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RESEARCH ARTICLE

Fitness consequences of plasticity in an extended phenotype

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ABSTRACT

Like regular phenotypes, extended phenotypes have demonstrable fitness advantages and their properties may vary plastically across environments. However, the fitness advantages of plasticity are only known for a select few extended phenotypes. It is known that the form and functions of spider orb webs can be manipulated by laboratory experiments. For instance, the physical and chemical properties of the spiral and gluey silks vary in property as protein intake varies. Orb web spiders thus represent good models for extended phenotypic plasticity studies. We performed experiments manipulating the protein intake of two vertically aligned orb web building spiders to determine whether variations in the chemical and physical properties of their spiral and gluey silk affect prey retention in their webs. We found in both spider species that individuals deprived of protein had a greater gluey silk glycoprotein core volume, and this correlated strongly with spiral thread stickiness and increased prey retention by the webs. Moreover, we found strong positive correlations between glue droplet volume and glycoprotein core volume for spiders in the protein-deprived treatment, but weaker correlations for protein-fed spiders. We interpreted these findings as the spiders investing more in glycoprotein when nutrient deprived. We attribute the associated increase in prey retention capacity as a fitness consequence of plasticity in the spiral properties.

KEY WORDS: Aggregate silk, Flagelliform threads, Plasticity, Spider orb webs, Physicochemical properties

INTRODUCTION

Animals build a wide range of structures to perform specific functions, including thermoregulation, mate attraction, and protection of eggs and hatchlings (Collias and Collias, 1984; Hansell, 2005). Given the importance of these functions in animal reproductive success and survivorship (Collias and Collias, 1984; Jones et al., 1994; Kolbe and Janzen, 2002), there are inevitably fitness costs and benefits associated with structure building (Hansell, 2005). The form and function of different animal structures are thus subject to Darwinian selection in a manner that is similar to other phenotypes (Turner, 2000). Accordingly, the structures that animals build can be regarded as 'extended phenotypes' (Dawkins, 1982; Turner, 2000).

Like regular phenotypes, extended phenotypes are not fixed but may vary in form and function across environments. For instance, the architectural traits of bird nests and bowers depend on a variety of environmental factors, including climatic conditions, levels of ultraviolet and other radiation, the availability of building materials, and the presence of predators, competitors and parasites (Collias and Collias, 1984). Likewise, the properties and architecture of caddisfly larvae cases vary with water flow and sediment types (Okano and Kikuchi, 2009; Okano et al., 2011, 2016), and the number and quality of fibrous threads incorporated into nests by sticklebacks, and the mass of substrate deposited onto their nests, varies with water depth and flow rate, and availability of materials (Rushbrook and Barber, 2008; Rushbrook et al., 2010).

Phenotypic plasticity describes variation in phenotypes across environments and is depicted by plots of some fitness parameters against an environmental factor; a so-called reaction norm (Schlichting and Pigliucci, 1995; Via et al., 1995; Nussey et al., 2007). Variations in extended phenotypes can likewise be depicted by plotting reaction norms if the fitness parameters associated with extended phenotypic plasticity can be determined (Laland and Sterelny, 2006; Blamires, 2010; Bailey, 2012). Unfortunately, the fitness effects of most plastic extended phenotypes are largely unknown (but see Okano et al., 2016).

Of the various animal structures, spider webs are one of the most intriguing and commonly used for extended phenotype research (Blamires, 2010; Nakata, 2012; Montiglio and Di Renzo, 2016; Blamires et al., 2017a). This is primarily due to their pervasiveness across most terrestrial biomes. In addition, their function as an aerial prey-catching structure is strikingly unique. Spiders utilize a complex toolkit of silks to construct their webs, each with remarkably unique chemical and physical properties (Vollrath and Knight, 2001; Blackledge and Hayashi, 2006; Townley and Tillinghast, 2013; Blamires et al., 2017b). Web silks are spun by spiders following simple behavioural rules (Krink and Vollrath, 1997). Most critically, however, spider webs have exceptional structural and functional plasticity across different environments (Herberstein and Tso, 2011; Boutry and Blamires, 2013; Hesselberg, 2015).

Undoubtedly, the most readily recognizable form of spider web is the orb web, with its characteristic two-dimensional circular-shaped capture area, a spiral capture thread circling outwards from the hub, and evenly distributed radial threads that span from the hub to the web periphery (Foelix, 2011). Most studies documenting spider web plasticity have measured shifts in the architecture or properties of the orb web or its constituent silks under changing climatic (e.g. wind, temperature; Vollrath et al., 1997; Wu et al., 2013; Stellwagen et al., 2014), physical (Harmer and Herberstein, 2009), or dietary (Blamires, 2010; Blamires et al., 2011, 2015a, 2017a) conditions or constraints. Although such studies have been invaluable for establishing spider webs as extended phenotypes exhibiting multiple forms of plasticity, they have not successfully measured any plausible fitness benefits for this plasticity.

The webs of modern orb web-building spiders (i.e. orb web spiders that spin viscous prey-capturing spiral threads into their webs) consist of spiral threads made up of a fibrous spiral (flagelliform) silken thread coated by an aqueous gluey

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(aggregate) silk. The gluey silk coating plasticizes the spiral thread and makes it soft and extensible (Guinea et al., 2010; Amarpuri et al., 2017). The high extensibility of the spiral thread enables it to absorb enormous amounts of kinetic energy imparted by flying prey, while the gluey silk serves to retain intercepted prey (Blackledge and Hayashi, 2006; Tarakanova and Buehler, 2012; Sahni et al., 2014). These properties are valuable for vertically aligned orb webs as prey and other flying animals may strike the webs while in full flight. Moreover, without any means of retention, the intercepted prey may easily fall out of the web owing to gravity (Blackledge and Zevenbergen, 2006; Opell and Schwend, 2007).

The stickiness of the spiral thread is conferred by the presence of a glycoprotein core within the gluey silk (Bonthrone et al., 1992; Sahni et al., 2010, 2011; Opell et al., 2013). Various salts and other compounds in the gluey silk render it hygroscopic and, as a consequence, it absorbs atmospheric water (Edmonds and Vollrath, 1992; Higgins et al., 2001; Sahni et al., 2011; Townley and Tillinghast, 2013; Stellwagen et al., 2014). The consequent increase in surface tension induces Rayleigh instability and the gluey silk forms into droplets (Vollrath and Edmonds, 1989, 2013; Edmonds and Vollrath, 1992; Opell et al., 2013), which position themselves along the thread to resemble a beads-on-a-string arrangement (Sahni et al., 2012; Wu et al., 2013; Torres et al., 2014; Blamires et al., 2017a).

