

Ontogenetic shift toward stronger, tougher silk of a web-building, cave-dwelling spider

D. Piorkowski¹, S. J. Blamires², N. E. Doran³, C.-P. Liao¹, C.-L. Wu⁴ & I.-M. Tso^{1,5}

¹ Department of Life Science, Tunghai University, Taichung, Taiwan

² Evolution and Ecology Research Centre, University of New South Wales, Sydney, NSW, Australia

³ Bookend Trust and the School of Biological Sciences, University of Tasmania, Sandy Bay, Tasmania, Australia

⁴ Center for Measurement Standards, Industrial Technology Research Institute, Hsinchu, Taiwan

⁵ Center for Tropical Ecology and Biodiversity, Tunghai University, Taichung, Taiwan

Keywords

cave-dwelling spider; *Hickmania troglodytes*; spider silk; mechanical properties; ontogeny; major ampullate silk; ontogenetic shift.

Correspondence

I-Min Tso, Department of Life Science, Center for Tropical Ecology and Biodiversity Tunghai University, Taichung 40704, Taiwan. Tel: 886 4 2359 0121 ext.32414; Fax: 886 4 2359 0296
Email: spider@thu.edu.tw

Editor: Gabriele Uhl

Received 2 May 2017; revised 6 July 2017; accepted 10 August 2017

doi:10.1111/jzo.12507

Introduction

Morphological traits such as tissue size, shape and functionality and body coloration can vary in animals across ontogeny as a means of meeting the changes in energetic demands and the novel resources and risks faced by animals across life stages (Werner & Gilliam, 1984; Booth, 1990; West, Brown & Enquist, 1997; Fish & Stayton, 2014). Web-building spiders are a diverse group of insectivorous terrestrial animals that build and reside in silken webs that they produce to capture prey (Eberhard, 1990; Blackledge *et al.*, 2009). Many web-building spiders display ontogenetic shifts in the shape, size, symmetry and dimensionality of their webs as their foraging and defensive demands vary across life stages (Witt, Rawlings & Reed, 1972; Eberhard, 1976, 1986; Opell, 1982; Japyassú & Ades, 1998; Blackledge, Coddington & Gillespie, 2003; Lopardo *et al.*, 2004; Eberhard, Barrantes & Madrigal-Brenes, 2008; Kuntner *et al.*, 2008; Hesselberg, 2010; Kuntner, Gregorič & Li, 2010a; Kuntner, Kralj-Fišer & Gregorič, 2010b). Few studies, nevertheless, have examined whether the material from which a spider's web is produced, silk, also varies across ontogeny (but see Ortlepp & Gosline, 2008; Sensenig,

Abstract

Animal morphological traits may vary across life stages. Web-building spiders are diverse insectivores that can display ontogenetic shifts in the design and properties of their webs. Nevertheless, we know little about how a critical component of their webs, major ampullate silk (MAS), varies in property across life stages, inferably owing to a difficulty in finding suitable model species. The Tasmanian cave spider *Hickmania troglodytes* presents as a good model as it is long-lived and grows to a large body size with overlapping generations. We collected MAS from the webs of different-sized *H. troglodytes* and performed tensile tests on MAS fibers collected from their webs to search for shifts in properties over life stages. We found that strength and toughness (i.e. ability to deform and absorb energy) of the MAS increased with spider carapace width and body length. We expect that such a shift in silk performance across life stages has distinctive advantages, including enhanced prey capture capabilities, an improvement in the economy of silk production and ability of the web to support the spider's larger body.

Agnarsson & Blackledge, 2011). As silk serves a multitude of functions for the spider, shifts in silk properties across ontogeny is likely to be associated with significant shifts in various aspects of spider biology, including the capacity to capture more or larger prey, the economy of web building and the ability of webs to support increasingly larger spiders (Ortlepp & Gosline, 2008; Kuntner *et al.*, 2010a; Sensenig *et al.*, 2011).

Spider major ampullate silk (MAS) constitutes the frame and supporting silks in most webs, as well as the radial threads (the threads primarily responsible for absorbing the impact energy of flying prey) in orb webs (Blackledge, Kuntner & Agnarsson, 2011; Foelix, 2011; Sensenig *et al.*, 2012). MAS has been of interest because its impressive combination of strength and extensibility surpasses that of most natural and artificial materials (Swanson, Blackledge & Hayashi, 2007; Vollrath, Porter & Holland, 2011; Blamires, Blackledge & Tso, 2017). Its mechanical properties are also highly variable, differing at interspecific, intraspecific and intra-individual levels as conditions such the reeling speed, or external temperature or humidity vary (Madsen, Shao & Vollrath, 1999; Vollrath, Madsen & Shao, 2001; Guinea *et al.*, 2005). However, only a

few studies (Ortlepp & Gosline, 2008; Sensenig *et al.*, 2011) have investigated how MAS mechanical properties vary ontogenetically, and these did not find significant shifts in the properties examined.

The above-mentioned studies used relatively short-lived (i.e. life cycles less than 12 months) orb web spiders (i.e. *Neoscona arabesca* and *Araneus diadematus*) and a jumping spider (*Salticus scenicus*), whose homogeneity in web building (orb web spiders), or lack of web building (jumping spiders), across instars and rapid growth rates may place constraints on the range of silk properties utilized across life stages. When animals live for a long time, however, they undergo, by necessity, significant changes in a multitude of structural and functional traits (Stearns, 1977). It might thus be hypothesized that long-lived (i.e. those with multiple years of activity) spiders experience significant shifts in their MAS properties across ontogeny, whereas short-lived species do not. Finding a suitable long-lived web-building spider for which experiments can readily be performed, nevertheless, provides an inherent difficulty for testing this hypothesis.

The Tasmanian cave spider *Hickmania troglodytes* (Araneae: Austrochilidae; Higgins & Petterd, 1883; Fig. 1) is a long-lived (individuals living several decades, Doran *et al.*, 1999; Doran, Richardson & Swain, 2001) web-building spider that displays a high degree of adult size variability. They can grow to about 2 cm in length with a leg span of approximately 18 cm (Hickman, 1928; Goede, 1967). They build large (~1 m in diameter) cribellate silk (dry sticky silk) laden sheet webs that are horizontally orientated (Hickman, 1928; Gertsch, 1958). These spiders tend to eat cave crickets and most other insects that are caught in their webs (Doran *et al.*, 1999, 2001). Like the webs of other austrochilid spiders (Lopardo *et al.*, 2004), *H. troglodytes* webs lack defined geometry and are maintained and periodically repaired over days to weeks.

