{1/2}$  and decreases with desiccation of the cuticle. Stiffness and strength of the tibia cuticle were measured using buckling and cantilever bending and increased with desiccation. A combination of the cuticle's high toughness with a relatively low stiffness of  $3.05 \text{ GPa}$  results in a work of fracture of  $5.56 \text{ kJ m}^{-2}$ , which is amongst the highest of any biological material, giving the insect leg an exceptional ability to tolerate defects such as cracks and damage. Interestingly, insect cuticle achieves these unique properties without using reinforcement by a mineral phase, which is often found in other biological composite materials. These findings thus might inspire the development of new biomimetic composite materials.**

**Key words:** cuticle, toughness, stiffness, strength, desiccation.

#### INTRODUCTION

Insect exoskeletons are made from cuticle, which, after wood, is the second most common natural composite material in the world. During the life cycle of an insect, this highly specialised exoskeleton has to fulfil a wide range of biological functions and is often subject to various high and repeated mechanical forces, including cyclic loading, impact and surface abrasion. Although the versatility and durability of arthropod cuticle has fascinated scientists [and even poets; for example, in Shakespeare's *Romeo and Juliet*, Mercutio describes details of Queen Mab's miniature fairy wagon: 'her wagon-spokes made of long spinners' legs, the cover of the wings of grasshoppers, [...] her whip of cricket's bone...' (Shakespeare, 1599)] for a long time, we still know very little about its fundamental properties (Vincent and Wegst, 2004).

So far, very limited coherent data on the static mechanical properties of cuticle are available. Over the past 60 years, different insect species and body parts have been tested by engineers and biologists, using various techniques, and it has been shown that the Young's modulus of cuticle can range from less than  $1 \text{ kPa}$  (intersegmental membranes of mature female locusts) to several  $\text{GPa}$  (tendons) (Vincent and Wegst, 2004). It has also been shown experimentally that variations of the cuticle's fibre alignment, water content and controlled 'tanning' of the material affect the cuticle's static mechanical properties, such as stiffness and hardness, and dynamic mechanical properties, such as damping, over several orders of magnitude (Müller et al., 2008; Klocke and Schmitz, 2011; Dirks and Dürr, 2011; Schöberl and Jäger, 2006; Göpfert and Robert, 2001).

Besides the frequently addressed question of stiffness or hardness, knowing a material's fracture toughness – the mechanical property that describes its resistance to the propagation of cracks – is crucial to understanding the principles of any load-bearing structure. However, surprisingly, until now no study has used standardised

fracture mechanics to actually quantify the fracture toughness of any arthropod cuticle. [This becomes clear when looking at an often cited graphical representation of cuticle toughness (see Fig. 5) (see Wegst and Ashby, 2004). The graph represents actually only the two data points  $1.4$  and  $0.4 \text{ kJ m}^{-2}$  measured from *Rhodnius* beetles, cited from Vincent (Vincent, 1980), who ultimately refers to personal communication.]

In addition, many previous studies were based on nano-indentation techniques, which often require elaborate pre-processing, drying and coating of the cuticle samples. As a consequence, until now there has been no complete data set on actual 'fresh' cuticle available, showing stiffness, strength and fracture toughness and their relationships to each other.

In this study we measured the fracture toughness of the hind tibiae from *Schistocerca gregaria* locusts, a typical and morphologically well-known model organism for insect biomechanics studies. Because of their biological function, the hind legs of locusts are of particular biomechanical interest. When jumping, the two tube-like metathoracic tibiae not only have to withstand repeated high bending forces of up to 20 times the locust's body mass (Bennet-Clark, 1975), they also temporarily store and release up to 10% of the jumping energy (Sutton, 2011; Katz and Gosline, 1992). Any defect in the thin-walled structure caused by, for example, local buckling or fatigue cracking, might affect jumping performance, affecting the fitness of the locust. It thus seems likely that the cuticle of the hind tibiae should show a high resistance to fracture, and in particular a high tolerance of defects.

To measure the defect tolerance, we induced small, defined notches into the tibia samples before loading them until failure in cantilever bending. We analysed our data using a standard fracture mechanics approach, which relates the load required for fracture by crack propagation to the size of the notch. Stiffness and strength of cuticle in the tibiae, required to calculate the work of fracture, were

