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Nanomechanical properties of wing membrane layers in the house cricket (*Acheta domesticus* Linnaeus)

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Abstract

Many insect wings change shape dynamically during the wingbeat cycle, and these deformations have the potential to confer energetic and aerodynamic benefits during flight. Due to the lack of musculature within the wing itself, the changing form of the wing is determined primarily by its passive response to inertial and aerodynamic forces. This response is in part controlled by the wing's mechanical properties, which vary across the membrane to produce regions of differing stiffness. Previous studies of wing mechanical properties have largely focused on surface or bulk measurements, but this ignores the layered nature of the wing. In our work, we investigated the mechanical properties of the wings of the house cricket (*Acheta domesticus*) with the aim of determining differences between layers within the wing. Nanoindentation was performed on both the surface and the interior layers of cross-sectioned samples of the wing to measure the Young's modulus and hardness of the outer- and innermost layers. The results demonstrate that the interior of the wing is stiffer than the surface, and both properties vary across the wing.

Keywords: wing, membrane, Young's modulus, hardness, nanoindentation

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1. Introduction

To sustain flight, many flying insects depend not only on the motion of their wings but also the wing deformations that occur over the course of the wingbeat cycle. These deformations may enhance lift or reduce drag according to computational modeling (Miller and Peskin, 2009; Mountcastle and Daniel, 2009; Vanella et al., 2009) and manipulation of bumblebee wings demonstrates that experimental stiffening reduces aerodynamic force production (Mountcastle and Combes, 2013). The twisting and bending of wing membranes can change the direction and magnitude of force produced, and in some species, the dynamic change in shape can produce a force asymmetry between half strokes (Young et al., 2009).

Unlike the wings of flying vertebrates, however, those of insects contain no internal musculature, limiting active control over wing shape to the flight muscles at the wing's base. Therefore, instead of arising solely from muscular actuation, much of the dynamic nature of the wing shape results from the passive response of the wing to both inertial and aerodynamic forces. This response partly reflects the architecture of the wing, in particular, the network of wing veins, and partly the inherent material properties of the wing. The network of veins is made up of longitudinal veins connected by shorter cross-veins. In wings with little depth out of the plane of the wing, or with few cross-veins, the longitudinal veins act like beams and resist bending and twisting. In corrugated wings with many crossveins, the longitudinal and cross-veins act together as a structural folded plate lattice. Veins are typically hollow tubular structures, of either circular or elliptical cross section; such tubular structures have a high moment of inertia relative to their mass, and in locusts, have been shown to increase wing fracture toughness by 50% by reducing crack propagation (Dirks and Taylor, 2012). The membranes are typically thin compared to the vein diameter. The membranes are typically thin compared to the vein diameter. The wing properties can vary across the wing, allowing it to achieve the complex threedimensional shapes that are observed during flight (Wootton, 1992).

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Understanding the mechanical properties of insect wings is essential to better understanding insect flight. The difficulty of performing accurate mechanical measurements on the small length-scale of the wing has limited tests until recently. Up until 2000, the only published measurement of the Young's modulus of the wing is 6.1 GPa for the calliphorid fly, although no information is given on how it was measured (Wainwright et al., 1976). Other early investigations of the mechanical properties of wing membrane focused on other properties (Reynolds, 1977; Elliott, 1981). The first well-documented measurements of the Young's modulus are those of Smith et al. (2000) who performed small-scale tensile testing on the hindwing of the desert locust. These tests found that the Young's modulus ranged between 0.73 GPa and 18.7 GPa at different locations on the wing. While this technique allows for measurements at the length-scale of the membrane cells, it is still limited to samples of 0.1-0.2 mm in size, preventing precise local measurements of properties.

The rise of nanoindentation as a characterization technique has led to even smaller scale measurements, allowing for testing at the micron and even nanometer scale. Nanoindentation allows for the measurement of both modulus and hardness in a single test and has already proved successful in assessing the mechanical properties of the wing membranes of a number of insects. Song et al. found the mean Young's moduli of the wings of a cicada (*Magicicada*) and a dragonfly (*Libellula basilinea* McLachlan) to be 3.72 ± 0.33 GPa and 2.85 ± 0.23 GPa (mean \pm s.d.), respectively, while the hardness values were 0.21 ± 0.72 GPa and 0.14 ± 0.04 GPa, respectively (Song et al. 2004; 2007).

Despite the advantages of nanoindentation, variations in the mechanical properties of the wing have so far been considered only in two dimensions, i.e., across the surface of the wing. There has been little consideration for the how they might vary into the thickness. However, as shown in Fig. 1, the wing is composed of two adjacent layers of integument that are further subdivided. On the interior side of these layers is a thin internal layer of epidermal cells, which produce the external cuticle that makes up the bulk of the wing (Chapman, 1998).

