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RESEARCH ARTICLE

Fatigue of insect cuticle

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SUMMARY

Many parts of the insect exoskeleton experience repeated cyclic loading. Although the cuticle of insects and other arthropods is the second most common natural composite material in the world, so far nothing is known about its fatigue properties, despite the fact that fatigue undoubtedly limits the durability of body parts *in vivo*. For the first time, we here present experimental fatigue data of insect cuticle. Using force-controlled cyclic loading, we determined the number of cycles to failure for hind legs (tibiae) and hind wings of the locust *Schistocerca gregaria*, as a function of the applied cyclic stress. Our results show that, although both are made from cuticle, these two body parts behave very differently. Wing samples showed a large fatigue range, failing after 100,000 cycles when we applied 46% of the stress needed for instantaneous failure [the ultimate tensile strength (UTS)]. Legs, in contrast, were able to sustain a stress of 76% of the UTS for the same number of cycles to failure. This can be explained by the difference in the composition and structure of the material, two factors that, amongst others, also affect the well-known behaviour of engineering composites. Final failure of the tibiae occurred *via* one of two different failure modes – crack propagation in tension or buckling in compression – indicating that the tibia is 'optimized' by evolution to resist both failure modes equally. These results are further discussed in relation to the evolution and normal use of these two body parts.

Key words: biomaterials, biomimetics, entomology, insect biomechanics.

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INTRODUCTION

Cuticle is the second most common natural composite material in the world, and one of the most versatile. All parts of the exoskeletons of insects and other arthropods are made from this fascinating material. Inevitably, during their lifetime, many parts of these exoskeletons are subject to repeated cyclic loadings. Wear and tear can be observed in the mouthparts, wings, stridulation organs, claws and adhesive organs of insects (Gorb, 2001; Vincent and Wegst, 2004). In general, however, the insect exoskeleton seems to show a quite remarkable durability for a biological composite material.

Particular examples of exoskeleton body parts subject to cyclic loading are the wings and legs of the locust *Schistocerca gregaria* (Forsskål 1775). During their migratory journeys across thousands of kilometres of deserts or oceans, their wings have to withstand many millions of cyclic loadings. The tibiae in the large hind legs experience stresses of more than half their tensile strength during jumping and kicking (Bayley et al., 2012; Taylor and Dirks, 2012). Mammalian bone subject to cyclic loading has been shown to resist fatigue failure by continuous repair, mediated by living cells (Martin, 1997; Taylor et al., 2007). Currently it is not clear whether this also happens in cuticle (see Johnson et al., 2011; Lai-Fook, 1968). In any case, the ability of cuticle to resist fatigue failure, whether by its composition and structure or by self-repair, will be a crucial factor in the durability of body parts and thus of the insect's fitness. Yet there are no published data on the fatigue of insect cuticle (Vincent and Wegst, 2004).

The phenomenon of fatigue is well known in the field of engineering materials, both for metallic materials (Stephens et al., 2001) and polymers and composites (Hertzberg and Manson, 1980). Recently, in particular driven by biomedical engineering, there has also been an increasing interest in the fatigue of biological materials (Taylor et al., 2007; Teoh, 2000). Almost all materials show some tendency towards fatigue, i.e. to fail after a number of cycles at an applied stress that is less than that required for instantaneous failure. However, behaviour differs considerably from one material to another, especially regarding the fatigue range, i.e. the extent to which fatigue failure occurs at reduced stresses and high numbers of cycles. Knowing more about the fatigue properties of insect cuticle could provide valuable entomological knowledge about morphological and evolutionary adaptations, as well as provide biomimetic inspiration for novel materials.

MATERIALS AND METHODS

Insects and sample preparation

Adult female *Schistocerca gregaria* locusts were taken from laboratory colonies approximately 3 weeks after final moult. Insects were fed fresh food *ad libitum* and kept at a controlled 12 h:12 h light:dark cycle with a maximum temperature of 25°C.

The tibiae of the hind legs were removed as close to the femur-tibia joint as possible. The proximal 5 mm was then embedded within the sample holder using fast-hardening cold-cure acrylic dental cement (Simplex ACR308, Kemdent, Swindon, UK) (Fig. 1A) (see Dirks and Taylor, 2012a). Dimensions for the legs were taken from previously published microCT measurements (cuticle thickness 53.9±7 μm, radius 596.2±40 μm) (Dirks and Taylor, 2012a).