We subsequently collected and performed tensile tests on the MAS fibers of a range of differently sized *H. troglodytes* to search for shifts in their properties across life stages. *H. troglodytes* is slow growing and individuals of different sizes can co-exist in the same cave ecosystem at a given time, thus providing a unique opportunity to examine the properties

of their MAS in multiple individuals across different instars at a single point in time. We expect a correlation between MAS mechanical performance and body size to provide evidence of silk property variation across ontogeny.

Materials and methods

Silk collection

We collected a single 50–300 cm² section from webs of 20 individual *H. troglodytes* ranging in size from small juveniles to the largest adult females from the entrances (0–50 m within) of Mystery Creek (−43.4634 latitude, 146.8468 longitude) and Bradley Chesterman (−43.4599 latitude, 146.8500 longitude) caves in Southwest National Park in southern Tasmania, Australia, in September 2015. Closely related Austrochiline web-building spiders add fresh silk to the front edges of their webs daily (Lopardo *et al.*, 2004). Therefore, the most accessible fresh silk near the web edges were sampled. The samples were attached to rectangular cardboard frames affixed with double-sided sticky tape. This method ensured the transportability and preservation of the samples as frames could be safely and easily extracted from webs often built in irregular rock formations within the cave. After web sampling, we captured the resident spider and measured its carapace width at the widest point and body length (cephalothorax + abdomen) using calipers (accurate to 0.5 mm) before returning the spider to its remaining web. These body size measurements are more practical to take in the field than other measurements, such as body weight, and generally correlate strongly with spider life stage (Jakob, Marshall & Uetz, 1996; Moya-Laraño *et al.*, 2008). Body weight is problematic as an indicator of spider age as it can be more variable within instar than other body size measurements (Higgins, 1992).

Sections of sampled *H. troglodytes* webs were immediately transported (taking up to 2 weeks in transit) from the field to the laboratory at Tunghai University, Taiwan, whereupon 1–2 undamaged and accessible MAS threads of 10–15 mm in length were sub-sampled from each web section. Samples of MAS were extracted from web sections across 10 mm wide U-shaped frames cut out of one end of 76 mm × 26 mm cardboard cards following Blamires *et al.* (2015). Original tension of the silk samples within webs was preserved using double-sided sticky tape applied to the edges of the U-shaped frames to adhere to the silk threads during extraction. Elmer's® glue was then applied atop the MAS thread affixed to the tape to further affix the silk thread to the frame; similar to how other studies have affixed gluey capture silks from orb webs and cobwebs to similar sized frames (Blackledge & Hayashi, 2006; Blamires *et al.*, 2015). Cyanoacrylate glue is sometimes used to affix silk threads to paper frames (Blackledge & Hayashi, 2006; Agnarsson, Kuntner & Blackledge, 2010). However, we did not use it to adhere the threads to the frames as it may dehydrate the silk samples. Transit time might have affected the overall quality of the MAS samples. Nevertheless, since we stored the samples in environmentally controlled containers (~20°C, ~35% RH) throughout and we were careful to test all samples at the same time upon return,

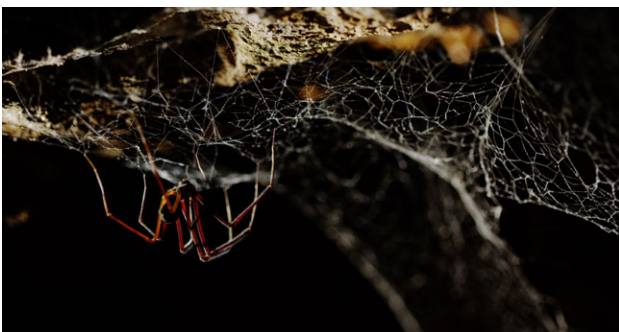


Figure 1 *Hickmania troglodytes* hanging upside down in its web in a cave in southern Tasmania. Copyright: SIXTEEN LEGS/Bookend Trust, photograph credit: Joe Shemesh. [Colour figure can be viewed at wileyonlinelibrary.com]

we were confident that the effect of transit on our results was minimal and uniform.

Mechanical testing

Determination of MAS fiber number and individual fiber diameter was determined using polarized light microscopy as described by Blackledge, Cardullo & Hayashi (2005), prior to mechanical testing. Three to five measurements of thread diameter were made per silk fiber along the axis with the average diameter determined as the mean value of the measurements. Most samples collected contained only a single MAS fiber, while some samples collected from adults contained two MAS fibers. Accordingly, cross-sectional area for single-fiber threads was calculated as:

$$A = \pi \left(\frac{d}{2}\right)^2$$

and for double-fiber threads as:

$$A = 2\pi \left(\frac{d}{2}\right)^2$$

where *d* is the fiber diameter. Samples that contained more than two fibers per thread were not tested.

The mechanical properties of 1–2 MAS threads sampled from the 20 individual *H. troglodytes* webs (37 silks were tested overall) were determined using a Nano Bionix® tensile tester (MTS Systems Corp., Eden Prairie, MN, USA) at the Industrial Technology Research Institute in Hsinchu, Taiwan, within 2 weeks of their collection. Load-extension data were generated for each MAS thread collected by pulling them to rupture (extension rate = 1.5% strain s⁻¹) using the tensile tester (Blackledge & Hayashi, 2006). All testing was conducted under controlled ambient temperature and humidity (20°C, 30% RH).