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Figure 1. Schematic of the half-thickness of the layered cuticle (not to scale); the full thickness includes a mirror image of the structure shown in the schematic. Cuticle is the main material that composes much of the wing exoskeleton and its structure is relatively well understood. In most areas of the body, it is divided into the external epicuticle and the internal procuticle. The epicuticle rarely exceeds 1.5 µm in thickness and is subdivided into the outer epicuticle (~18 nm) of polymerized hydrocarbons and the inner epicuticle (0.5-2.0 µm) of mostly proteins with polyphenols and lipids (Neville, 1975; Filshie, 1982). These proteins are often sclerotized, the process by which certain components of the cuticle are cross-linked. The hardness of the cuticle is linked to the degree and nature of the sclerotization (Sugamaran, 2010).

The procuticle is much thicker, sometimes up to a few millimeters, and is a composite material consisting of chitin microfibrils in a matrix of proteins (Vincent, 1980). In many regions of the body, the procuticle may further be partitioned into the hard, sclerotized exocuticle and the inner, soft endocuticle. An intermediate layer known as the mesocuticle may also be present. While the exact structure of the wing cuticle is unknown, Smith et al. (2000) found no evidence of chitin and, therefore, of procuticle in desert locust wings they studied. However, no further work on the mechanical properties of cuticle layers of insect wing has been performed.

Due to the differences in structure and composition between the layers of the wing, it is likely that each layer exhibits different mechanical properties. Indeed, in their indentation tests, Song *et al*

(2004) identified an increase in the material parameter P/S^2 (where P is the indentation load and S the contact stiffness) with indentation depth, although they attributed it to the effect of the substrate to which the wing was bonded. In this study, we focus on the mechanical properties of the forewing of the house cricket, *Acheta domesticus*, with respect to the different layers of the wing membrane. We used nanoindentation to investigate the reduced Young's modulus and hardness of both the surface and interior of the wing membrane, allowing us to determine differences between the layers of the wing.

2. Materials and Methods

2.1 Sample Preparation

House crickets, *Acheta domesticus*, were bought from Petco as adults and kept in a temperaturecontrolled environment at 23°C. Food and water were provided *ad libitum*. Before measurements were taken, seven crickets were sacrificed and the forewings removed and allowed to dry. Female crickets were studied since males use their forewings for sound production. Insect wings are known to lose mass rapidly due to dehydration (Mengesha et al., 2011), so the masses of three wings were measured and averaged as a function of time, as shown in Fig. 2. The initial average mass of the wings was 6.2 ± 0.2 mg. At first, the wings lose mass rapidly, but the mass stabilizes at 3 hours. Therefore, to reduce variation among measurements, all mechanical tests were performed between 3 and 48 hours after sacrifice. Although the mechanical properties will have changed due to dehydration, Song *et al.* (2007) found a 4% difference in the moduli and a 29% difference in the hardness between living and dead wing membrane; in both cases, the mean values of the living and dead wings were within one standard deviation of each other.



Figure 2. Drying profile of Acheta domesticus wings.

2.2 Scanning Electron Microscope (SEM) Imaging

Cricket wing membranes were observed and measured in an SEM (JSM-6610/LV, JEOL, Tokyo, Japan). To prepare surface samples for the SEM, the wing was attached to an SEM stub with carbon tape. Cross-section samples were prepared by cutting the wing with a razor and keeping it upright by lightly securing the base in a vice. Due to the nonconductive nature of these samples, they were sputter-coated with gold to a thickness of 5-10 nm and then observed in the SEM. Images were recorded and the layer thicknesses measured using the JEOL SEM software.

2.3 Roughness measurements

A Tencor P16 surface profilometer was used to measure the surface roughness of the wing membrane. Sections of membrane were adhered with double-sided tape to a glass slide with the dorsal side facing up. A 2 μ m stylus tip was used with 2 mg of force and a scanning rate of 50 μ m/s to measure linear scans of 1 mm in length. Scans were taken on the membrane cells of ten different wings, and the resulting profiles were analyzed by the Tencor software to produce arithmetic average and root-mean-square average roughness values.

2.4 Mechanical Testing

Mechanical tests were performed using a nanoindenter (Triboindenter, Hysitron, Minneapolis, MN, USA) with a Berkovich diamond tip. Cross-sectional samples for nanoindentation are typically prepared by mounting the specimen in epoxy. However, any epoxy absorbed by the wing may have an effect on the mechanical properties measured, so the ventral surface of the wings were first adhered to double-sided tape and then mounted on one side in epoxy so that the dorsal surface was still exposed. Each region of the wing to be tested was then sliced through the entire thickness with a razor blade into two sections. The section on one side of the slice was mounted with the dorsal surface facing upwards while the section on the other side was mounted with the cross-section facing upwards to allow for measurements of both the surface and interior properties in approximately the same location. A diagram of the prepared sample is shown in Fig. 3.