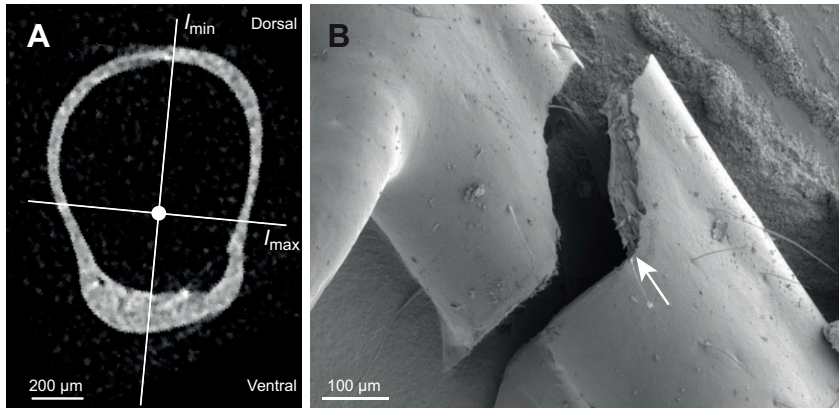


Fig. 1. (A) Post-processed microCT scan of a desiccated *Schistocerca gregaria* locust tibia used to measure morphological features. The two lines represent the maximum and minimum second moments of area  $l_{\max}$  and  $l_{\min}$ , respectively. (B) Scanning electron microscopy image of desiccated tibia showing the initial straight notch induced using a scalpel (left towards arrow) and the crack resulting from fracture of the tibia through cantilever bending.

measured by: (1) experimentally inducing buckling by axial compression and (2) cantilever bending. We also systematically varied the time between tibia ablation and mechanical testing to quantify the effect of water loss on the cuticle's mechanical properties.

## MATERIALS AND METHODS

### Insects

Desert locusts (*Schistocerca gregaria* Forsskal 1775) were bought at fifth instars and kept in a controlled 12 h (35°C):12 h (20°C) day:night cycle. Insects were fed with fresh plants and dried cereals *ad libitum*. Adult female locusts were used for trials 2 weeks after the final moult. For all experiments, the tibiae of live adult locusts were cut off directly below the femur–tibia joint. Tarsal segments were cut off within the distal 1 to 2 mm of the tibia, leaving the tibia samples in the form of hollow tubes, open at the proximal and distal ends.

### MicroCT imaging and SEM

Calculating stiffness and toughness from buckling and cantilever bending tests requires detailed knowledge of the samples' geometric features. Micro-computer tomography (microCT) scanning of biological samples allows a fast, accurate and almost non-destructive three-dimensional reconstruction of these features and has been previously used for studies on insects (e.g. Metscher, 2009). Sections (42 µm) of freshly cut tibia were scanned in air using a MicroCT (µCT 42, Scanco Medical AG, Brüttisellen, Switzerland) with a peak voltage of 70 kV, a current of 114 µA and a resolution of 6 × 6 × 6 µm voxel size (Fig. 1). To reduce desiccation, the sample tubes were sealed during the scanning process. Geometrical features such as second moment of area, tube wall thickness and radius were measured from representative 2.5 mm sections (416 slices, excluding spines and other distortions) of the tibia using ImageJ with the BoneJ plug-in (Doube et al., 2010).

To determine the ratio of exocuticle to endocuticle from MicroCT scans, we scanned fresh and dried legs from freshly moulted young adults. There is only very little endocuticle present in the locust tibia immediately after the final moult. As the exocuticle thickness does not increase during maturation of the adult insect (Jensen and Weis-Fogh, 1962), measurements of wall thickness from fully desiccated 'young' legs allowed us to estimate the ratio of endocuticle to exocuticle.

Samples used for scanning electron microscopy (SEM) imaging were fixed using 3% glutaraldehyde, washed with de-ionized water, gradually dehydrated to 100% ethanol and critically point dried. All samples were then mounted and sputter-coated with 20 nm gold. Images were taken using a Zeiss Ultra Plus scanning electron microscope (5 kV; Oberkochen, Germany).

### Desiccation

Initial experiments showed great variability in mechanical properties, which we suspected was due to rapid loss of water immediately *ex vivo*. To quantify this desiccation, the mass per millimetre length of freshly cut tibia samples was continuously recorded for 180 min using an analytical balance (XS 205, Mettler Toledo, Giessen, Germany; ±0.01 mg, 2 Hz recording, see Fig. 2). The size of the enclosed weighing compartment allowed unrestricted water evaporation at room temperature (21°C) whilst still preventing draught.

To differentiate between desiccation and other effects of 'ageing' of the cuticle, samples for cantilever bending were divided into three desiccation states: 'fresh' samples, which were tested immediately (within 15 min); 'medium' samples, which were kept in closed micro tubes for 180 min restricted desiccation; and 'dry' samples, which involved unrestricted desiccation for 180 min before testing.

### Mechanical tests

Nano-indentation has been frequently used to measure the Young's modulus and hardness of isolated cuticle samples (Klocke and Schmitz, 2011; Schöberl and Jäger, 2006). However, though also used extensively on brittle materials such as engineering ceramics, nano-indentation has also recently been the subject of much criticism (Kruzic et al., 2009). In this study we chose to test the locust metathoracic leg using standardised experimental techniques, which take into account the leg as a whole structure and are more similar to the actual biomechanical stresses during the locust jumping.

### Buckling

Measuring the maximal force supported by a structure before failure by buckling allows one to determine the Young's modulus (or elastic modulus) of the material,  $E$ . For a column loaded in compression,  $E$  is calculated as:

$$E = \frac{Fl_{\text{col}}^2}{\pi^2 I}, \quad (1)$$

where  $F$  is the maximum force supported before buckling,  $I$  is the sample's second moment of area and  $l_{\text{col}}$  is the length of the column.

