

RESEARCH ARTICLE

Diet-induced co-variation between architectural and physicochemical plasticity in an extended phenotype

Sean J. Blamires^{1,*}, Matthew Hasemore¹, Penny J. Martens² and Michael M. Kasumovic¹

ABSTRACT

The adaptive benefits of extended phenotypic plasticity are imprecisely defined due to a paucity of experiments examining traits that are manipulable and measurable across environments. Spider webs are often used as models to explore the adaptive benefits of variations in extended phenotypes across environments. Nonetheless, our understanding of the adaptive nature of the plastic responses of spider webs is impeded when web architectures and silk physicochemical properties appear to co-vary. An opportunity to examine this co-variation is presented by modifying prey items while measuring web architectures and silk physicochemical properties. Here, we performed two experiments to assess the nature of the association between web architectures and gluey silk properties when the orb web spider *Argiope keyserlingi* was fed a diet that varied in either mass and energy or prey size and feeding frequency. We found web architectures and gluey silk physicochemical properties to co-vary across treatments in both experiments. Specifically, web capture area co-varied with gluey droplet morphometrics, thread stickiness and salt concentrations when prey mass and energy were manipulated, and spiral spacing co-varied with gluey silk salt concentrations when prey size and feeding frequency were manipulated. We explained our results as *A. keyserlingi* plastically shifting its foraging strategy as multiple prey parameters simultaneously varied. We confirmed and extended previous work by showing that spiders use a variety of prey cues to concurrently adjust web and silk traits across different feeding regimes.

KEY WORDS: *Argiope keyserlingi*, Extended phenotype, Gluey silk, Orb web architecture, Plasticity, Predatory trap, Silk properties, Stickiness

INTRODUCTION

Plasticity in developmental, physiological, behavioural or morphological traits facilitates animal adaptations to variable environments (Houston and McNamara, 1992; Pigliucci, 2005; Uller, 2008; Chevin et al., 2010; Wund, 2012; Snell-Rood, 2013). However, traits that determine fitness may not necessarily form part of the individual. Traits expressed external to the body are dubbed extended phenotypes (Dawkins, 1982). Beaver dams, termite mounds, bee hives, bird nests and spider webs are examples of

extended phenotypes that can moderate fitness (Dawkins, 1982; Healy et al., 2008; Blamires, 2010; Bailey, 2012). As plastic responses in extended phenotypes are not measurable at a somatic level, they may go unnoticed as important adaptive responses in variable environments (Plague and McArthur, 2003; Borges, 2008; Blamires, 2010; Bailey, 2012). Thus experiments using suitable model extended phenotypes are urgently required to better understand the adaptive benefits of trait plasticity (Bailey, 2012; Herberstein and Hebets, 2013).

Of the many extended phenotypes examined, only spider webs fit the requisites of a good model for plasticity studies (Herberstein and Hebets, 2013). This is because spiders and their webs fit all of the following criteria: (i) they keep well in laboratory conditions, (ii) they change measurably in response to stimuli over short time periods, (iii) they consist of different correlated architectural features (e.g. radials, capture area, spiral spacing, and decorations/stabilimenta), and most importantly (iv) there is a broad accumulation of information available regarding the behaviours, physiology, ecology, adaptability and potential trade-offs associated with building webs (Blamires, 2010; Baba et al., 2012; Nakata, 2012; Herberstein and Hebets, 2013; Blamires et al., 2016). Several studies show that a range of web architectural features and silk physicochemical properties co-vary in response to changes in environmental factors such as humidity, ultraviolet (UV) radiation, prey type and nutrients (see reviews by Blackledge et al., 2011; Herberstein and Tso, 2011; Scharf et al., 2011; Boutry and Blamires, 2013). Nevertheless, despite the abundant and variable information available on spider web plasticity, research has so far only confounded and contradicted rather than illuminated our understanding of extended phenotypic plasticity. Specifically, research has not yet discriminated between the relative importance of plasticity in spider web architectural and physicochemical properties and how these features dynamically trade off with one another as a function of environmental variability. It is thus pertinent that studies going forward dissect the specific relationships and relative importance of the factors that induce spider web architectures and silk physicochemical properties to plastically co-vary.

The benefit of carefully dissecting the relationship between environmental cues and architectural properties was demonstrated in a study that systematically assessed the influence of diet on multiple web architectural features (Blamires, 2010). In this study, the diet of the orb web spider *Argiope keyserlingi* Karsch 1878 was manipulated by feeding them different prey types (cockroaches or crickets) or prey of different sizes (adult or juvenile crickets). Under these feeding regimes some prey parameters were manipulated while others were held constant. For instance, prey type, mass and energy consumed were manipulated when spiders were fed cockroaches or crickets, while prey size and feeding frequencies were manipulated when spiders were fed adult or juvenile crickets. By examining the corresponding changes in web architectural parameters such as decoration (also called stabilimenta) length

¹Evolution & Ecology Research Centre, School of Biological, Earth & Environmental Sciences D26, The University of New South Wales, Sydney, NSW 2052, Australia.

²Graduate School of Biomedical Engineering, Samuels Building F25, The University of New South Wales, Sydney, NSW 2052, Australia.

*Author for correspondence (s.blamires@unsw.edu.au)

 S.J.B., 0000-0001-5953-3723

(ascertained as the sum of the length of each arm of the X-shaped decoration), capture area, spiral spacing (the distance between spiral threads; what Blamires referred to as ‘mesh size’), and number of radial threads, Blamires (2010) identified that certain web parameters co-varied in accordance with variations in specific prey parameters. Decoration length and web capture area, for instance, varied when mass and energy consumed were manipulated, while spiral spacing and the number of radials used varied when prey size and feeding frequencies were manipulated (Blamires, 2010). The study thus demonstrated that extended phenotypic traits are affected by the availability of resources and that animals trade off their investment into different traits to maximize their fitness.

An ancillary impediment to our understanding of extended phenotypic plasticity using a spider web model is the confounding influence of web architectural and silk physicochemical property variability. For instance, the properties of gluey silks and spiral threads within orb webs may co-vary with web architectures when humidity, wind speed or dietary regimes change (Townley et al., 2006; Opell et al., 2013; Wu et al., 2013; Blamires et al., 2014; Stellwagen et al., 2014). To identify the adaptive benefits associated with extended phenotypic plasticity we thus need to discern whether the physicochemical properties of silks co-vary with web architectural properties as part of an overall shift in foraging strategy.

Spiral threads within orb webs consist of a fibrous silk spun from the flagelliform glands coated by an aqueous gluey silk spun from the aggregate glands. The flagelliform thread is plasticized by the gluey silk coating, and is thus highly extensible (Guinea et al., 2010). The extensibility of the flagelliform thread enables it to absorb the kinetic energy of flying prey, while the sticky aggregate silk serves to retain intercepted prey (Blackledge and Hayashi, 2006; Tarakanova and Buehler, 2012; Sahni et al., 2014a,b). In addition to water, the aggregate silk contains dissolved proteins, glycoproteins and low molecular weight organic (e.g. gabamide) and inorganic (e.g. HPO_4 and KNO_3) salts (Vollrath et al., 1990; Vollrath and Tillinghast, 1991; Townley et al., 2012; Townley and Tillinghast, 2013). The stickiness of the spiral threads is conferred by the presence of the glycoproteins (Bontrone et al., 1992; Sahni et al., 2010, 2011; Opell, et al., 2013). The salts induce cohesive forces that cause the aggregate silk to coalesce along the flagelliform thread into droplets that resemble beads on a string (Sahni et al., 2014b; Opell et al., 2015). The salts also facilitate water uptake from the environment into the droplets (Vollrath et al., 1990; Sahni et al., 2010, 2011; Townley and Tillinghast, 2013), which keeps the droplets hydrated, and thus solvates the glycoproteins (Sahni et al., 2011, 2014a).