We assume constant thread volume during all tensile testing, that is, the silks did not “neck” during pulling (Guinea *et al.*, 2006). We calculated true stress and strain values using the equation:

$$A_0L_0 = A_fL_f$$

where *A*₀ represents initial cross-sectional area, *L*₀ represents initial gage length, *A*_f represents final cross-sectional area and *L*_f represents final gage length. True stress values were calculated from the load values using the formula:

$$\sigma_t = \frac{F}{A}$$

where *F* is force applied and *A* is the instantaneous cross-sectional area. True strain values were calculated from extension data using the formula:

$$\varepsilon_t = \ln\left(\frac{L}{L_0}\right)$$

where *L* is instantaneous gage length and *L*₀ is initial gage length. Using the true stress and strain data we determined: (1) fiber strength (MPa) as the ultimate true stress, (2) fiber extensibility ln (mm/mm) as ultimate true strain, (3) initial resistance to deformation or Young’s modulus (GPa) calculated as the initial linear slope of the stress–strain curve before yielding and (4) work to break or toughness (MJ m⁻³) calculated as the area under the stress–strain curves.

Statistical analyses

All data used for our analyses were natural log transformed to account for potential non-linear relationships, as this is typically observed between the biological features of an organism, and to allow for better fitting of linear functions to the data (West *et al.*, 1997). Simple linear regression was thence used to test the relationship between *H. troglodytes* body length and carapace width and we found them to be positively correlated (*t* = 9.62, *P* < 0.001, *R*² = 0.84, Fig. 2, Table 1), indicating that both could be used as a measure of overall size in our subsequent analyses. Allometric analyses were first carried out to determine the growth rates of these features, and we found that the slope of the relationship was significantly less than an isometric slope of *m* = 1 (*t* = -3.96, *P* = 0.001, Fig. 2, Table 1). This implies that carapace width changed more slowly than body length as the spiders grew. Accordingly, we conducted our analysis of MAS properties using both body length and carapace width as fixed factor measurements.

We tested the relationship between the above-mentioned fixed factors, thread diameter and MAS mechanical properties using two separate general linear mixed models by R package

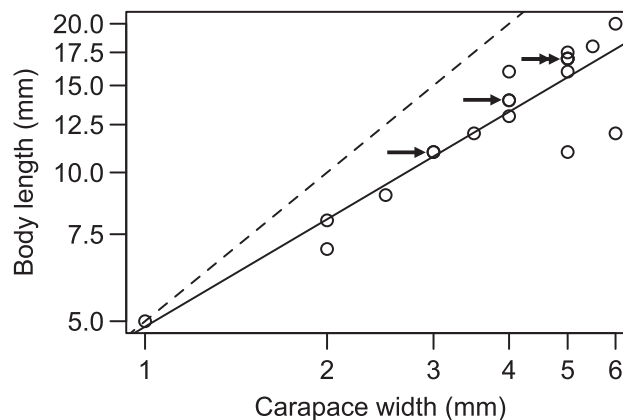


Figure 2 Relationship between body length and carapace width of *Hickmania troglodytes* individuals (simple linear model, log vs. log, *P* < 0.001, *R*² = 0.84). The dashed line represents an isometric slope of *m* = 1. The slope of the regression line (solid line, *m* = 0.72, 95% C.I. = [0.562, 0.880]) is significantly smaller than isometry (*P* < 0.001). Double-headed arrow indicates three overlapping data points and single-headed arrows indicate two overlapping data points.

Table 1 Results of simple linear regression of natural log-transformed data determining changes in body length with carapace width of *Hickmania troglodytes*^a

	Est.	SE	d.f.	<i>t</i>	<i>P</i>	<i>R</i> ²	95% bootstrapped C.I.
Intercept	1.58	0.1	18	15.23	<0.001	–	[1.402, 1.65]
Carapace width*	0.72	0.08	18	9.62	<0.001	0.83	[0.562, 0.880]

^aBody length and carapace width measured in units of mm.

*Results testing against $m = 1$ as null hypothesis give $t = -3.69$ and $P = 0.001$.

'blme' (Chung *et al.*, 2013) with spider individual as a random factor. We tested the ln-transformed data against a null hypothesis of an isometric slope of $m = 1$, which was chosen since we were determining the ln/ln relationships between three linear measures (West *et al.*, 1997). We assumed deviation in the growth rate from an isometric slope to signify that physiological factors produced disproportional allometric growth. Since spider body length and carapace width were considered independent in the different regression models, Bonferroni corrections were applied to the mixed models resulting in a significance level of $\alpha = 0.025$ being used. Model assumptions of normality of residuals (or random factor effects) and heteroscedasticity of residual variation were checked by using Anderson & Darling (1952) and Breusch & Pagan (1979) tests, respectively. The degrees of freedom for the independent variables were based on Satterthwaite approximations to cope with heteroscedasticity of residual variation. Confidence intervals for the coefficients of the mixed model results were generated using 2000 iterations using a bootstrap method to verify our statistical inferences and achieve model robustness when the assumption of residual normality was violated (Davison & Hinkley, 1997). Conditional and marginal R^2 values for the mixed models were generated according to Nakagawa & Schielzeth (2013).

Results

We found that MAS thread diameter increased with both carapace width ($t = 7.50$, $P < 0.001$, cond. $R^2 = 0.94$) and body length ($t = 7.74$, $P < 0.001$, cond. $R^2 = 0.94$) (Fig. 3; Table 2 and 3). Thread diameter also increased isometrically with both carapace width ($t = 0.36$, $P = 0.73$) and body length ($t = 1.97$, $P = 0.068$) as the regression line of the mixed model in both cases did not significantly differ from an isometric slope (Fig. 3, Table 2 and 3).

The mechanical performance of *H. troglodytes* MAS varied across both carapace width and body length (Figs 4 and 5, Table 2 and 3). We found both ultimate stress ($t = 2.79$, $P = 0.01$, cond. $R^2 = 0.42$) and toughness ($t = 2.71$, $P = 0.02$, cond. $R^2 = 0.42$) to significantly increase (by ~50% and ~100% respectively) with *H. troglodytes* carapace width (Fig. 4, Table 2). Ultimate strain ($t = 1.80$, $P = 0.09$, cond. $R^2 = 0.45$) increased only marginally and Young's modulus ($t = 0.73$, $P = 0.48$, cond. $R^2 = 0.41$) did not significantly vary (Fig. 4, Table 2). We also found ultimate stress ($t = 2.68$, $P = 0.02$, cond. $R^2 = 0.48$) and toughness ($t = 2.92$, $P = 0.01$, cond. $R^2 = 0.41$) to increase with spider body length by ~50% and ~100% respectively (Fig. 5, Table 3). Ultimate strain ($t = 1.92$,

