Dynamic Mechanical Properties of Resilin

by

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Abstract

Resilin is an almost perfect elastic protein found in many insects. It can be stretched up to 300% of its resting length and is not affected by creep or stress relaxation. While much is known about the static mechanical properties of resilin, it is most often used dynamically by insects. Unfortunately, the dynamic mechanical properties of resilin over the biologically relevant frequency range are unknown. Here, nearly pure samples of resilin were obtained from the dragonfly, *Libellua luctuosa*, and dynamic mechanical analysis was performed with a combination of time-temperature and time-concentration superposition to push resilin through its glass transition. The tensile properties for resilin were found over five different ethanol concentrations (65, 70, 82, 86 and 90% by volume in water) between temperatures of -5°C and 60°C, allowing for the quantification of resilin's dynamic mechanical properties over the entire master curve. The glass transition frequency of resilin in water at 22°C was found to be $10^{6.3}$ Hz. The rubber storage modulus was 1.6 MPa, increasing to 30 MPa in the glassy state. At 50 Hz and 35% strain over 98% of the elastic strain energy can returned each cycle, decreasing to 81% at the highest frequencies used by insects (13 kHz). However, despite its remarkable ability to store and return energy, the resilin tendon in dragonflies does not act to improve the energetic efficiency of flight or as a power amplifying spring. Rather, it likely functions to passively control and stabilize the trailing edge of each wing during flight.

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Introduction

The rubber-like protein resilin is a vital component of insect biomechanics from locomotion, to feeding and respiration. First described by Weis-Fogh (1960) in samples from the flight systems of locusts and dragonflies, resilin was shown to have remarkable mechanical properties. In 1960 Weis-Fogh subjected resilin to many different mechanical and chemical tests and found that resilin was not affected by creep or relaxation even after weeks of bearing load. Weis-Fogh (1960) also found that resilin could be strained up to 250% - 300% without breaking, but that its rubbery nature was dependent upon resilin's state of hydration and pH level. Since its first description, resilin has been found to be ubiquitous in insects, present in the salivary pump of assassin bugs (Edwards, 1960), the feeding pump of triatomid bugs (Bennet-Clark, 1963), the mechanism responsible for flea jumping (Bennet-Clark and Lucey, 1967), and the elastic element in the sound-producing tymbal mechanism in cicadas (Fonseca and Bennet-Clark, 1998). These examples demonstrate the functional diversity of resilin and also show the wide range of frequencies over which it is used. In the salivary pump and feeding pump of the assassin bug and triatomid bug, respectively, resilin is used as a spring antagonist to a muscle around a frequency of 3-10 Hz (Bennet-Clark, 1963, Bennet-Clark and Lucey, 1967). Locusts and dragonflies use resilin in conjunction with their wings, which beat at frequencies ranging from 15-50 Hz (Jensen and Weis-Fogh, 1962). A flea releases the energy stored storage in resilin for jumping in 1 ms and, though it is not a cyclic action, the rate of release can be equated to a frequency on the order of 500 Hz (Bennet-Clark and Lucey, 1967). Lastly, and probably most extremely, cicadas use resilin in creating resonant sound pulses at over 13 kHz (Fonseca and Bennet-Clark, 1998). While the presence and wide range of resilin functions in different insect species is a well reported topic, the dynamic mechanical properties of resilin have yet to be studied in detail.

The only published paper on the dynamic mechanical properties of resilin is inadequate for analyzing resilin over the frequency ranges presented above. Jensen and Weis-Fogh (1962) used dynamic mechanical analysis (DMA) on locust resilin and presented the viscoelastic properties of resilin within the biological range of a locust and beyond (10-200 Hz). Andersen and Weis-Fogh (1964) analyzed Jensen and Weis-Fogh's (1962) data and found that resilin begins to lose resilience at 100 Hz. According to viscoelastic theory, rubbery materials that are subjected to increasingly higher frequencies often show a reduction in resilience during the shift

from the rubber state to the glass state (Ferry, 1980). Although there are different equations used to express resilience, it is commonly defined as the ability of a rubber to store elastic energy. Here we were interested in the percent of the stored elastic energy returned by resilin, which is equal to the area under the unloading curve divided by the area under the loading curve of a force versus displacement graph. This is equal to:

$$R = 100e^{\left(-\frac{\pi}{2}\tan\delta\right)} \quad (1)$$

where *R* is percent resilience and tan δ is the ratio between the imaginary and real components of the complex modulus as defined by Ferry (1980). Andersen and Weis-Fogh (1964) showed a reduction of the resilience of resilin at 100 Hz and thus implied the beginning of its transition from rubber to glass. However, if this were true, a cicada would not be able to use resilin in a rubbery state at 13 kHz with a total energy loss of under 20% (Bennet-Clark, 1997). Resilin's dynamic mechanical properties need to be expanded beyond the work of Jensen and Weis-Fogh (1962), and Andersen and Weis-Fogh (1964) in order to better understand this discrepancy.

The technique of time temperature superposition principle (TTSP) is used for finding the dynamic mechanical properties of polymeric materials over multiple decades of frequencies with experiments that only range over 1-2 decades of frequencies. Temperature and time (or its reciprocal frequency) are usually varied in order to shift several trials of polymeric dynamic mechanical data into one long curve, called a master curve, that spans multiple decades of time/frequency (Ferry, 1980). The superposition principle has also been successfully expanded to include other techniques that allow the shifting of data, without varying temperature, but by varying diluent concentration, stress level, or polymer blending ratios (Brinson and Brinson, 2007). Knauss and Emri (1981) state that the time-multiplying factor *a* that allows data to be shifted into a master curve depends on temperature *T*, solvent concentration *c*, and mechanical dilatation θ.

$$a = a(T, c, \theta) \quad (2)$$

In addition, Doolittle expressed the shift factor in terms of the free volume by:

$$\log a = \frac{B}{2.303}\left(\frac{1}{f} - \frac{1}{f_o}\right) \quad (3)$$

where f is the fractional free volume in terms of the total volume, f_o is the fractional free volume at a reference condition and B is a material constant. Using equations 2 and 3, it is possible to conclude that fractional free volume must depend on the variables of temperature, solvent concentration and mechanical dilatation.

$$f = f(T, c, \theta) \quad (4)$$

Equation 4 suggests that one of the underlying principles behind superposition is that the time-multiplying factor for shifting data on a master curve is directly related to the amount of free volume in the polymeric material allowing for rheological motion (Knauss and Emri, 1981).

Understanding that the superposition principles are based on internal free volume, and not only on time and temperature, is important when working with bio-polymeric materials like resilin. Using only TTSP is difficult with resilin since its properties can only be tested between temperatures around 0 °C to a maximum of 80 °C. Resilin cannot be tested much below 0 °C because the water molecules inside the resilin matrix begin to freeze and the DMA is testing the properties of frozen water instead of resilin. Conversely, resilin cannot be tested above 80 °C as the chitin apodemes used to hold the resilin during testing begin to degrade. Since TTSP alone does not produce a master curve for resilin containing the full transition from a rubbery regime to a glassy regime, it was necessary to vary the hydration level of the resilin using ethanol solutions of varying concentrations. Using different values of frequency, temperature, and hydration level it was possible to force resilin from its usual rubbery state into the glass regime and better understand how the mechanical viscoelastic properties change during the transition.

The purpose of this paper is to determine the mechanical properties of resilin across a large range of frequencies and to evaluate resilin's role in the energetics and control of insect flight. In order to produce a material properties master curve for resilin, samples from the dragonfly, *Libellula luctuosa*, were subjected to dynamic mechanical analysis under varying conditions of temperature and hydration. Once known, these properties were used to evaluate the function of resilin in insect flight. One hypothesis is that elastic energy storage is essential to

reducing the energy cost of flight. Since resilin has such an incredible ability to return almost all of the strain energy input (Weis-Fogh, 1960), some have speculated that it must contribute, along with other elastic systems inside the thoraces of insects, to energy efficient flight (Weis-Fogh, 1972, Weis-Fogh, 1973, Alexander and Bennet-Clark, 1977). On the other hand, several researchers question the need for elastic storage during flight at all (Dickinson & Lighton, 1995, Wakeling & Ellington, 1997, Dudley and DeVries, 1990) stating that the work of decelerating the wing does not need to be stored in elastic elements but instead could be used directly as a source of aerodynamic power during the second half of each stroke. This paper will propose an alternate hypothesis that resilin is used to passively control and stabilize the wing trajectory during flight.

Methods

Dynamic mechanical analysis

Libellua luctuosa dragonflies were collected in and around the Blacksburg, Va (VADGIF permit #: 037528) in the summer of 2009. Intact specimens were frozen and maintained at -20 °C until dissected. The nearly pure (Andersen, 2003) resilin sample tested in this experiment is from a sausage-like tendon inserted in the apodeme of the muscle IIIpm3 (muscle nomenclature from Hatch, 1966). Identifying where the resilin tendons are and dimensioning them was done using a Leica Microsystems Inc. M165FC microscope with the Leica Application Suite software. Seven individual samples were tested with a mean length of 0.307 mm (±0.018mm) and mean cross-sectional area of 0.00934 mm^2 (±0.00152 mm^2). Filter set UV for Leica MZ16 F/FA was also used to make it easier to correctly identify resilin, since resilin was found to fluoresce under UV light by Weis-Fogh (1960). Each dragonfly has four samples of resilin tendons, one at the base of each wing, but only the fore wing resilin tendons were used since their long chitin apodemes made them easier to attach to the DMA setup (Figure 1). Resilin samples that were not immediately tested were either dried and refrozen or placed in a bath of 0.02% NaN_3 (w/v). Both of these preservation methods helped reduce the possibility of bacterial degradation while awaiting testing.