Several studies have established that the physical and chemical properties of modern orb web spiral and gluey silks vary with changing climatic or dietary conditions (Wu et al., 2013; Stellwagen et al., 2014, 2016; Blamires et al., 2014, 2017a). As the function of the spiral thread is to retain any intercepted prey, it might be hypothesized that a fitness consequence of any changes in spiral and/or gluey silk properties is the enhancement or diminishment of prey retention (Opell et al., 2017), assuming that the spiders consume all prey retained in the web, as previous observations have suggested they do (Blamires et al., 2017a). We tested this hypothesis here on two species of orb web spider by performing dietary manipulations known to induce physical and chemical property variations in orb web spiral and gluey silks (Blamires et al., 2014, 2017a). We measured the spiral and gluey silk property variations induced by the dietary manipulations before placing prey onto their webs and measuring the prey retention capacity. We interpreted correlations between the spiral and gluey silk properties and spiral thread prey retention capacity as confirmation that prey retention represents a fitness consequence associated with changes in the spiral and gluey silk properties. A lack of a correlation was assumed to indicate that there are no discernible fitness consequences for changes to the silk's properties.

MATERIALS AND METHODS

Spider collection and pre-feeding

For the following experiments we used two species of modern orb web building spider: *Argiope keyserlingi* Karsch 1878 (Araneae, Araneidae) and *Nephila plumipes* (Latreille 1804) (Araneae, Nephilidae). Both of these spiders build large, vertically aligned orb webs that may be required to intercept and retain prey in full flight. We collected 40 sub-adult females of these spiders from various locations between Sydney and Ballina, New South Wales, Australia, during trips made between October 2014 and January 2015. To ensure that spiders of approximately equal size were used, we measured each spider's body length to ±0.1 mm, using digital Vernier callipers (Calliper Technologies, Mountain View, CA, USA), and mass to ±0.001 g, using an electronic balance (Ohaus, Pine Brook, NY, USA), upon collection before placing them in 115×45 mm (width×height) plastic circular containers. The containers had perforated wire mesh lids with a 20-mm-long slit cut into them using a Stanley knife to facilitate feeding with a 50 µl micropipette. We pre-fed the spiders 20 µl (*A. keyserlingi*) or 50 µl (*N. plumipes*) of a 30% (w/v) glucose solution daily over 5 days to standardize the diet of the spiders prior to experimentation. We reweighed the spiders after the pre-feeding treatment and any individuals who lost >50% of their initial mass (two *A. keyserlingi* and four *N. plumipes*) were not used any further.

Feeding experiment

As a previous experiment found that protein intake or deprivation affected the physical and chemical properties of other modern orb web spiral and gluey silks (Blamires et al., 2014), we randomly divided the 40 or so spiders from each species equally into two groups and fed them either one of two solutions over 10 days: a protein-rich (the 'P' treatment) or protein-deprived solution (the 'N' treatment). Before commencing the feeding experiment the spiders were placed within upright 300×300×50 mm (height×width×thickness) (*A. keyserlingi*) or 500×500×70 mm (*N. plumipes*) enclosures (these sizes were chosen so that enclosure size approximated the size of the web of each species in the field), and we waited approximately 3 days for them to all build orb webs. These webs were subsequently used for the pre-feeding between treatment comparisons of spiral and gluey silk properties, stickiness and prey retention capacity.

Once the spiders had built webs, they were removed from the enclosures and placed back into their plastic circular containers for the feeding experiment. The protein-rich solution used was identical to the protein-rich treatment used by Blamires et al. (2014). The protein-deprived solution used was 8 g of sucrose in 30 ml of water. We fed the spiders by placing a 20 µl (*A. keyserlingi*) or 50 µl (*N. plumipes*) droplet of solution onto their chelicerae using a 50 µl micropipette (see Blamires et al., 2014, 2015b) once per day over 10 days. Because protein and carbohydrates contain approximately similar energy densities (~4 kJ g^{-1}) solutions of similar energy concentrations were fed to all of the spiders. We chose this housing and feeding regime because we found the spiders readily feed this way in the laboratory, so it is an effective way to deliver known nutrients to spiders. Furthermore, it was effectively used previously to induce physical and chemical property changes in the gluey silks of other modern orb web spiders (Higgins et al., 2001; Blamires et al., 2014).

Once the feeding experiment was completed, we placed the spiders back into their respective enclosures and waited approximately three further days for them to all rebuild orb webs, upon which the following measurements were made under controlled temperature (25.0±0.2°C) and humidity (50.0±2.7% RH) in still air.

Spiral thread collection and property measurements

We cut 11×11 mm (width×depth) U-shaped openings into the short side of 75×25 mm (length×width) cardboard cards onto which we collected six spiral threads from the lower portion of each spider's web, as this is the only location on the webs where spirals with at least 11 mm between radii are found. We collected each thread by lightly touching the tips of the U-shaped openings to a length of spiral thread and allowing the thread to adhere to the cardboard at the tips of the U-shaped openings. We freed the11 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 cardboard. Three of the six collected threads were used to measure the thread width, droplet

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volume, number of droplets per 0.5 mm of thread, droplet surface area and glycoprotein core volume. The remaining three spiral threads were used to measure spiral stickiness.

To measure thread width, droplet volume, droplets per 0.5 mm of thread, droplet surface area and glycoprotein core volume, 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 and randomly selected droplets under 100× and 400× magnification using a light microscope (CKX41, Olympus, Tokyo, Japan) connected to a SPOT Idea 5 megapixel digital camera (Spot Imaging Solutions, Sterling Heights, MI, USA). From the 100× magnification photographs we counted the number of gluey droplets per 0.5 mm of thread. We used the 400× magnification photographs to measure the length and width of the droplets using the program ImageJ (National Institutes of Health, Bethesda, MD, USA). We removed the dowels so that the samples touched the slides they were mounted on. The consequent flattening of the droplets rendered the glycoprotein core and underlying thread visible (Opell et al., 2017). The glycoprotein core appeared approximately ellipsoid, as depicted in Opell et al. (2017). We viewed and photographed the flattened droplets under 400× magnification. We measured the width of the flattened droplets, and the radii of the underlying threads and the glycoprotein cores, using ImageJ.