The hind wings were cut off close to the wing base. Samples from the mid-section of the wing (mean thickness 3.05±0.71 μm)

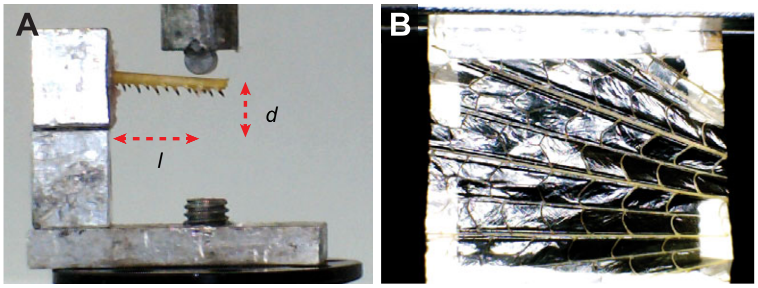

Fig. 1. Experimental setup for cyclic loading of locust legs and wings. (A) The proximal end of the tibia is fixed, whilst the actuator presses down a deflection amplitude *d* at a distance *l* from the clamping. (B) The aluminium foil frame with the wing section is mounted between two standard tensile clamps. Before the test, the connecting parts of the frame are cut. Both tibia and wing samples were fully submerged in water during the tests (container not shown).

(see Dirks and Taylor, 2012b) were then glued to a supporting aluminium foil frame using small amounts of fast-drying super glue (Fig. 1B) (Loctite, Henkel, Hatfield, Herts, UK) cut to fit and dimensions measured.

As desiccation has been shown to strongly affect the mechanical properties of insect cuticle (Dirks and Dürr, 2011; Dirks and Taylor, 2012a), all mounted samples were immediately fully immersed in Millipore-filtered water for the duration of the tests. Covering the water with a thin layer of vegetable oil prevented evaporation of water during the experiments. To test for the effect of long-term immersion in water on the fatigue properties, control experiments were performed where some samples were immersed in water for at least 24 h before the beginning of the fatigue tests.

Fatigue tests

All fatigue tests were performed using a Bose Electroforce 3100 tensile machine (Bose, Gillingham, UK) equipped with a standard 22 N load cell in force-control mode. Legs were loaded in cantilever bending (Fig. 1A) (see Dirks and Taylor, 2012a); stress was calculated as the maximum bending stress, which occurs close to the fixed end and is equal in magnitude for tension (on the top of the leg) and compression (on the bottom). The wings were loaded in uniform tension (Fig. 1B) (see Dirks and Taylor, 2012b).

During the fatigue tests, legs were cycled with a sinusoidal variation of load at a frequency of 2 Hz; wings were cycled at a frequency of 3 Hz, which was the maximum frequency at which the machine could reliably operate. In preliminary tests neither body part showed a notable effect of cycling frequency on material stiffness (0.1–2 Hz experimental limitation for legs, wings tested 0.1–5 Hz).

The maximal loads were varied for each test between 0.1 and 3 N (legs) and 0.05 and 1.3 N (wings), with the cyclic load amplitude ratio (load min/max) always set to 0.1. The actual cyclic stress was then calculated from the respective sample dimensions and loads. The ultimate tensile strength (UTS) of the samples was calculated by pooling the maximum stress of samples that failed after fewer than 50 cycles. Following the convention used for engineering materials, the data were presented on a logarithmic plot showing the number of cycles to failure as a function of the maximum stress in the cycle, normalised by the UTS.

RESULTS

The measured UTS of the legs (76.67±12.46 MPa) and of the wings (30.89±2.67 MPa), defined here using samples with very low numbers of cycles to failure (<50), were in good agreement with the values we previously measured for legs and wings using simple monotonic loading tests [fresh legs 72.05±30.5 MPa (Dirks and Taylor, 2012a), wings 27.79±6.34 (Dirks and Taylor, 2012b)].

Cyclic tests demonstrated that fatigue failure occurs in both legs and wings, with the number of cycles to failure increasing as the cyclic stress was reduced (Fig. 2). Data from wing samples fell onto a sigmoidal curve (which is typical for many engineering materials), starting at small numbers of cycles near the UTS, and beginning to level off at very high numbers of cycles, approaching one million (R^2=0.88). The straight-line portion of the curve corresponds to the following equation relating the number of cycles to failure (N_f) to the stress range ($\Delta\sigma$):

$$N_f = \frac{C}{(\Delta\sigma)^m}, \quad (1)$$

where C and m are constants, the exponent m having a value of 12.6.

Data from the tibiae showed quite different results, with a much shallower curve, giving an exponent m=33.3 (R^2=0.44). As a result, the cuticle in the tibiae was able to withstand a much higher cyclic stress (in relation to its UTS) for large numbers of cycles than the cuticle in the wing. For example, at 100,000 cycles to failure, the average stress for the tibiae was 76% of the UTS, compared with 46% of the UTS for the wings. There was a considerable amount of scatter in the value of N_f for a given applied stress, which is quite common in other materials, especially when the slope parameter m is high. However, our results show that there is a significant fatigue effect in both materials, i.e. that the stresses for large N_f (greater than 100,000 cycles) are significantly lower than the UTS (14.34 MPa wings, t-test, t_{13}=12.04, P<0.001; 61.72 MPa tibiae, t-test, t_{10}=2.39, P<0.05). We were also able to confirm a statistically significant difference between the two materials at high N_f (t-test, t_{10}=16.16, P<0.001).