After removal from the dragonfly the tendon was dimensioned at its rest length in water and then dried. Since both ends of the fore resilin tendon are attached to very stiff chitin apodemes, either end could then be glued (Loctite Super Bonder 409) to the tip of an insect pin. As chitin's stiffness is roughly 20 GPa and resilin's is around 1 MPa it was assumed that any changes in elongation during testing was transferred solely to the resilin and not into the chitin apodemes (Ker, 1977, Weis-Fogh, 1961). Figure 1 shows both the fluorescence of resilin under UV light and the chitin apodemes on either end of the tendon that were used for gluing.

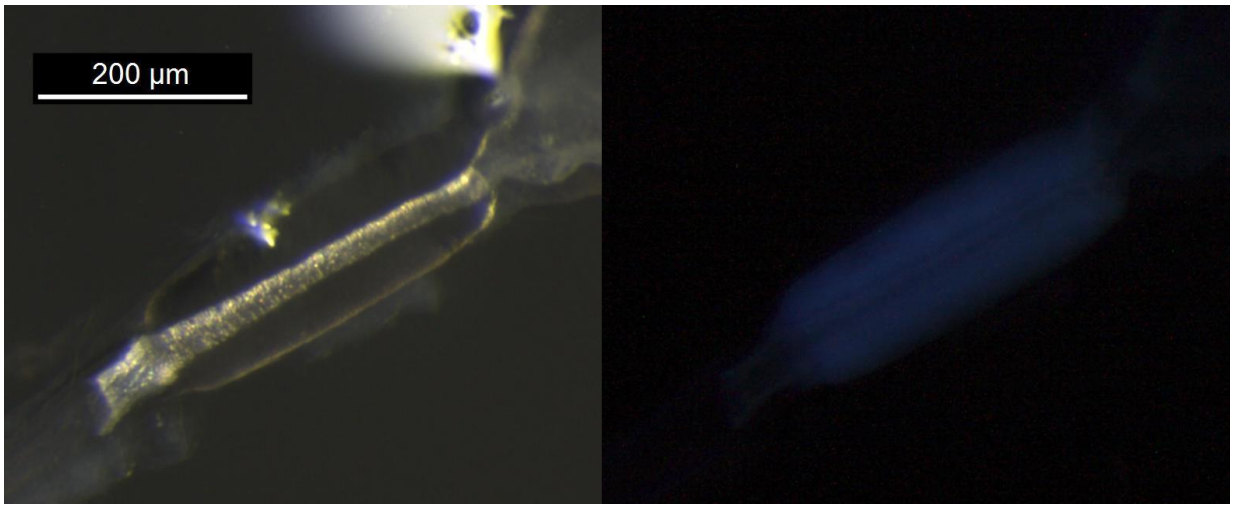

Figure 1: *(Left)* Picture of a fore dragonfly resilin tendon in water, note the chitin apodeme at either end of the sample used for attachment to the DMA setup. *(Right)* The same tendon glowing blue under UV light. The hollow tube down the center of the resilin is lined with tightly bunched epithelial cells arranged like an accordion to not break when resilin is stretched up to 300%.

The insect pin, with the resilin attached, was then clamped to the shaft of an electromagnetic oscillator (V203) from LDS Test and Measurement, Royston, UK. The mounting process was finished once the free end of the resilin was glued to an AE801 force gauge from Kronex Technologies Corp., Oakland, CA (linear N/V= 0.035, R^2= 0.99, Resolution= $1.19*10^{-4}$ mV). The tendon was then extended to a pre-strained of approximately 35%.

The dynamic mechanical properties of the resilin were then measured by driving the oscillator with a custom swept sine LabVIEW program (Nation Instruments, Austin, TX) and recording the resulting displacement and induced forces with a simultaneous sampling data acquisition card (PCI-4461, Nation Instruments). Strain was around 2% peak to peak for all samples from the rubbery zone through the glass transition and beyond. Displacement was measured using a custom transducer attached to the oscillator shaft via a stainless steel cantilever beam with two strain gauges bonded in a half bridge configuration. The output of the displacement gauge was a linear 7.01 mV/µm (R^2=0.99) with a resolution of $1.19*10^{-4}$ mV and a natural frequency of 528 Hz. Both the displacement gauge and force gauge signals were amplified through a Vishay Micro-Measurements Strain Gauge Conditioner (2120B). A diagram of the experimental setup can be found in Figure 2.

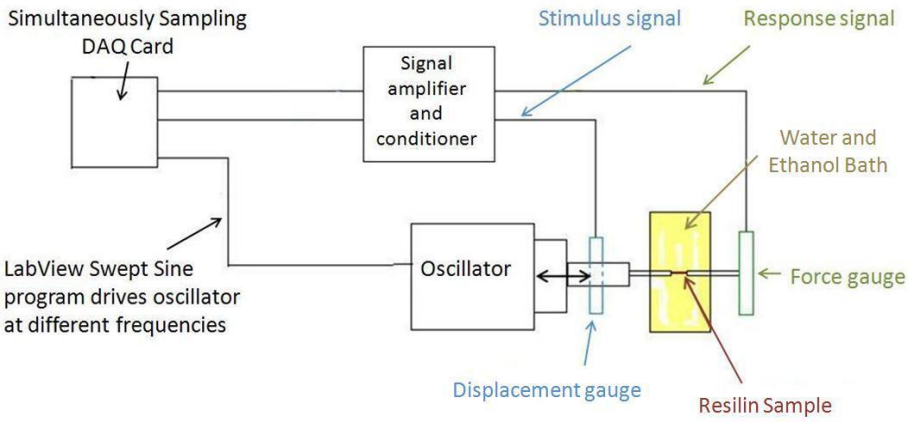

Figure 2: Diagram of the dynamic mechanical experiment setup. The LabView program both drives the oscillator and converts the outputs of the force and displacement gauges into viscoelastic moduli.

Simultaneously sampled data from both the displacement and force gauges were used to find the frequency response of the complex modulus and phase between the two signals. This data was used to find the viscoelastic properties of storage modulus, loss modulus, and tan δ over the frequency (ω) range of the test, 10 to 140 Hz (Ferry, 1980). For this experiment we defined the frequency of glass transition or the temperature of glass transition (T_g) as where the peak of the tan δ curve appears and equates to the point at which resilin is the least resilient.

Data was collected with three varying parameters: frequency, temperature and ethanol concentration. Temperature was controlled using a LAUDA RE206 temperature bath with a resolution of 0.1 °C and recorded using a Honywell S&C NTC bead thermistor calibrated to the temperature bath. All ethanol concentrations were measured as a percentage of total volume in combination with distilled water. The ethanol data was collected from an average of 19 different ethanol concentrations ranging from 0% ethanol (pure water) to 100% ethanol (catalog number: A405F, Fisher Scientific). All ethanol tests were performed at room temperature (22 °C) and were referred to as the ethanol sweep data. The temperature data was collected at up to 15 temperatures ranging from -5 and 60 °C at constant ethanol concentrations and was referred to as the temperature sweep data. The temperature was increased in increments of roughly 4 °C between each test and ample time was allotted between tests for the tendon to equilibrate to each

new temperature. A total of seven individual resilin tendons were exposed to an ethanol sweep as well as 3-6 temperature sweeps.

Resilin elongation *in vivo*

In order to estimate the elongation of resilin during flight it was necessary to be able to view resilin's attachment points as the wings were manually manipulated. Four *L.luctuosa* were carefully dissected to remove the exoskeleton without damaging the resilin and muscles of interest. This allowed both the fore and hind resilin tendons to be viewed inside the dragonfly while keeping all the internal muscles and tendons intact. With the dragonfly resilin exposed, both the fore and hind wing were articulated over the range of an average dragonfly wing stroke and the total length of the resilin, muscle and apodeme, referred to as the muscle tendon unit (MTU), from origin to insertion was recorded. Since resilin became difficult to view during certain angles of wing manipulation the estimate of resilin's elongation was assumed to be the difference between the passive muscle and apodeme length compared to the total MTU length at the manipulation angle. The elongation of both the fore and hind resilin samples was estimated in this way along with an additional assumption that passive muscle length could be contracted a maximum of 15% during flight (Weis-Fogh and Alexander, 1977).

Results

Dynamic mechanical analysis

For each DMA trial, consisting of various combinations of ethanol and temperature, the storage modulus, loss modulus and tan δ were calculated and plotted against frequency on a log-log plot. Using the principles of time-temperature and time-concentration superposition the results showed that the viscoelastic properties of resilin are affected by frequency, temperature and ethanol concentrations.

Temperature shift

Two kinds of master curves were obtained through the experiments, a temperature master curve and a hygral master curve. Five different ethanol concentrations (65, 70, 82, 86 and 90%) were selected for trials where a swept sine frequency input was run at temperatures between -5 °C and 60 °C. Each trial was analyzed using superposition. As an example, the data for 65% ethanol (or 35% water) is presented below, but data from all ethanol concentrations showed similar trends. Figure 3 shows twelve storage and loss modulus curves collected from the ethanol concentration of 65% over a range of temperatures from -2 to 42 °C.