We used the above measurements to determine the mean droplet volume (DV), assuming the droplets conformed to an ellipsoid shape (Fig. S1), using the formula (Liao et al., 2015): $DV=[16\pi(h)^2b]/15$, where h is half the width of the droplet, and b is half the length of the droplet. We then calculated the total droplet volume along an arbitrary 0.5 mm length of thread (DV/0.5 mm) (Opell and Hendricks, 2007). We calculated the approximate surface area of the droplets (DSA) using the formula: $DSA=(4\pi hb)/3$. We used this formula as it was used previously to approximate the surface area of orb web glue droplets (Wu et al., 2013; Blamires et al., 2015a), not because it is a proven measure of glue droplet surface area. Indeed, such a proof does not exist and is urgently required. Nevertheless, as we applied the same formula to all glue droplets, the formula is inconsequential to the outcome of our between-treatment comparisons.

The droplet volume to surface area ratio (DV:DSA) was determined as the droplet surface area divided by droplet volume. We divided DV by the flattened droplet area (calculated as π×droplet radius squared) to ascertain the thickness of the flattened droplet. We calculated the glycoprotein core volume (GCV) as the flattened droplet thickness multiplied by the surface area of the flattened core, which we ascertained from the photographs of the flattened droplets using ImageJ. The droplet volume to glycoprotein core volume ratio (DV:GCV) was then calculated.

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 (~5 days) had negligible effects on any subsequent property 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 facing downwards) within the uppermost grips of an Instron 5543 tensile testing machine (Instron Machines, Melbourne, Australia) with a resolution of approximately 2 µN (Blamires et al., 2017a). A 6×2 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, before being pulled up at 0.1 mms^{-1} until the thread detached from the stage. The force (µN) required to pull the thread off the stage was measured using the program Bluehill 3.0 (Instron Machines) and used as a proxy of thread stickiness (Opell, 1989). We repeated this procedure 10 times using a different part of the stage each time, cleaning the stage with an ethanol swab prior to each repeated measurement, and obtained an average value per thread.

Prey retention measurements

Upon sampling of spiral threads, we simultaneously placed one live (mass: 0.316±0.133 g) and one dead (mass: 0.298±0.129 g) cricket randomly onto the left and right upper portion of each spider's web and two observers timed how long it took for each of them to escape/fall from the web using digital stopwatches. The time recorded was the time taken for the crickets to permanently or temporarily wriggle or fall free. Any subsequent recapture of the live/dead cricket by other spirals in the web was ignored. The dead crickets were from the same laboratory-reared stock as the live crickets, and were killed immediately prior to experimentation by lethal exposure to CO_2. The dead crickets were thus physically identical to the live crickets with the exception that they did not struggle to escape when placed in the web. We called the time it took the dead cricket to fall from the web the 'functional stickiness' of the spiral thread, as it is a product of the stickiness of the thread, surface features of the cricket cuticle, any movements of the thread, and the downward force induced by the carcass falling under gravity (Opell and Schwend, 2007). We called the time taken for the struggling live cricket to escape the 'prey retention capacity' and it is a product of the same factors inducing the 'functional stickiness' of the thread and the ability of the glue to withstand the forces exerted against it by the struggling prey (Opell et al., 2017).

Table 1. Results of Friedman's pairwise multivariate ANOVAs and Tukey's *post hoc* analyses for *Argiope keyserlingi*

Variable	Friedman's statistic	Kendall's co-efficient of concordance	Average rank (*r*)	*P*	*Post hoc* comparison
Spiral thread width	0.793	0.012	0.050	0.379	–
Droplet volume	0.954	0.016	0.066	0.335	–
Droplet volume/0.5 mm thread	0.043	0.001	0.038	0.835	–
Droplet volume to surface area ratio	1.446	0.002	0.063	0.237	–
Glycoprotein core volume	16.638	0.476	0.892	<0.0001*	N>P
Droplet to glycoprotein core volume ratio	12.257	0.549	0.710	<0.0001*	N>P
Thread stickiness	6.785	0.258	0.236	0.013*	N>P
Functional stickiness	11.925	0.331	0.315	0.001*	N>P
Prey retention capacity	34.124	0.932	0.831	<0.0001*	N>P

N, protein-derived treatment; P, protein-rich treatment. d.f.=1,36. Asterisks indicate significance at $P<0.05$.

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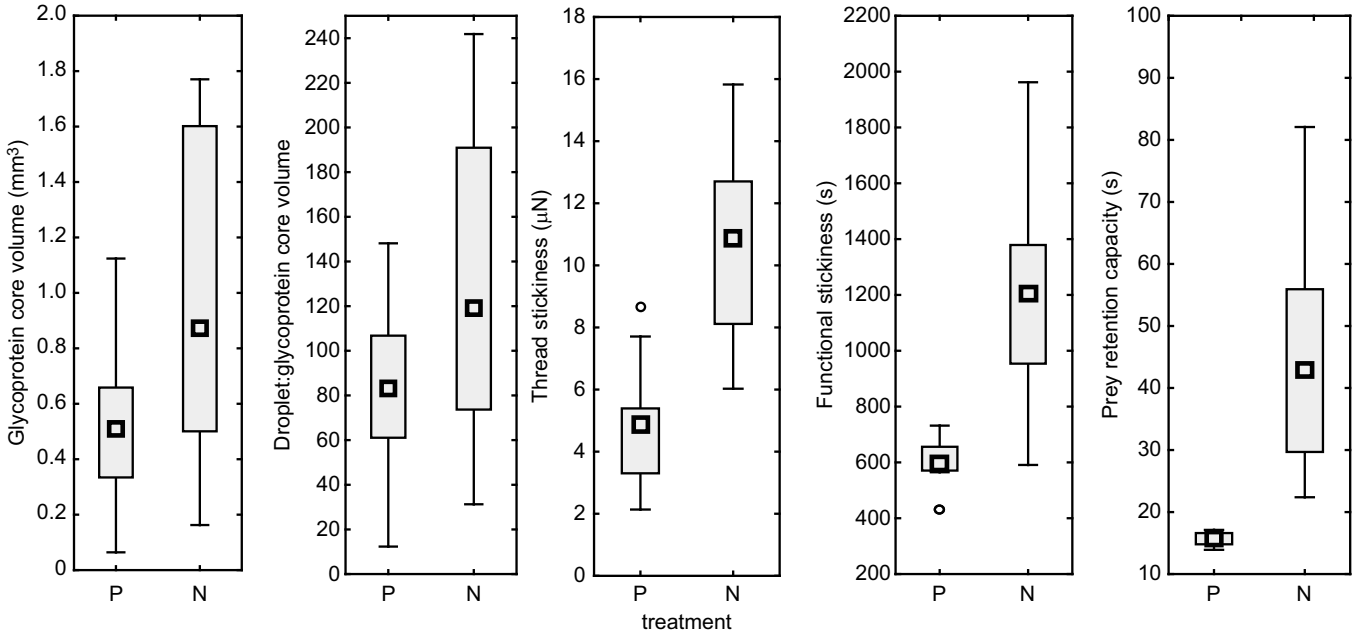

