Supporting Online Material for:

Mechanical Properties of the Beetle Elytron, a Biological Composite Material

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Supplement Contents:

S.1 Introduction

This supplementary material provides additional information about the physical properties of the beetle elytra examined in this study and their measurement. It also presents the tabulated data in bar chart form to help illustrate the trends in mechanical properties with tanning time, and to provide a comparison between *Tribolium castaneum* and *Tenebrio molitor*.

S.2 Physical Properties of Elytra

Electron Microscopy

Samples of fully tanned *Tribolium castaneum* elytra were imaged by scanning electron microscopy to identify the primary submillimeter features in the elytra being tested. Elytra samples from 5d-old adults were prepared for SEM by flash-freezing in liquid nitrogen and fracturing. The SEM samples were coated by the Technics Hummer II (Gold Source) sputter coater to achieve a thin ~10 nm conducting gold layer on the surface. Evenly spaced “ribs” that run in a straight line parallel to each other in the longitudinal direction of the elytra can be seen on the dorsal surface (Figure S1A). During static mechanical failure tests, it was observed that these structures interrupted tearing of partially tanned samples.

A cross sectional view of an elytron is shown in Figure S1B. The existence of multiple layers is clearly seen. The mechanical tests performed in this study used the entire elytra and thus the mechanical properties are the overall average properties of these different layers.

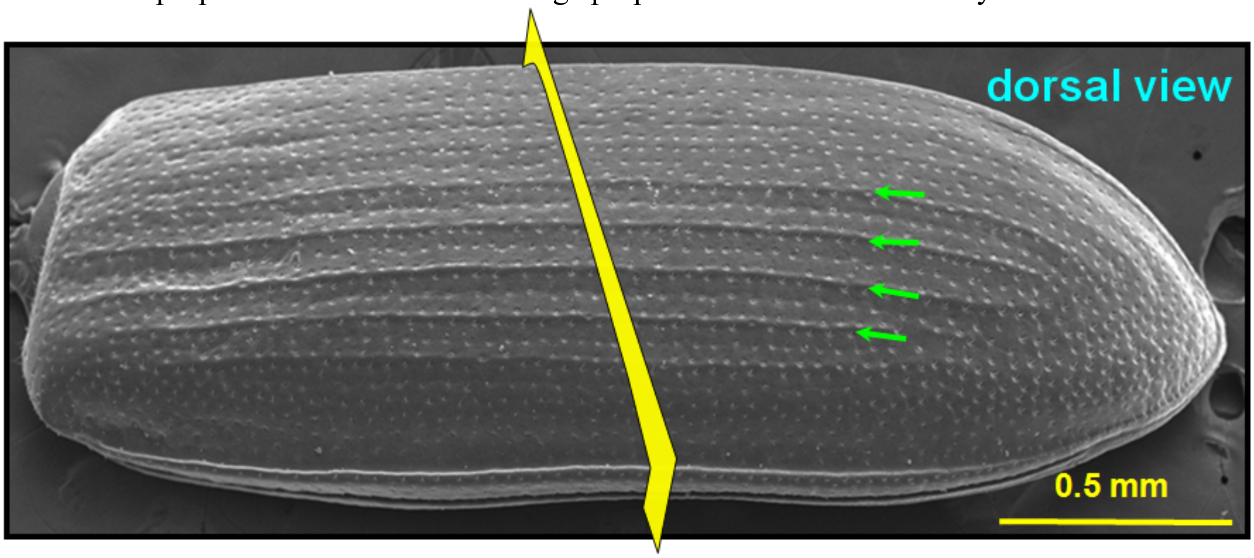

Figure S1A. Scanning electron micrographs of the dorsal view of an elytron from mature adults. Arrows indicate the rib structures on the dorsal surface.