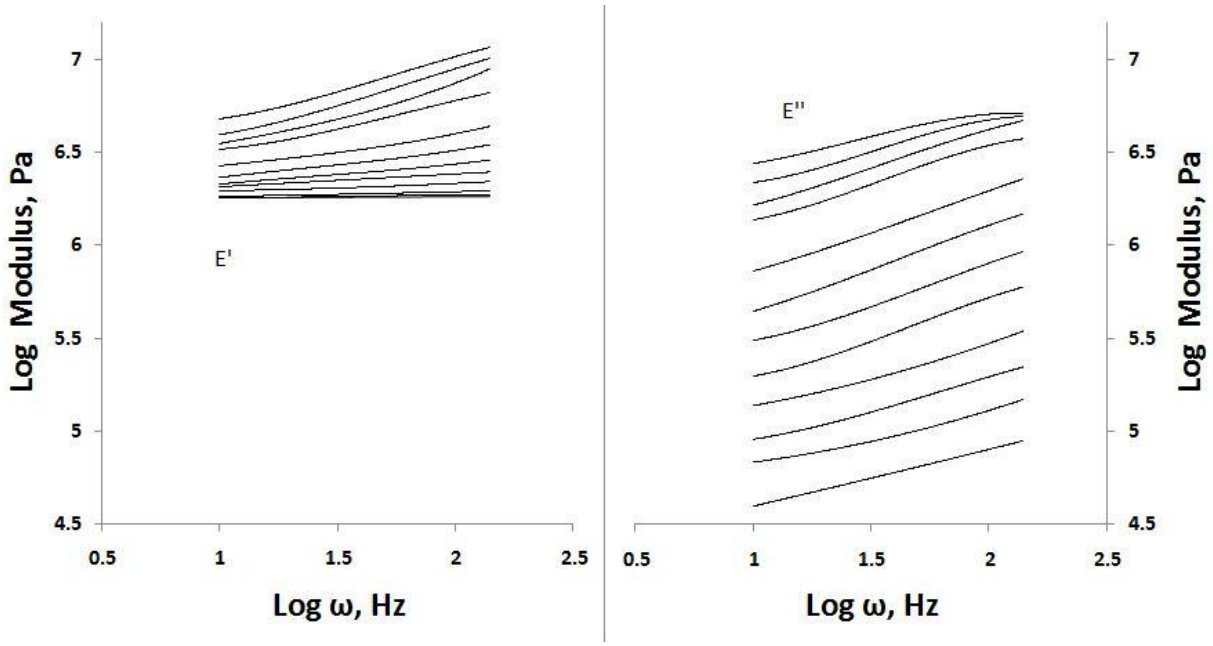

Figure 3: Storage (E') and loss modulus (E'') data for resilin in 65% ethanol (by volume in water), -2 °C is at the top and 42 °C is at the bottom.

It is clear that the viscoelastic properties of resilin changed significantly with temperature over the range of the frequencies tested. The storage modulus starts in the rubber regime with a value around 1.6 MPa and moves partially through the glass transition to a reach a final value of 11.6 MPa. The different data sets from Figure 3 were then used to estimate a temperature master curve at 65% ethanol. Each individual curve was shifted horizontally along the log frequency axis a distance a_T until they overlapped into one long master curve. This was accomplished by satisfying the two main requirements of Ferry's method of reduced variables (1980). The first requirement is that the storage modulus, loss modulus and tan δ are simultaneously shifted to ensure overlap with all three respective adjacent temperature curves, thereby guaranteeing a more accurate estimation of a_T. The second is that the temperature dependence of a_T must have a reasonable form consistent with experience, in this case the Williams-Landel-Ferry (WLF) equation (Ferry, 1980). Using 22 °C as the reference temperature (log a_T = 0) a plot of shift factor versus temperature was made to illustrate how far each temperature shifted the data (Figure 4).

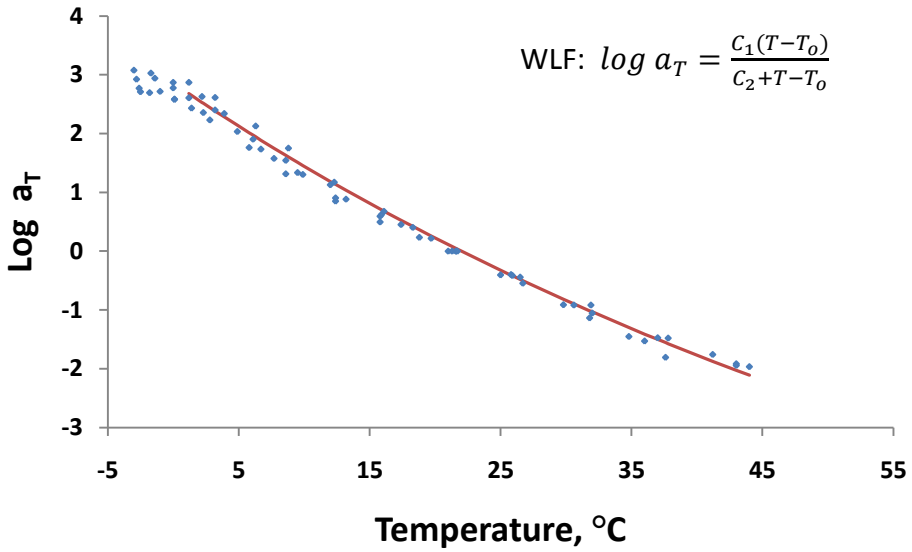

Figure 4: Temperature shift factor a_T plotted against temperature for Figure 3 along with all 65% ethanol trials (blue dots). The WLF equation is displayed and fit to the data (red line) with C_1 = 16.1, C_2= 145.8 and T_o= 22 °C.

The data from Figure 4 shows not only the relation between shift factor a_T and temperature but also the fit of the WLF equation. The WLF fit is sufficient to estimate the values of log a_T until it approaches temperature values close to the glass transition temperature of resilin in 65% ethanol. At this point some of the assumptions of WLF are no longer valid. The master

curve based on 65% ethanol at 22 °C shows the behavior of resilin from $10^{-0.8}$ to $10^{4.8}$ Hz, (Figure 5).

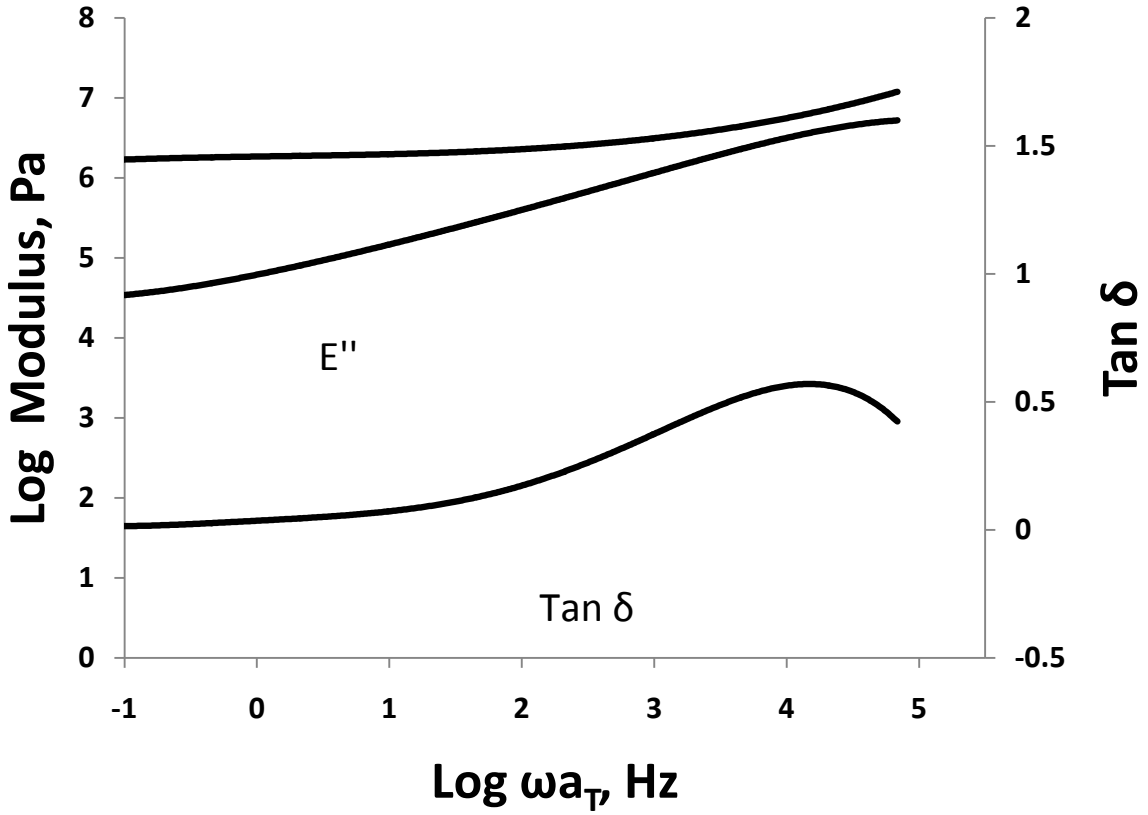

Figure 5: Master curve for resilin in 65% ethanol with E', E'' and tan δ (damping) based on data from Figure 3 and 4, 22°C reference temperature.