In this paper we performed dietary manipulations similar to those of Blamires (2010) using the orb web spider *A. keyserlingi* and measured the resultant web architectural parameters, droplet morphometrics, spiral thread stickiness and gluey silk salt concentrations. We hypothesized that the spiral silk physicochemical properties (droplet morphology, spiral thread stickiness and/or salt concentrations) change in a similar manner to web architectural parameters as part of a shift in foraging strategy suited to the prey environment that the spiders encounter. This hypothesis would be confirmed by a strong correlation between the various traits. Conversely we assumed a lack of an association between spiral silk physicochemical properties and web architecture to indicate that diet-induced plasticity in the spiral silk properties of *A. keyserlingi* is functionally independent of diet-induced web architectural plasticity.

MATERIALS AND METHODS

Spider collection, pre-treatment and web measurements

We performed two experiments lasting approximately 4 weeks each; one was conducted in October 2014 and one in January 2015. For both experiments we collected adult female *A. keyserlingi* from suburban Sydney and immediately brought them back to the laboratory at the University of New South Wales, Kensington, NSW, Australia. We weighed each spider on an electronic balance (OHAUS Corporation, Pine Brook, NY, USA) and selected 40 individuals of approximately equal mass for each experiment (experiment 1: 0.151 ± 0.018 g; experiment 2: 0.146 ± 0.017 g).

To habituate the spiders to the laboratory and remove the influence of any variation in previous diet between individuals before commencing either experiment, we placed the spiders within $300 \times 300 \times 50$ mm enclosures and pre-fed them by placing one house fly (*Musca domestica*) onto their web every second day over 10 days. After the pre-treatment feeding we removed all spiders from their webs, destroyed their webs, and reweighed them. Any spiders that lost more than 10% of their initial body mass ($N=4$ in October and $N=3$ in January) were excluded from the experiment as we considered these individuals unable to adapt to the laboratory. All of the remaining spiders were randomly allocated into one of two feeding treatments (see details below) and placed back into their enclosure to build new webs over the next 3 days. We noted the presence or absence of any decorations and measured the decoration length, capture area, spiral spacing and number of radii of these new webs following the protocols of Blamires (2010). We also collected the spiral threads to measure their physicochemical properties (see the ‘Spiral thread collection and measurements’, below, for details). These measurements represented the pre-feeding architectures and spiral thread properties, and we considered the experimental period to have started once all of the spiders had built this pre-feeding web.

Experiments

For the experiment conducted in October (experiment 1), 18 spiders were each allocated into either the cricket or cockroach feeding treatment. These represented prey of similar size, but cockroaches were the more energy-dense prey (Blamires, 2010). Spiders in the cricket feeding treatment were fed one adult cricket (*Acheta domestica*) every other day for 20 days, while those in the cockroach feeding treatment were fed one Australian cockroach (*Panesthia australis*) every other day for 20 days. The crickets and cockroaches were of similar mass (crickets: 0.316 ± 0.133 g; cockroaches: 0.346 ± 0.165 g) and were fed a similar diet of carrot slices and a protein supplement (Gutload, Pisces, Brisbane, Australia) *ad libitum*. We did not measure cricket or cockroach energy content. However, as we followed identical insect housing, feeding and handling protocols to those of Blamires (2010) we were confident that prey type, mass and energy consumed were manipulated in this experiment, while prey size and feeding frequencies were held constant.

For the experiment conducted in January (experiment 2), 18 spiders were allocated into an adult cricket feeding treatment, while 19 were allocated into a juvenile cricket feeding treatment. These represented prey of similar energy density, but the adult crickets were larger (Blamires, 2010). As we measured adult crickets (mean = 0.316 ± 0.133 g) as being approximately 1.3 times larger than the juvenile crickets (mean = 0.245 ± 0.080 g) and approximately equal dry mass proportions ($81 \pm 9\%$) of all crickets were consumed, we balanced mass consumption over the 20 days of the experiment by feeding the spiders in the adult cricket feeding treatment one adult cricket every 3 days while spiders in the juvenile cricket

feeding treatment received one juvenile cricket every 2 days. We fed the adult and juvenile crickets an identical diet as that fed to the crickets and cockroaches in experiment 1. We followed the cricket housing, feeding and handling protocols of Blamires (2010). Accordingly, we were confident that prey size and feeding frequencies were manipulated in this experiment, while prey type and mass, and therefore energy consumed, were held constant.

Both of the experiments were terminated after 20 days. Upon termination of the experiments we destroyed all of the webs, leaving the spiders within their enclosure for a further 48 h to build new webs. We subsequently measured the capture area, spiral spacing, number of radii and decoration length of these webs as described by Blamires (2010). We also collected the spiral threads of these new webs and measured their properties as described below.

Spiral thread collection and measurements

The following measurements were all made under controlled temperature ($25.0 \pm 0.2^\circ\text{C}$) and humidity ($50.0 \pm 2.7\%$ RH) conditions in still air.

We cut 11 mm (wide) \times 11 mm (deep) V-shaped openings into the short side of 75 mm (long) \times 25 mm (wide) cardboard cards onto which we collected eight spiral threads from the lower portion of each web, from which all of the proceeding droplet morphometrics and gluey silk properties were made. We only collected spiral threads from the bottom half of the orb webs, as only here were spirals with at least 11 mm between radii found.

To collect each thread we lightly touched the tips of the V-shaped openings to a length of spiral thread to allow the spiral thread to adhere to the cardboard frames at the tips of the V-shaped openings. We freed the 11 mm of spiral thread across the openings from the rest of the web using a hot soldering iron. We used a single drop of Elmer's glue (Elmer's Products, Westerville, OH, USA) to reinforce the thread to the attachment sites. We used four of the spiral threads collected from each web to measure the thread width, droplet volume, droplet volume per 0.5 mm of thread and droplet surface area to droplet volume ratio (collectively called 'glue droplet morphometrics'). The remaining four spiral threads from each web were used to measure spiral stickiness.

We gently placed the cards containing spiral threads onto parallel wooden dowels that were 20 mm apart on a microscope slide, ensuring that the threads and their droplets had no contact with any surface that could distort their shape. We viewed and photographed the spirals under 100 \times and 400 \times magnification using a light microscope (CKX41, Olympus, Tokyo, Japan) connected to a SPOT Idea 5.0 MP digital camera (Spot Imaging Solutions, Sterling Heights, MI, USA). From the photographs we calculated the number of glue droplets per 0.5 mm of thread and measured the length and width of ten randomly selected droplets using the program ImageJ (NIH, Bethesda, MD, USA). We removed the dowels, placed the samples back on the slides and re-photographed the threads with the droplets flattened to render the underlying thread visible. We then measured the width of the thread using ImageJ.

We used the above measurements to determine the mean droplet volume (DV), assuming an approximately ellipsoid shape, using the formula (Liao et al., 2015):

$$DV = \frac{2\pi(h)^2b}{15},$$

where h is half the width of the droplet and b is half the length of the droplet. We then calculated the droplet volume across a measured 0.5 mm length of thread (DV/0.5 mm) (Opell and Hendricks, 2007,

2009). We calculated the surface area of the droplets (DSA) using the formula (Opell and Hendricks, 2009; Wu et al., 2013):

$$DSA = \frac{4\pi hb}{3}.$$

The droplet volume to surface area ratio (DV:DSA) was determined as the droplet surface area divided by droplet volume. This provided an indication of droplet shape, with a greater DV:DSA indicating more spheroid-shaped droplets. All measurements were done as soon as possible after collection and the treatments were sampled in a random order. Given that orb web glue droplets retain their shape and stickiness for several months when stored under standard laboratory conditions (Opell and Schwend, 2008), the time taken after collection to perform these measurements (~ 3 –6 days) had negligible effects on any variations between treatments.