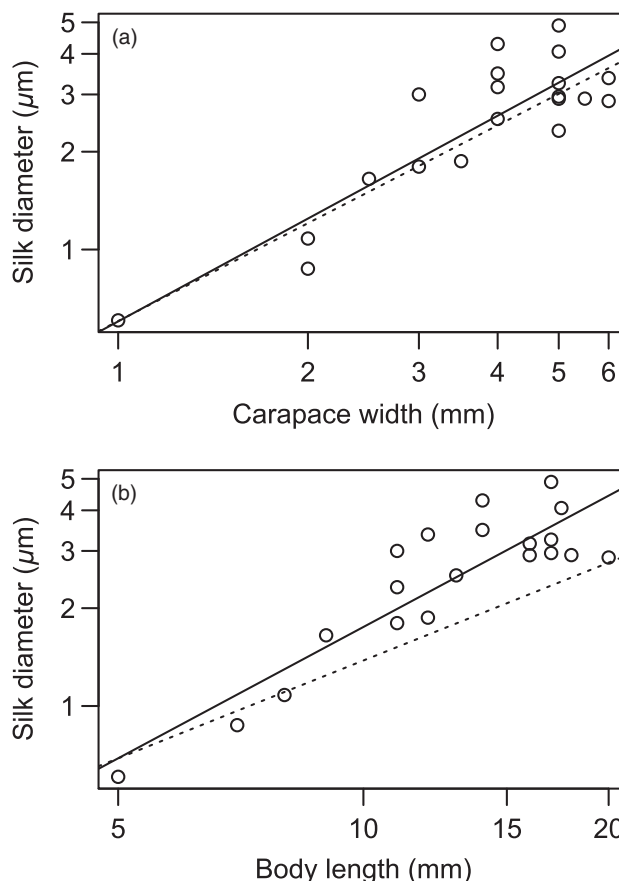


Figure 3 Relationship between single fiber diameter size and carapace width (a) and body length (b) for *Hickmania troglodytes* individuals (linear mixed model, log vs. log, carapace width: $P < 0.001$, marginal $R^2 = 0.738$, body length: $P = 0.001$, marginal $R^2 = 0.749$). Lines representing an isometric slope of $m = 1$ (dashed lines) falls within both 97.5% confidence intervals of the slopes of regression lines (solid lines) for carapace width ($m = 1.050$, C.I. = [0.735, 1.354], $P = 0.726$) (a) and body length ($m = 1.342$, C.I. = [0.968, 1.726], $P = 0.068$) (b).

$P = 0.07$, cond. $R^2 = 0.45$) on the other hand increased only marginally, while Young's modulus ($t = 0.47$, $P = 0.64$, cond. $R^2 = 0.41$) did not significantly vary with carapace width or body length (Fig. 5, Table 3). All of our analyses were verified by bootstrapped confidence intervals (Table 2 and 3).

We found that the stress versus strain relationship of *H. troglodytes*' MAS (Fig. 6) generally displayed

Table 2 Results of linear mixed model determining changes in physical properties with carapace width of *Hickmania troglodytes*

DV ^a	IV	Est.	SE	d.f.	<i>t</i>	<i>P</i> ^b	Cond. <i>R</i> ²	Marg. <i>R</i> ²	97.5% bootstrapped C.I.
Diameter*	Intercept	-0.51	0.19	14.26	-2.63	0.019	–	–	[-0.933, -0.079]
	Carapace width	1.05	0.14	14.42	7.50	<0.001	0.94	0.74	[0.735, 1.354]
Young's modulus	Intercept	9.29	2.07	13.33	4.50	<0.001	–	–	[4.646, 14.017]
	Carapace width	0.36	0.49	13.77	0.73	0.480	0.41	0.02	[-0.762, 1.473]
Toughness	Intercept	54.41	49.03	12.99	1.11	0.287	–	–	[-56.732, 163.545]
	Carapace width	31.63	11.66	13.54	2.71	0.017	0.42	0.21	[5.406, 57.450]
Ultimate stress	Intercept	0.78	0.24	13.06	3.25	0.006	–	–	[0.227, 1.330]
	Carapace width	0.16	0.06	13.56	2.79	0.014	0.48	0.23	[0.027, 0.288]
Ultimate strain	Intercept	0.17	0.07	13.81	2.54	0.024	–	–	[0.016, 0.319]
	Carapace width	0.03	0.02	14.27	1.80	0.094	0.45	0.11	[-0.007, 0.063]

^aUnits of measure: body length (mm), diameter (μm), Young's modulus (MPa), toughness (MJ m^{-3}), true stress (GPa), true strain $\ln(\text{mm}/\text{mm})$.

^bBold indicates a significant effect of carapace width with Bonferroni correction ($P < 0.025$).

*Results testing against isometric slope of $m = 1$ as null hypothesis give $t = 0.36$ and $P = 0.73$.

(–) indicates data not available.

Table 3 Results of linear mixed model determining changes in physical properties with body length of *Hickmania troglodytes*

DV ^a	IV	Est.	SE	d.f.	<i>t</i>	<i>P</i> ^b	Cond. <i>R</i> ²	Marg. <i>R</i> ²	97.5% bootstrapped C.I.
Diameter*	Intercept	-2.53	0.44	14.82	-5.72	0.839	–	–	[-3.530, -1.559]
	Body length	1.34	0.17	14.87	7.74	<0.001	0.94	0.75	[0.968, 1.726]
Young's modulus	Intercept	9.64	2.37	13.81	4.07	0.001	–	–	[4.207, 14.722]
	Body length	0.08	0.17	14.04	0.47	0.6442	0.42	0.009	[-0.293, 0.467]
Toughness	Intercept	29.37	53.89	12.54	0.55	0.595	–	–	[-93.156, 151.884]
	Body length	11.41	3.91	12.88	2.92	0.012	0.41	0.23	[2.647, 19.799]
Ultimate stress	Intercept	0.71	0.27	13.17	2.58	0.023	–	–	[0.064, 1.332]
	Body length	0.05	0.02	13.44	2.68	0.019	0.48	0.21	[0.010, 0.101]
Ultimate strain	Intercept	0.15	0.07	13.58	1.97	0.071	–	–	[-0.015, 0.321]
	Body length	0.01	0.005	13.83	1.92	0.076	0.45	0.12	[-0.002, 0.022]

^aUnits of measure: body length (mm), diameter (μm), Young's modulus (MPa), toughness (MJ m^{-3}), true stress (GPa), true strain $\ln(\text{mm}/\text{mm})$.