The above formula applies if the ends of the column are free to rotate. To ensure this, metal spheres (ball-gearing spheres, Ø1.5 mm) were attached to each end. The spheres were held in place by the hollow shape of the tibia, and were also fixed using small amounts of fast-drying super glue (Loctite, Henkel Ltd, Hatfield, Herts, UK). To test the effect of desiccation, the time between cutting-off the tibia and attachment of the spheres was varied between 15 and 180 min.

Immediately after preparation, the samples were aligned between two parallel metal plates of a tensile testing machine (5N load cell, Zwick Roell, Ulm, Germany) and compressed at a constant speed of 15 mm min<sup>-1</sup> until failure by buckling occurred.

### Cantilever bending

Measuring the deflection  $d$  of a cantilever beam with one fixed end and a load  $F$  applied at a distance  $l_{\text{fix}}$  from the fixation point, the Young's modulus of the beam can be calculated using:

$$E = \frac{Fl_{\text{fix}}^3}{3dI} \quad (2)$$

The proximal 5 mm of the tibiae samples were embedded in fast-hardening cold-cure acrylic dental cement (Simplex ACR308, Kemdent, Swindon, UK). Samples were then bent at a distance of 9 or 10 mm from the fixation point (preventing interference with the cuticle spines) at a constant speed of 15 mm min<sup>-1</sup> using a standard tensile testing machine (see above). A custom made load-cell 'tip' ensured a defined point of contact during the bending and lateral self-alignment of the sample. Samples were bent in the dorsal-ventral plane, loading either from the dorsal side or from the ventral side to test for a possible effect of bending direction.

To calculate the bending strength  $S$ , we used the maximum force supported by the sample before failure ( $F$ ), the distance  $l_{\text{fix}}$  from the fixation point, the mean radius  $r$  of the sample and the mean second moment of area  $I$  for cantilever bending (see Fig. 3):

$$S = \frac{Fl_{\text{fix}}r}{I} \quad (3)$$

### Fracture toughness and work of fracture

To measure the fracture toughness of the cuticle, small notches were induced into the dorsal ('spiny') side of the tibia 10 min after curing of the cement. These notches were created using a sharp scalpel and were oriented perpendicular to the longitudinal axis of the tube. The lengths of the induced notches ranged from 0.4 to 1.2 mm. Samples were then bent as described above, with the notch located on the tensile side of the bend. The exact length and position of the notch along the tibia was measured after the experiments from SEM images (Fig. 1B), which also confirmed that failure had occurred by crack propagation.

The standard formula for fracture toughness,  $K_c$ , is:

$$K_c = F_b l_{\text{bend}} \frac{r}{I} \sqrt{(\pi a) Q} \quad (4)$$

where  $2a$  is the length of the induced notch,  $l_{\text{bend}}$  is the bending distance between the notch and the loading point, and  $F_b$  is the maximum force before fracture.  $Q$  is a constant that depends on the geometry of the sample and of the notch, and also on the type of loading. Our sample was slightly complex in shape, but could be approximated to a circular cylinder of constant radius  $r$  and

wall thickness  $t$ , for which values of  $Q$  are available (Takahashi, 2002). In this case,  $Q$  was found to be a function of the normalised quantities  $a/r$  and  $t/r$ .

The work of fracture (also called the strain energy release rate),  $G_c$ , is another measure of toughness: it is defined as the energy needed for the notch to propagate a given amount (in units of energy per unit increase in crack area).  $G_c$  is directly related to the fracture toughness  $K_c$  and the elastic modulus of the material  $E$  by:

$$G_c = \frac{K_c^2}{E} \quad (5)$$

### Statistics

Statistical tests [ANOVA, Fisher's least significant difference (LSD) and paired  $t$ -tests] were performed where applicable using SPSS (Version 19, IBM, Armonk, NY, USA). If not stated otherwise, all values shown are means  $\pm$  s.d.; box-and-whisker plots show standard quartiles. Fits were calculated using customised MATLAB scripts (The MathWorks, Natick, MA, USA) to determine the minimum scalar function of the variables.

### RESULTS

MicroCT scans were used to measure geometric features of the tibia samples and to quantify the effect of desiccation on the tibial cuticle. Buckling and cantilever bending experiments were used to measure the stiffness, strength and fracture toughness of the samples.

#### General morphology of the metathoracic tibia cuticle

The geometrical features measured from microCT scans of 'fresh' and 'dry' (180 min) tibia samples are summarised in Table 1. Freshly cut and desiccated paired tibia samples showed a non-significant reduction of radius (596.2 $\pm$ 40  $\mu$ m fresh vs 591.7 $\pm$ 30  $\mu$ m dry; 180 min, paired  $t$ -tests,  $N=8$ ,  $P>0.05$ ) but a significant decrease of the average cuticle wall thickness from 53.9 $\pm$ 7 to 46.2 $\pm$ 12  $\mu$ m ( $N=8$ ,  $P<0.05$ ). The second moment of area varied around the cross section: Table 1 shows the maximum and minimum values, both of which were significantly reduced by drying (all  $P<0.001$ ). Dry control samples scanned that were dried once showed no difference with dry samples scanned twice (fresh and dry), excluding any notable desiccation effect of the microCT scans.