To measure spiral thread stickiness we placed each card containing a thread upside down (i.e. with the openings containing a thread faced downwards) within the uppermost grips of an Instron 5543 tensile testing machine (Instron Machines, Melbourne, Australia). A 6 mm \times 2 mm stainless-steel stage was mounted securely in the lowermost grips. We then lowered the card at 0.01 mm s^{-1} until the thread touched the stage. The specimen was held with the thread in contact with the stage for 60 s to allow it to adhere to the stage, before being pulled up at 0.1 mm s^{-1} until the thread detached from the stage. The force (μN) required to pull the thread off the stage was measured as a proxy of thread stickiness (Opell, 1989; Sahni et al., 2011) using the program Bluehill 3.0 (Instron Machines). We repeated this procedure 10 times using a different part of the stage each time, obtaining an average value per thread.

Upon collection of the spiral threads on cardboard frames we wound the entire web onto 200 μl plastic pipette tips. The tips were immediately taken to the Solid State Elemental Unit of the Mark Wainwright Analytical Centre at the University of New South Wales for potassium and phosphorus composition determination using inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) procedures. As potassium and phosphorus are only found in orb webs as a component of the inorganic salts HPO_4 , and KNO_3 within the glue droplets, measuring the concentrations of these elements allowed us to estimate the relative concentrations of HPO_4 and KNO_3 within the gluey silks.

Data analyses

We proceeded by initially comparing (i) the web architectures (decoration length, web capture area, spiral spacing or number of radii), (ii) droplet morphometrics (spiral thread width, droplet volume, droplet volume per 0.5 mm, droplet volume to surface area ratio), and (iii) gluey silk properties (thread stickiness, and potassium and phosphorus concentrations) from webs of pre-treated spiders allocated to the cricket or cockroach feeding treatments (experiment 1), or adult and juvenile cricket feeding treatments (experiment 2).

Both experiment 1 and experiment 2 pre-treatment data pertaining to web architectural parameters had heterogeneous variances (Levene's tests; $P < 0.05$) and did not conform to normality (Kolmogorov–Smirnov tests; $P > 0.05$), even upon transformation (\log_{10} , SQRT, or arcsine), so parametric procedures such as MANOVAs or ANOVAs could not be performed (Sokal and Rohlf, 1995). We accordingly used multivariate Kruskal–Wallis tests (Katz and McSweeney, 1980) to

Table 1. Results of a Friedman's multivariate ANOVA and Tukey's *post hoc* analyses for experiment 1

	Friedman's statistic	Kendall's co-efficient of concordance	Average rank (<i>r</i>)	<i>P</i>	<i>Post hoc</i> comparison
Web architecture					
Capture area	55.389	0.769	0.763	<0.001*	Cricket>cockroach
Spiral spacing	1.279	0.133	0.087	0.258	
Number of radii	5.662	0.173	0.166	0.059	
Decoration length	53.261	0.740	0.732	<0.001*	Cricket>cockroach
Droplet morphometrics					
Thread width	54.222	0.753	0.476	<0.001*	Cricket>cockroach
Droplet volume	2.003	0.061	0.047	0.464	
DV/0.5 mm	4.500	0.556	0.450	0.002*	Cockroach>cricket
DV: DSA	16.001	0.644	0.528	0.001*	Cockroach>cricket
Gluey silk properties					
Stickiness	53.657	0.765	0.759	<0.001*	Cockroach>cricket
P concentration	21.778	0.604	0.593	<0.001*	Cricket>cockroach
K concentration	56.741	0.788	0.782	<0.001*	Cricket>cockroach

Spiders were fed either cricket or cockroaches, comparing web architectures and spiral thread properties across treatments (i.e. cricket versus cockroach feeding). *Denotes a significant difference was detected between treatments.

compare the pre-treated web architectures and spiral thread properties between the allocated treatments.

Decoration length and droplet volume per 0.5 mm differed between pre-treated spiders allocated to the cricket feeding treatments compared with those allocated to the cockroach feeding treatment prior to the commencement of experiment 1 (Table S1). Likewise, droplet volume, droplet volume per 0.5 mm, droplet surface area to volume ratio and thread stickiness differed between pre-treated spiders allocated to the adult cricket feeding treatments and those allocated to the juvenile cricket feeding treatment (Table S1). Hence we deduced that our pre-treatments did not induce spiders allocated to the different feeding treatments to produce webs with similar architectures or spiral thread properties prior to commencing either experiment. We thus performed Friedman's pairwise multivariate ANOVAs with Tukey's *post hoc* analyses (Sokal and Rohlf, 1995) for both experiments to compare pre- and post-treatment web architectures, droplet morphometrics and gluey silk properties across treatments.

All web architectural and spiral physicochemical property parameters that were found to significantly differ across treatments for each experiment were used to develop a series of generalized mixed models. These models tested for biological associations between large numbers of multiple predictor and response variables (Burton et al., 1998). The spiral physicochemical properties were considered the response variables while web architectural parameters were the predictor variables in the models, with different models created for each significantly varying web architectural parameter from each experiment. All of the models had Poisson response distributions, owing to the large number of zeros for the 'decoration length' data, and log link functions, as this function fitted the data for both experiments (experiment 1: Pearson goodness of fit test, $\chi^2=0.737$, d.f.=2, $P=0.590$; experiment 2: Pearson goodness of fit test, $\chi^2=1.352$, d.f.=2, $P=0.342$). We included the identity of each individual spider as a categorical random factor in all of the models.

RESULTS

Experiment 1

Web capture areas and decoration lengths differed between the spiders fed cockroaches and those fed crickets (Table 1, Fig. 1), with the spiders fed crickets building webs with larger capture area and more and longer decorations (Fig. 2). This result is in agreement with the findings reported by Blamires (2010) who fed these same species these same feeding treatments.

We found that all of the droplet morphometrics and gluey silk properties that were measured from the webs of the spiders fed cockroaches and spiders fed crickets differed between treatments