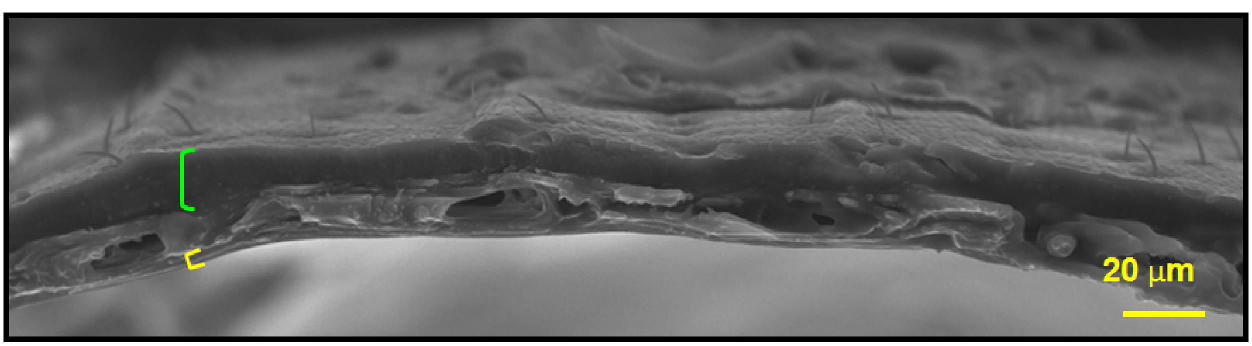

Figure S1B. Scanning electron micrographs of the cross-sectional view of an elytron from mature adults. Green and yellow brackets indicate highly tanned dorsal- and less tanned ventral cuticle layers, respectively.

Thickness and water content measurements

The thickness and water content of *Tribolium* and *Tenebrio* elytra from Tables 1a and 1b of the paper are shown below in bar chart form (Figures S2 and S3) to aid comprehension of the trends. These figures show that the elytra thicken upon tanning, and that even fully tanned elytra have a notable water content, which can be removed by drying in dry air or nitrogen.

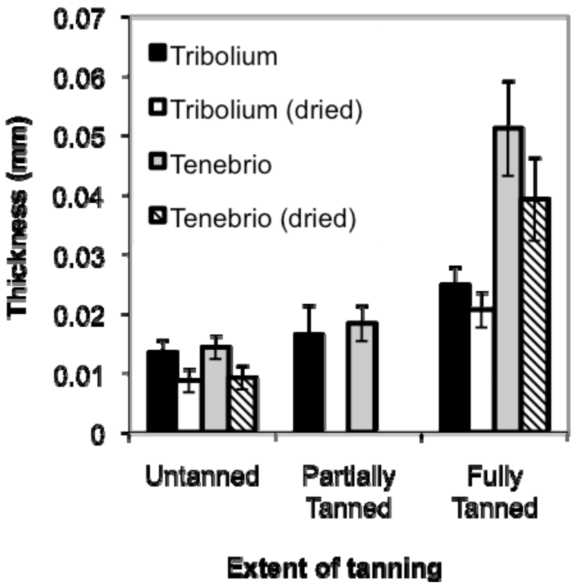

Figure S2. Thickness of *Tribolium* and *Tenebrio* elytra at different stages of tanning, measured by SEM.

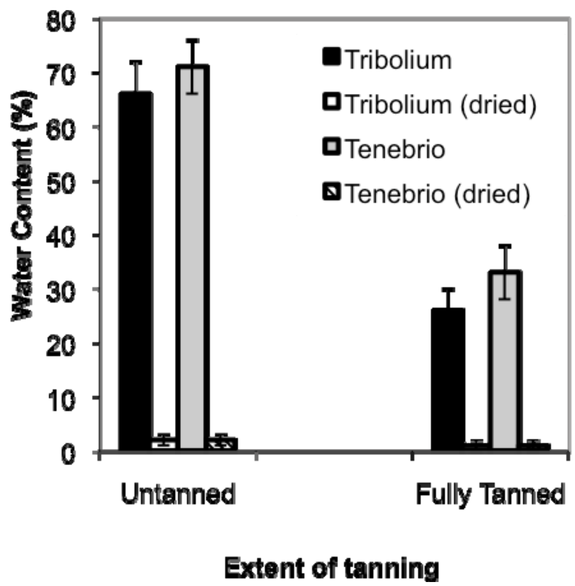

Figure S3. Water content of *Tribolium* and *Tenebrio* elytra measured by mass change upon drying.

S.3 Static Mechanical Analysis

To aid comprehension of the trends in the static mechanical properties, data provided in Tables 2a and 2b are plotted in bar chart form in Figures S4 – S7 below. These figures illustrate the trends in fracture strain, fracture stress, energy to failure, and Young's modulus, and are presented as a function of tanning for *Tribolium* and *Tenebrio* elytra.