Paired scans from freshly moulted adult insects showed that desiccation significantly decreased the mean wall thickness from 42.9 $\pm$ 12 to 27.5 $\pm$ 6  $\mu$ m (paired  $t$ -test,  $t_4=3.67$ ,  $N=5$ ,  $P<0.05$ ). Assuming an almost complete desiccation of the endocuticle, this indicates a mean exocuticle thickness of 27.5  $\mu$ m for adult locust tibia cuticle.

Individual samples could not be subjected to both microCT scanning and testing owing to the time involved, so for the mechanical property calculations we used constant mean values of the geometrical parameters, pooling results from the fresh and dry samples (see Table 1).

Table 1. Geometrical features of fresh and dried locust cuticle tibia segments from microCT measurements

	Fresh	Dry	Standard for buckling	Standard for cantilever
Cuticle thickness ( $\mu$ m)	53.9 $\pm$ 7	46.2 $\pm$ 12	50	50
Radius ( $\mu$ m)	596.2 $\pm$ 40	591.7 $\pm$ 30	594	594
$l_{\text{max}}$ (mm <sup>4</sup> )	0.041 $\pm$ 0.011	0.034 $\pm$ 0.008	—	0.038
$l_{\text{min}}$ (mm <sup>4</sup> )	0.016 $\pm$ 0.004	0.014 $\pm$ 0.003	0.015	—
$l_{\text{mean}}$ (mm <sup>4</sup> )	0.029 $\pm$ 0.007	0.025 $\pm$ 0.006	—	—

Values are means  $\pm$  s.d., all  $N=8$ . For buckling experiments, we used the minimum second moment of area  $l_{\text{min}}$ ; for cantilever bending we used the maximum second moment of area  $l_{\text{max}}$  in accordance to the bending direction.

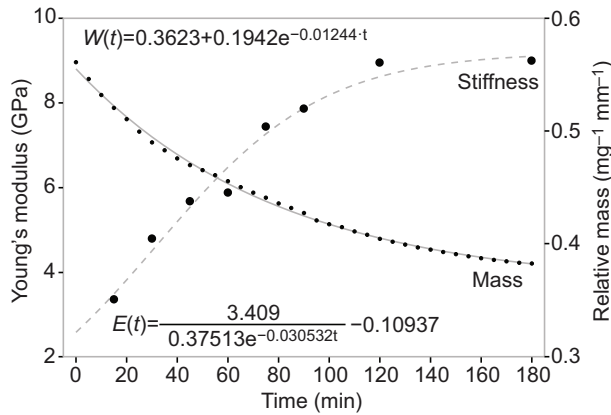


Fig. 2. Variation of sample mass and stiffness (Young's modulus, measured from buckling tests) of *S. gregaria* tibiae over time *ex vivo*, with a sigmoidal fit for elastic modulus and an exponential fit for mass loss.

For buckling experiments we used a second moment of area of  $0.015 \text{ mm}^4$ , which is the mean of  $I_{\min}$  and determines the buckling behaviour of the leg. We used a wall thickness of  $50 \mu\text{m}$  and a radius of  $594 \mu\text{m}$  ( $N=16$ ). Cantilever bending was carried out in the dorsal–ventral plane, which had the maximum  $I$  value for the cross-section ( $I_{\max}$  in Table 1). The standard leg for cantilever bending thus had an effective second moment of area of  $0.038 \text{ mm}^4$ , a wall thickness of  $50 \mu\text{m}$  and a radius of  $594 \mu\text{m}$  ( $N=16$ ).

#### Mass loss through desiccation

Cut-off tibia segments with a length of  $18.43 \pm 1.7 \text{ mm}$  had an initial mass of  $10.42 \pm 1.7 \text{ mg}$ , giving a mean mass of  $0.56 \pm 0.03 \text{ mg mm}^{-1}$  tibia ( $N=5$  tibia from different insects). Within the first 180 min after cutting off, the leg segments lost a considerable amount of mass, levelling out at approximately  $0.38 \text{ mg mm}^{-1}$ . The mass loss per mm tibia length was in excellent agreement with a simple exponential decay model ( $R^2 > 0.99$ ; Fig. 2). This model predicts a theoretical cuticle dry mass of  $0.36 \text{ mg mm}^{-1}$  (64% of overall mass) for adult tibia cuticle with an estimated maximum water content of  $0.20 \text{ mg mm}^{-1}$  (36%).

For samples with 'restricted' desiccation, paired  $t$ -tests showed a significant reduction in mass per mm from the 'fresh' to the 'medium' condition ( $t_7=24.19$ ,  $P<0.001$ ) and from the 'fresh' to the 'dry' condition ( $t_7=23.28$ ,  $P<0.001$ ).