Fig. 1. Box-and-whisker plots of median glycoprotein core volume, droplet to glycoprotein core volume ratio, thread stickiness, functional stickiness and prey retention capacity of *Argiope keyserlingi* spiral threads across the protein-rich and protein-deprived feeding treatments. P, protein-rich treatment; N, protein-deprived treatment. The open square symbol denotes the median, the white box denotes the 25–75% range, and the whiskers denote the non-outlier range.

The prey retention capacities of orb webs are influenced by a combination of the spiral thread properties, gravity, insect surface properties, and the number and width (often referred to as 'mesh height' or 'spiral spacing'; Blamires, 2010; Blamires et al., 2017a,b) of the successive spiral thread turns (Blackledge and Zevenbergen, 2006; Opell and Schwend, 2007). We thus measured the web height and width, and counted the number of times the spiral thread crossed selected radial threads running in an upward, downward, rightward and leftward direction to calculate the 'spiral spacing' according to Herberstein and Tso (2000).

Analyses

We first checked that the pre-treatment feeding effectively standardized all of the spiral thread properties and prey retention capacities across treatments for all spiders. The pre-feeding data of both *A. keyserlingi* and *N. plumipes* failed tests for normality (Table S1) and heterogeneity of variances (Table S2), even after repeated transformations. We therefore used rank-based multivariate Mann–Whitney tests, an analysis that performs similarly to a Kruskal–Wallis one-way ANOVA comparison of means (Choi and Marden, 1997; Konietschke et al., 2012), to compare, for each species, the pre-feeding spiral thread and prey retention properties between treatment allocations. We found that thread width, DV:DSA, DV:GCV and prey retention capacity differed between treatment allocations for *A. keyserlingi* (Table S3A), and droplet volume, DV:GCV, functional stickiness and prey retention capacity differed between treatment allocations for *N. plumipes* (Table S3B).

Our analyses of the pre-feeding data (see above) indicated that our pre-feeding regimes failed to standardize the various spiral thread properties and prey retention capacities across allocated treatments for all of the spiders. The experimental data also failed tests for normality (Table S4) and heterogeneity of variances (Table S5) even after transformation. We therefore performed Friedman's pairwise multivariate ANOVAs with Tukey's *post hoc* analyses for each species to compare the mean spiral thread properties and prey retention capacities across treatments (Sokal and Rohlf, 1995).

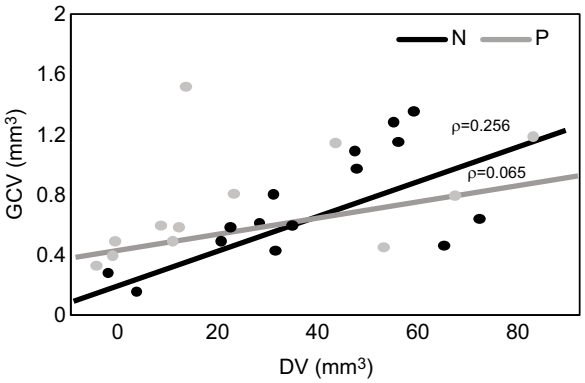

Fig. 2. Linear regression curves for droplet volume and glycoprotein core volume, showing correlation coefficients across P and N treatments for *A. keyserlingi*. DV, droplet volume; GCV, glycoprotein core volume; ρ, correlation coefficient; P, protein-rich treatment; N, protein-derived treatment.

Table 2. Results of the generalized mixed models for *A. keyserlingi*, assigning spiral spacing and glycoprotein core volume as the predictor variables and thread stickiness, functional stickiness and prey retention capacity as the response variables

		Spiral spacing		Glycoprotein core volume	
	d.f.	Wald's statistic	*P*	Wald's statistic	*P*
Intercept	3	11.088	0.002*	4.879	0.014*
Thread stickiness	3	0.114	0.737	4.633	0.009*
Functional stickiness	3	0.024	0.872	5.841	0.003*
Prey retention capacity	3	0.672	0.418	10.271	0.003*
Treatment	3	0.611	0.451	0.423	0.514

Asterisks indicate significance at $P<0.05$.

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As a result, we built generalized mixed models for both species. These models can reliably ascertain any associations among large numbers of predictor and response variables (Burton et al., 1998). We assigned 'spiral spacing', and any thread properties (i.e. thread width, droplet volume, droplet volume/0.5 mm, droplet volume to surface area ratio, glycoprotein core volume and/or droplet volume to glycoprotein core volume ratio) that our analyses found to differ between treatments as the predictor variables, and thread stickiness and/or any prey retention parameters (i.e. functional stickiness and/or prey retention capacity) that we found to differ between treatments as the response variables. Our models had normal response distributions and log link functions, as these fitted the data for both species (*A. keyserlingi*: Pearson goodness-of-fit test, χ^2=0.856, d.f.=2, *P*=0.648; *N. plumipes*: Pearson goodness-of-fit test, χ^2=3.570, d.f.=2, *P*=0.167). We included individual spider identity as a categorical random factor in our models to control for any within-individual differences.

RESULTS

For *A. keyserlingi*, glycoprotein core volume, thread stickiness, droplet to glycoprotein core volume ratio, functional stickiness, and prey retention capacity differed between treatments (Table 1; see Table S6 for values) with the N treatment being greater than the P treatment for all of these parameters (Fig. 1). We found droplet to glycoprotein core volume to differ between treatments without a corresponding significant difference in droplet volume (Table 1), which indicated to us that the increase in glycoprotein investment by the protein-deprived spiders was not necessarily a consequence of them producing larger droplets. To confirm this interpretation of the data, we checked the relationship between droplet volume and glycoprotein core volume using multiple linear regression and found that the relationships differed across treatments (Table S7A,B). Indeed, a significant positive relationship existed between spiral droplet volume and glycoprotein core volume for the protein-deprived spiders (*P*=0.007), whereas there was no relationship (*P*=0.280) between these parameters for the spiders fed the protein-rich solution (Fig. 2).