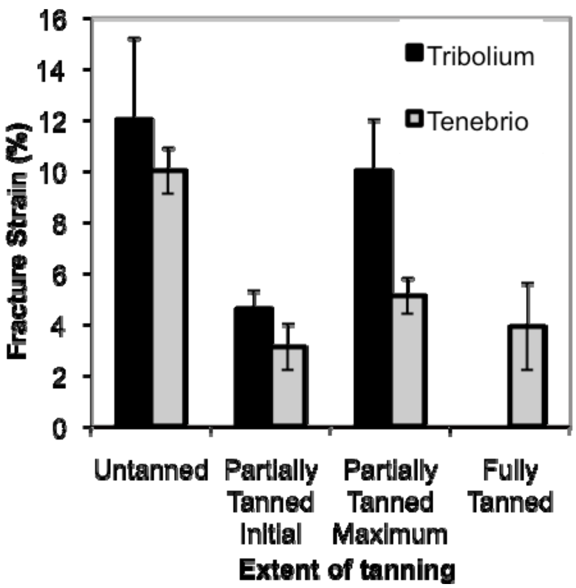

Figure S4. Fracture strain for *Tribolium* and *Tenebrio* elytra.

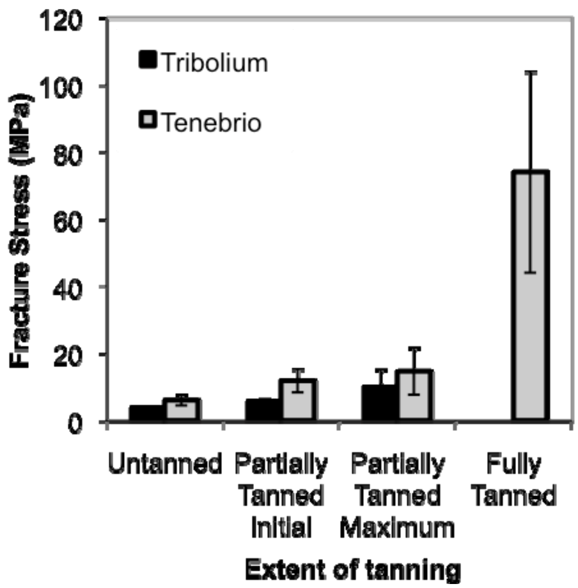

Figure S5. Fracture stress for *Tribolium* and *Tenebrio* elytra.

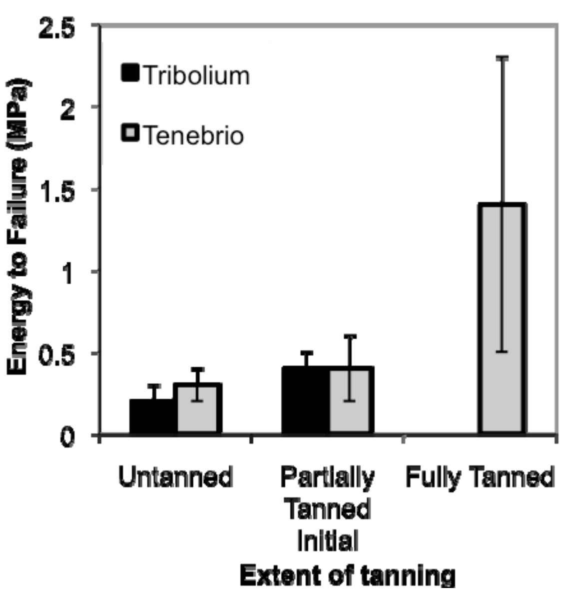

Figure S6. Toughness (work to failure) for *Tribolium* and *Tenebrio* elytra.