#### Stiffness from buckling

The stiffness of tibia segments measured by buckling increased by a factor of 2.6 during desiccation, from  $3.36 \pm 1.2 \text{ GPa}$  at  $t=15 \text{ min}$

to a maximum of  $8.94 \pm 0.4 \text{ GPa}$  at  $t=120 \text{ min}$ , levelling out thereafter at a mean of  $8.88 \pm 0.7 \text{ GPa}$  (Fig. 2). The increase of stiffness of locust tibia cuticle over time can be very well described by a simple sigmoid correlation ( $R^2=0.98$ ; Fig. 4). This gives an estimated minimal elastic modulus at  $t_0$  of  $2.58 \text{ MPa}$  for 'fresh' locust tibiae.

#### Stiffness and strength from cantilever bending

Cantilever bending tests gave a mean stiffness of  $3.05 \pm 0.6 \text{ GPa}$  for 'fresh' ( $N=10$ ),  $4.15 \pm 0.3 \text{ GPa}$  for 'medium' ( $N=7$ ) and  $6.17 \pm 0.7 \text{ GPa}$  for 'dry' tibial samples ( $N=8$ , see Table 2). Univariate ANOVA showed a significant effect of desiccation state on the stiffness ( $F_{2,25}=79.127$ ,  $P<0.001$ ), but no significant effect of deflection direction ( $F_{1,25}=2.611$ ,  $P=0.123$ ). *Post hoc* tests showed significant differences in stiffness between all desiccation groups (Fisher's LSD, all  $P \leq 0.001$ ; Fig. 3).

A previous study has shown the presence of a small lightly sclerotized 'buckling' region located on the proximal dorsal part of the locust tibia, possibly affecting the structural stiffness of the leg (Heitler, 1977). The non-significant effect of bending direction on the stiffness demonstrates that the embedding of the proximal 5 mm of the tibia in cement removed any potential effect of this buckling region.

Desiccation also had a significant effect on the bending strength of the tibia samples ( $F_{2,25}=41.593$ ,  $P<0.001$ ). 'Fresh' and 'medium' (partially dry) samples had a mean bending strength of  $72.05 \pm 30.5 \text{ MPa}$  ( $N=10$ ) and  $85.14 \pm 24.9 \text{ MPa}$  ( $N=7$ ), respectively, but were not significantly different from one another; 'dry' samples had a significantly higher strength of  $217.41 \pm 48.2 \text{ MPa}$  (Fisher's LSD,  $N=8$ , both  $P \leq 0.001$ ). Microscopic observation during testing showed that failure occurred by buckling on the part of the sample that was loaded in compression, rather than by fracture on the tensile side.

#### Fracture toughness and work of fracture

'Fresh' samples had a mean fracture toughness of  $4.12 \pm 0.4 \text{ MPa m}^{1/2}$  ( $N=9$ ). Desiccation significantly decreased the fracture toughness to  $2.06 \pm 0.6 \text{ MPa m}^{1/2}$  ( $t_{1,17}=8.83$ ,  $N=9$ ,  $P<0.001$ ).

The work of fracture  $G_c$  was calculated from the previously obtained values of  $K_c$  and  $E$ , using the  $E$  values from the bending experiments. Because desiccation caused a decrease in  $K_c$  and an increase in  $E$ , the corresponding change in  $G_c$  was very large, from  $5.56 \text{ kJ m}^{-2}$  for 'fresh' samples ( $E=3.05 \text{ GPa}$ ,  $K_c=4.12 \text{ MPa m}^{1/2}$ ) to  $0.68 \text{ kJ m}^{-2}$  for 'dry' samples ( $E=6.17 \text{ GPa}$ ,  $K_c=2.06 \text{ MPa m}^{1/2}$ ).

#### DISCUSSION

First, we will briefly discuss our measurements of stiffness and strength of the locust tibia cuticle, two 'standard' mechanical parameters of a material. We will then discuss the fracture toughness

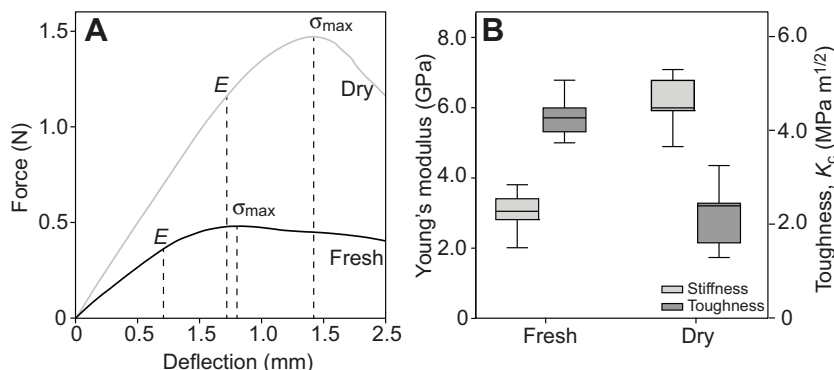


Fig. 3. Stiffness and toughness measured from cantilever bending of 'fresh' and 'dry' *S. gregaria* locust tibiae. (A) Force–deflection curve from cantilever bending of a fresh and a desiccated locust tibia. (B) Desiccated locust tibiae had significantly higher structural stiffness (Young's modulus) but significantly lower fracture toughness ( $K_c$ ) than fresh tibiae.



