



The web architecture of *Latrodectus hesperus* black widow spiders (Araneae: Theridiidae) shows genetic variation and sexual dimorphism, but no plasticity according to the experience of the site of prey capture

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Abstract

Animals create extended phenotypes to adaptively modify and interact with their environment. Animals also respond to changes in the environment by adaptively modifying their behavior or body in a process called phenotypic plasticity. Extended phenotypes might be especially prone to plasticity as they are often the products of behavior. We tested for plasticity in black widow web architecture in response to long-term variation in prey capture location. We also estimated genetic variation, genetic variation in plasticity, and sexual dimorphism in web architecture. We found no overall plasticity in response to variation in prey capture location. However, web architecture differed between spider families, and spider families differed in their plastic response. Web architecture also differed between male and female spiders, and male and female spiders differed in their plastic response. We conclude that black widows do not adjust web architecture in response to long-term inputs from the external environment and that internal inputs are responsible for more of the variation in web architecture than external inputs. We suggest that in order to fully understand variation in extended phenotypes, it is important to investigate potential environmental and physiological or genetic sources of variation, and to examine these potential sources at different time scales.

Significance statement

Animals can alter their extended phenotypes in response to short- or long-term variation in the external environment, or to internal sources of variation, such as genetic variation or sexual dimorphism. Studies asking how extended phenotypes vary at more than one time scale and that simultaneously test for variation due to different sources of variation are rare. Here, we ask how an extended phenotype varies in response to environmental variation at short and long time scales, whether the extended phenotype has a genetic component of variation, and whether the extended phenotype is sexually dimorphic. We found that the extended phenotype did not respond to long-term environmental variation but did differ in response to all tested internal inputs. These results suggest that variation in extended phenotypes should be considered at multiple time scales and across external and internal sources of variation.

Keywords Extended phenotype · Plasticity · Spider · Variation

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Introduction

Extended phenotypes are traits that are expressed outside the body of the animal and are often the product of the animal's behavior (Dawkins 1982; Schaedelin and Taborsky 2009; Blamires 2013). They constitute adaptive modifications of the animal's environment and serve a variety of functions, from providing shelter to furnishing signaling platforms (Dawkins 1982; Hill et al. 2006;

Schaedelin and Taborsky 2009; Blamires 2010; Bailey 2012; Mhatre and Bertram 2018).

Extended phenotypes often persist much longer than the behavior that produced them (Schaedelin and Taborsky 2009) but are less permanent (or more modifiable) than other aspects of animals' phenotypes, such as their morphology. In terms of adjustability, extended phenotypes may occupy an intermediate position between traits that exhibit "developmental plasticity" and those that have "activational plasticity" (Snell-Rood 2013). It is therefore interesting to analyze the extent and time scale at which extended phenotypes may afford the advantages of plasticity in terms of adaptive adjustment to changes in the environment, and whether this varies with the type of environmental variable involved (Ghalambor et al. 2007; Nussey et al. 2007). These variables will in turn influence the extent to which extended phenotypes may evolve novel forms (and novel forms of plasticity) due to selection of the mechanisms that regulate their expression (i.e., respond with genetic accommodation, genetic assimilation, or canalization) (West-Eberhard 2003, 2005; Flatt 2005; Ghalambor et al. 2007).

Some extended phenotypes have been shown to be plastic in response to changes in the environment. For example, yellow meadow ants (*Lasius flavus*) alter the architecture of their nests in response to differences in the layering of soil (Minter et al. 2012). Orb-weaving spiders alter the architecture of their webs in response to variation in prey capture location, prey type, prey nutrition, and prey vibratory stimuli (Schneider and Vollrath 1998; Blamires 2010; Blamires et al. 2010; Nakata 2012; Blamires et al. 2018). There is also evidence of limits to phenotypic plasticity in extended phenotypes, however. For example, although black widow spiders alter web architecture in response to changes in body condition induced by feeding or the production of an egg case (Blackledge and Zevenbergen 2007; Zevenbergen et al. 2008; DiRienzo and Aonuma 2018), they make only slight and seemingly non-adaptive changes in web architecture in response to short-term experience of the site of prey capture (Thompson et al. 2020).

Extended phenotypes can also be adjusted at both short and long time scales. Adjustments in black widow web architecture in response to changes in body condition and to the production of an egg case happen on a scale of days to weeks (Blackledge and Zevenbergen 2007; Zevenbergen et al. 2008; DiRienzo and Aonuma 2018). Adjustments in web architecture to other factors, such as long-term differences in prey abundance, happen on a scale of weeks to months (DiRienzo and Montiglio 2016a). Spider web architecture also changes ontogenetically in multiple taxa, and these changes occur on a scale of weeks to months (Eberhard et al. 2008; Gregoric et al. 2013).