^bBold indicates a significant effect of carapace width with Bonferroni correction ($P < 0.025$).

*Results testing against isometric slope of $m = 1$ as null hypothesis give $t = 1.97$ and $P = 0.07$.

(–) indicates data not available.

characteristics similar to orb-weaving spiders, such as *Argiope argentata* (Blackledge & Hayashi, 2006), with: (1) an initial linear relationship, (2) a well-defined yield on the curve marked by a change in slope and (3) strain hardening near failure (defined by a non-linear increase in slope). Post-yield modulus was the most qualitatively variable aspect of MAS mechanical behavior between differently sized *H. troglodytes* spiders. We observed a more rapid increase in the post-yield slope of the stress–strain curve in the MAS of smaller spiders compared to similar sized spiders (Fig. 6).

Discussion

We tested whether MAS fiber properties vary with body size in the long-lived, large bodied, cave-dwelling spider *H. troglodytes*. We observed that larger individuals had stronger and tougher MAS in their webs than smaller individuals (Table 2 and 3, Figs 4 and 5). We interpret this correlation between silk mechanical performance and body size as evidence of variation in MAS properties across ontogeny, a

physiological phenomenon not previously reported in long-lived spiders.

Variations in spider web architectures and construction behaviors across ontogeny are well described (Witt *et al.*, 1972; Eberhard, 1976, 1986; Opell, 1982; Japyassú & Ades, 1998; Blackledge *et al.*, 2003; Lopardo *et al.*, 2004; Eberhard *et al.*, 2008; Kuntner *et al.*, 2008; Hesselberg, 2010; Kuntner *et al.*, 2010a,b). Spider webs appear to be variable when a spider grows and when they encounter variable environments (Boutry & Blamires, 2013), so may be considered plastic extended phenotypes adaptable to the changing demands of a growing spider. Variability in the silk's mechanical properties over ontogeny has nonetheless received less attention, so we currently do not know the relative roles of web architectures and silk properties in modulating web performance across spider life stages.

Previous studies using faster growing orb-weaving spiders and a jumping spider did not detect a similar ontogenetic change in MAS property (Ortlepp & Gosline, 2008; Sensenig *et al.*, 2011). However, similar to these studies, and others (Madsen *et al.*, 1999; Vollrath *et al.*, 2001; Guinea *et al.*,

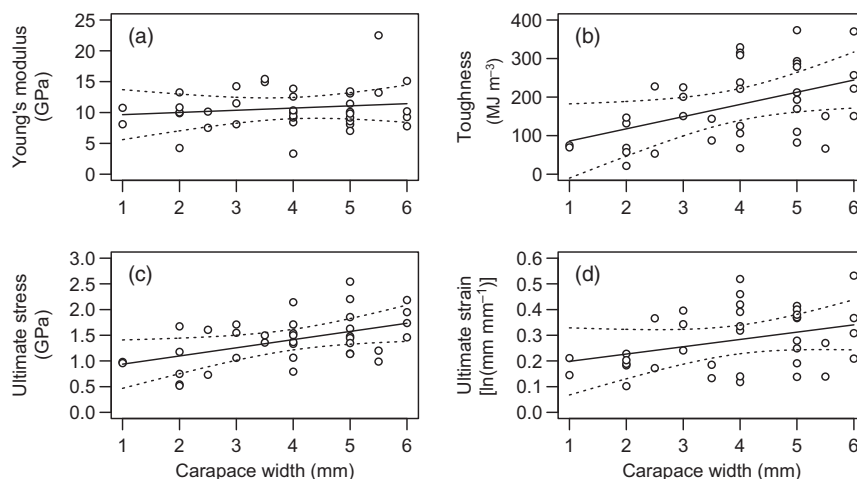


Figure 4 Relationship between MAS tensile properties and body length of *Hickmania troglodytes* individuals. Solid lines represent regression lines of a mixed model analysis and dashed lines represent 97.5% confidence band. Ultimate stress ($P = 0.019$, marginal $R^2 = 0.211$) and toughness ($P = 0.012$, marginal $R^2 = 0.0228$) increased with body size. Ultimate strain increased marginally ($P = 0.076$, marginal $R^2 = 0.124$) but not significantly, and Young's modulus did not vary ($P = 0.644$, marginal $R^2 = 0.009$).

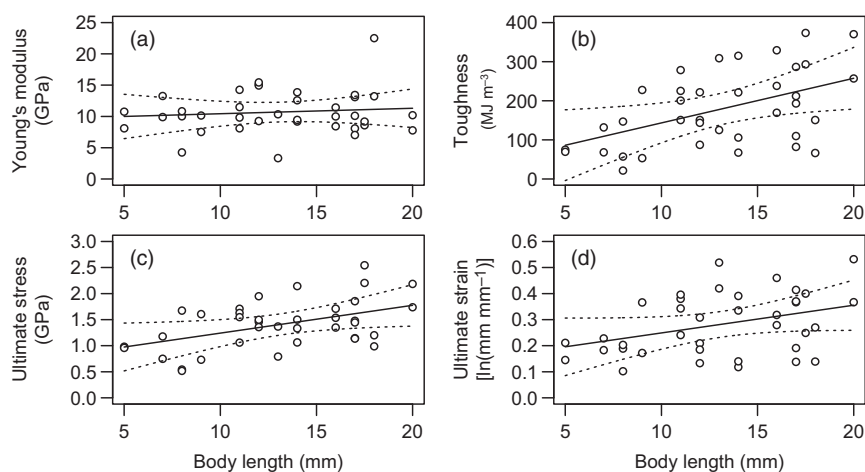


Figure 5 Relationship between MAS tensile properties and carapace width of *Hickmania troglodytes* individuals. Solid lines represent regression lines of a mixed model analysis and dashed lines represent 97.5% confidence band. Ultimate stress ($P = 0.015$, marginal $R^2 = 0.227$) and toughness ($P = 0.017$, marginal $R^2 = 0.208$) increased with body size. Ultimate strain increased marginally ($P = 0.094$, marginal $R^2 = 0.112$) but not significantly, and Young's modulus did not vary ($P = 0.480$, marginal $R^2 = 0.020$).