The process described above, for generating a master curve, was used for all temperature sweep trials at each of the different ethanol concentrations. The master curves generated for the other four ethanol concentrations matched the shape and viscoelastic values measured at 65%, except that as ethanol concentration increased the resulting master curves shifted horizontally left along the frequency axis. For comparison of all ethanol concentrations values of C_1, C_2, T_o, frequency of glass transition and the temperature range can be found in Table 1, the universal WLF constants can also be found here.

Table 1: The WLF constants established for the different ethanol concentrations along with the range of temperatures used during testing and frequency of glass transition.

% Ethanol	Temp. Range (°C)	C_1	C_2	Reference Temperature T_o (°C)	Frequency of glass transition (Hz)
65	-3 to 44	16.1	145.8	22	$10^{4.1}$
70	-5 to 47	16.1	135.2	22	$10^{3.6}$
82	1 to 57	14.1	74.4	22	$10^{1.1}$
86	17 to 56	27.7	123.5	22	$10^{-0.7}$
90	18 to 56	28.4	90.9	22	$10^{-2.5}$
Universal WLF constants C_1 = 17.4 C_2 = 51.6 (Ferry, 1980)					

Hygral shift

The same method used to find the temperature shift factor a_T was then used to find the hygral shift factor a_C, using ethanol sweeps at a constant temperature (22 °C), (Figure 6). The hygral shift factors, a_C, were minimal for ethanol concentrations of between 0% and 20%. From 40% ethanol and above the shift factors began to rise until finally leveling off at log a_C =10 at 100% ethanol. Figure 7a shows the collected data from the seven different resilin tendons tested, while Figure 7b shows the averaged master curve from all ethanol sweeps.

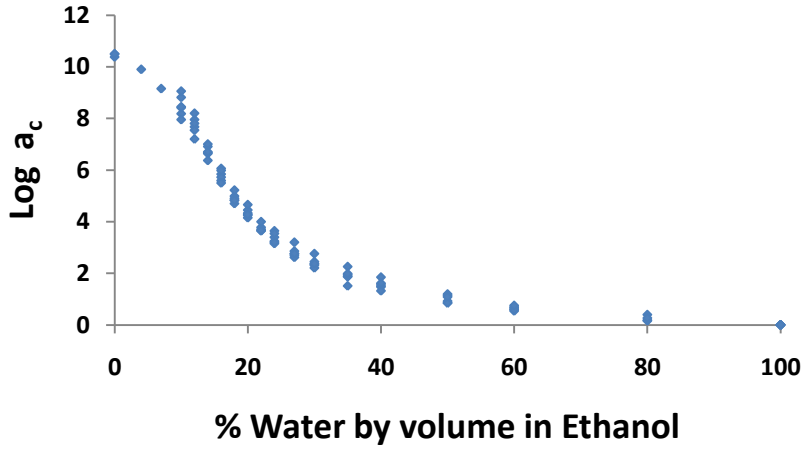

Figure 6: Hygral shift factor a_c plotted against % Water concentration for all trials, reference ethanol concentration of 0% (100% Water concentration)

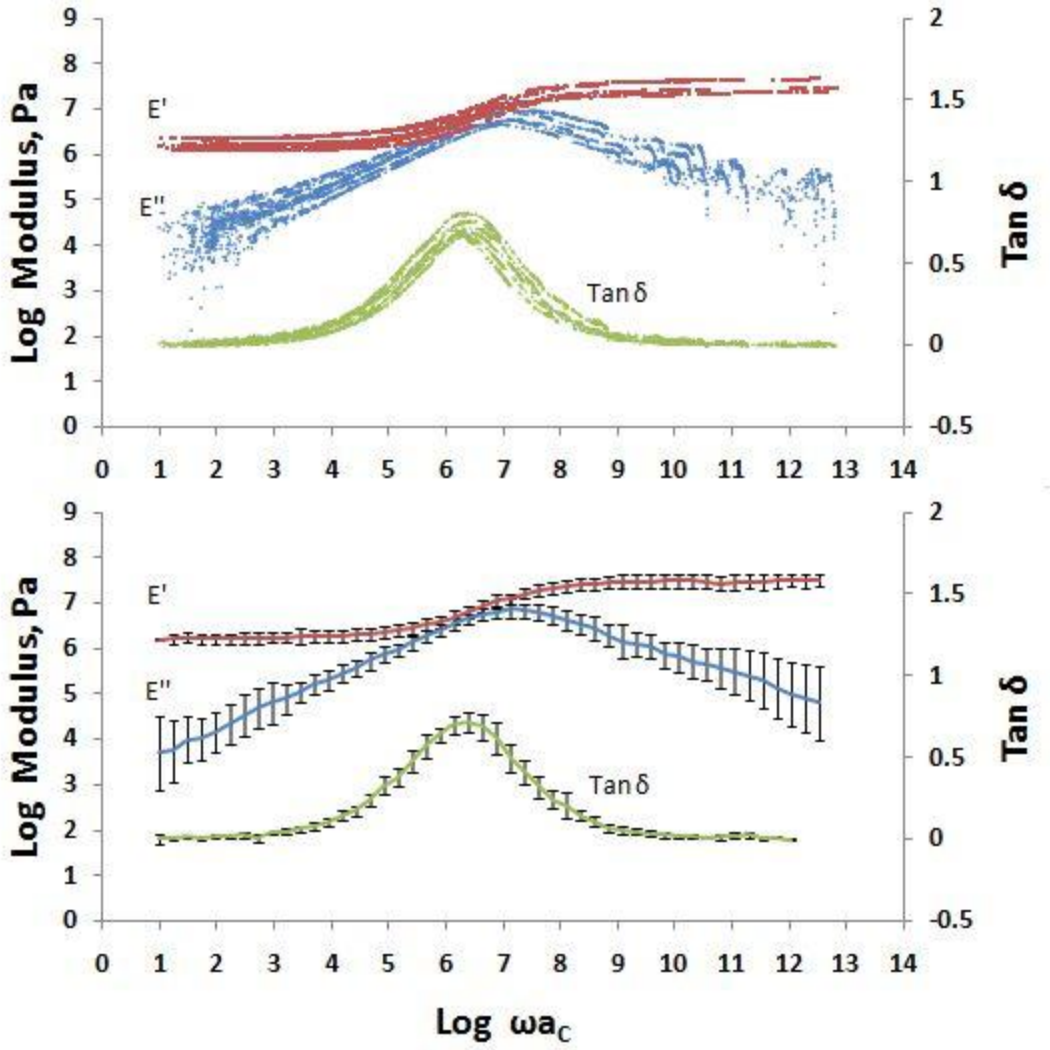

Figure 7: (a) Storage and Loss modulus with Tan δ for all seven resilin tendons during ethanol sweep. (b) Master curve obtained by averaging trials with standard deviation error bars. Both have a reference ethanol concentration of 0% and 22 °C.

Figure 7b represents the full range of material properties of resilin at 22 °C in water, from frequencies of around 10 to 10^{12} Hz. The glass transition frequency of resilin is around $10^{6.3}$ (~2 million Hz). The curves in Figure 7b can be used to predict any of the viscoelastic properties if the total shift, log $a_C a_T$, is known. The total shift for five different ethanol concentrations was calculated by adding the measured values of log a_C from the ethanol sweeps to each value of log a_T from the temperature sweeps. The relationship between the total shift factor for different ethanol concentrations as a function of temperature is show in Figure 8.

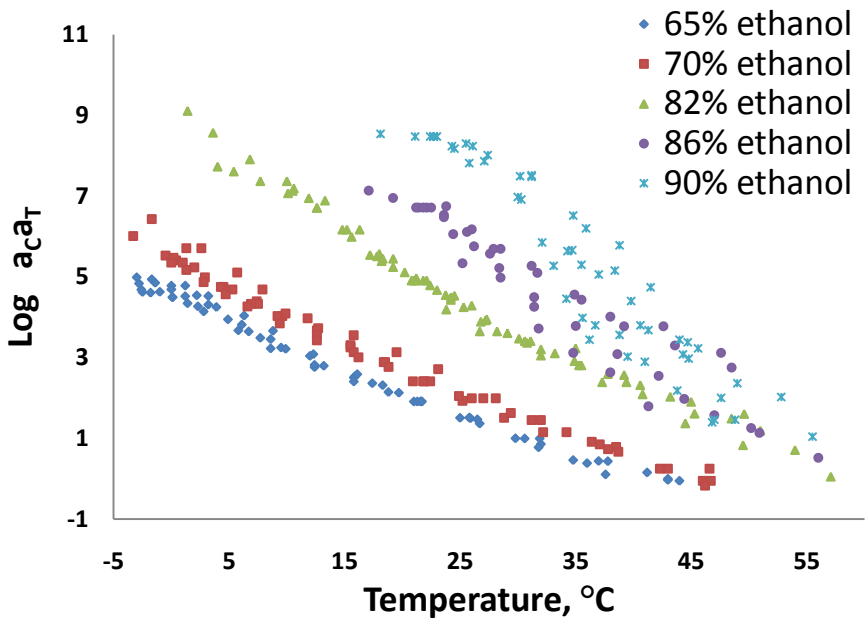

Figure 8: Total shift factor $a_C a_T$ plotted against temperature for five different ethanol concentrations.