Figure 3. Cross-sectional diagram and SEM image of sample preparation.

Indentations were performed in controlled-displacement mode. To minimize substrate effects, no indent should go to a depth greater than 10% of the thickness of the material to be tested, but to compromise with the effects of surface roughness, surface indents were taken to a depth of 250 nm (18% of thickness of surface layer). Interior indents were also performed at a depth of 250 nm to avoid

indentation size effects and to allow for comparison with surface indent values. Surface indents were taken between 10 and 20 μ m from the edge to provide comparability with interior indents while avoiding edge effects. The center of the interior layer was found by measuring the cross-section thickness with the Triboscan software and identifying the interior layer midpoint for sampling. Each reported measurement is the average of the results of a line of 8-10 indents at the mid-plane, each separated from one another by approximately 5 μ m, as shown in Fig. 4.



Figure 4. Schematic of indent locations on surface and cross-sectioned samples.

3. Results

3.1 Microstructural Measurements

Scanning electron microscopy (SEM) images of the wing surface reveal micro-scale surface patterning. In particular, the dorsal surface of the wings of *Acheta domesticus* exhibits scale-like features as shown in Fig. 5. Surface roughness measurements give values of the arithmetic average and root-mean-square average roughness of 402 ± 78 nm and 532 ± 118 nm (mean \pm s.d.), respectively. Additionally, the SEM micrographs of the cross-section of the wing reveal three visible layers, here referred to as the dorsal, interior, and ventral layers. The mean thickness of the three layers was $1.33 \pm$

0.07 $\mu m,$ 4.60 \pm 0.25 $\mu m,$ and 0.99 \pm 0.35 $\mu m,$ respectively, and the mean total thickness of the wing

membrane was $6.94 \pm 0.23 \ \mu m$.



Figure 5. SEM micrographs of (a) surface and (b) cross-section of *Acheta domesticus* wing. *3.2 Mechanical Tests*

Fig. 6 shows a typical plot of load versus indentation depth measured in a nanoindentation test. These curves were analyzed using the Oliver-Pharr method (Oliver and Pharr, 1992) to determine the reduced Young's modulus and hardness; we refer to the reduced Young's modulus as the Young's modulus throughout the remainder of the paper.



Figure 6. Typical indentation response, plotting indentation force against indentation depth, showing the fit to the unloading data (thin curve) using the Oliver-Pharr method and the linear fit to the initial unloading (thin line).

The testing locations and corresponding Young's moduli measured from the seven wings tested are shown in Fig. 7. The Young's modulus of the interior layer range from 0.92 GPa to 3.98 GPa across the wing $(1.78 \pm 0.81$ GPa, mean \pm s.d.). The Young's moduli for the dorsal surface are significantly lower, ranging from 0.12 GPa to 0.62 GPa (0.37 \pm 0.14 GPa, mean \pm s.d., two-tailed *p* < 0.0001 for paired t-test of interior vs. surface). Additionally, measurements were taken at the same location in the lower middle portion of the wing for four different specimens (1, 2, 4, and 7), and these show substantial variation among individuals. The surface Young's moduli of specimens 1 and 7 are similar but nearly four times those of 2 and 4. The interior Young's modulus similarly varies over a wide range, from a minimum of 1.00 ± 0.66 GPa to a maximum of 2.29 ± 0.59 GPa.



Figure 7. Forewing micrograph composite showing locations and values of measurements of reduced Young's modulus (GPa) from seven wings. For each location, the top value corresponds to the surface measurement (mean +/- s.d.) and the bottom value corresponds to the interior measurement (mean +/- s.d.).

The values of the hardness determined through nanoindentation are shown in Fig. 8, with the locations from which they were measured. The range of the hardness values of the dorsal surface is 26.4

MPa to 108 MPa, with a mean \pm s.d value of 58.0 \pm 18.8 MPa. The hardness of the interior layer is comparable, with a range of 31. 5 MPa to 184 MPa and a mean \pm s.d of 82.4 \pm 47.7 MPa. Comparing these two sets of values with a paired t-test shows that the difference is again statistically significant, although with a much higher p-value of 0.027.



Figure 8. Forewing micrograph composite showing locations and values of measurements of hardness (MPa) from seven wings. For each location, the top value corresponds to the surface measurement (mean +/- s.d.) and the bottom value corresponds to the interior measurement (mean +/- s.d.).