2005), we do detect high variation in MAS properties at the intra- and inter-individual levels. Sensenig *et al.* (2011) found evidence of an ontogenetic change in only breaking strength in the sticky capture silk of the orb weaver *N. arabesca*. In previous studies, spiders built webs under laboratory conditions and recycled webs daily allowing researchers to control stress history of the web (Sensenig *et al.*, 2011). By contrast, while we collected the freshest outer edge of webs from natural conditions, we cannot ascertain previous stress history of these webs that may persist for weeks (Lopardo *et al.*, 2004). Whether *H. troglodytes* is a unique case owing to its exceptionally slow growth or we have uncovered just one of a suite of strategies used by spiders is not known and we hesitate to speculate.

Clearly, more comparative analyses are required to shed light on this.

The shift toward stronger and tougher MAS fibers within the webs of larger *H. troglodytes* likely influences spider fitness. This could be important for supporting the increase in spider body mass (Kuntner *et al.*, 2010a), to mitigate the high energetic cost of producing silk (Craig *et al.*, 1999) or compensate for a less than isometric increase in available thread volume (Gregorič, Kuntner & Blackledge, 2015). MAS is also the primary contributor to the absorption of the kinetic energy of flying prey in webs, often performing 100% of the total work done (Sensenig *et al.*, 2012). Accordingly, large-bodied web-building spiders produce webs with higher prey stopping

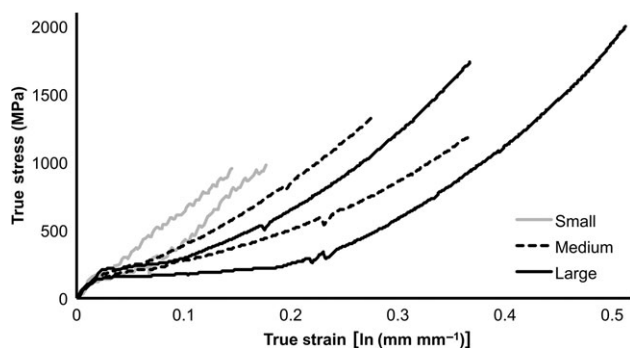


Figure 6 Qualitative depiction of mechanical performance of MAS fibers of *Hickmania troglodytes* across spider size. Two exemplar curves from three arbitrarily assigned body size classes are shown. Gray lines represent small size spiders defined as carapace width <3 mm and body length <12 mm. Dashed lines represent medium size spiders defined as carapace width 3–4.5 mm and body length 13–16.5 mm. Solid black lines represent large spiders defined as carapace width >5 mm and body length >17 mm.

potential and higher quality silk (Sensenig, Agnarsson & Blackledge, 2010). Fitness benefits resulting from variation in MAS properties cannot be fully attributed to ontogeny, however, as intra- and inter-individual variation could be a result of differences in foraging history, spinning conditions or external environmental conditions (Madsen *et al.*, 1999; Vollrath *et al.*, 2001; Guinea *et al.*, 2005; Blamires, Chao & Tso, 2010; Blamires, Wu & Tso, 2012). Ultimately, within a species, we have identified ontogenetic variation in MAS properties as an additional level of variation, but the mechanism still needs to be identified.

To date, virtually nothing is known about the chemical composition and molecular configuration of *H. troglodytes* MAS. In general, MAS is a proteinaceous fiber composed of several highly repetitive glycine and alanine sequences inducing nanoscale crystalline β -sheet and amorphous domain formations during spinning (Hinman & Lewis, 1992; Simmons, Michal & Jelinski, 1996; Van Beek *et al.*, 2002). It may be reasonable to deduce that concurrent variations in spinning processes, silk gland duct size, or silk gene expression across body sizes, act on silk protein structures and are responsible for the mechanical property variations that we measured (Vollrath *et al.*, 2001; Pérez-Rigueiro *et al.*, 2005; Davies, Knight & Vollrath, 2013; Babb *et al.*, 2017; Blamires *et al.*, 2017). However, it is also possible that differential responses of silks to external factors such as temperature, humidity or previous stress history of webs may also explain observed variation in mechanical properties. Nevertheless, further work needs to be conducted to identify the mechanism(s) of MAS property variation across ontogeny in *H. troglodytes* and other large bodied web-building spiders.

In conclusion, we examined the physical properties of the MAS of a large-bodied, long-lived, cave-dwelling web-building spider, *H. troglodytes*, across a range of body sizes and found MAS fiber strength and toughness to increase with size. Our

study was the first to find shifts in MAS properties across ontogeny in a spider. Acquiring a large body is usually associated with shifts in the properties of structural biomaterials, for example: bone and scutes, in vertebrates (Schmitt, Zumwalt & Hamrick, 2010; Fish & Stayton, 2014). We thus expect that the observed shifts in properties bestow larger spiders with fitness benefits, which include: an increased capacity for the web to absorb high prey kinetic energy, improvement of the economics of its silk production, support the spider's body weight or other structural purposes. We expect our results to inspire further investigations into the mechanisms controlling the changes in silk property over ontogeny, which will be useful for better understanding silk variability in a broader context to understand how spiders produce such an impressive and sought after material.

Acknowledgments

Our research was conducted as permitted by the Tasmanian Department of Primary Industries, Parks, Water and the Environment (permit no. FA 15189). We thank Hamish Craig, Simon Bischoff and Serena Benjamin for assistance during field collections. This research was funded by an ARC DECRA post-doctoral grant (DE140101281) to SJB and a Ministry of Science and Technology, Taiwan grant (MOST 103-2621-B-029-002-MY3) to IMT. We declare no conflict of interest.