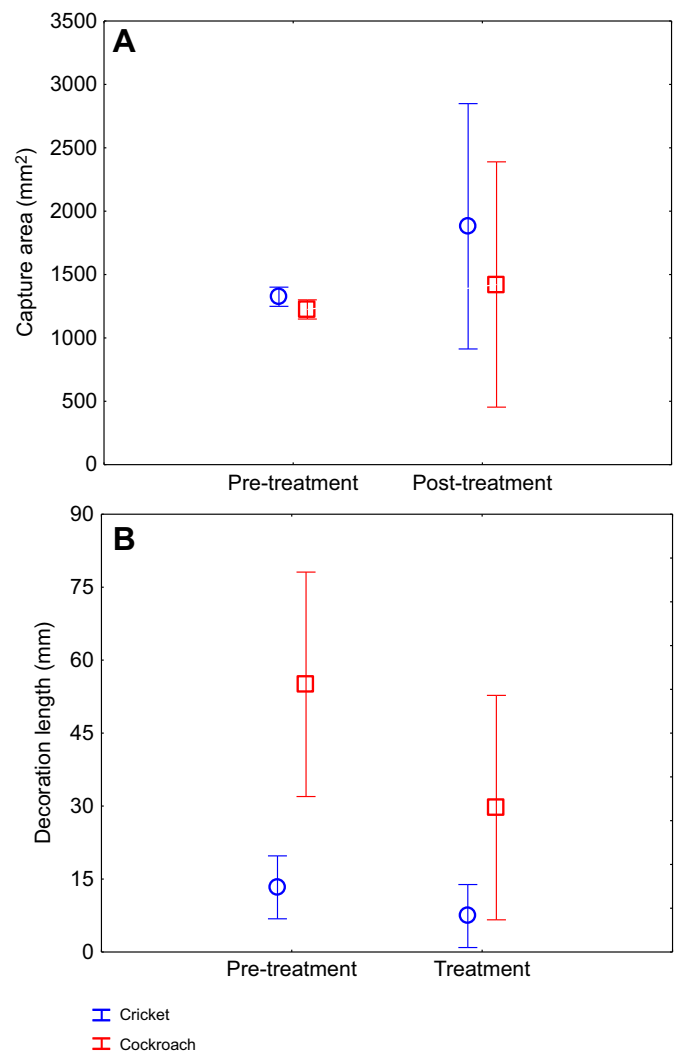


Fig. 1. Scatterplot of pre-treatment compared with post-treatment web architectural differences across the cricket and cockroach feeding treatments in the orb web spider *Argiope keyserlingi*. (A) Differences in capture area; (B) differences in decoration length.

(Table 1). The webs of the spiders fed crickets had wider spiral threads that were associated with fewer, smaller, less spheroid-shaped gluey silk droplets (Figs 2A–C and 3). The droplets also had greater concentrations of HPO_4 and KNO_3 , as indicated by the greater phosphorus and potassium concentrations (Fig. 2E,F). We expect that the higher salt concentrations are a consequence of the smaller droplet volumes. The spiral silks of the spiders fed cockroaches were stickier than those of the spiders fed crickets (Fig. 2D).

Our subsequent generalized mixed model found that all of the droplet morphometrics and gluey silk properties varied when web capture areas varied, while none varied when decoration length varied (Table 2). We interpreted these results as indicating that *A. keyserlingi* functionally co-varies its spiral silk properties with web capture area as a component of an overall shift in foraging strategy when prey type, mass and energy consumed varies. However, our modelling suggested that gluey silk properties varied independently of decoration length.

Experiment 2

In this experiment we found that of the architectural parameters measured, only spiral spacing differed between the webs of spiders fed adult or juvenile crickets (Table 3, Fig. 4A). In contrast to Blamires (2010) we did not find that the number of radials within webs differed between these feeding treatments.

All of the droplet morphometrics and gluey silk properties that we measured differed between the webs of spiders fed adult and juvenile crickets (Fig. 4), with the exception of spiral thread width and DV:DSA. The spiders fed juvenile crickets produced larger gluey silk droplets (Fig. 5) with higher HPO_4 and KNO_3 concentrations (Fig. 4E,F). Their spiral threads were also stickier (Fig. 4D). The spiders fed adult crickets produced a greater number of smaller droplets with greater HPO_4 concentrations. Our generalized mixed model found only the inorganic salt

Table 2. Results of a generalized mixed model for experiment 1

	d.f.	Capture area		Decoration length	
		Wald's statistic	P	Wald's statistic	P
Intercept	1	6.214	0.012*	3.396	0.065
Thread width	1	10.635	0.001*	0.365	0.425
DV/0.5 mm	1	5.735	0.016*	0.151	0.697
DSA:DV	1	4.001	0.045*	0.020	0.884
Stickiness	1	11.247	<0.001*	0.002	0.903
P concentration	1	14.391	<0.001*	0.002	0.877
K concentration	1	4.653	0.030*	0.001	0.927
Treatment	1	0.814	0.358	0.424	0.514

The model determined the influence of variation in the web architectural parameters capture area and decoration length on spiral property variations across treatments (i.e. cricket versus cockroach feeding). The models developed Poisson response distributions and log link functions. *Denotes a significant difference was detected between treatments. In this experiment, spiders were fed either cricket or cockroaches.

concentrations to vary in congruence with spiral spacing (Table 4). We accordingly concluded that *A. keyserlingi* co-varies the inorganic salt composition of its spiral gluey silks along with spiral spacing as a component of a shift in foraging strategy when prey size and feeding frequencies vary.

DISCUSSION

Herein we performed experiments showing that the orb web spider *A. keyserlingi*, whose webs and silk represent a model extended phenotype, concurrently adjusts web architectural, spiral droplet morphometric, and/or its gluey silk properties when fed crickets or cockroaches (where mass and energy consumed were manipulated), or adult or juveniles crickets (where prey size and feeding frequencies were manipulated). Our subsequent generalized mixed models showed that certain web architectural, i.e. web capture area (experiment 1) or spiral spacing (experiment 2), and silk physicochemical properties co-varied under particular

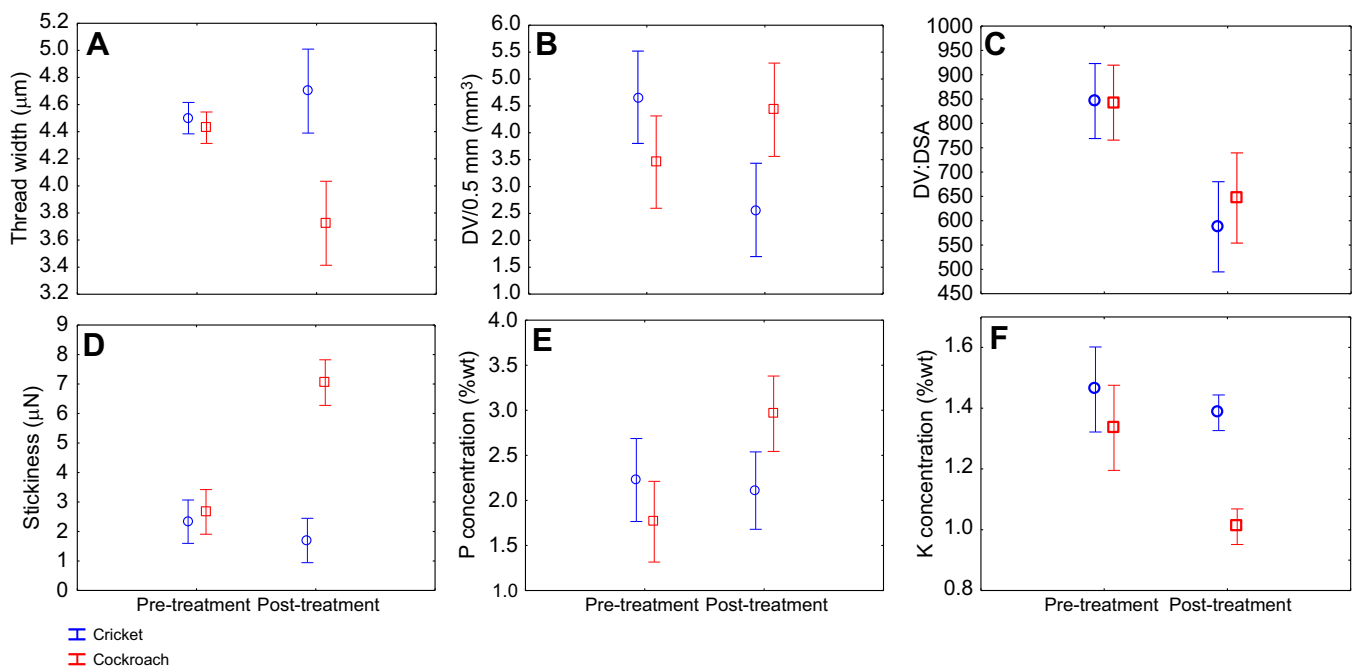


Fig. 2. Scatterplot of pre-treatment compared with post-treatment differences in spiral droplet morphometrics and gluey silk properties across the cricket and cockroach feeding treatments. (A) Spiral thread widths; (B) droplet volume per 0.5 mm of thread (DV/0.5 mm); (C) droplet volume to droplet surface area ratio (DV:DSA); (D) thread stickiness; (E) glue phosphorus (denoted as 'P') concentration; and (F) glue potassium (denoted as 'K') concentration.