We subsequently built generalized mixed models for the predictor variables 'spiral spacing' and glycoprotein core volume, and found that functional stickiness and prey retention capacity varied with glycoprotein core volume across treatments (Table 2). The parameters thread stickiness, functional stickiness and prey retention capacity did not vary with 'spiral spacing'. These results, and the across-treatment reaction norms for glycoprotein core volume, droplet to glycoprotein core volume ratio, functional stickiness and prey retention capacity for the spiral threads of *A. keyserlingi* (Fig. 3), indicated that the prey retention capacity of *A. keyserlingi* webs was enhanced by glycoprotein core volume plastically enlarging when the spiders were deprived of protein.

For *N. plumipes*, spiral thread width, glycoprotein core volume, droplet to glycoprotein core volume ratio, thread stickiness, functional stickiness and prey retention capacity differed between treatments (Table 3) with the values for the N treatment being consistently greater than for the P treatment (Fig. 4). Again, between-treatment differences in glycoprotein core volume without corresponding significant differences in droplet volume (Table 3) prompted us to check the relationship between droplet volume and glycoprotein core volume using multiple linear regression. As with *A. keyserlingi*, we found that the relationship of these parameters differed across treatments (Table S6C,D). Again, a significant

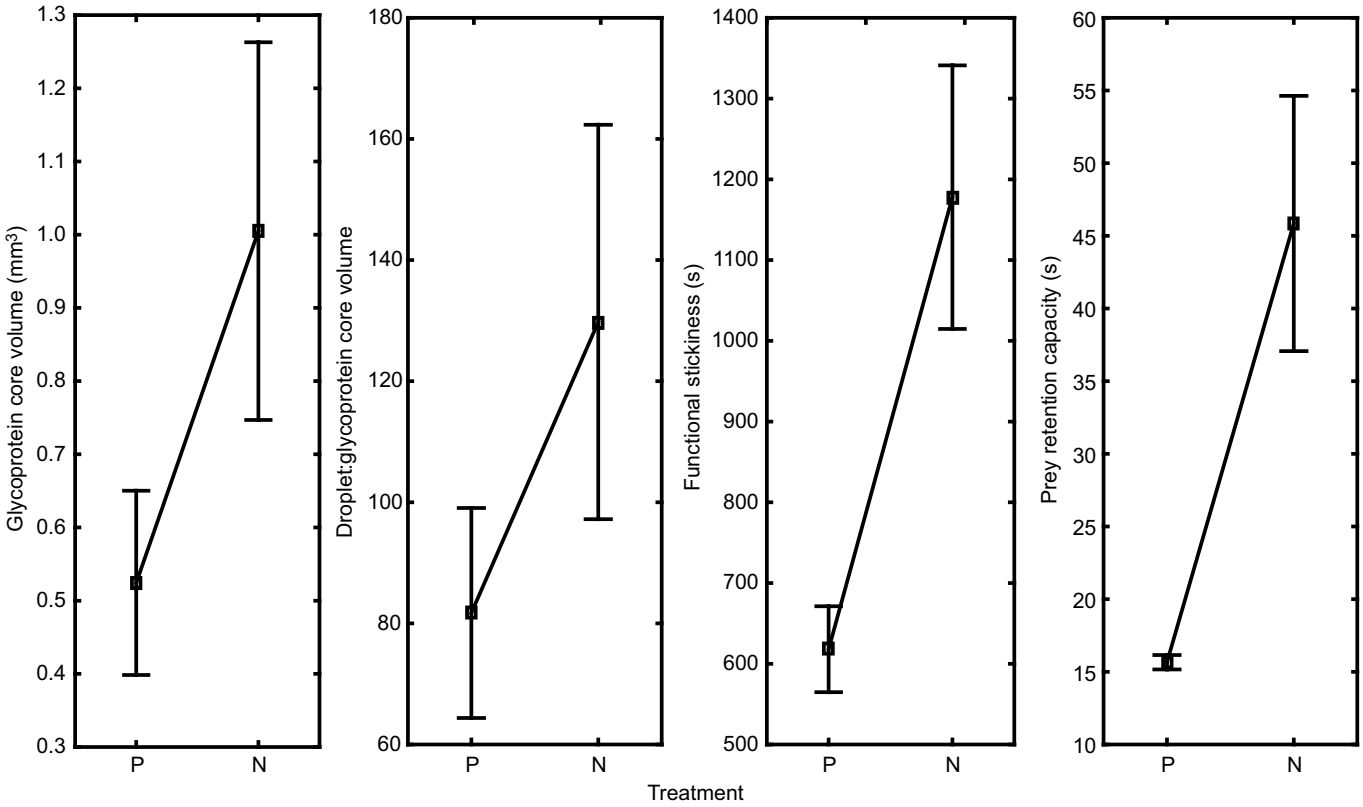

Fig. 3. Reaction norms of mean glycoprotein core volume, droplet to glycoprotein core volume ratio, functional stickiness and prey retention capacity of *A. keyserlingi* spiral threads across the protein-rich and protein-deprived feeding treatments. P, protein-rich treatment; N, protein-derived treatment. The square symbols denotes the mean, the whiskers denote ±0.95 confidence interval.

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Table 3. Results of a Friedman's pairwise multivariate ANOVA and Tukey's *post hoc* analyses for *Nephila plumipes*

Variable	Friedman's statistic	Kendall's co-efficient of concordance	Average rank (*r*)	*P*	*Post hoc* comparison
Spiral thread width	5.475	0.103	0.329	0.025*	N>P
Droplet volume	0.722	0.044	0.088	0.401	–
Droplet volume/0.5 mm thread	0.022	0.006	0.009	0.952	–
Droplet volume to surface area ratio	0.050	0.009	0.014	0.823	–
Glycoprotein core volume	14.352	0.750	0.535	<0.0001*	N>P
Droplet to glycoprotein core volume ratio	10.115	0.624	0.627	<0.0001*	N>P
Thread stickiness	42.173	0.901	0.839	<0.0001*	N>P
Functional stickiness	28.288	0.871	0.442	<0.0001*	N>P
Prey retention capacity	27.423	0.811	0.383	<0.0001*	N>P

N, protein-derived treatment; P, protein-rich treatment. d.f.=1,34. Asterisks indicate significance at $P<0.05$.

positive relationship existed between spiral droplet volume and glycoprotein core volume for the protein-deprived spiders ($P<0.001$). However, in the case of *N. plumipes*, we also found a significant positive relationship between spiral droplet volume and glycoprotein core volume ($P<0.023$) for the protein-fed spiders. The correlation coefficients of the regression curves (Fig. 5), nevertheless, indicated that this relationship is not as pronounced as that for the protein-fed spiders.