The above examples demonstrate that extended phenotypes can vary within populations. This variation may follow from differences in their response to variation in the external environment as well as to internal (physiological or genetic)

differences in the organism that creates the extended phenotype. Physiological differences may in turn arise from factors such as genetic variation or sexual dimorphism. The above examples also show that variation in extended phenotypes can occur at different time scales, with some variation occurring immediately in response to environmental or physiological changes, and some variation occurring at much longer time scales, such as over weeks or months. Although tests of variation in extended phenotypes at a single time scale and in response to a single input are common, tests comparing variation in extended phenotypes at multiple time scales and types of input are rare (Schaedelin and Taborsky 2009). It is therefore of interest to analyze whether and how extended phenotypes respond to different causes of variation that may provide inputs at different time scales (Schaedelin and Taborsky 2009).

Here, we report on a study of external environmental and internal physiological and genetic causes of variation in an extended phenotype, the web of western black widow spiders (*Latrodectus hesperus*). We analyzed variation in web architecture in terms of allocation of silk to two web components with a suite of complementary hypotheses: We tested for plasticity according to differences in the spiders' experience of the site of prey capture on the web (plasticity hypothesis in Table 1). We also tested for genetic variation in web architecture and for genetic variation in the plastic response to the experience of the site of prey capture (genetic variation and genetic variation in plasticity hypotheses in Table 1). Finally, we tested for sexual dimorphism in web architecture and sexual dimorphism in the plastic response to the experience of the site of prey capture (sexual dimorphism and sexual dimorphism in plasticity hypotheses in Table 1).

Some spiders have been shown to modify their web architecture in response to variation in the external environment (Schneider and Vollrath 1998; Blamires 2010; Nakata 2012; Blamires 2013). Black widows modify their web architecture and behavior in response to long-term differences in body condition, resulting from differences in amount of prey captured (DiRienzo and Montiglio 2016a). However, in the case of black widows, it is not clear whether variation in the external environment causes the spiders to adjust web architecture. Our test for a response to an external input focused on whether black widows adjust their webs according to variation in their experience of the site of prey capture on the web at different time scales.

Black widow spiders create space-filling cobwebs (Fig. 1) (Benjamin and Zschokke 2003; Blackledge and Zevenbergen 2007; Zevenbergen et al. 2008). The main foraging components of black widow webs are a forest of gumfooted lines that are anchored to the substrate and adhere to prey that walk beneath the web. The gumfooted lines descend from the sheet of the web, which is a tangled mass of silk that serves to support the gumfooted lines and provide defensive structure to the web. Black widows also construct a retreat, where they

Table 1 The four hypotheses about the causes of variation in the architecture of black widow spiders that we tested and their corresponding predictions. Names of hypotheses in quotes

Hypothesis	Prediction
“Plasticity”—web architecture is plastic in response to prey location.	Spiders will allocate more silk in future webs to area of web where they experienced prey capture.
“Genetic variation”—web architecture has a genetic component of variation.	Spiders will build webs that are more similar to webs built by siblings than webs built by unrelated spiders.
“Genetic variation in plasticity”—plasticity has a genetic component of variation.	Spider families will differ in their response to past prey capture location experience.
“Sexual dimorphism”—web architecture differs between sexes.	Male and female spiders will differ in allocation of silk to sheet or gumfooted lines.
“Sexual dimorphism in plasticity”—plastic response is sexually dimorphic.	Male and female spiders will differ in their response to prey capture location.

wait when not foraging or constructing the web (Blackledge and Zevenbergen 2007).

In the field, black widows capture a mixture of terrestrial prey (with the gumfooted lines) and flying prey (with the sheet) (Salomon 2011). Although the flying prey makes up a minority of prey capture events, variation in the relative abundance of flying prey may result in individual variation in these proportions (Salomon 2011).

In a prior study with adult female black widows, we varied the spiders’ experience of the site of prey capture over 4 weeks and compared the size of that effect with the magnitude of individual variation in web components (Thompson et al. 2020). We found that plasticity in web components was of small effect size, barely significant, and did not appear to be an adaptive response to variation in site of prey capture, whereas repeatability in one web component (the number of gumfooted lines) was of large effect size and easily detectable. These results suggest that black widows do not respond with adaptive plasticity in web architecture after experiencing short-term differences in prey capture location (Thompson et al. 2020). However, it is possible that the adult spiders we used in our previous study had already been exposed to differences in prey capture location in the field prior to being collected. It is also possible that the duration of our treatment was too short to induce a plastic response in web architecture or that adult black widows are generally less responsive to changes in prey capture location than juveniles.