Table 3. Results of a Friedman's multivariate ANOVA and Tukey's *post hoc* analyses for experiment 2

	Friedman's statistic	Kendall's co-efficient of concordance	Average rank (<i>r</i>)	<i>P</i>	<i>Post hoc</i> comparison
Web architecture					
Capture area	2.189	0.059	0.330	0.138	
Spiral spacing	7.811	0.211	0.189	0.005*	Adult crickets>juvenile crickets
Number of radii	0.471	0.012	0.014	0.492	
Decoration length	1.058	0.028	0.001	0.303	
Droplet morphometrics					
Thread width	0.243	0.007	0.021	0.628	
Droplet volume	5.513	0.650	0.734	<0.001*	Juvenile crickets>adult crickets
DV/0.5 mm	65.261	0.854	0.844	<0.001*	Adult crickets>juvenile crickets
DSA:DV	2.432	0.095	0.089	0.082	
Gluey silk properties					
Stickiness	46.162	0.758	0.752	<0.001*	Juvenile crickets>adult crickets
P concentration	14.567	0.223	0.199	0.032*	Adult crickets>juvenile crickets
K concentration	19.828	0.243	0.222	0.027*	Juvenile crickets>adult crickets

Web architectures and spiral thread properties were compared across treatments (i.e. adult cricket versus juvenile cricket feeding). *Denotes a significant difference was detected between treatments. In this experiment, spiders were fed adult crickets or juvenile crickets.

circumstances. We concluded that spider web architectures and silk physicochemical properties constitute co-varying extended phenotypes that are plastically adjusted under different feeding regimes. Our work extends that of Blamires (2010) by measuring the concomitant changes in spiral droplet morphology and gluey silk physicochemical properties, finding significant shifts in foraging strategies when multiple prey cues simultaneously varied. While our experiments did not directly address whether spiders were able to make decisions about the type of webs they built when they fed on different prey, or whether there are

physiological and behavioural constraints on web and silk properties, we made deductions (see below) based on the extensive body of literature that discusses spider web building behaviours, constraints and plasticity.

Studies using the orb web spider *Nephila pilipes* have shown that the nutritional and tactile cues elicited by prey induce spiders to co-vary their web architectures and silk physicochemical properties (Tso et al., 2007; Blamires, 2010, 2011, 2015). Furthermore, mapping the web and silk properties for that particular species across macronutrient space showed that web architectural features and silk physicochemical properties vary with lipid and protein intake (Blamires et al., 2016). We expanded those findings herein by showing that a larger suite of prey cues, including prey mass, energy content, size, and feeding frequencies, induces the orb web spider *A. keyserlingi* to concurrently adjust its web architectures, droplet morphometrics and gluey silk properties. Moreover, our experiments found significant differences across treatment variations in spiral adhesiveness, gluey silk morphology and salt concentration that were congruent with shifts in the web architectural parameters, web capture area and spiral spacing. Our subsequent generalized mixed models found that these adjustments represented shifts in the spider's foraging strategy and were not a consequence of coincidentally varying parameters.

Our model based on our findings for experiment 1 showed that while droplet morphology, gluey silk physicochemical properties and decoration length co-varied across treatments they co-varied independently. An explanation for this finding might be that decoration length varied in response to a multitude of biological and environmental cues (Herberstein et al., 2000) that may have varied subtly across treatments. Moreover, while decoration function has attracted a lot of attention and is somewhat controversial (Herberstein et al., 2000; Herberstein and Tso, 2011; Yeh et al., 2015), it does not seem to be directly related to intercepting and retaining prey in orb webs (Herberstein et al., 2000), so appears to be a functionally different type of web architectural component from the rest of those measured herein.

In our first experiment we fed the spiders crickets or cockroaches, i.e. prey of similar size, with the cockroaches more energy dense. We found *A. keyserlingi* built webs with a larger capture area and more and longer decorations when fed crickets. Our subsequent model found that only web area co-varied with droplet morphometrics and gluey silk properties. The same across-treatment differences in the architecture of *A. keyserlingi* webs

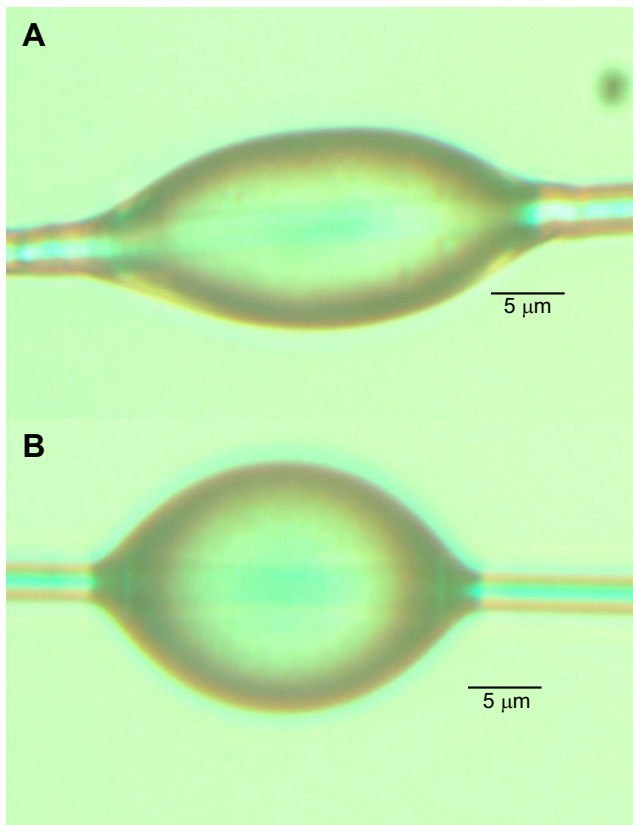


Fig. 3. Representative light microscope images of glue droplets (400× magnification). (A) Represents droplets taken from the webs of spiders fed crickets, and (B) represents droplets taken from the webs of spiders fed cockroaches. Scale bar, 5 µm.

Table 4. Results of a generalized mixed model for experiment 2

	d.f.	Mesh size	
		Wald's statistic	P
Intercept	1	55.195	<0.001*
Droplet volume	1	1.583	0.214
DV/0.5 mm	1	0.615	0.433
Stickiness	1	0.018	0.891
P concentration	1	5.800	0.016*
K concentration	1	38.363	<0.001*
Treatment	1	21.437	<0.001*

The model determined the influence of variation in spiral spacing on sticky spiral property variations across treatments (i.e. adult cricket versus juvenile cricket feeding). The models developed Poisson response distributions and log link functions. *Denotes a significant difference was detected between treatments. In this experiment, spiders were fed either adult cricket or juvenile crickets.

were found by Blamires (2010) who fed the same spiders a similar cricket or cockroach diet. We deduced from our results, and from those of other studies (Herberstein et al., 2000; Prokop and Gryglakova, 2005; Harmer et al., 2015; Yeh et al., 2015), that building larger webs with more decorations is a strategy that spiders use to enable them to capture more prey. We accordingly deduced that the spiders fed crickets were probably hungrier post-feeding than those fed cockroaches, and so they proceeded to build webs aimed at catching more prey.