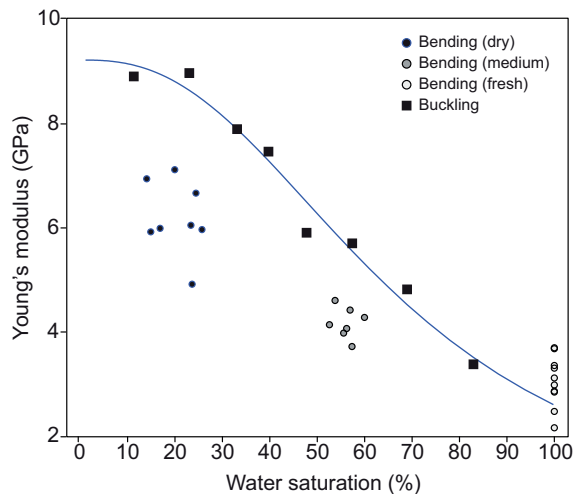


Fig. 4. Correlation between water saturation and stiffness in *S. gregaria* locust tibiae measured from both buckling and cantilever bending. The line shows the sigmoidal model based on buckling experiments. The values for water saturation are based on a tibia cuticle dry mass of  $0.20 \text{ mg mm}^{-1}$  tibia and are calculated from drying times (buckling) or mass/length (cantilever).

and work of fracture of the same material, the correlation of the mechanical properties and possible biomechanical implications.

#### Stiffness and strength

Stiffness and strength are often the first mechanical parameters to be determined for a 'new' biological material, as they are often seen to be relatively straightforward to measure. However, as most engineering textbooks point out, the choice of mechanical tests and sample preparation have a notable impact on the accuracy of the results.

The few earlier studies on insect cuticle have used a wide variety of mechanical tests, sample treatments, terminology, insect species or body parts. A very comprehensive review on this matter has been written by Vincent and Wegst, who summarised and discussed the mechanical properties of insect cuticle of different species (Vincent and Wegst, 2004). It has also been shown that even in the same locust species, the mechanical properties of the tibia can vary and scale with age, gender and size of the locust (Katz and Gosline, 1992). This variability makes the quantitative comparison of previous results to findings of our study very difficult and, in particular with respect to the water contents, not necessarily reasonable.

Just looking at the metathoracic tibiae of adult female *S. gregaria*, Jensen and Weis-Fogh measured an often-cited structural stiffness of  $9.0 \text{ GPa}$  and a strength of  $94 \text{ MPa}$  from buckling experiments (Jensen and Weis-Fogh, 1962). These earlier results are in good agreement with the stiffness and strength measured from buckling and cantilever bending of the 'dry' samples in our study, which indicates that Jensen and Weis-Fogh, and several later studies, presumably underestimated the effect of desiccation on their samples.

Recently, the awareness of the sensitivity of cuticle to water content has increased, and desiccation has become more of a concern when designing mechanical tests on insect cuticle. An interesting trend becomes apparent: recent results show that the stiffness of 'fresh' wing membranes [typically between  $1$  and  $5 \text{ GPa}$  (Smith et al., 2000)], 'fresh' mandibles [approximately  $3.5 \text{ GPa}$  (Schöberl and Jäger, 2006)] and 'rewetted' sternal plates [approximately  $2 \text{ GPa}$

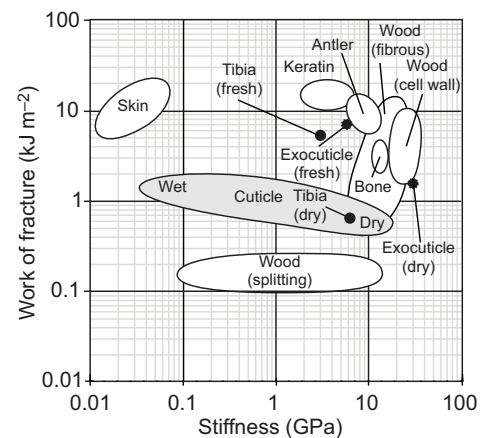


Fig. 5. Work of fracture and stiffness of selected biological materials. Data are based on Wegst and Ashby (Wegst and Ashby, 2004), with our own results for fresh and dry *S. gregaria* locust tibia added for comparison (solid circles). The dark grey 'cuticle' area refers to data from *Rhodnius* beetles (Vincent, 1980).

(Klocke and Schmitz, 2011)] from *S. gregaria* locusts are very similar to the stiffness of tibia cuticle samples ( $3.05 \text{ GPa}$ , present study). This indicates that the range of exoskeleton cuticle stiffness, at least within one insect species, might be relatively small, and fine-tuning of the material's properties could be happening in a much smaller range. Future mechanical experiments on other body parts of *S. gregaria* could help to clarify this point and help to build up a more comprehensive material data sheet for one 'model' species.