4. Discussion

From the SEM images of the cross-section, we observe that the wing is composed of three layers, a thick interior layer and two thinner surface layers. This structure is similar to that observed in the dragonfly *Libellula basilinea* McLachlan (Song et al., 2007), although in the case of *Acheta domesticus*, the dorsal surface has scale-like features rather than columnar structures. These scales resemble those previously observed on the elytra of some beetles (Sun et al. 2012), and it is likely that they serve a similar purpose of increasing the hydrophobicity of the surface. Furthermore, the overall mean thickness of 6.94 µm is greater than that of the dragonfly (2.8 µm) and desert locust (3.71 µm) but less than that of

the cicada (12.2 μm) (Smith et al., 2000; Song et al., 2004; Song et al., 2007). There appears to be little correlation between body mass and wing thickness, with female crickets lighter than both the female desert locusts and cicadas (0.37g versus 2.0g and 0.59g, respectively) (Davey, 1954; Heckel and Keener, 2007).

The 1.33 μ m and 0.99 μ m thickness of the two surface layers suggests that they are the two epicuticle layers, which rarely exceed 1.5 μ m in thickness. While Smith et al. demonstrated that the desert locust wings lack chitin are thus composed entirely of epicuticle, the epicuticle layers are too thin to comprise the cricket wing in its entirety. Therefore, the interior layer, which makes up the majority of the thickness, is likely the procuticle. The presence of this layer in the cricket wings may account for the greater thickness of these wings than those of the desert locust.

Although intraspecific variation does not allow for direct comparison among locations on the wing, comparing surface and interior values at each measurement site demonstrates that the middle layer is stiffer than the dorsal surface at all anatomical locations. The mean value of the Young's modulus of the middle layer is 1.78 GPa, which falls on the low end of the 1-20 GPa range expected for sclerotized cuticle (Vincent and Wegst, 2004). This implies some degree of sclerotization, so it is likely that the exocuticle dominates the mechanical behavior of the procuticle, although it may be mediated by the endocuticle, if present.

The mean value of the Young's modulus for the dorsal surface is 0.37 ± 0.14 GPa, approximately 20% of the magnitude of the middle layer. Although this value falls below the typical values of sclerotized cuticle, which is unusual for heavily sclerotized epicuticle, it is still well above the range for soft cuticle of 1 kPa to 50 MPa (Vincent and Wegst, 2004), suggesting that some degree of sclerotization is likely present. The low value may be due in part to contributions from the cuticular wax layer, which can be around 110-130 nm in thickness (Lockey, 1959).

Unlike the measured Young's moduli, the hardness values of both the inner and outer layers display a wide range of values with extensive variation at some of the locations on the wing, with mean values of the hardness of the inner and outer layers of 82.4 ± 47.7 and 58.0 ± 18.8 MPa, respectively, putting them at around half of the hardness values observed in cicada and dragonfly wings. Although the middle layer is generally harder than the corresponding dorsal surface, there are a number of exceptions and both values tend to fall well within each other's uncertainties. Thus, no conclusion about a relationship between the surface and interior values can be drawn from these data. Although this differs from the clear trend in Young's moduli, we emphasize that moduli and hardness are different mechanical properties. Indeed, the stiffness of cuticle is correlated with the hydrophobicity of the matrix proteins whereas hardness is correlated with the degree of sclerotization of the cuticle (Hackman, 1974). Thus, the variation in hardness could arise from local variations in sclerotization. On the other hand, variation in the Young's moduli, especially in those values measured at the same location in different specimens, likely arises from different compositions of matrix proteins, which can vary among the epidermal cells that secrete them (Smith et al., 2000).

Additionally, variation may arise from the limitations of the nanoindentation technique. In nanoindentation tests, both the reduced modulus and hardness depend on the area of the indent, which may be affected by surface roughness of the specimen (Kim et al, 2006; Wang et al., 2013). However, if surface roughness is the primary cause of the variation we observed, we would expect more scatter in the surface measurements than in the interior measurements, as the interior specimens were cut with a razor blade. Instead, the coefficients of variation for measurements made on the inner and outer specimens are similar, with slightly higher values for the inner specimens. In addition, the scatter in Young's modulus is relatively low compared with that observed in many biological materials. Furthermore, the derivation of the equations used to calculate modulus and hardness from nanoindentation data assumes that the material is isotropic. Chitin fibrils within the procuticle may be highly oriented and confer substantial anisotropy, which might result in the nanoindentation technique we employed further underestimating true variation in modulus. However, beetle elytra possess

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anisotropic fibrous structure but are nonetheless functionally isotropic (Hepburn and Ball, 1973), so the isotropy assumption we employed may be reasonable.

The results of this study should be viewed as preliminary; we took measurements at only a limited number of locations in the membrane and none in the vein. However, the findings demonstrate that mechanical properties vary among wing layers, and a full understanding of the mechanical behavior of the wing requires improved understanding of this layered nature. Future investigations may continue to better characterize the mechanical properties of the individual layers, furthering our knowledge of insect flight.

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