At face value it seems unclear how constructing larger webs with less sticky spiral threads might enhance the foraging success of the spiders fed crickets. Nonetheless, we found that the spiral threads of these spiders were significantly thicker and were associated with fewer, smaller gluey silk droplets containing greater concentrations of inorganic salts (i.e. HPO_4 and KNO_3). The thicker threads should serve to absorb more energy when prey impact the web (Tatham and Shewry, 2002; Tarakanova and Buehler, 2012), so may enable the

interception of larger prey without the web breaking, thus enhancing foraging success despite a reduction in stickiness. Our finding in experiment 1 of smaller and less spheroid gluey silk droplets in the webs of the spiders fed crickets compared with those fed cockroaches was probably a consequence of more water being lost to the atmosphere. This may explain why the spiral threads in the webs of the spiders fed crickets were less sticky than those of spiders fed cockroaches (Edmonds and Vollrath, 1992; Sahni et al., 2014b; Amarpuri et al., 2015; Opell et al., 2015). Water loss may also explain the greater concentration of inorganic salts in the spiral threads of the spiders fed crickets compared with those of the spiders fed cockroaches (Sahni et al., 2014b; Amarpuri et al., 2015).

Many of the salts found in the aggregate gluey silk are metabolically costly to synthesize (Townley et al., 2006; Blamires et al., 2014), so might have been down-regulated by the spiders when fed cockroaches because they were under some degree of nutritional stress owing to the higher lipid:protein composition of the cockroach body. It is the glycoprotein composition that primarily determines stickiness in orb web gluey silks (Sahni et al., 2014b). Henceforth the differential expression of aggregate glycoprotein (Choresh et al., 2009; Vasanthavada et al., 2012; Collin et al., 2016) remains a plausible alternative explanation for the differences in spiral stickiness between spiders in the cricket and cockroach feeding treatments. Spread and extension of the glycoproteins under load is, nevertheless, enhanced if the glycoproteins are sufficiently hydrated (Stellwagen et al. 2014; Opell et al., 2015). Hence a reduction in the amount of hygroscopic salts in the gluey silk droplets of the cricket-fed spiders may be responsible for the differences in adhesion regardless of whether or not they produced droplets with more or less glycoproteins than the cockroach-fed spiders.

Our second experiment found that the spiders fed juvenile crickets built webs with greater spiral spacing than the spiders fed adult crickets. How to rationalize this result is a matter of conjecture.

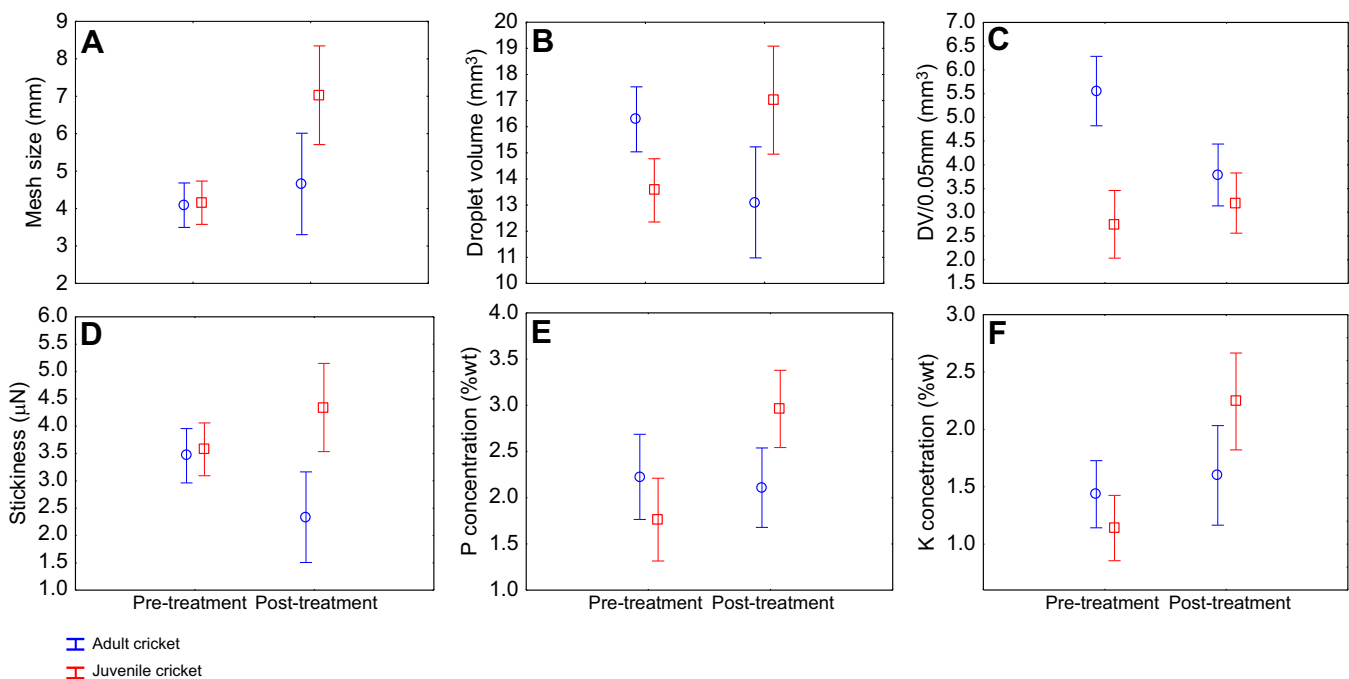


Fig. 4. Scatterplot of pre-treatment compared with post-treatment differences in web architecture, spiral droplet morphometrics and gluey silk properties across the adult cricket and juvenile cricket feeding treatments. (A) Spiral spacing; (B) droplet volume; (C) droplet volume per 0.5 mm of thread (DV/0.5 mm); (D) thread stickiness; (E) glue phosphorus (denoted as 'P') concentration; and (F) glue potassium (denoted as 'K') concentration.

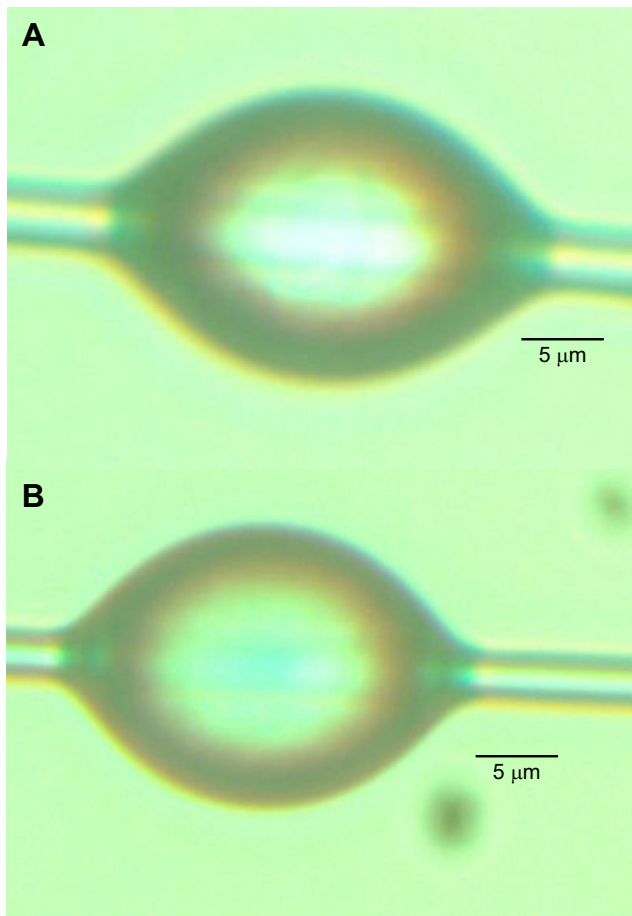


Fig. 5. Representative light microscope images of glue droplets (400× magnification). (A) Represents droplets taken from the webs of spiders fed adult crickets, and (B) represents droplets taken from the webs of spiders fed juvenile crickets. Scale bar, 5 µm.