#### Fracture toughness and work of fracture

Fracture toughness is a measure of a material's 'defect tolerance', its ability to withstand the presence of defects such as cracks without significant loss of strength. This is very relevant for insects, whose exoskeletons may sustain cracks as a result of impact damage or fatigue. As a consequence, one can assume that the locust hind leg cuticle, subject to repeated and high loads during jumps and defensive kicks, should show high fracture toughness.

Indeed, our results show that with a fracture toughness of  $4.12 \text{ MPa m}^{1/2}$ , fresh locust tibia cuticle is comparable to several natural composites such as bone, nacre, wood, as well as artificial fibre-reinforced composites and ceramics (Boccaccini et al., 2005; Wegst and Ashby, 2004; Gogotsi, 2003). However, the properties of cuticle are remarkable, considering that, unlike these other materials, insect cuticle does not contain a reinforcing mineral phase.

An additional approach to describe the fracture toughness of a material is to look at the strain energy release rate  $G_c$ , which describes the amount of energy required to form new surfaces during crack propagation (Janssen et al., 2004). The higher  $G_c$  is, the more energy is needed during crack growth. Because of its relatively high fracture toughness combined with relatively low stiffness (compared with bone, nacre, wood, etc.), fresh locust tibia cuticle shows a remarkably high  $G_c$  value of  $5.56 \text{ kJ m}^{-2}$ . This makes cuticle one of the toughest natural composite materials known: tougher than bone and similar to materials such as antler and horn (Vincent and Wegst, 2004).

Comparing the biological function of antler, horn and locust metathoracic legs, one notices that these structures all need to withstand repeated high power impacts, antlers and horn through

Table 2. Stiffness, strength, fracture toughness  $K_c$  and work of fracture  $G_c$  for fresh and desiccated tibia cuticle from *Schistocerca gregaria* locusts

	Structural stiffness (GPa)		Strength (MPa)	$K_c$ (MPa m <sup>1/2</sup> )	$G_c$ (kJ m <sup>-2</sup> )
	Buckling	Bending			
Fresh	3.36±1.2	3.05±0.6	72.05±30.5	4.12±0.4	5.56
Desiccated	8.88±0.7	6.17±0.7	217.41±48.2	2.06±0.6	0.68

Data are means ± s.d. For details see Results.

usage as a weapon or tool, and insect legs during jumping and kicking. Previous studies have indeed shown that during jumping locust tibiae have to withstand loads with a peak power of approximately 0.74 W (Bennet-Clark, 1975; Sutton and Burrows, 2008). The high work of fracture indicates that the hind tibiae of locusts are very well adapted to withstand the high energy involved in jumping.

The only other study that has measured the fracture toughness of insect cuticle is that of Sun and Tong (Sun and Tong, 2007), who measured a  $K_c$  value of 1.56 MPa m<sup>1/2</sup> for cuticle from dung beetle elytra (hardened forewings) using nano-indentation. However, looking at the Young's modulus of 6.2 GPa obtained by Sun and Tong, and the samples' low fracture toughness, it seems likely that their samples had a low water content, especially at the surface where the indentation was applied.

Both mechanical tests used in this study, bending and compression (buckling), gave similar results for the stiffness of locust tibia cuticle. However, inaccuracies can arise in buckling experiments from the non-ideal geometry of the sample (Jensen and Weis-Fogh, 1962; Katz and Gosline, 1992). We therefore consider our cantilever bending experiments to be more accurate than the buckling experiments, though the similarity of the two results is encouraging.

Furthermore, the geometry of the locust leg is very close to, but not exactly, that of a hollow thin-walled tube, which is a limitation of our study (see Fig. 1) and previous studies (Jensen and Weis-Fogh, 1962; Katz and Gosline, 1992). For our calculations, we used average values for the second moment of area and cuticle thickness of fresh and dry legs. However, if we were to adjust our calculations for the small changes of cuticle thickness and second moment of area, the observed differences between 'fresh' and 'dry' legs become even bigger, illustrating that our simplification is a conservative approach.

A current limitation of our study is that we consider the tibia cuticle to be one material, when in fact it consists of two major layers – an outer exocuticle and a softer, inner endocuticle – with distinct mechanical properties (Klocke and Schmitz, 2011). However, given that in fresh cuticle the exocuticle is the harder, stiffer component, it may be bearing most of the stress during loading. If we assume that it bears all the stress (reducing the effective cross-section of the leg), the stiffness of fresh exocuticle increases to 5.97 GPa, its fracture toughness  $K_c$  to 6.59 MPa m<sup>1/2</sup> and its  $G_c$  value even to 7.27 kJ m<sup>-2</sup> (Fig. 5B). In future studies, we hope to address and model the effect of the distinct cuticle layers in more detail, in particular involving histological data obtained from microCT measurements.