Control experiments with two leg samples and two wing samples immersed in water for 24 h before the test showed no significant difference in the number of cycles to failure compared with the other

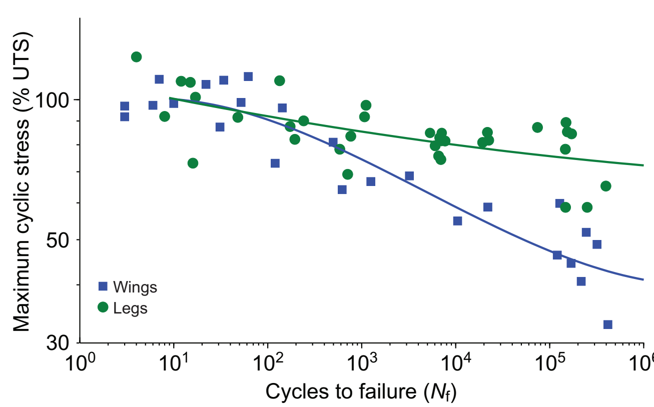

Fig. 2. Fatigue test results, showing the number of cycles to failure as a function of the maximum stress in the cycle, expressed as a percentage of the ultimate tensile strength (UTS). The lines present the best fit for a typical sigmoidal fatigue curve model (for details, see Results).

data, implying that immersion in water, in itself, does not change the fatigue behaviour.

DISCUSSION

The mechanical properties and the versatility of insect cuticle has fascinated and inspired biologists and materials scientists for several decades (Vincent and Wegst, 2004). Recently, insect cuticle has started to inspire the development of novel biomimetic materials (Bhushan, 2009; Fernandez and Ingber, 2012; Miessner et al., 2001; Vincent, 2008). However, we still know very little about its basic mechanical properties, such as stiffness, strength or toughness (Dirks and Taylor, 2012a; Klocke and Schmitz, 2011), and nothing about fatigue (Vincent and Wegst, 2004). To our knowledge, this study shows the first published fatigue data for insect cuticle.

The results of our experiments demonstrate that different body parts of the insect exoskeleton can show very different fatigue properties. There is no 'general' insect cuticle fatigue behaviour. Legs and wings of locusts have notably different behaviour when subject to cyclic loading.

Wing material failed by fatigue at stresses much less than the UTS. Limited by the biological 'stability' of the cuticle samples over time, we did not continue the tests beyond 400,000 cycles. Some materials show a distinct 'fatigue limit' around one million cycles, below which fatigue does not occur. Extrapolating the trend line through our data suggests that there may be a fatigue limit, but further work would be needed to confirm this. In any case, the material displayed fatigue failure at stress levels as low as 33% of the UTS. Though not completely unprecedented, this is unusual: most engineering materials will endure one million cycles or more at 50% of the UTS (Hertzberg and Manson, 1980; Stephens et al., 2001).

Much better fatigue endurance was displayed by the locust tibiae, which not only have a much higher UTS than the wings, but also will probably survive one million cycles at ~70% of UTS (extrapolating our data slightly; see Fig. 2). A likely explanation for this fatigue behaviour lies with the structure of the cuticle of the legs and wings. Cuticle in the tibiae contains a high proportion of chitin fibres, arranged in a complex layered pattern with a predominance of fibres lying parallel to the longitudinal axis of the leg (Neville, 1965). This ensures good resistance to the dominant type of loading, which is axial bending during jumping (Taylor and Dirks, 2012). This fibrous structure, like wood and some other natural materials, is very resistant to cracking when loaded parallel to its grain direction. This is an effective way to prevent the gradual crack growth which is the most common mechanism of fatigue failure. The same strategy is employed in fibre composite materials such as carbon fibre reinforced polymers, and gives rise to materials with a similarly small fatigue range (Hertzberg and Manson, 1980). Wing membrane cuticle, in contrast, is probably not reinforced with chitin fibres (Smith et al., 2000) and as a result, is weaker, more isotropic and less able to resist crack propagation. In a previous study we showed that cracks growing through this material in monotonic loading tests were hindered in their progress mostly by veins in the wings, which are spaced ~1 mm apart (Dirks and Taylor, 2012b). These veins probably also provide some resistance to fatigue, but evidently not as much as the fibrous, anisotropic structure of the tibial cuticle.

The locust tibia experiences its most strenuous type of loading during jumping, the mechanics of which has been investigated previously (Bennet-Clark, 1975; Sutton and Burrows, 2008). We used these results to estimate the ground reaction force and thus calculate the maximum stress in the tibia during jumping (Taylor and Dirks, 2012), obtaining a result of 42.2 MPa, which is 55% of the UTS. Such estimates are inevitably approximate in nature given the various sources of error involved. However, this figure coincides well with our fatigue results, which suggests that this material can withstand many repeated jumps at this stress level without failing.