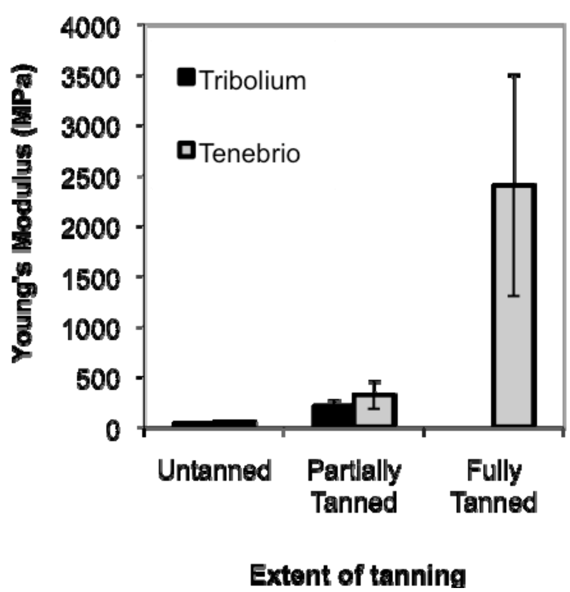

Figure S7. Young's modulus for *Tribolium* and *Tenebrio* elytra.

S.4 Dynamic Mechanical Analysis

Dynamic Mechanical Properties. The storage moduli, loss moduli and tan δ values from Tables 3a and 3b of the paper are presented in bar chart form in Figures S8 - S10 below to aid comprehension of trends seen in these data.

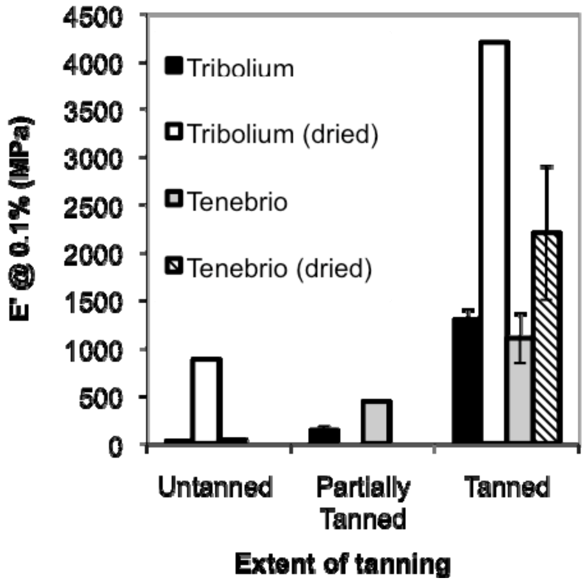

Figure S8. Dynamic storage moduli for *Tribolium* and *Tenebrio* elytra obtained from a strain sweep at 1 Hz.

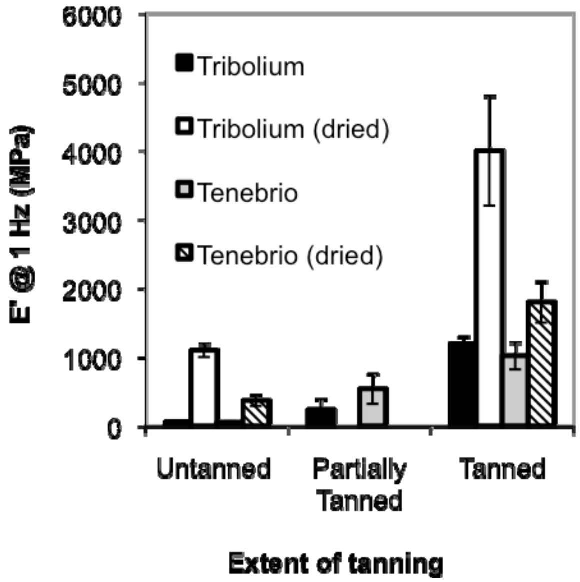

Figure S9. Dynamic storage moduli for *Tribolium* and *Tenebrio* elytra obtained from a frequency sweep at 0.1% strain.