We built generalized mixed models for the predictor variables 'spiral spacing', thread width and glycoprotein core volume, and found that thread stickiness, functional stickiness and prey retention capacity varied across treatments with glycoprotein core volume (Table 4).

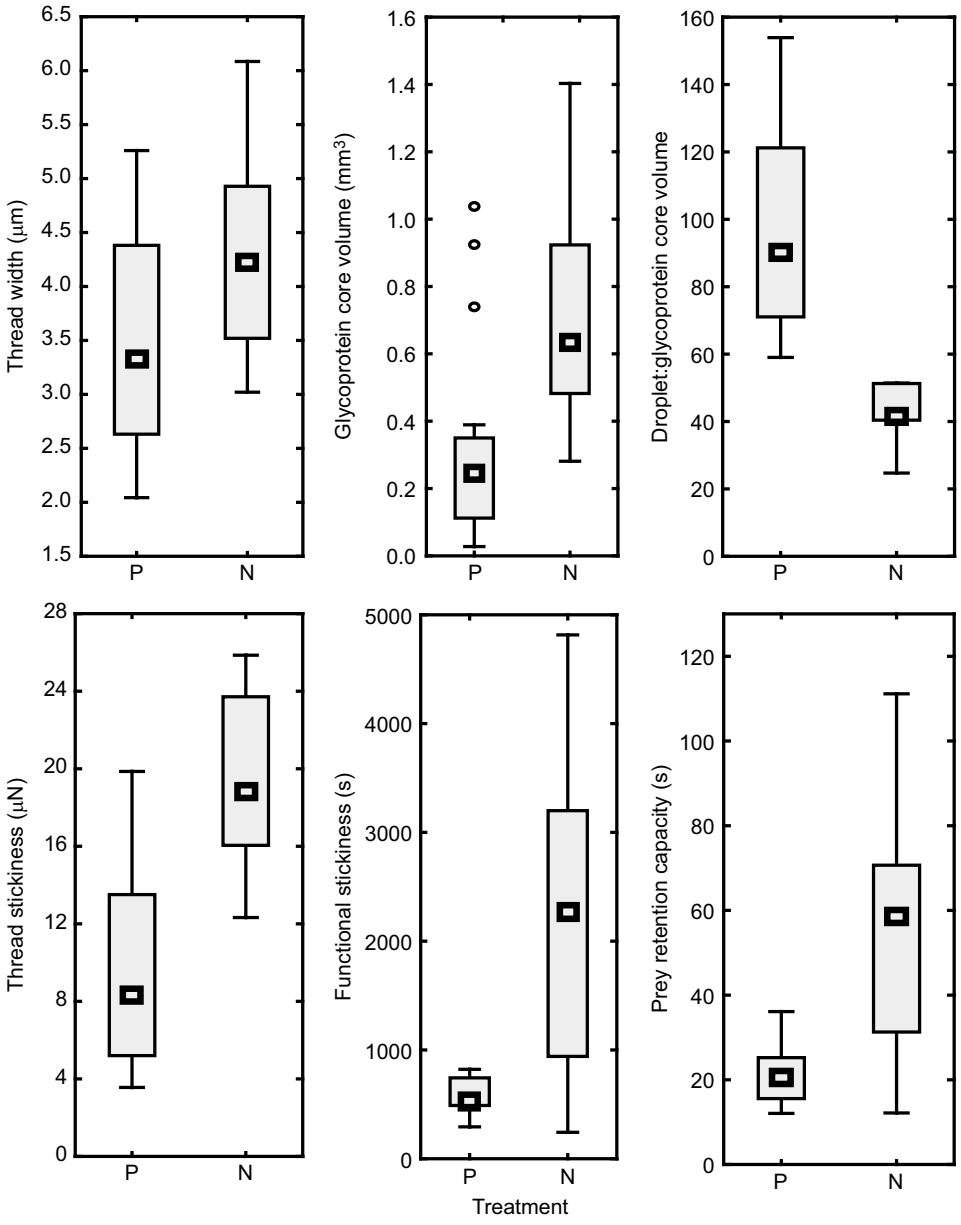

Fig. 4. Box-and-whisker plots of median glycoprotein core volume, droplet to glycoprotein core volume ratio, thread stickiness, functional stickiness and prey retention capacity of *Nephila plumipes* spiral threads across the protein-rich and protein-deprived feeding treatments. P, protein-rich treatment; N, protein-derived treatment. The open square symbol denotes the median, the white box denotes the 25–75% range, and the whiskers denote the non-outlier range.

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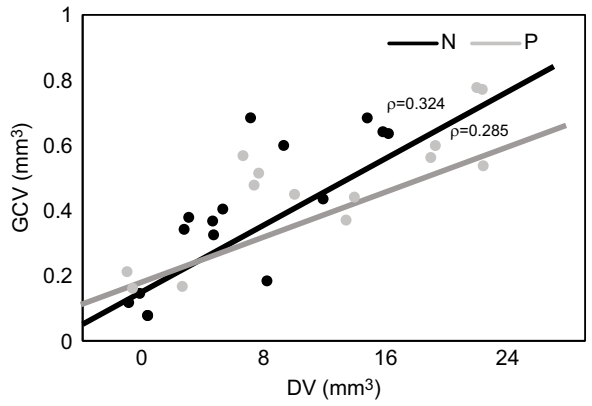

Fig. 5. Linear regression curves for droplet volume and glycoprotein core volume showing correlation coefficients across P and N treatments for *N. plumipes*. DV, droplet volume; GCV, glycoprotein core volume; ρ, correlation coefficient; P, protein-rich treatment; N, protein-derived treatment.

None of the response variables (i.e. spiral stickiness, functional stickiness or prey retention capacity) varied with 'spiral spacing' or thread width. Thus, like *A. keyserlingi*, our results and the resultant reaction norms (Fig. 6) indicated that the prey retention capacity of the spiral threads of *N. plumipes* was enhanced as the glycoprotein core volume plastically enlarged when the spiders were deprived of protein.