There are two possibilities: (1) that juvenile crickets are generally more active so need more glue to retain them, or (2) the spiders built webs with a finer spiral spacing because they are more efficient at catching the much larger adult crickets (Murakami, 1983; Sherman, 1994; Blackledge and Eliason, 2007; Blackledge et al., 2011; Harmer et al., 2015). Our modelling showed that the inorganic salt concentrations varied with spiral spacing when prey size and feeding frequencies varied. Spiral thread stickiness, however, was not significantly affected by feeding treatment so the second possibility seems the most likely. A greater concentration of HPO_4 but lower concentration of KNO_3 in the spiral glues of the webs of spiders fed adult crickets is somewhat difficult to explain, primarily because so little is known about the costs, benefits and functional significance of these salts within orb web gluey silks (Sahni et al., 2014a). A tentative explanation is that because nitrates are needed for other body functions (Frost et al., 2005) KNO_3 becomes more sparingly used as more spiral threads, and consequently more gluey silk, are deposited (Opell et al., 2013). A reduction in the KNO_3 concentration within the glue might have been counteracted by an increase in HPO_4 concentration in order to retain droplet hygroscopy and prevent droplet desiccation (Vollrath et al., 1990).

To summarize, we experimentally demonstrated here that the orb web spider *A. keyserlingi* adjusts web architectural parameters concurrently with droplet morphometrics and gluey silk properties when prey type, size or feeding frequencies vary. Our experiments

indicated that the spiders adjust multiple web parameters and spiral silk properties as a component of an overall switch in foraging strategy. The broader implication of our research is that it uncovered some of the web and silk properties that appear to be traded off against each other, such as web spiral spacing and gluey silk salt concentrations, when the spider's diet becomes compromised. Considering the complex toolkit of silks used by orb web spiders, more studies are clearly required to ascertain exactly how and why such parameters are traded off. Our findings also provided important insights into the adaptive benefits of extended phenotypic plasticity, and highlighted the importance of using manipulable models for furthering our understanding of its adaptive benefit.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.J.B. and M.M.K. conceived the study. M.H. performed the feeding experiments. S.J.B. and M.H. performed the web, spiral thread and droplet measurements. S.J.B., M.H. and P.J.M. performed the thread stickiness measurements. All authors analysed the data. S.J.B., P.J.M. and M.M.K. wrote the paper.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.150029.supplemental>

References

- Amarpuri, G., Zhang, C., Diaz, C., Opell, D. B., Blackledge, T. A. and Dhinojwala, A. (2015). Spiders tune glue viscosity to maximize adhesion. *ACS Nano* **9**, 11472–11478.
- Baba, Y. G., Osada, Y. and Miyashita, T. (2012). The effect of host web complexity on prey-stealing success in a kleptoparasitic spider mediated by locomotor ability. *Anim. Behav.* **83**, 1261–1268.
- Bailey, N. W. (2012). Evolutionary models of extended phenotypes. *Trends Ecol. Evol.* **27**, 561–569.
- Blackledge, T. A. and Eliason, C. M. (2007). Functionally independent components of prey capture are architecturally constrained in spider orb webs. *Biol. Lett.* **3**, 456–458.
- Blackledge, T. A. and 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., Kuntner, M. and Agnarsson, I. (2011). The form and function of spider orb webs: evolution from silk to ecosystems. *Adv. Insect Physiol.* **41**, 175–262.
- Blamires, S. J. (2010). Plasticity in extended phenotypes: orb web architectural responses to variations in prey parameters. *J. Exp. Biol.* **213**, 3207–3212.
- Blamires, S. J., Chao, I.-C., Liao, C.-P. and Tso, I.-M. (2011). Multiple prey cues induce foraging flexibility in a trap-building predator. *Anim. Behav.* **81**, 955–961.
- Blamires, S. J., Sahni, V., Dhinojwala, A., Blackledge, T. A. and Tso, I.-M. (2014). Nutrient deprivation induces property variations in spider gluey silk. *PLoS ONE* **9**, e88487.
- Blamires, S. J., Piorkowski, D., Chuang, A., Tseng, Y.-H., Toft, S. and Tso, I.-M. (2015). Can differential nutrient extraction explain property variations in a predatory trap? *R. Soc. Open Sci.* **2**, 140479.
- Blamires, S. J., Tseng, Y.-H., Wu, C.-L., Toft, S., Raubenheimer, D. and Tso, I.-M. (2016). Spider web and silk performance landscapes across nutrient space. *Sci. Rep.* **6**, 26383.
- Bonthrone, K. M., Vollrath, F., Hunter, B. K. and Sanders, J. K. M. (1992). The elasticity of spiders' webs is due to water-induced mobility at a molecular level. *Proc. R. Soc. B Biol. Sci.* **248**, 141–144.
- Borges, R. M. (2008). Plasticity comparisons between plants and animals. *Plant Sign. Behav.* **3**, 367–375.
- Boutry, C. and Blamires, S. J. (2013). Plasticity in spider webs and silk: an overview of current evidence. In *Spiders: Morphology, Behavior and Geographic Distribution* (ed. M. Santerre), pp. 1–46. New York: Nova.