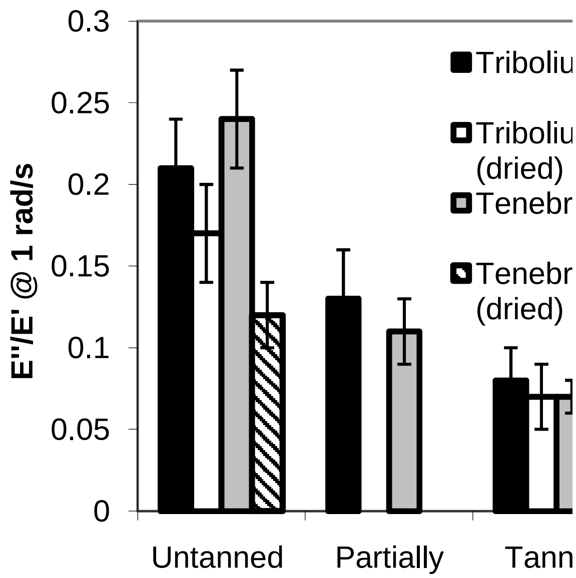

Figure S10. Tan δ (E"/E') values for *Tribolium* and *Tenebrio* elytra obtained from a frequency sweep at 0.1% strain.

S.5 Validation of Testing Methods

Reproducibility of Dynamic Mechanical Measurements
The dynamic mechanical property figures and tables in the paper present the averages of the measurement of multiple samples. Figures S11 and S12 provide an example of a complete data set obtained from frequency sweeps of elytra of six fully tanned, freshly harvested *Tribolium* samples of the same tanning stage. It is evident in Fig. S11 that while there is variation in the magnitude of the storage modulus, the dependence of the sample modulus on frequency is quite consistent. That is, the dependence on frequency is very reproducible. Thus, while the actual magnitudes of E' and E" tend to vary, the overall shape remains the same for the same type of elytra.

Figure S12 shows that the variability in tan δ is much less than in E'. As discussed in the paper, reproducibility of modulus is affected by the sample-to-sample variability in dimensions, while tan δ measurements are less affected by dimensional variability as tan δ is a ratio of the moduli E"/E' and as such, the actual cross-sectional areas cancel out. Other variations in sample dimensions such as curvature, naturally occurring specimen-to-specimen variability, and slight differences in sample preparation and mounting likely account for smaller variations observed in Fig. S12.

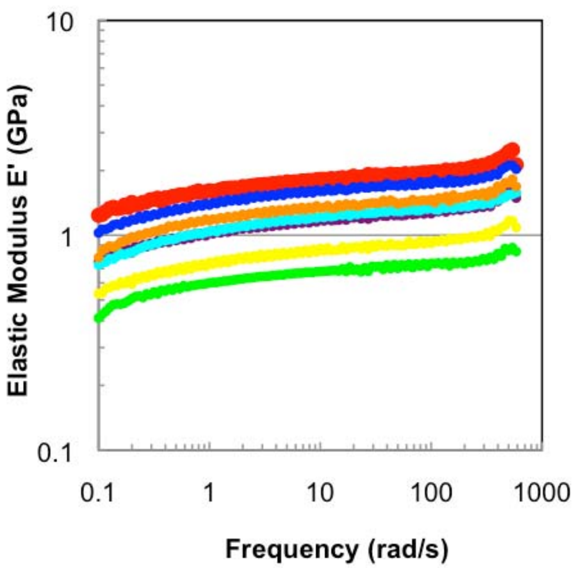

Figure S11. Six representative frequency sweep measurements of the elastic modulus at 0.1% strain of fully tanned, fresh *Tribolium* elytra. The magnitudes vary but the shapes of the curves remain constant.

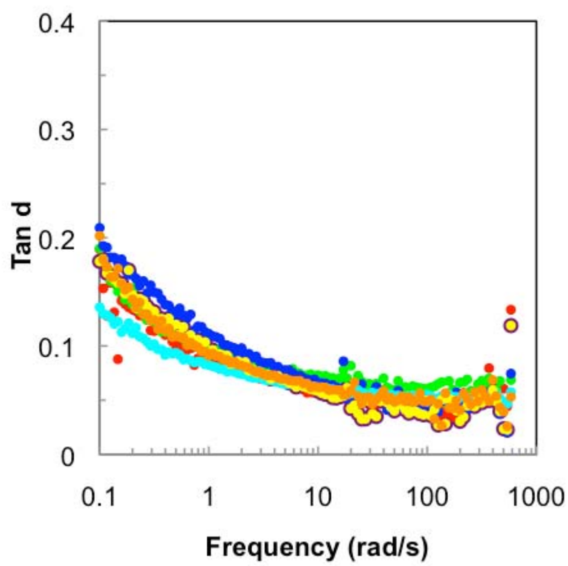

Figure S12. Six representative frequency sweep measurements of the viscous modulus to elastic modulus E"/E' (tan δ) at 0.1% strain of fully tanned, fresh *Tribolium* elytra. The values are reproducible.