References

- Agnarsson, I., Kuntner, M. & Blackledge, T.A. (2010). Bioprospecting finds the toughest biological material: extraordinary silk from a giant riverine orb spider. *PLoS ONE* **5**, e11234.
- Anderson, T.W. & Darling, D.A. (1952). Asymptotic theory of certain "goodness-of-fit" criteria based on stochastic processes. *Ann. Math. Stat.* **23**, 193–212.
- Babb, P.L., Lahens, N.F., Correa-Garhwal, S.M., Nicholson, D.N., Kim, E.J., Hogenesch, J.B., Kuntner, M., Higgins, L., Hayashi, C.Y., Agnarsson, I. & Voight, B.F. (2017). The *Nephila clavipes* genome highlights the diversity of spider silk genes and their complex expression. *Nat. Genet.* **49**, 895–903.
- Blackledge, T.A. & Hayashi, C.Y. (2006). Silken toolkits: biomechanics of silk fibers spun by the orb web spider *Argiope argentata* (Fabricius 1775). *J. Exp. Biol.* **209**, 2452–2461.
- Blackledge, T.A., Coddington, J.A. & Gillespie, R.G. (2003). Are three dimensional spider webs defensive adaptations? *Ecol. Lett.* **6**, 13–18.
- Blackledge, T.A., Cardullo, R.A. & Hayashi, C.Y. (2005). Polarized light microscopy, variability in spider silk diameters, and the mechanical characterization of spider silk. *Invertebr. Biol.* **124**, 165–173.
- Blackledge, T.A., Scharff, N., Coddington, J.A., Szűts, T., Wenzel, J.W., Hayashi, C.Y. & Agnarsson, I. (2009).

- Reconstructing web evolution and spider diversification in the molecular era. *Proc. Natl. Acad. Sci. USA* **106**, 5229–5234.
- Blackledge, T.A., Kuntner, M. & Agnarsson, I. (2011). The form and function of spider orb webs: evolution from silk to ecosystems. *Adv. Insect Physiol.* **41**, 175.
- Blamires, S., Chao, I.C. & Tso, I.M. (2010). Prey type, vibrations and handling interactively influence spider silk expression. *J. Exp. Biol.* **213**, 3906–3910.
- Blamires, S.J., Wu, C.L. & Tso, I.M. (2012). Variation in protein intake induces variation in spider silk expression. *PLoS ONE* **7**, e31626.
- Blamires, S.J., Piorkowski, D., Chuang, A., Tseng, Y.H., Toft, S. & Tso, I.M. (2015). Can differential nutrient extraction explain property variations in a predatory trap? *R. Soc. Open Sci.* **2**, 140479.
- Blamires, S.J., Blackledge, T.A. & Tso, I.M. (2017). Physicochemical property variation in spider silk: ecology, evolution, and synthetic production. *Annu. Rev. Entomol.* **62**, 443–460.
- Booth, C.L. (1990). Evolutionary significance of ontogenetic colour change in animals. *Biol. J. Linn. Soc.* **40**, 125–163.
- Boutry, C. & Blamires, S.J. (2013). Plasticity in web architecture and silk mechanics. In *Spiders: morphology, behavior and geographic distribution*: 1–46. Santerre, M. (Ed). New York: Nova Science.
- Breusch, T.S. & Pagan, A.R. (1979). A simple test for heteroscedasticity and random coefficient variation. *Econometrica* **47**, 1287–1294.
- Chung, Y., Rabe-Hesketh, S., Dorie, V., Gelman, A. & Liu, J. (2013). A nondegenerate penalized likelihood estimator for variance parameters in multilevel models. *Psychometrika* **78**, 685–709.
- Craig, C.L., Hsu, M., Kaplan, D. & Pierce, N.E. (1999). A comparison of the composition of silk proteins produced by spiders and insects. *Int. J. Biol. Macromol.* **24**, 109–118.
- Davies, G.J., Knight, D.P. & Vollrath, F. (2013). Structure and function of the major ampullate spinning duct of the golden orb weaver, *Nephila edulis*. *Tissue Cell* **45**, 306–311.
- Davison, A.C. & Hinkley, D.V. (1997). *Bootstrap methods and their application, Chapter 5-6*. Cambridge: Cambridge University Press.
- Doran, N.E., Kiernan, K., Swain, R. & Richardson, A.M. (1999). *Hickmania troglodytes*, the Tasmanian cave spider, and its potential role in cave management. *J. Insect Conserv.* **3**, 257–262.
- Doran, N.E., Richardson, A.M. & Swain, R. (2001). The reproductive behaviour of the Tasmanian cave spider *Hickmania troglodytes* (Araneae: Austrochilidae). *J. Zool.* **253**, 405–418.
- Eberhard, W.G. (1976). The webs of newly emerged *Uloborus diversus* and of a male *Uloborus* sp. (Araneae: Uloboridae). *J. Arachnol.* **4**, 201–206.
- Eberhard, W.G. (1986). Ontogenetic changes in the web of *Epeirotypus* sp. (Araneae, Theridiosomatidae). *J. Arachnol.* **14**, 125–128.
- Eberhard, W.G. (1990). Function and phylogeny of spider webs. *Annu. Rev. Ecol. Syst.* **21**, 341–372.
- Eberhard, W.G., Barrantes, G. & Madrigal-Brenes, R. (2008). Vestiges of an orb-weaving ancestor? The “biogenetic law” and ontogenetic changes in the webs and building behavior of the black widow spider *Latrodectus geometricus* (Araneae Theridiidae). *Ethol. Ecol. Evol.* **20**, 211–244.
- Fish, J.F. & Stayton, C.T. (2014). Morphological and mechanical changes in juvenile red-eared slider turtle (*Trachemys scripta*) shells during ontogeny. *J. Morphol.* **275**, 391–397.
- Foelix, R. (2011). *Biology of spiders*. 3rd edn. Oxford: Oxford University Press.
- Gertsch, W.J. (1958). The spider family Hypochilidae. *Am. Mus. Novit.* **1912**, 1–28.
- Goede, A. (1967). Tasmanian cave fauna: character and distribution. *Helicite* **5**, 71–86.
- Gregorič, M., Kuntner, M. & Blackledge, T.A. (2015). Does body size predict foraging effort? Patterns of material investment in spider orb webs. *J. Zool.* **296**, 67–78.
- Guinea, G.V., Elices, M., Pérez-Rigueiro, J. & Plaza, G.R. (2005). Stretching of supercontracted fibers: a link between spinning and the variability of spider silk. *J. Exp. Biol.* **208**, 25–30.
- Guinea, G.V., Pérez-Rigueiro, J., Plaza, G.R. & Elices, M. (2006). Volume constancy during stretching of spider silk. *Biomacromol* **7**, 2173–2177.
- Hesselberg, T. (2010). Ontogenetic changes in web design in two orb-web spiders. *Ethology* **116**, 535–545.
- Hickman, V.V. (1928). Studies in Tasmanian spiders. Part 2. *Pap. Proc.* **1927**, 158–175.
- Higgins, L.E. (1992). Developmental plasticity and fecundity in the orb-weaving spider *Nephila clavipes*. *J. Arachnol.* **1**, 94–106.
- Higgins, E.T. & Petterd, W.F. (1883). Description of a new cave-inhabiting spider, together with notes on mammalian remains from a recently discovered cave in the Chudleigh district. *Pap. Proc.* **1883**, 191–192.
- Hinman, M.B. & Lewis, R.V. (1992). Isolation of a clone encoding a second dragline silk fibroin. *Nephila clavipes* dragline silk is a two-protein fiber. *J. Biol. Chem.* **267**, 19320–19324.
- Jakob, E.M., Marshall, S.D. & Uetz, G.W. (1996). Estimating fitness: a comparison of body condition indices. *Oikos* **1**, 61–67.
- Japyassú, H.F. & Ades, C. (1998). From complete orb to semi-orb webs: developmental transitions in the web of *Nephilengys cruentata* (Araneae: Tetragnathidae). *Behaviour* **135**, 931–956.
- Kuntner, M., Haddad, C.R., Aljančić, G. & Blejec, A. (2008). Ecology and web allometry of *Clitaetra irenae*, an arboricolous African orb-weaving spider (Araneae, Araneoidea, Nephilidae). *J. Arachnol.* **36**, 583–594.
- Kuntner, M., Gregorič, M. & Li, D. (2010a). Mass predicts web asymmetry in *Nephila* spiders. *Naturwissenschaften* **97**, 1097–1105.
- Kuntner, M., Kralj-Fišer, S.I. & Gregorič, M. (2010b). Ladder webs in orb-web spiders: ontogenetic and evolutionary patterns in Nephilidae. *Biol. J. Linn. Soc.* **99**, 849–866.