In our fatigue tests we loaded the tibiae in cantilever bending, similar to the loading during jumping. This creates tensile stress on one side of the leg and compressive stress of similar magnitude of the other side. Two distinct modes of failure were observed during fatigue testing. The first was similar to the mode of fatigue failure found in many engineering materials: crack initiation on the tensile side, followed by gradual crack propagation before final fast fracture (Fig. 3A,B). The second observed mode of failure was buckling (Fig. 3C), which occurred on that part of the tibia loaded in compression. Buckling is a common mode of failure in thin-walled tubes; it is essentially an elastic mode of failure, which in this case probably occurred after some cycling, rather than instantaneously, owing to viscoelasticity in the material. Samples that buckled showed no sign of cracking, and *vice versa*. The two failure modes occurred in approximately equal numbers and the failure mode was not affected by the stress level or number of cycles to failure.

In a previous publication we presented a theoretical analysis that predicted that the locust tibia had evolved to an optimal design, in relation to its dimensions of length, diameter and thickness (Taylor and Dirks, 2012). One aspect of that analysis was the prediction that the two failure modes of tensile fracture and compressive buckling would be equally likely to occur. The present results are thus in agreement with our prediction.

So far very little is known about the actual stresses the wing membrane experiences during flight (Herbert et al., 2000). The wings are needed for long-distance migratory flights and, as repair mechanisms are likely to be very limited in insect cuticle (Lai-Fook, 1968), should be adapted to withstand high-cycle fatigue. During normal flight, the locust's wings can beat up to a frequency of 17.3 Hz (Weis-Fogh, 1956). To travel a 'typical' distance of 100 km at a speed of 3.85 m s^{-1} (Kennedy, 1951; Weis-Fogh, 1956), this adds up to 449,350 wing beats. Our results suggest that in order to survive cycles above 400,000, stresses would have to be less than 30% of the UTS, which is approximately 9 MPa.

Another physiological parameter affecting fatigue properties could be the presence of water, as hydration has been previously shown to strongly affect the biomechanics of cuticle (Dirks and Dürr,

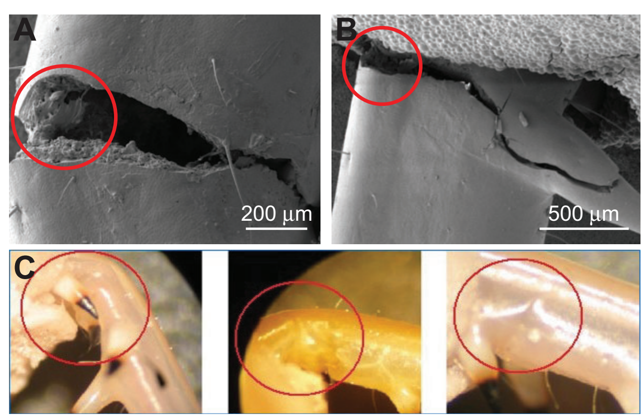

Fig. 3. Selection of failure modes of locust tibiae. (A,B) Scanning electron microscopy images showing failure by cracking on the tensile side of tibiae after fatigue testing. The circles indicate the area of crack initiation. (C) Evidence of failure by buckling on the compressive side of tibia samples.

2011; Dirks and Taylor, 2012a; Klocke and Schmitz, 2011). To prevent desiccation, we performed all experiments with fully submerged samples. Comparing the UTS of the submerged samples from this study with the previously measured values for 'fresh' leg and wing samples shows that submerging did not notably affect the mechanical properties of the samples. The performance of the 'pre-submerged' samples also excludes any notable effect of the long-term water immersion on the outcome of the experiment. The failure of the leg and wing samples observed in these tests thus was only a result of mechanical fatigue of the samples and not of desiccation.

Conclusions

This study has shown, for the first time, that insect cuticle suffers from fatigue failure when loaded repeatedly at stresses less than that required for instantaneous failure. Cuticle from two different body parts – wings and legs – behaved very differently, the wing material displaying a much wider range of stresses over which fatigue can occur. This difference might be explained in relation to the composition and structure of the two materials, especially the fibrous and strongly anisotropic nature of the leg material. Leg segments (tibiae) showed two different failure modes, showing an equal likelihood of failing in tension or compression. This finding may be useful in relation to the biomechanics of insects, because fatigue failure will limit the maximum stresses that these body parts can repeatedly sustain.

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AUTHOR CONTRIBUTIONS

J.H.D., E.P. and D.T. designed the study, interpreted the results and wrote the article. J.H.D. and E.P. performed the experiments and analysed the results.

COMPETING INTERESTS

No competing interests declared.

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