On inspection of Figure 8 it is evident that the lower concentrations of ethanol produced minimal scatter occurred over all trials, while the higher concentrations had a much greater degree of scatter. This issue will be addressed later in the discussion section of the paper. The total shift factor follows an overall trend of reducing with temperature, but the higher level of ethanol concentration seems to have a much greater effect on shift factor over a smaller range of temperatures. For example, the total shift factor for 65% ethanol goes from a value of log $a_C a_T$ = 5 down to 0 over a range of 50 °C, whereas the total shift factor for 90% ethanol begins at log $a_C a_T$ = 9 and reduces around 2 over a range of 35 °C.

Isoshift factors

The data from Figure 8 was re-plotted into an isoshift factor plot, where each line represents the integer values of log $a_C a_T$ from 1 to 8, (Figure 9). The isoshift factor plot estimates the total horizontal shift for different combinations of ethanol concentration and temperature. The isoshift curves from Figure 9 can also be used with frequency to represent all the viscoelastic properties. For example, if isoshift curve 1 were assigned a frequency of 1 Hz (i.e. log f$a_C a_T$ = 1) the storage modulus would have a value of 1.6 MPa from Figure 7b. In the same way, if isoshift curve 3 was assigned a value of 100 Hz, and curve 5 a value of 1 Hz, both curves would represent a storage modulus of 12.6 MPa. The same would be true if curve 1 were assigned a value of 1 MHz and curve 8 a value of 1 mHz. Ultimately the surface created by Figure 9 can be used to define how the three different parameters (ethanol concentration, temperature and frequency) can be combined to represent unique values of the storage modulus, loss modulus or tan δ over the master curve.

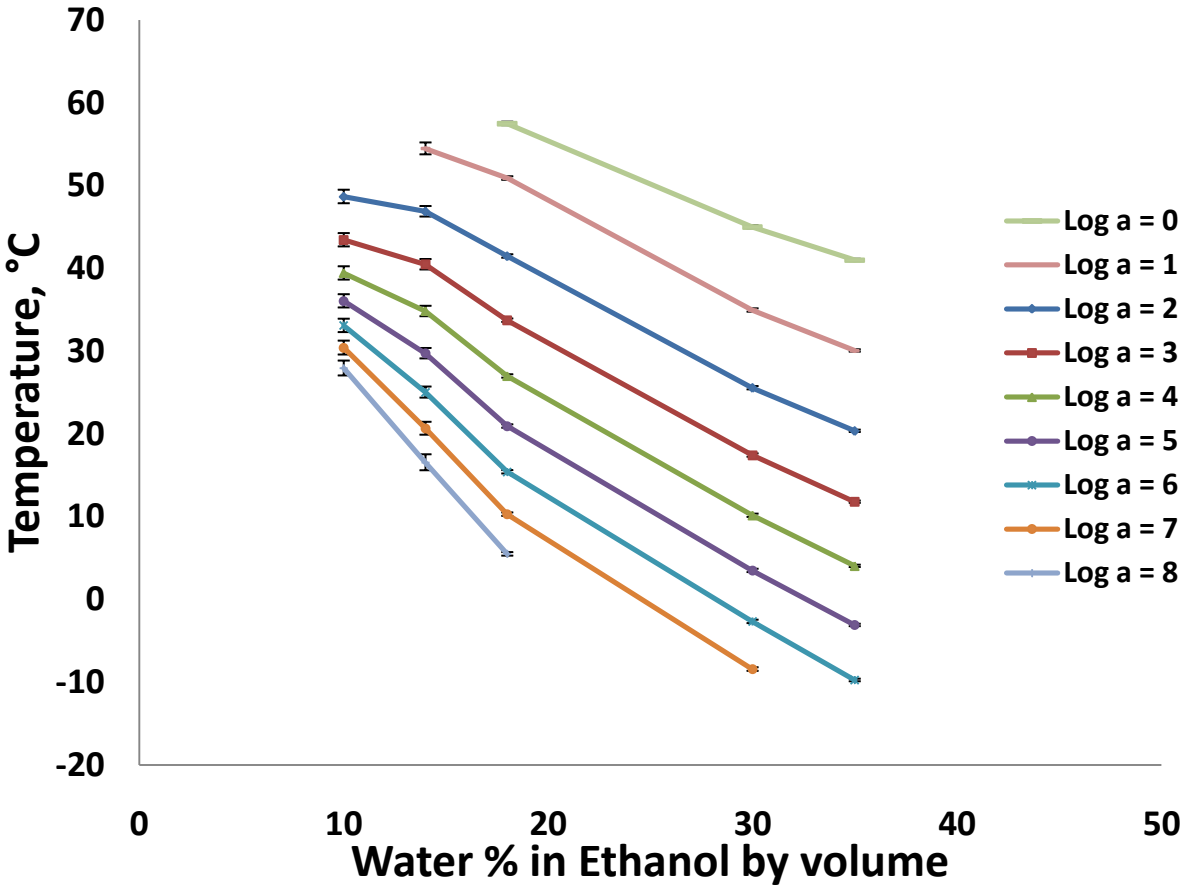

Figure 9: Isoshift factor curves from 0 to 8 fit to the data from Figure 8 with error bars to show standard error.

Resilin elongation *in vivo*

Estimates of *in vivo* resilin elongation show that, at maximum wing abduction (80°), the fore resilin could elongate up to a strain of 252% and the hind resilin could elongate up to 164%. Using these figures the average strain for all resilin at peak wing abduction becomes 208%, but may be as low as 87%. The results from four different *L.luctuosa* dissections were averaged to obtain Table 2.

Table 2: The percent elongation of resilin during manual manipulation of wing angle with an assumed 15 % muscle contraction (Weis-Fogh and Alexander, 1977). Angle measured as wings horizontal is 0° and wings nearly touching above dorsal midline at 80°

	Wing angle (degrees)	Resilin-muscle length (mm)	Passive length of muscle (mm)	Total apodeme (mm)	Passive length of resilin (mm)	Length of resilin without muscle contraction (mm)	Length of resilin with muscle contraction (mm)	Range of resilin Percent elongation
Hind Resilin	5	-	-	-	-	-	-	-
	45	2.962	2.504	0.353	0.33	0.105	0.480	0 - 46%
	80	3.352	2.504	0.353	0.33	0.495	0.870	50 - 164%
Fore Resilin	5	3.407	2.808	0.419	0.33	0.18	0.601	0 - 82%
	45	3.718	2.808	0.419	0.33	0.491	0.912	48 - 176%
	80	3.967	2.808	0.419	0.33	0.74	1.161	124 - 252%

In the table above, the passive length is the measured length of the muscle or resilin without any visible tension or compression, the muscle length is assumed to be unchanged *in vivo* other than the contraction of 15%. The value of percent elongation for the hind resilin at 5° was not possible to estimate due to a small viewing area and the wing obscuring the view of the resilin. This was not a problem for the fore resilin as the viewing area was much larger. Though it is difficult to assume the elongation for the 5° hind resilin estimate, it is clear that the fore resilin tendon seems to be under more strain over wing stroke above 0°. This table also shows that the fore resilin's maximum elongation is slightly less than double that of the hind resilin's maximum elongation at the same wing angle. It appears that the fore resilin tendon is on average under a larger amount of strain for more of the wing cycle than the hind resilin tendon.

Discussion

Resilin mechanical properties

Major findings

The results of the DMA tests showed that over the range of biological use (1 Hz to 13 kHz) resilin behaves as a highly resilient rubber with a glass transition of around $10^{6.3}$ Hz. This would indicate that the frequency of glass transition is roughly two decades away from the highest known biological frequencies resilin is used at. Using Figure 7b the resilience of resilin at 13 kHz, tan δ =0.13, is about 81% which supports Bennet-Clark's (1997) claim of a total energy loss of under 20%. Results also showed that dragonfly resilin transitions to glass at a much higher frequency than locust resilin.

Experiment limitations

There are a few limitations relating to the reported results of the DMA experiments that should be addressed. The first of these is the noticeably large scatter in the loss modulus in Figure 7a at both the low and high ends of the master curve. This limitation is present due to the way in which the loss modulus is calculated. The loss modulus, E'', is equal to the complex modulus multiplied by the sin of δ, but since δ is very small at the beginning and end of the master curve any amount of electrical noise can cause the value of δ to greatly affect the loss modulus. For instance, if the true value of δ is 0.005 and the electrical noise variation is around 0.005 δ can easily fluctuate between 0.001 and 0.00001 causing a two decade decrease in loss modulus on the log modulus axis (see Figure 7a). The electrical noise only affected very small δ values and did not affect the legitimacy of the DMA tests other than the lowest and highest ends of the loss modulus. Another limitation of the experiment was the effect of evaporation of ethanol at the higher ethanol concentrations and temperatures. This explains the more random nature of the 86% and 90% ethanol tests in comparison to the lower ethanol tests in Figure 8. Another notable limitation was the possible effect of prolonged ethanol exposure on resilin. Though there was no obvious mechanical trend that accompanied long term exposure to ethanol, several pieces of preserved resilin did not fluoresce as brightly in UV light as newly removed resilin samples implying some change in optical properties. Lastly, there was some concern in resilin's shift from 1.6 MPa in the rubber regime to only 30 MPa in the glass regime. Most

materials change three orders of magnitude between the rubber and glass regime which leaves possible concerns about the compliance of the experimental setup as resilin became more glassy. In summary, the limitations discovered during the experiment caused a few inaccuracies in the results, however there is strong confidence in the major findings of the DMA tests of resilin.