DISCUSSION

We hypothesized here that, as the spiral threads of vertically aligned orb webs function to intercept and retain flying prey, a fitness consequence of plasticity in their spiral and gluey silk properties is a change in the web's prey retention capacity. Our subsequent experiments found significant correlations between the spiral thread and gluey silk properties and the web's prey retention capacity in two species of spider that build vertically aligned orb webs (*A. keyserlingi* and *N. plumipes*), thereby confirming our hypothesis.

We took our analyses further and created generalized linear models to tease apart the environmental (protein intake), extended phenotype (spiral thread properties) and fitness (prey retention) effects for *A. keyserlingi* and *N. plumipes*, and found that spiral thread glycoprotein core volume varied with prey retention in both species under protein deprivation. The significance of our findings is that they unequivocally show a fitness effect for plasticity in an extended phenotype. Our conclusion, however, assumes that the spiders consume all of the prey retained by their webs. We think that this is a reasonable assumption as we had observed previously that *A. keyserlingi* consumes any prey caught in its web if they are retained long enough to be attacked and wrapped (Blamires et al., 2017a).

For both species we found greater prey retention in the spiral threads as glycoprotein core volume plastically enlarged under protein deprivation. It makes sense to us that prey retention capacity should co-vary with glue droplet volume and glycoprotein core volume, as it is the glycoprotein component of orb web glues that has been shown to confer their stickiness (Opell and Hendricks, 2010; Opell et al., 2011a; Townley and Tillinghast, 2013; Sahni et al., 2014). Spiral thread adhesion is, however, not exclusively reliant on the volume of glycoproteins. Hygroscopic salts in the glue droplets mobilize the glycoproteins and promote the absorption of atmospheric water to keep them pliable (Edmonds and Vollrath, 1992; Opell et al., 2011b; Sahni et al., 2011, 2014). Although we did not detect any differences is droplet shape or volume across the N and P feeding treatments, suggesting there was little or no difference in the amount of atmospheric water absorbed into the glue droplets produced by spiders on the different treatments, our multiple regressions did find a difference in the association between droplet volume and glycoprotein core volume between treatments in both spiders. We inferred this finding as indicative that the spiders invested in more glycoproteins as they produced more voluminous droplets under nutrient deprivation, presumably to significantly enhance the prey retention capacities of their webs.

As water absorption into the glue droplets is driven by the concentration of hygroscopic salts (Edmonds and Vollrath, 1992; Sahni et al., 2011), it seems as though salt composition in the spider's gluey silks did not change significantly across treatment. This contrasts with the results of Blamires et al. (2017a), who found greater potassium and phosphate salt concentrations in *A. keyserlingi* gluey silk when on different diets, and Blamires et al. (2014) who found the gluey silk droplets of *Nephila clavipes* to enlarge when they were protein deprived. The hygroscopic potential of the gluey silks of different orb web spiders is highly variable and humidity specific (Opell et al., 2013). Accordingly, the relative humidity that the experiments were performed at (i.e. 50.0±2.7%) may have been too high or low to induce humidity-induced changes in glue droplet volume for either of these spiders regardless of any changes to the concentration of the glue's salts.

An increased glycoprotein core volume with an unchanged droplet volume when the spiders were fed the protein-rich solution indicates that water may have been lost from the glue droplets and the resultant increase in glycoprotein viscoelasticity rather than volume per se was responsible for thread adhesiveness. A possible explanation for the strong association between glycoprotein core volume and prey retention capacity is that an enlargement of the gluey silk glycoproteins exposed their chitin-binding protein fragment, which is responsible for their toughness and persistent adhesiveness to insect surfaces (Opell and Schwend, 2007; Opell et al., 2011b; Vasanthavada et al., 2012; Amarpuri et al., 2015). Our finding that the functional stickiness, which is a product of the stickiness of the thread, the interaction between cuticle surface features and the thread, any movements of the thread, and the downward gravitational force, also varied with glycoprotein core volume across treatments, rendering the abovementioned

Table 4. Results of the generalized mixed models for *N. plumipes*, assigning spiral spacing, spiral thread width and glycoprotein core volume as the predictor variables, and thread stickiness, functional stickiness and prey retention capacity as the response variables

		Spiral spacing		Spiral thread width		Glycoprotein core volume	
	d.f.	Wald's statistic	*P*	Wald's statistic	*P*	Wald's statistic	*P*
Intercept	3	0.365	0.696	1.407	0.281	30.835	<0.0001*
Thread stickiness	3	1.584	0.319	0.631	0.432	19.963	<0.001*
Functional stickiness	3	0.948	0.530	2.429	0.129	9.834	0.003
Prey retention capacity	3	0.535	0.678	0.621	0.411	5.791	0.023
Treatment	3	2.079	0.129	0.007	0.978	14.973	0.001*

Asterisks indicate significance at $P<0.05$.