Consequently, in the current study, we assessed the effect of long-term (average 10 weeks) experience of variation in the site of prey capture for juvenile black widows.

Methods

We tested the above hypotheses (Table 1) with a full-sibling, split-family rearing experiment. We reared a sample of *L. hesperus* full-sib families in long-term treatments of experiencing prey capture either at gumfooted lines only, sheet only, or alternating between gumfooted lines and sheet.

We collected adult female black widow spiders in June 2017 and June 2018 in Medford, Oregon. We maintained them in 0.47-L plastic cups and fed them one cricket (approximately 1-cm body length) every 2 weeks. We monitored the spiders for the production of egg cases and transferred any egg cases laid to 0.47-L plastic cups (one egg case per cup). We consider that the offspring born from each egg case of a given female are full siblings for the following reasons: we collected the females near the beginning of the breeding season; males of other *Latrodectus* species (and possibly *L. hesperus* too) avoid mating with previously mated females; and in *L. hesperus*, mated females do not re-advertise receptivity to mating until several months after their first mating (Stoltz et al. 2007; Perampaladas et al. 2008). Thus, it is unlikely that the

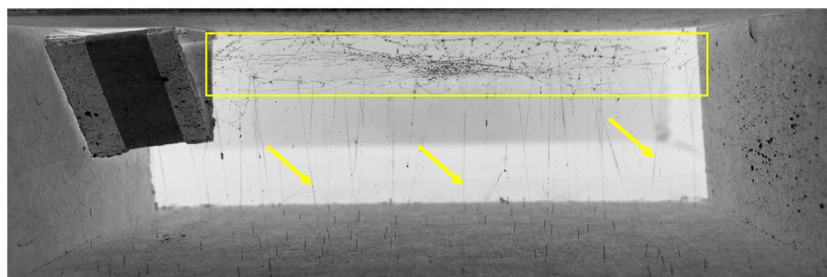


Fig. 1 Typical black widow spider web. This web was constructed in a cardboard frame that includes a triangular prism spiders use as a retreat. The web sheet (yellow rectangle) extends from the retreat and is the major structural and defensive component of the web. Gumfooted lines

(examples marked with yellow arrows) descend from the sheet and are anchored to the substrate. Gumfooted lines are the primary foraging component of black widow webs

females had mated with more than one male when we collected them. However, this possibility is not zero, and we recognize that our estimate of whether there is genetic variation only approximates broad-sense heritability (H^2). We further note that H^2 includes not only additive genetic variance but also non-additive components such as dominance variance and maternal effects (Lynch and Walsh 1998). Our experimental design thus provides only a rough approximation of whether there is a genetic variation component.

Black widow spiderlings spend approximately 2 weeks on their mother's web before dispersing (Johnson et al. 2010). We therefore retained newly hatched spiderlings in their container for 14 days before separating the spiderlings into individual 0.47-L plastic cups. To standardize spiderling condition (given the possibility of sibling cannibalism), we excluded spiderlings that were notably larger than their siblings (abdomen approx. $2 \times$ larger). We used this exclusion criterion to decrease the likelihood of including spiders that had already experienced eating in the experiment.

We then randomly assigned 30 individuals from each egg case (hereafter referred to as family) to one of the three treatments that varied the experience of the site of prey capture (at gumfooted lines only, sheet only, or alternating between gumfooted lines and sheet) (10 spiderlings/family/treatment). We sustained the treatments until males were in their penultimate instar (determined by the appearance of the pedipalpal bulb) and females had grown to a body size that matched that of the males. Thus, spiders experienced the treatments for an average of 10 weeks (range 6–13 weeks). (In "Discussion", we compare the results of these treatments to a prior study, in which we sustained the same differences in experience of the site of prey capture for 4 weeks; Thompson et al. 2020.)

The plastic cups in which we placed each spider contained a single piece of bamboo garden stake that spanned from the bottom corner of the cup to the top corner on the opposite side. Spiders used the pieces of bamboo and the bottom of the cups to construct webs with clearly defined sheets and gumfooted lines anchored to the bottom. We fed each spider one cricket per week, starting 1 week after we separated them into individual cups. We visually size-matched crickets so that the cricket body length was equal to the spider body length.