Consistency between Testing Methods and Static and Dynamic Moduli

Figure S13 shows static data for fully tanned *Tenebrio*. The blue and green data are for dry samples and the red is for fresh samples. The two sets of data for dry samples were obtained from two different testing methods. One was gripped directly and tested immediately (green) and one was mounted to a test frame using epoxy (blue), as described in the methods of the paper. However, the stress strain curves of the two sets appear similar, and the Young's modulus, fracture stress, fracture strain and toughness are all the same within uncertainty.

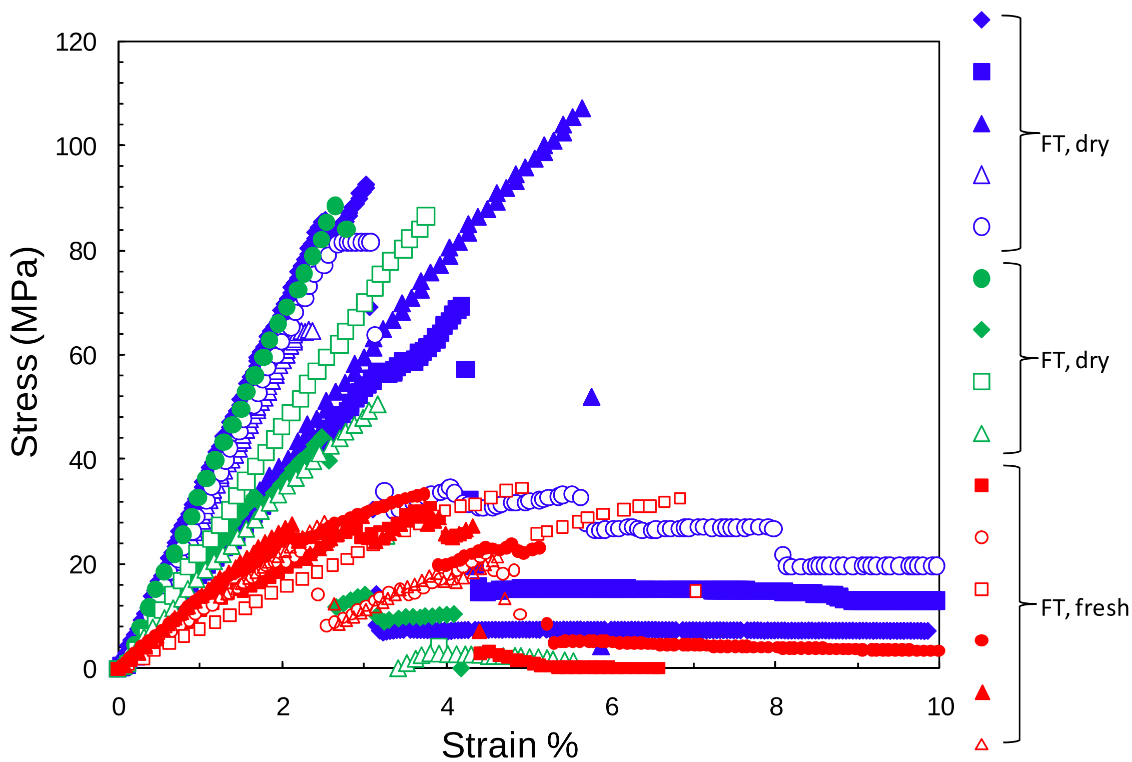

Figure S13. Static tests for fully tanned *Tenebrio elytra*. The blue and green data sets are dry elytra tested using 2 different mounting techniques. Results are comparable. The Young's modulus for each of these data sets matches the moduli obtained with dynamic testing.

For both the fresh and dry data in Figure S13, the Young's modulus values are also consistent with the E' values obtained from strain sweeps and frequency sweeps (see Table S1 below). The static data for fresh, fully tanned elytra (red data in Figure S13), were obtained by gripping the samples and testing immediately (within 5 minutes), and the modulus is comparable to the dynamic modulus which required up to an hour for testing. The comparison confirms that water loss due to lag time between mounting and testing the samples is negligible (see Table S1).