Comparison of resilin to other elastic proteins

Resilin

Although Jensen and Weis-Fogh (1962) were the first to report dynamic tests on materials containing resilin, they were not completely representing the true viscoelastic behavior of pure resilin. Andersen and Weis-Fogh (1964) later converted Jensen and Weis-Fogh's (1962) locust prealar arm data into the standard terms of storage modulus, loss modulus, and tan δ (Figure 10). Andersen and Weis-Fogh (1964) reported that the resilin containing locust prealar arm has a resilience of 95% at 15 Hz and 85% at 50 Hz, (tan δ = 0.035 and tan δ = 0.1, respectively). In comparison the data presented here show that dragonfly resilin has a resilience of 99% at 15 Hz and 98% at 50 Hz, (tan δ = 0.007 and tan δ = 0.01, respectively). Additionally, Andersen and Weis-Fogh (1964) reported locust resilin's resilience of 80% at 200 Hz (tan δ = 0.14), whereas dragonfly resilin has a resilience of 96% at 200 Hz (tan δ = 0.022). The results of locust resilin testing from Andersen and Weis-Fogh's study (1964) imply that at frequencies above 200 Hz the resilience of resilin begins to drop, suggesting the beginning of resilin's glass transition. Based on this if cicada use resilin at 13 kHz to produce sound (Fonseca and Bennet-Clark, 1998) the resilin would most likely be in glass transition, if not fully glass, and thus at risk of fatigue failure, since materials in a glass state are much less fatigue resistant than the same material in the rubber state (Mars, 2004).

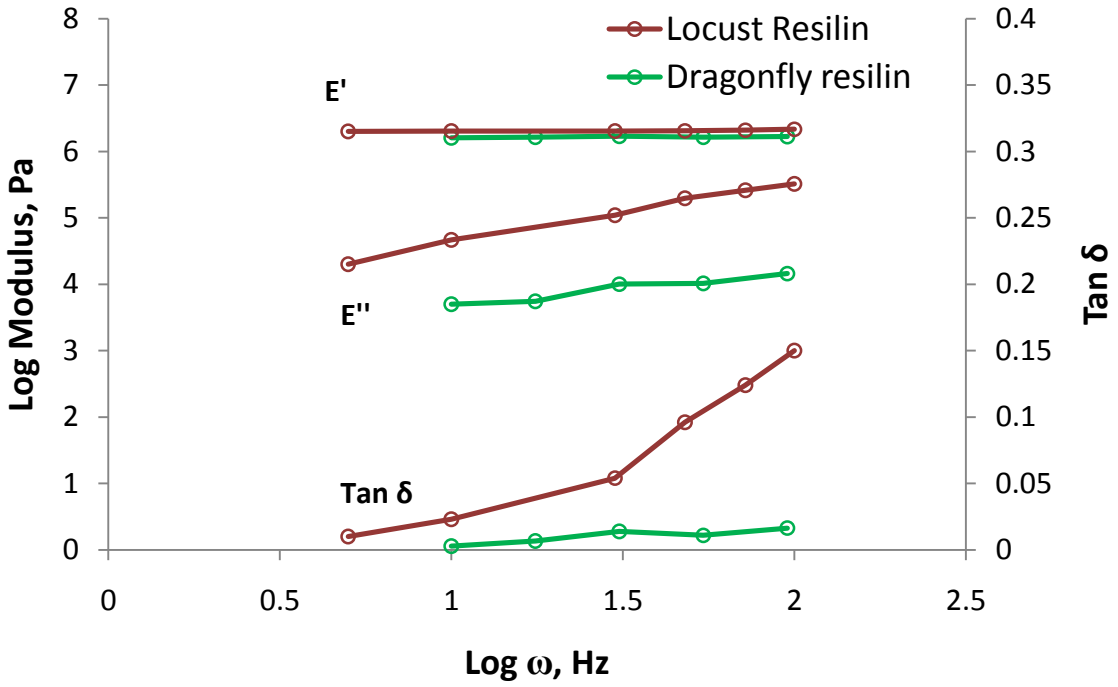

Figure 10: Comparison of the dynamic mechanical properties of locust prealar arm resilin and dragonfly tendon resilin from 5- 100 Hz.

The difference in frequency response between locust and dragonfly resilins is likely due to the structures tested. The locust prealar arm is almost 23% chitin and 77% resilin making locust prealar arm a combination of two materials(Andersen, 2003), whereas the resilin in dragonfly tendons consists of almost pure resilin with chitin apodemes only at the ends. The affect of chitin in locust resilin appears to be a shift of the viscoelastic properties of resilin toward a more glassy state and this could explain the difference between the results reported here and those of Andersen and Weis-Fogh (1964).

However, even though there is a difference in the makeup of the two resilins they do behave similarly at low frequencies (10 Hz). Results from both this paper and Andersen and Weis-Fogh (1964) show that dragonfly resilin and locust prealar arm have similar stiffness at around 1.6 MPa. This may be due to minimal chitin interference with resilin at low frequencies and possibly suggests that if the locust resilin was separated from chitin it may have properties more akin to dragonfly resilin.

Elastin

Elastin is a rubber-like protein found in all vertebrates and is an essential part of many tissues including the skin, the lungs, the arterial walls, muscle tendons, bone ligaments, etc. (Lillie and Gosline, 1990, Matsuda *et al.*, 1987). The static and dynamic mechanical properties of mammalian arterial elastin have been well characterized (Lillie and Gosline, 1990, Lillie *et al.*, 1996, Gosline *et al.*, 2002). In Lillie and Gosline (1990) they performed DMA tests on arterial elastin under different frequency, temperature and hydration levels to better understand the affects of all three on elastin. Elastin behaves similar to resilin under similar conditions of frequency, temperature, and hydration. Lillie and Gosline (1990) also found that elastin is sensitive to changes in material hydration, a total shift factor $a_C a_T$ could be determined when combining the effects of hydration and temperature, and that an isoshift surface could be created to represent a combination of frequency, temperature and hydration level shift factors. It is interesting to note that several of the figures shown in this experiment match the trend of elastin under similar conditions in Lillie and Gosline (1990).

However, there are some major differences between the viscoelastic properties of elastin and resilin (Table 3). Resilin is more resilient than elastin during both the rubber and glass regimes. While storage modulus for elastin changes three orders of magnitude between theses regime, resilin's storage modulus only changes by a single order of magnitude. Other differences between elastin and resilin include the way temperature affects their mechanical properties.

Table 3: Comparison of viscoelastic properties between elastin (Lillie and Gosline, 1990) and resilin

	Storage modulus		Tan δ		
	Rubber regime	Glass regime	Rubber regime	Glass regime	Maximum
Elastin at 37 °C under physiological water content	0.3 Mpa	100 MPa	0.08	0.25	0.76
Resilin at 22 °C in 100% water	1.6 MPa	30 MPa	0.02	0.02	0.7

The effect of temperature on elastin is much greater than on resilin over the range of 1 to 37 °C. In elastin at physiological hydration this range of temperature causes a shift value of a_T = 0 at 37 °C (reference temperature) and a_T = 4 at 1 °C (Lillie and Gosline, 1990), whereas in the resilin tests in water over the same range only resulted in an a_T = 0 at 37 °C and an a_T = 1 at 1 °C. However, it should be pointed out that the elastin tests were performed in a closed system that did not allow a change in water content with changes in temperature while resilin was tested here in an open system, allowing for changes in hydration with temperature. Though there is a discrepancy in testing we are confident that resilin in a closed system over this range of temperatures would still have a much smaller a_T at 1 °C than elastin. Elastin is a hydrophobic protein that visibly swells in an open system as temperature decreases (Gosline and French 1979). In contrast, resilin is a hydrophilic protein with minimal swelling in an open system regardless of temperature (Weis-Fogh, 1960). Based on this observation, it was assumed there would be little to no difference between the mechanical properties of resilin in either an open or a closed system.

Another difference between elastin and resilin is the frequency of glass transition. The frequency of glass transition for elastin is on the order of 10^4 (estimated from Fig 7b of Lillie and Gosline, 1990), whereas resilin's glass transition frequency is on the order of 10^6 (Fig 7b). The maximum biological use of elastin is on the order of 10^2 for vertebrates operating at 37 °C (Lillie and Gosline, 1990), which means that elastin is two orders of magnitude away from the glass transition frequency at 37 °C. However, some vertebrates, like fish in the polar regions, operate at temperatures close to 0 °C, suggesting that without elastin's ability to swell in cold temperatures it would be in an almost glassy state with a resilience of 29% at 200 Hz in 0 °C (tan δ =0.67) (Clarke and Johnston, 1999, Lillie and Gosline, 1990). Luckily for polar vertebrates the swelling of elastin at cold temperatures increases the free volume inside the protein and allows for resilience above 29% (Gosline and French, 1979). In comparison, resilin has a maximum biological frequency on the order of 10^4 in a cicada and a maximum frequency of 10^3 in most other insects (Fonseca and Bennet-Clark, 1998, Bennet-Clark and Lucey, 1967). The results obtained in this study suggest that, like elastin in vertebrates, insects use resilin two orders of magnitude away from the glass transition frequency. Still, if a cicada chose to use resilin at 13 kHz on a 0 °C day the resilin would still be an order of magnitude away from glass transition frequency whereas most other insects at 0 °C would be over two orders of magnitude away from

glass transition, tan δ = 0.35 R = 56% for cicada at 0 °C and tan δ = 0.12 R = 83% for other insects at 0 °C. This shows that resilin does not require swelling at cold temperatures, like elastin does, as resilin is never used close to its glass transition frequency. This suggests that resilin may have more free volume than elastin to allow for the amino acid chains that make up the bio-polymer to be more mobile even at very high frequencies.