We used forceps to offer freshly killed crickets to spiders in their assigned web component. To give prey in a gumfooted line, we visually identified a gumfooted line attached to the bottom of the cup, held the cricket against the gumfooted line, and manually moved the cricket using small jerking motions to simulate prey that walked into a gumfooted line. To give prey in the sheet, we held the cricket against the top of the sheet and manually moved the cricket using small jerking motions to simulate prey that had become tangled in the threads of the sheet. We continued moving crickets until the spiders approached and began flicking silk at the crickets, which is a normal prey capture behavior (Enders 1975).

During some attempted feedings, spiders would not react to the offered cricket. In these cases, we continued to move the cricket for 60 s, and then left the cricket attached to the gumfooted line or tangled in the sheet. Spiders readily accepted crickets when left in this manner, and consumed the crickets within 1 day.

When male spiders were one molt from maturity and females were visually the same size as penultimate males, we transferred them to cardboard frames (Fig. 1), in which they constructed the webs we used in our analyses (Quade et al. 2019). We placed these frames inside 5.7-L plastic shoeboxes. We coated the interior of the shoeboxes with petroleum jelly to ensure that the spiders only attached silk threads to the cardboard frame. We allowed spiders 1 week to construct webs. This procedure therefore pinpoints the effect of prey capture treatments to the newly built webs at the end of the period of accumulated experience and excludes any gradual modifications spiders may have made to their webs during the period of implementation of the treatments.

At the end of the web-building period, we removed spiders by luring them to the edge of the sheet and scooping them off the web in a plastic vial. We then lightly coated each web with flour to make web threads visible against a black background and then photographed each web inside a dark box lined with matt black construction paper. We placed a ruler next to the bottom edge of each frame to allow setting the scale of images during the analysis. We took two photographs of each web, one with a perspective looking through the forest of gumfooted lines between the sheet and bottom of the frame (Fig. 2a) and one with a perspective looking through the sheet of the web (Fig. 2b).

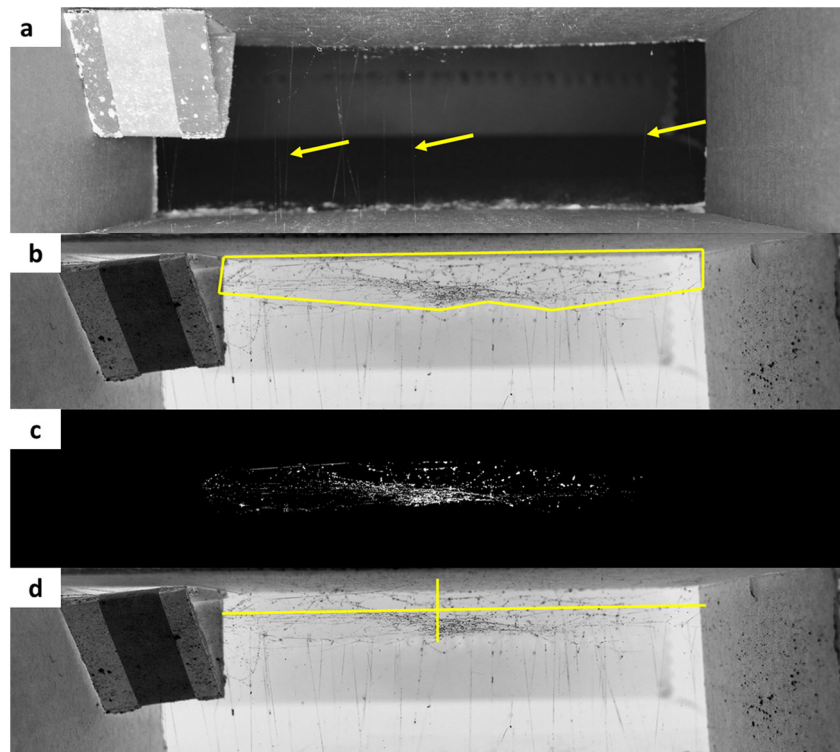
Quantifying web components

To analyze causes of variation in black widow web architecture, we began by quantifying the web components of interest—number of gumfooted lines, cross-sectional area of the sheet, and shape of the sheet (sheet height/sheet width).

To count the number of gumfooted lines, we used the photographs taken looking through the center of the cardboard frame (Fig. 2a). We used ImageJ (National Institute of Health, Bethesda, MD, USA) to convert each image into black and white, which enhanced the contrast between the gumfooted lines and the background of each image (Fig. 2a). We then manually counted the number of gumfooted lines in each web.