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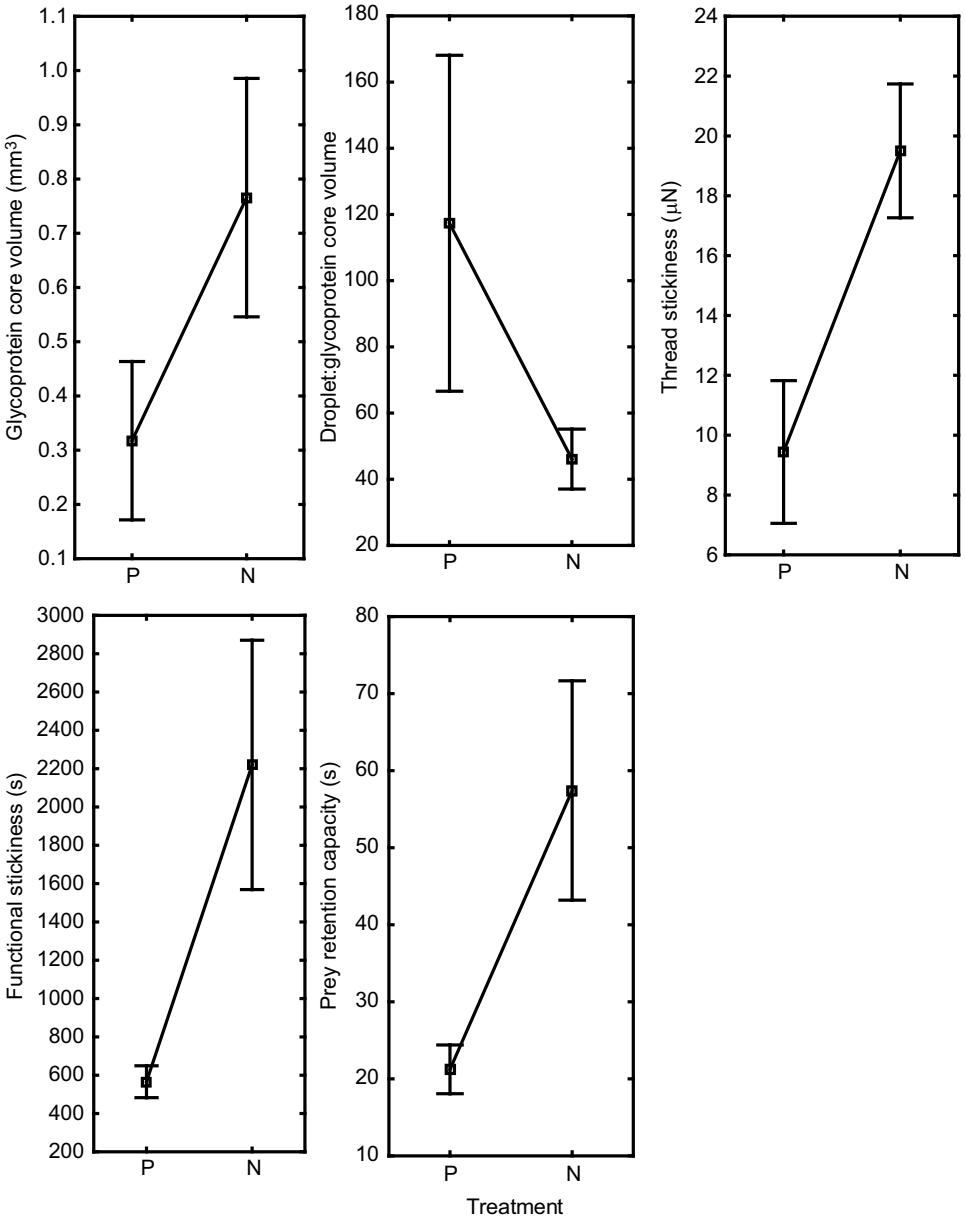

Fig. 6. Reaction norms of mean glycoprotein core volume, droplet to glycoprotein core volume ratio, functional stickiness and prey retention capacity of *N. plumipes* spiral threads across the protein-rich and protein-deprived feeding treatments. P, protein-rich treatment; N, protein-deprived treatment. The square symbols denotes the mean, the whiskers denote ±0.95 confidence interval.

proposition likely. Whatever the mechanism, it seems that the enhanced prey retention provided by the enhanced glycoprotein core volume provides a fitness benefit to the spider in the form of greater prey retention. Our findings suggest that if a spider experiences a reduction in prey interception or retention rates with subsequent nutritional depletion, it can adjust its glycoprotein investment within its gluey silk to enhance its prey capture success. In the wild, however, there may be a multitude of fluctuating environmental factors, e.g. prey availability, temperature, humidity, wind and UV radiation, all of which can further affect spider spiral and gluey silk functional morphology and biochemistry (Vollrath et al., 1997; Opell et al., 2011b, 2013; Wu et al., 2013; Stellwagen et al., 2014, 2016).

Our study significantly expands on previous studies of spider web architectural and property plasticity. Some studies have shown that starved spiders construct larger orb webs and/or orb webs with greater investment in spiral threads, which has been presumed to be an adaptation to enhance prey interception (Mayntz et al., 2003; Blamires et al., 2015a, 2017a). Other studies have shown gluey silk droplet properties to vary with diet (Blamires et al., 2014, 2015a, 2017a) or environmental changes (Opell et al., 2013; Wu et al., 2013; Stellwagen et al., 2016), albeit without subsequent investigations of the fitness benefits for the spider. Our experiments showed that orb web spiders are able to vary the volume of the glycoproteins in their gluey droplets in order to enhance prey capture. This ability probably comes at the expense of other uses for the requisite proteins, such as growth or investment in other silks (Higgins, 2006; Blamires et al., 2017b). We used two species of spider that build vertically aligned orb webs, webs from which large struggling prey often readily escape (Blackledge and Zevenbergen, 2006), and found similar trends. Deployment of greater glycoprotein content into the gluey silks may thus be a way that these spiders actively counteract high prey escape rates.

Additional studies have shown that spider silk and web properties vary dynamically across ‘nutrient space’ (Blamires et al., 2015a, 2016) as well as across prey types (Blamires, 2010; Blamires et al., 2017a). Our study expands these concepts by suggesting that spiders can manipulate their web and silk properties to optimize their

eventual nutrient uptake. Alternative explanations for the results of our study include the idea that protein intake serves as a signal to invest in certain types of silks or webs in order to alter the prey retention capacity of the web, or that the investment in mechanisms that increase spiral thread stickiness is so costly that they are only invested in when it is considered absolutely necessary (e.g. when completely depleted of protein). Evidently, more studies are needed to differentiate among the alternative explanations.

Various fitness effects have been ascribed for specific extended phenotypes, e.g. bird nests and bowers and crab pillars and hoods (Collias and Collias, 1984; Christy et al., 2002, 2003; Doucet and Montgomerie, 2003). However, detailed assessments of the fitness consequences of variations in extended phenotypes across environments are lacking. Some studies have identified potential consequences of extended phenotypic plasticity (e.g. Okano et al., 2016; Opell et al., 2017), but they could not differentiate amongst environmental, extended phenotypic, and fitness effects, and their interactions. Here we constructed models for the orb web spiders *A. keyserlingi* and *N. plumipes* and assigned parameters such as 'spiral spacing' and various thread properties as predictor variables, and fitness parameters such as thread stickiness and prey retention as response variables, to differentiate between environmental, extended phenotypic, and fitness effects. We categorically show a fitness benefit for orb web spiral thread plasticity when nutrient intake differed. Further studies need to be done to fully understand how multiple factors interact to induce fitness effects in other plastic extended phenotypes.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: S.J.B., M.M.K.; Methodology: S.J.B., P.J.M.; Software: S.J.B., P.J.M.; Validation: S.J.B., P.J.M.; Formal analysis: S.J.B., P.J.M., M.M.K.; Investigation: S.J.B., M.M.K.; Resources: S.J.B., P.J.M., M.M.K.; Data curation: S.J.B., P.J.M., M.M.K.; Writing - original draft: S.J.B.; Writing - review & editing: S.J.B., P.J.M., M.M.K.; Project administration: S.J.B., P.J.M., M.M.K.; Funding acquisition: S.J.B.

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