- Burton, P., Gurrin, L. and Sly, P. (1998). Extending the simple linear regression model to account for correlated responses: an introduction to generalized estimating equations and multi-level mixed modelling. *Stat. Med.* **17**, 1261–1291.
- Chevin, L.-M., Lande, R. and Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* **8**, e1000357.
- Chohesh, O., Bayarmagnai, B. and Lewis, R. V. (2009). Spider web glue: two proteins expressed from opposite strands of the same DNA sequence. *Biomacromolecules* **10**, 2852–2856.
- Collin, M. A., Clarke, T. H., Ayoub, N. A. and Hayashi, C. Y. (2016). Evidence from multiple species that spider silk glue component ASG2 is a spidroin. *Sci. Rep.* **6**, 21589.
- Dawkins, R. (1982). *The Extended Phenotype: The Long Reach of the Gene*. Oxford: Oxford University Press.
- Edmonds, D. T. and Vollrath, F. (1992). The contribution of atmospheric water vapour to the formation and efficiency of a spider's capture web. *Proc. R. Soc. B Biol. Sci.* **248**, 145–148.
- Frost, P. C., Evans-White, M. A., Finkel, Z. V., Jansen, T. C. and Matzek, V. (2005). Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos* **109**, 18–28.
- Guinea, G. V., Cerdeira, M., Plaza, G. R., Elices, M. and Pérez-Rigueiro, J. (2010). Recovery in viscid line fibers. *Biomacromolecules* **11**, 1174–1179.
- Harmer, A. M. T., Clausen, P. D., Wroe, S. and Madin, J. S. (2015). Large orb-webs adapted to maximise total biomass not rare, large prey. *Sci. Rep.* **5**, 14121.
- Healy, S., Walsh, P. T. and Hansell, M. (2008). Nest building by birds. *Curr. Biol.* **18**, R271–R273.
- Herberstein, M. E. and Hebets, E. A. (2013). Behaviour: why are spiders good models for research? In *Spider Research in the 21st Century: Trends and Perspectives* (ed. D. Penny), pp. 230–250. Manchester: SIRC Scientific Publishing.
- Herberstein, M. E. and Tso, I. M. (2011). Spider webs: evolution, diversity and plasticity. In *Spider Behaviour: Flexibility and Versatility* (ed. M. E. Herberstein), pp. 57–98. Cambridge: Cambridge University Press.
- Herberstein, M. E., Craig, C. L., Coddington, J. A. and Elgar, M. A. (2000). The functional significance of silk decorations of orb-web spiders: a critical review of the empirical evidence. *Biol. Rev.* **75**, 649–669.
- Houston, A. I. and McNamara, J. M. (1992). Phenotypic plasticity as a state-dependent life-history decision. *Evol. Ecol.* **6**, 243–253.
- Katz, B. M. and McSweeney, M. (1980). A multivariate Kruskal–Wallis test with post hoc procedures. *Multivariate Behav. Res.* **15**, 281–297.
- Liao, C.-P., Blamires, S. J., Hendricks, M. L. and Opell, B. D. (2015). A re-evaluation of the formula to estimate the volume of orb web glue droplets. *J. Arachnol.* **43**, 97–100.
- Murakami, Y. (1983). Factors determining the prey size of the orb-web spider, *Argiope amoena* (L. Koch) (Argiopidae). *Oecologia* **57**, 72–77.
- Nakata, K. (2012). Plasticity in an extended phenotype and reversed up-down asymmetry of spider orb webs. *Anim. Behav.* **83**, 821–826.
- Opell, B. D. (1989). Measuring the stickiness of spider prey capture threads. *J. Arachnol.* **17**, 112–114.
- Opell, B. D. and Hendricks, M. L. (2007). Adhesive recruitment by the viscous capture threads of araneoid orb-weaving spiders. *J. Exp. Biol.* **210**, 553–560.
- Opell, B. D. and Hendricks, M. L. (2009). The adhesive delivery system of viscous capture threads spun by orb-weaving spiders. *J. Exp. Biol.* **212**, 3026–3034.
- Opell, B. D. and Schwend, H. S. (2008). Persistent stickiness of viscous capture threads produced by araneoid orb-weaving spiders. *J. Exp. Zool.* **309A**, 11–16.
- Opell, B. D., Karinshak, S. E. and Sigler, M. A. (2013). Environmental response and adaptation of glycoprotein glue within the droplets of viscous prey capture threads from araneoid spider orb-webs. *J. Exp. Biol.* **216**, 3023–3034.
- Opell, B. D., Andrews, S. F., Karinshak, S. E. and Sigler, M. A. (2015). The stability of hygroscopic compounds in orb-web spider viscous thread. *J. Arachnol.* **43**, 152–157.
- Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends Ecol. Evol.* **20**, 481–486.
- Plague, G. R. and McArthur, J. V. (2003). Phenotypic plasticity of larval retreat design in a net-spinning caddis fly. *Behav. Ecol.* **14**, 221–226.
- Prokop, P. and Gryglakova, D. (2005). Factors affecting the foraging success of the wasp-like spider *Argiope bruennichi* (Araneae): role of web design. *Biologia* **60**, 165–169.
- Sahni, V., Blackledge, T. A. and Dhinojwala, A. (2010). Viscoelastic solids explain spider web stickiness. *Nat. Commun.* **1**, 19.
- Sahni, V., Blackledge, T. A. and Dhinojwala, A. (2011). Changes in the adhesive properties of spider aggregate glue during the evolution of cobwebs. *Sci. Rep.* **1**, 41.
- Sahni, V., Miyoshi, T., Chen, K., Jain, D., Blamires, S. J., Blackledge, T. A. and Dhinojwala, A. (2014a). Direct solvation of glycoproteins by salts in spider silk glues enhances adhesion and helps to explain the evolution of modern spider orb webs. *Biomacromolecules* **15**, 1225–1232.
- Sahni, V., Dhinojwala, A., Opell, B. D. and Blackledge, T. A. (2014b). Prey capture adhesives produced by orb-weaving spiders. In *Biotechnology of Silk* (ed. T. Asakura and T. Miller), pp. 203–217. Dordrecht: Springer.
- Scharf, I., Lubin, Y. and Ovadia, O. (2011). Foraging decisions and behavioural flexibility in trap-building predators: a review. *Biol. Rev.* **86**, 626–639.
- Sherman, P. M. (1994). The orb-web: an energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. *Anim. Behav.* **48**, 19–34.
- Snell-Rood, E. C. (2013). An overview of the evolutionary causes and consequences of behavioural plasticity. *Anim. Behav.* **85**, 1004–1011.
- Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*, 3rd edn. New York, NY: W. H. Freeman.
- Stellwagen, S. D., Opell, B. D. and Short, K. G. (2014). Temperature mediates the effect of humidity on the viscoelasticity of glycoprotein glue within the droplets of an orb-weaving spider's prey capture threads. *J. Exp. Biol.* **217**, 1563–1569.
- Tarakanova, A. and Buehler, M. J. (2012). The role of capture spiral silk properties in the diversification of orb webs. *J. R. Soc. Interface* **9**, 3240–3248.
- Tatham, A. S. and Shewry, P. R. (2002). Comparative structures and properties of elastic proteins. *Philos. Trans. R. Soc. B Biol. Sci.* **357**, 229–234.
- Townley, M. A. and Tillinghast, E. K. (2013). Aggregate silk gland secretions of Araneoid spiders. In *Spider Ecophysiology* (ed. W. Nentwig), pp. 283–302. Berlin: Springer.
- Townley, M. A., Tillinghast, E. K. and Neefus, C. D. (2006). Changes in composition of spider orb web sticky droplets with starvation and web removal, and synthesis of sticky droplet compounds. *J. Exp. Biol.* **209**, 1463–1486.
- Townley, M. A., Pu, Q., Zercher, C. K., Neefus, C. D. and Tillinghast, E. K. (2012). Small organic solutes in sticky droplets from orb webs of the spider *Zygiella atrica* (Araneae: Araneidae): β -alaninamide is a novel and abundant component. *Chem. Biodivers.* **9**, 2159–2174.
- Tso, I.-M., Chiang, S.-Y. and Blackledge, T. A. (2007). Does the giant wood spider *Nephila pilipes* respond to prey variation by altering web or silk properties? *Ethology* **113**, 324–333.
- Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends Ecol. Evol.* **23**, 432–438.
- Vasanthavada, K., Hu, X., Tuton-Blasingame, T., Hsia, Y., Sampath, S., Pacheco, R., Freemark, J., Falick, A. M., Tang, S., Fong, J. et al. (2012). Spider glue proteins have distinct architectures compared with traditional spidroin family members. *J. Biol. Chem.* **287**, 35986–35999.
- Vollrath, F. and Tillinghast, E. K. (1991). Glycoprotein glue beneath a spider web's aqueous coat. *Naturwissenschaften* **78**, 557–559.
- Vollrath, F., Fairbrother, W. J., Williams, R. J. P., Tillinghast, E. K., Bernstein, D. T., Gallagher, K. S. and Townley, M. A. (1990). Compounds in the droplets of the orb spider's viscid spiral. *Nature* **345**, 526–528.
- Wu, C.-C., Blamires, S. J., Wu, C.-L. and Tso, I.-M. (2013). Wind induces variations in spider web geometry and sticky spiral droplet volume. *J. Exp. Biol.* **216**, 3342–3349.
- Wund, M. A. (2012). Assessing the impacts of phenotypic plasticity on evolution. *Integr. Comp. Biol.* **52**, 5–15.
- Yeh, C.-W., Blamires, S. J., Liao, C.-P. and Tso, I.-M. (2015). Top down and bottom up selection drives variations in frequency and form of a visual signal. *Sci. Rep.* **5**, 9543.