Abductin

Abductin is another rubber-like protein found in the shells of scallops and other mollusks that has shown different mechanical properties in different species. The mechanical properties of abduction from two species of scallops, one from the Antarctica and one from Prince Edward Island, Canada, have been compared by Denny and Miller (2006). Denny and Miller's (2006) results showed that Antarctic scallops have a resilience of 90% at 0 °C but that Canadian scallops have a resilience of only 70% at 0 °C. This study helps to illustrate how the same bio-polymer from one species can be altered, by structure or subtle protein changes, to develop different characteristics better suited for the mechanical requirement in different environments. Just like abductin, resilin differs from species to species in both structure and composition. It has been shown that the amino acid sequence for resilin in locusts, cockroaches, and fruit flies are indeed different (Ardell and Andersen, 2001, Lombardi and Kaplan, 1993) and that the resilin in the locust prealar arm has chitin mixed in, whereas the dragonfly tendon does not. It is interesting to speculate on the notion that every insect species may have tailored resilin with different amino acid compositions and structures to better suit their mechanical needs.

Resilin function in Dragonflies

Resilin can be found in many structures inside a dragonfly but only the resilin found as a tendon in series with the pleural muscle 3, PM3, will be evaluated for functionality (Hatch, 1966). The resilin tendon will be shown to have a minimal role in the energetics required for flight, a limited ability for power amplification, and possible uses for wing stability and passive control.

Elastic storage in flight

It has been claimed by many people that resilin is an almost perfect rubber (Weis-Fogh, 1960, Bennet-Clark and Lucey, 1967, Bennet-Clark, 1963, Jensen and Weis-Fogh, 1962, Dickinson and Lighton, 1995). In light of this fact there has been speculation that insects must use resilin as part of the elastic storage system to reduce the power required for flight (Weis-Fogh, 1972, Weis-Fogh, 1973, Alexander and Bennet-Clark, 1977). However, there is some debate over the need for an elastic system for power conservation at all (Dickinson & Lighton, 1995, Wakeling & Ellington, 1997, Dudley and DeVries, 1990). The main focus of the debate concerns whether or not flying insects use an elastic storage system to reduce the inertial power required to accelerate and decelerate the wing mass along with the added mass of the air displaced by the wing (Ellington, 1984).

It has been hypothesized that insect flight is impossible without elastic energy storage and return mechanisms (Weis-Fogh, 1972). Inside the dragonfly Weis-Fogh attributes this energy storage to three different materials: The solid skeletal cuticle, resilin and an elastic component in the myofibrils, later identified as the elasticity of the cross-bridges of active muscle (Huxley & Simmons 1971, Rack & Westbury 1974). Weis-Fogh (1972) claims that 75% of the inertial energy is stored in and released from passive-elastic elements within this system. Data from locusts also supports the idea that an elastic system is needed to aid flight (Alexander and Bennet-Clark, 1977). As in the dragonfly, large quantities of elastic strain energy are stored in cuticular structures made of resilin and in the elasticity of muscle during locust flight. It was later shown that the analysis of dragonfly hovering flight (Weis-Fogh, 1972) is flawed and that these conclusions are not fully supported by the data (Ellington, 1984). However, Ellington ultimately concluded that some of the kinetic energy of oscillating the wing must be absorbed and returned during flight (Ellington, 1984).

While these results support the hypothesis that some insects can improve the energetic efficiency of flight by storing and returning elastic strain energy, for some insects no amount of energy storage can reduce the total power required to fly. Dickinson and Lighton (1995) suggest that there are two ways to minimize the cost of flight but an insect cannot take advantage of both at the same time as they are mutually exclusive. The first option is akin to the arguments of Weis-Fogh (1972, 1973) and Alexander and Bennet-Clark (1977) and requires that the elastic elements in an insect stretch and decelerate the wings during the second part of a half stroke. Then during the beginning of the next half stroke the strain energy stored in the elastic elements is used to overcome the inertial power requirement to reverse the direction of the wing, thus returning the stored elastic strain energy into the current half stroke. The second option suggests that the work of decelerating the wing is converted directly into aerodynamic power during the second half of each stroke, in that if the total inertial power required to accelerate the wing mass during the first half of a half-stroke was less than the aerodynamic power over the same interval then the kinetic energy of the oscillating wing mass can be used to satisfy aerodynamic power requirements in the second half of the half-stroke (Wakeling and Ellington, 1997, Dickinson and Lighton, 1995, Dudley and DeVries, 1990). This method of improving flight efficiency was proven to have very limited benefit from any elastic storage, even perfect elastic storage, in butterflies (Dudley and DeVries, 1990) and fruit flies (Dickinson and Lighton, 1995). In these insects, where the aerodynamic power was higher than the inertial power, muscle efficiency during flight tests was the same regardless of elastic storage assumptions. However, both Wakeling and Ellington (1997), and Dickinson and Lighton (1995) state that even though elastic storage does not seem to be essential for small insects, like *Drosophila*, there is a possibility that larger insects could benefit from an elastic storage system due to larger inertial power requirements.

It has been shown in the case of hawk moths that larger hawk moths require more elastic storage than smaller hawk moths (Bartholomew and Casey, 1978). Bartholomew and Casey (1978) claimed that as hawk moth body mass increases the flight muscle mass decreases. This meant that larger hawk moths must either have more efficient flight muscles or a more effective elastic storage system in order to remain airborne with less flight muscles. However, since all the hawk moths studied were of the same family, it is unlikely for there to be an increase in muscle efficiency and instead larger moths must have a more effective elastic storage system

(Bartholomew and Casey, 1978, Casey 1981). Additionally, Casey and Ellington (1989) showed that the muscle efficiency, assuming perfect elastic storage, for 1000 mg euglossine bees could achieve a maximum value of 16% muscle efficiency, whereas 80 mg euglossine bees could only achieve a value of 4% muscle efficiency. These cases seem to lend weight to the argument that large insects do benefit from some elastic storage to assist them during flight.

The significance of this data is that while elastic energy storage may be unimportant for small insects, it is probably important for larger insects like dragonflies. Assuming dragonflies can improve flight efficiency via elastic energy storage, what contribution do the four resilin tendons make? In order to estimate what percentage of the total inertial power all four resilin tendons can store in the *L.luctuosa,* we used Wakeling and Ellington's (1997) data of mechanical power during flight in the dragonfly *Sympetrum sanguineum* (see Table 4) and the results from Table 2.

Table 4: Components of mechanical power during flight. Reproduced from Wakeling and Ellington (1997) with an additional column of muscle mass-specific inertial power

Flight	m (mg)	m_m*	P_{ind} (mW)	P_{pro} (mW)	P_{acc} (mW)	P_{aero} (mW)	P^*_{aero} (W kg^{-1})	P_{acc} / m (W kg^{-1})
SSan2.1	121.9	0.492	3.85	2.52	3.49	6.92	115.3	28.63
SSan2.3	121.9	0.492	1.92	1.49	2.61	3.65	60.9	21.41
SSan5.1	133.0	0.483	4.70	2.86	3.83	7.84	122.1	28.80
SSan5.2	133.0	0.483	1.16	1.00	2.71	2.27	35.4	20.38
SSan6.1	111.5	0.479	4.60	3.20	5.73	8.36	156.2	51.39
SSan6.2	111.5	0.479	2.71	2.50	3.24	5.58	104.3	29.06
SSan9.1	139.3	0.489	2.88	1.94	2.68	5.20	76.3	19.24
Average	124.6	0.485	3.12	2.22	3.47	5.69	95.8	27.85

m, mass; m_m*, non-dimensional muscle mass; P_{ind}, induced power; P_{pro}, profile power; P_{acc}, inertial power; P_{aero} aerodynamic power; P^*_{aero}, muscle mass-specific aerodynamic power, P_{acc} / m, mass-specific inertial power. Ssan, *Sympetrum sanguineum.*

In order to calculate the power output of the resilin tendons we first need to determine their elastic storage capacity. This depends on the cross-sectional area (0.0103 ±0.00164 mm^2, N=10), initial length (0.331 ±0.015mm, N=10), storage modulus (1.6 MPa, Figure 7b), and average strain (87% – 208%, Table 2) for all four resilin tendons. The elastic strain energy of all

four resilin tendons for each half stroke ranges from 0.019 mJ - 0.045 mJ depending on whether the PM3 muscle contracts isometrically or concentrically. With an average dragonfly wing beat frequency of 25 Hz (Weis-Fogh and Alexander, 1977) we find the average power generated by all four resilin tendons to be between 0.475 mW to 1.1 mW.