- Lopardo, L., Ramírez, M.J., Grismado, C. & Compagnucci, L.A. (2004). Web building behavior and the phylogeny of austrochiline spiders. *J. Arachnol.* **32**, 42–54.
- Madsen, B., Shao, Z.Z. & Vollrath, F. (1999). Variability in the mechanical properties of spider silks on three levels: interspecific, intraspecific and intraindividual. *Int. J. Biol. Macromol.* **24**, 301–306.
- Moya-Laraño, J., Macías-Ordóñez, R., Blanckenhorn, W.U. & Fernández-Montraveta, C. (2008). Analysing body condition: mass, volume or density? *J. Anim. Ecol.* **77**, 1099–1108.
- Nakagawa, S. & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142.
- Opell, B.D. (1982). Post-hatching development and web production of *Hyptiotes cavatus* (Hentz) (Araneae, Uloboridae). *J. Arachnol.* **10**, 185–191.
- Ortlepp, C. & Gosline, J.M. (2008). The scaling of safety factor in spider draglines. *J. Exp. Biol.* **211**, 2832–2840.
- Pérez-Rigueiro, J., Elices, M., Plaza, G., Real, J.I. & Guinea, G.V. (2005). The effect of spinning forces on spider silk properties. *J. Exp. Biol.* **208**, 2633–2639.
- Schmitt, D., Zumwalt, A.C. & Hamrick, M.W. (2010). The relationship between bone mechanical properties and ground reaction forces in normal and hypermuscular mice. *J. Exp. Zool. A Ecol. Genet. Physiol.* **313**, 339–351.
- Sensenig, A., Agnarsson, I. & Blackledge, T.A. (2010). Behavioural and biomaterial coevolution in spider orb webs. *J. Evol. Biol.* **23**, 1839–1856.
- Sensenig, A.T., Agnarsson, I. & Blackledge, T.A. (2011). Adult spiders use tougher silk: ontogenetic changes in web architecture and silk biomechanics in the orb-weaver spider. *J. Zool.* **285**, 28–38.
- Sensenig, A.T., Lorentz, K.A., Kelly, S.P. & Blackledge, T.A. (2012). Spider orb webs rely on radial threads to absorb prey kinetic energy. *J. R. Soc. Interface* **9**, 1880–1891.
- Simmons, A.H., Michal, C.A. & Jelinski, L.W. (1996). Molecular orientation and two-component nature of the crystalline fraction of spider dragline silk. *Science* **271**, 84.
- Stearns, S.C. (1977). The evolution of life history traits: a critique of the theory and a review. *Annu. Rev. Ecol. Syst.* **8**, 145–171.
- Swanson, B.O., Blackledge, T.A. & Hayashi, C.Y. (2007). Spider capture silk: performance implications of variation in an exceptional biomaterial. *J. Exp. Zool. A Ecol. Genet. Physiol.* **307**, 654–666.
- Van Beek, J.D., Hess, S., Vollrath, F. & Meier, B.H. (2002). The molecular structure of spider dragline silk: folding and orientation of the protein backbone. *Proc. Natl. Acad. Sci. USA* **99**, 10266–10271.
- Vollrath, F., Madsen, B. & Shao, Z. (2001). The effect of spinning conditions on the mechanics of a spider's dragline silk. *Proc. Roy. Soc. Lond. Ser. B.* **268**, 2339–2346.
- Vollrath, F., Porter, D. & Holland, C. (2011). There are many more lessons still to be learned from spider silks. *Soft Matter* **7**, 9595–9600.
- Werner, E.E. & Gilliam, J.F. (1984). The ontogenetic niche and species interactions in size-structured populations. *Annu. Rev. Ecol. Syst.* **15**, 393–425.
- West, G.B., Brown, J.H. & Enquist, B.J. (1997). A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122–126.
- Witt, P.N., Rawlings, J.O. & Reed, C.F. (1972). Ontogeny of web-building behavior in two orb-weaving spiders. *Am. Zool.* **12**, 445–454.