#### Effect of water content on mechanical properties

Our results show that stiffness and strength of insect cuticle were significantly affected by the water content (Table 2). This is in good agreement with several previous studies, demonstrating the effect of desiccation on the static and dynamic mechanical properties of cuticle from various insect species (Dirks and Dür, 2011; Klocke and Schmitz, 2011; Mills and Krolak, 1986; Göpfert and Robert,

2001; Schöberl and Jäger, 2006) and other biological composite materials such as bone, squid beaks or even equine hoof walls (Nyman, 2006; Miserez, 2008; Bertram, 1987).

It is likely that the increase in stiffness and strength of locust tibia with decreasing water content is a combination of both structural effects and material effects. The mechanical properties of cuticle, like those of most biological composite materials, are determined by the volume fraction and the mechanical properties of its components. In particular, the viscoelasticity and plasticity of the protein matrix play an important role, allowing the material to dissipate energy, trap cracks and redistribute stress (Ji and Gao, 2010). However, desiccation of the cuticle can lead to additional cross-linking of the chitin and the proteins (Vincent, 2009; Moussian, 2010; Klocke and Schmitz, 2011), reducing the material's ability to dissipate energy and withstand crack propagation.

The comparison of stiffness from tibia segments with 'restricted' evaporation ('medium') with samples with the same desiccation time but 'unrestricted' evaporation ('dry'; Fig. 3) demonstrates that the observed changes in mechanical properties are not due to decay of the cuticle or underlying tissue over time, but are in the main an effect of water desiccation. By minimising the time between dissection from the insect body and mechanical tests, we can therefore assume that our measurements for 'fresh' cuticle were very close to the actual *in situ* properties of the locust cuticle.

#### Correlation of stiffness, strength and toughness

Looking at the biomechanics of an insect exoskeleton, one could wonder which evolutionary constraints led to this combination of stiffness, strength and toughness. As our results and those of previous experiments (Klocke and Schmitz, 2011; Schöberl and Jäger, 2006) suggest, small reductions of the water content would increase the stiffness and strength of locust tibia cuticle, potentially providing better protection, and less wear. However, the insect is clearly going to some effort to maintain hydration of its cuticle, which tends to dehydrate very quickly *ex vivo*, and presumably even faster in the dry environments in which desert locusts normally live. Why don't insects increase stiffness and strength of their exoskeleton by 'simply' reducing the cuticle's water content by a few percent?

Our results show that increasing stiffness and strength of cuticle by water loss comes with a significant mechanical cost: it reduces the fracture toughness, making the legs more brittle with a reduced strain to fracture. With a poorer defect tolerance, in particular the jumping legs experiencing high and repeated loading are more likely to fail as a result of small cracks. Our results showed that the bending strength of the 'fresh' tibia was not reduced at all by the presence of a crack provided that the total crack length ( $2a$ ) was less than 0.9 mm, which is a considerable proportion of the leg's circumference (approximately 3.7 mm), indicating a very high defect tolerance. For dry cuticle, in contrast, the critical crack length was only 0.06 mm.

Besides the increased durability, another possible reason for maintaining cuticle with low stiffness is its ability to store and quickly release more energy, thus for example allowing high-power

insect jumps (Burrows et al., 2008; Katz and Gosline, 1992). These correlations illustrate a trade-off for the evolutionary biomechanical optimisation for the mechanical properties of insect cuticle.

The quantitative changes in stiffness from fresh to dried cuticle shown in this study are interestingly in very good agreement with the results of Klocke and Schmitz (Klocke and Schmitz, 2011), who showed that rewetting of dry sternal exocuticle decreased its stiffness by a factor of 2.4. This indicates that the effects of desiccation and rewetting of some parts of locust exoskeletal cuticle are almost fully reversible, even from fresh to dry (present study) and between dry and cyclic 'rewetted' cuticle (Klocke and Schmitz, 2011). A histological adaptation like this would not only allow an insect to initially 'fine-tune' the exoskeleton's mechanical properties during its ontogenesis by determining the water content, but would also allow a 'cyclic' re-tuning of the cuticle's properties, depending on metabolic or environmental water abundance.

Such a dynamic adaptation of cuticle properties could play an important role in the biomechanical short-term modification of body parts. By adjusting, for example, the water content of their wings, locusts could potentially regulate mechanical parameters such as wing stiffness, strength or fracture toughness before long-term flights, thus controlling or reducing the effect of wing desiccation.

### Conclusions

This study has established values of the fracture toughness and crack propagation energy for locust cuticle, showing that they are amongst the highest of any biological material, and allow the insect leg to sustain significant amounts of damage without loss of strength. An interesting feature of insect cuticle is that it has achieved its high toughness (of all material groups only metals could be said to have significantly higher values) without using reinforcement by a ceramic, mineral phase (as occurs in bone and in engineering fibre composites), thus giving a useful combination of high toughness with relatively low stiffness (Fig. 5). From an engineering point of view, the information obtained in this study may be useful in the future development of biomimetic materials inspired from cuticle.

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