The inertial power requirements for flight in an *L.luctuosa* dragonfly were estimated using Table 4. From the table we find the average mass-specific inertial power of the *S.sanguineum* to be 27.85 W/kg. Assuming that inertial power, P_{acc}, scales linearly with dragonfly body mass and an average *L.luctuosa* weight of 390 mg (M. May, personal communication) we estimate the average inertial power required for flight in the *L.luctuosa* to be 10.9 mW. With these calculations it is possible to see that a somewhere between 4.4% and 10.4% of the inertial power required for flight can be storage in the resilin tendons. However, it is unlikely inertial power requirements increase linearly with mass (Dickinson and Lighton, 1995, Wakeling and Ellington, 1997). This then suggests that the 4.4% to 10.4% inertial power saving is an over estimate of the actual inertial power savings in an *L.luctuosa* dragonfly.

Weis-Fogh (1972), Alexander and Bennet-Clark (1977) both assume that due to resilin's amazing mechanical properties it must be part of the elastic system for storing inertial energy. However, it has been shown above that the resilin tendon could only store a very small percentage of the inertial power required for flight. In summary, this paper agrees with Weis-Fogh, Alexander and Bennet-Clark's findings in that resilin has the incredible potential to be used for elastic storage, but it has been shown that the resilin tendons could only support a maximum of 4.4% to 10.4% of the total inertial power requirements for flight and can not significantly improve the energetic efficiency of flight without amplifying their power output in some way.

Power amplification

Power amplification in animals and insects is best described as the ability of a system to release stored work faster than it was acquired, increasing the power output of a system beyond its usual capabilities (Hof *et al.*, 1983). Due to resilin's elastic nature it is possible that it could be used for power amplification during takeoff or quick flight movements. Power amplification is common in terrestrial animals including dogs, wallabies and humans and it is the combination of power amplification and resilin in fleas that allows for their impressive jumping ability (Sawicki *et al.*, 2009, Alexander and Bennet-Clark, 1977, Bennet-Clark and Lucey, 1967). The following section will identify the two different types of power amplification, compare the principles of power amplification systems to resilin in dragonflies, and conclude with whether or not the resilin tendon in series with PM3 can be used for power amplification during flight.

There are two types of power amplification systems, one with and one without a catch mechanism. Both require an elastic element in series with a muscle, referred to as a muscle-tendon unit (MTU). The Achilles tendon and calf muscles, in humans, increase metabolic efficiency and amplify mechanical power during the push-off phase of locomotion (Sawicki *et al.*, 2009). It is important to note that the two muscles of the calf are pennated muscles, muscle fibers orientated at an angle, meaning they are stronger and when isometrically contracted can produce considerably more force compared to fusiform muscles, muscle fibers lined from end to end, of the same size (Guyton and Hall, 2006). During the stance phase of walking the calf muscle contracts to a constant length (isometric contraction) allowing the Achilles tendon to store energy that is then released during push-off of the foot (Fukunaga *et al.*, 2001). During this push-off phase, power peaks can be observed that are higher than the power used to drive the calf muscles, indicating a slight amplification of power (Hof *et al.*, 1983). An added benefit to this MTU system is a noticeable increase in metabolic efficiency due to the reduced metabolic requirements of isometric contractions (Hof *et al.*, 1983). When amplification occurs without a catch mechanism only a maximum of two times power amplification is possible (G. Sawicki, personal communication), however MTU systems with a catch mechanism can amplify power to a much greater extent.

An MTU with a catch mechanism requires that the muscle pulls the MTU into an anatomical latch, once latched the tendon is then stretched and stored energy builds in the tendon

allowing for 10-20 times the power amplification of the attached muscle (Bennet-Clark and Lucey, 1967). When an animal wishes to release the energy the tendon is unlatched and all the energy is released at once. In a flea the energy is stored in a pad of resilin and when it is discharged it can allow the flea to jump up to 40 times its body length (Bennet-Clark and Lucey, 1967). Now that the two amplification systems have been defined the possibility of power amplification in dragonflies can be discussed.

Through personal observations during *L.luctuosa* dissections and through detailed dragonfly muscle systems illustrations from Hatch (1966), it is clear that all four resilin tendons are attached in series with the four PM3 muscles. The structure of the resilin tendon and PM3 muscle in series suggests an MTU system with the potential for power amplification. As for the type of power amplification neither Hatch nor my own observations show any evidence of a catch mechanism around the resilin-PM3 MTU making it more akin to the Achilles-calf catch-less MTU system than the flea MTU system. The similar structures of the Achilles-calf MTU and the resilin-PM3 MTU suggest that, just like the calf muscle in humans, dragonflies could isometrically contract PM3 in order to reduce the metabolic demand. This could also allow the resilin tendon to store and release energy during wing cycles in addition to possibly amplifying the power of the PM3 muscle. However, there is a major difference between the Achilles-calf and resilin-PM3 MTU's. One of the reasons the power amplification in the Achilles-calf MTU is significant is that the muscles of the calf are quite powerful due to their size and pennation angle, whereas in a dragonfly the PM3 muscle is a very small fusiform muscle (Hatch, 1966). From both my own dissections and Hatch's illustration there appear to be several muscles whose clear function is to drive the wings during flight and PM3 is around 1/20 the size of many of these muscles, suggesting that PM3 is not a major contributor to the overall power required for flight.

Since resilin is attached in series to the PM3 muscle it is possible for it to amplify PM3's power output. However, without a catch mechanism the maximum amplification of the PM3 muscle is limited to 1-2 times its own power. While it is theoretically possible for resilin to amplify the power of the PM3 muscle, with limited amplification and small muscle size it is unlikely that resilin is amplifying the power of PM3 enough to contribute any notable power towards to total power required for flight during takeoff, quick flight maneuvers or even steady state flying.

Stability and Control

Despite their capacity for energy storage, dragonfly resilin tendons do not appear to be used for power amplification or for improving energetic efficiency of flight. Hatch (1966) suggests a possible use of the PM3 muscles and resilin tendons that does not entail elastic storage or power amplification. Hatch (1966) suggests that there are a few small muscles, including all four PM3 muscles, whose primary function is not powering aerodynamic lift generation but instead stabilizing the wings during flight. In his view, they could be used to adjust twisting wings in turbulent air to keep the dragonfly on course. In the same line of thought it may be possible that the resilin tendon helps passively control the wing. Assuming a constant length of the PM3 muscle the resilin tendon may help to anchor the trailing edge of a wing during up stroke, absorbing and damping out extra motions of the trailing edge of the wing caused by turbulent flow from wind gusts without needing any response from the nervous system. This aspect of the resilin tendon's potential function will be an interesting area for some further study.

One aspect of the PM3 muscles that Hatch does not mention is that all sets of PM2 and PM3 muscles attach at the same point at the posterior part of the wing base but have two different origins. All PM3 muscles attach directly to the side wall of the thorax instead of the abdomen like all the other flight muscles. It is interesting that the most posterior part of the wing base can be contracted by muscle with two different angle of actuation, one of those muscles, PM3, having a very elastic tendon and the other, PM2, with a regular stiff tendon. Is there any benefit to this dual angle contraction for flight stability and control or does it have another use? Another possible explanation for the unique structure of the resilin tendons could be for the passive control of inspiration. As previously mentioned, the origin of all four PM3 muscle is on the side of the thorax but the two fore PM3 muscles attach directly above the 2nd spiracle of the dragonfly on either side of the thorax. Is there any significance in this? Could the fore PM3 muscles and resilin tendons assist with dragonfly inspiration? The use of the resilin tendon in a dragonfly is still unclear and continued research in this area is suggested in order to understand its function.

Conclusions and further work

Weis-Fogh named resilin from the Latin word *resilire*, to spring back, and the results of this paper show that pure dragonly resilin is even more resilient in dynamic tests than first reported by Jensen and Weis-Fogh in 1962. The major findings of this paper show the ability of fully hydrated resilin at 22 °C to have a resilience of 99% at 15 Hz, only decreasing to 94% at 1000 Hz, and a glass transition frequency of around $10^{6.3}$ Hz. At similar reference conditions the glass transition frequency of resilin is two decades higher than for elastin, suggesting that resilin is a more mobile bio-polymer. Despite such remarkable resilience, it is unlikely that resilin tendons play a significant role in improving flight efficiency or amplifying power during flight. Rather, it may simply be used for passively stabilizing the trailing edge of the wing during flight.

While a few questions about resilin and its role in a dragonfly's physiology have been addressed here, there are several other important issues that could be investigated in the future to obtain a more complete understanding of resilin. First, an accurate measure of the effect of ethanol concentration on water hydration within resilin should be developed to better compare the results in this paper to other bio polymers in known hydration states. Secondly, experiments on the actual hydration levels of resilin *in vivo* would help to better understand what range of viscoelastic properties insects are actually using. In vertebrates it was shown that the fatigue life of elastin closely matched the life span of the animal from which the elastin originated (Gosline *et al.*, 2002). It would be interesting to find out if the same was true for resilin in insects. Finally, there may be much future work to be done in the area of synthetic resilin (Elvin *et al.* 2005). Using the data presented in this paper, it would be possible to measure and compare the viscoelastic properties of any synthetic resilin with those of true resilin as a way of verifying how similar the two polymers are. The investigation of synthetic resilin from different insect species may lead to a deeper understanding of the link between amino acid sequences and composition and their influence on mechanical properties.

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