Table S1. Comparison of static and dynamic moduli for *Tenebrio*.

Elytral Sample	Young's Modulus *MPa*	E' (Strain Sweep) @ 1Hz, 0.1% *MPa*	E' (Frequency Sweep) @ 1Hz, 0.1% *MPa*
Untanned	51 ± 12	40	52 ± 13
Partially Tanned *(24 h)*	320 ± 140	440	540 ± 220
Fully tanned *(~7 d), fresh*	1000 ±210	1100 ± 260	1020 ± 200
Fully tanned *(~7 d), dry*	2300 ±810	2200 ± 700	1800 ± 300

Sample Integrity During Frequency Sweep Experiments

Frequency sweep experiments apply small strains in a sinusoidally varying fashion. To demonstrate that the elytra do not break down due to fatigue during testing or because of testing, successive frequency sweeps have been performed on the same samples. Figures S14 and S15 show the results of repeated tests on a fully tanned, freshly harvested elytron. A frequency sweep was run from 0.1 - 628 rad/s at 0.1% strain, which was then immediately repeated over the same frequency range. As seen in Figures S14 and S15, the E' and tan δ values overlap, indicating that the elytral structure remains intact over the tested range of frequencies. Other experiments have been run from low frequency to high and back to the starting low frequency with no evidence of significant hysteresis. Thus, the sample properties obtained during frequency sweeps appear unaffected by the test itself.

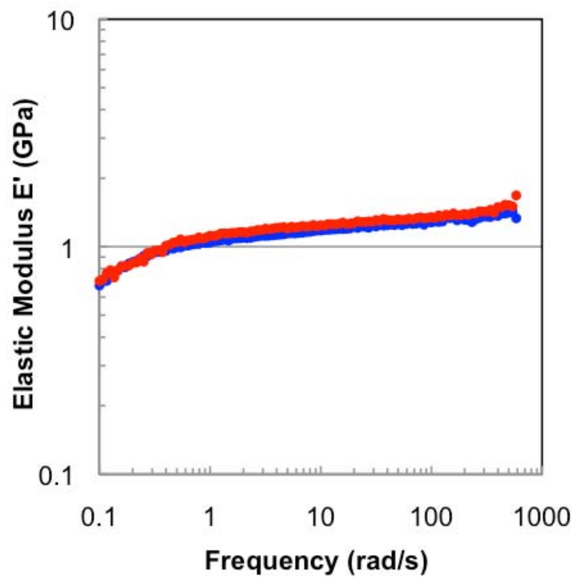

Figure S14. Two frequency sweep measurements of the elastic modulus at 0.1% strain of the same fully tanned, fresh *Tribolium* elytra. The curves overlap, indicating that the structure does not break down during the test.

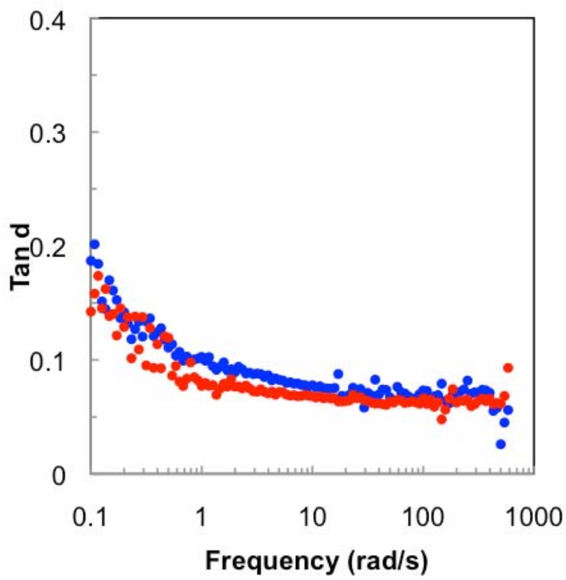

Figure S15. Two frequency sweep measurements of the viscous modulus to elastic modulus E"/E' (tan δ) at 0.1% strain of the same fully tanned, fresh *Tribolium* elytra. The values also overlap.