To measure the cross-sectional area of web sheets, we used the protocol of Thompson et al. (2020). We imported the images taken perpendicular to the sheet in ImageJ. We used the set scale tool to measure the ruler in each image and set the scale of each image in centimeters. We then converted each image into an 8-bit black and white image. Next, we used the freehand select tool to outline the area of the sheet

Fig. 2 Quantification of the components of a black widow web. **a** Example of image used to count gumfooted lines (examples marked with yellow arrows). **b** Image showing the sheet outlined using the free hand select tool in ImageJ before being cut and pasted into a new image. **c** Example of a sheet that has been cut and pasted into an image with a black background. Image shows the sheet after adjusting using the threshold function in ImageJ. **d** Example of image used to measure sheet height (vertical yellow line) and sheet width (horizontal yellow line). All images have been cropped and do not show the ruler used to set the scale of images used for measuring sheet cross-sectional area



(Fig. 2b). We then cut the sheet out of the original image and pasted it into a new image with a black background. We used the threshold function to change the whiteness threshold of the image, so that only the pixels filled by sheet silk were white and the rest of the image was black (Fig. 2c). Finally, we used the measure tool to measure the area of white pixels in the sheet in square centimeters. The cross-sectional sheet areas obtained using this procedure are proportional to the mass of silk used in sheet construction (Blackledge and Zevenbergen 2007).

To measure the maximum height and width of web sheets, we used the images of each web that were taken from the perspective perpendicular to the sheet. We then set the scale of the image as above using the ruler included in each image. We then used the line segment tool to draw a line across the tallest part of the sheet from edge to edge and across the widest part of the sheet from edge to edge (Fig. 2d). We then used the measure tool to measure the length of drawn line segments in centimeters.

All measurements were conducted blind to the treatment and sex of the spiders by BM, WP, and CS. Before measuring the full set of web images, we began with a training round consisting of repeated measures of 10 different images. The repeatability of these measures was high (gumfooted line counts by WP $r = 0.996$; sheet cross-sectional area estimates by BM $r = 0.956$, sheet height and sheet width measurements by CS $r = 0.983$ and $r = 0.996$, respectively).

Statistical analysis

We used linear mixed effects models fit with the lme4 package in R (R Core Team 2018; Bates et al. 2015). We used a separate test for each of the following dependent variables: number of gumfooted lines, cross-sectional area, and web shape (sheet height/sheet width). We checked whether these variables were correlated with one another and found that they were not (gumfooted lines with sheet area $r = -0.15$, $p = 0.091$, $n = 92$; gumfooted lines with sheet shape $r = -0.10$, $p = 0.316$, $n = 92$; sheet area with sheet shape $r = 0.13$, $p = 0.210$, $n = 92$).

For each dependent variable, the explanatory variables were experience treatment, family, sex, the interaction between family and treatment, and the interaction between sex and treatment; we fit the family and family \times treatment terms as random variables. For the number of gumfooted lines, we used a general linear mixed effects model fit with a Poisson error distribution. We fit three models: one with all of the above explanatory variables, one without the family \times treatment term, and one without family and family \times treatment terms. To estimate the significance of the family \times treatment interaction, we used ANOVA to test the significance of the full model compared with the model with the family \times treatment term removed. To estimate the significance of the family term, we used ANOVA to test the significance of the model with the family \times treatment term removed compared with the model with the family and family \times treatment terms removed.

We were not able to fit a full model with all predictors as above when testing for effects on sheet area and sheet shape, because these models did not have enough degrees of freedom to run using lme4 in R (Bates et al 2015). Instead, we fit models with treatment, family, sex, and treatment by sex terms to estimate the fixed effects. To estimate the random effects, we fit models with only treatment, family, and family \times treatment; models with only treatment and family; and models with only treatment. We then used ANOVA to compare the models to estimate the significance of the family and family \times treatment terms.

We used the lmerTest package in R to calculate degrees of freedom and p values for the fixed effects in each of our models (Kuznetsova et al. 2017). We used the effectsize package to calculate the percent variance explained for each of the fixed terms in our models (Makowski et al. 2019). The lmerTest output for the Poisson model did not include denominator degrees of freedom, so to estimate the size of the fixed effects on the number of gumfooted lines, we used $n-1$ as the denominator degrees of freedom to provide the most conservative estimate of the effect sizes (Kuznetsova et al. 2017). We do not report effect sizes for the random effects in our models, because best practices are currently under debate. Therefore, we only estimate whether there is a genetic component of variation and do not provide quantitative estimates of heritability. Finally, we calculated the coefficients of determination for our full models using the MuMIn package in R (Barton 2019). For our analyses, we used all spiders that completed the experimental procedure for a total of 130 spiders from 21 families (range 1–18 spiders per family, average 6 spiders per family). Our family and within-family sample sizes are adequate for detecting mid effect size heritability (Lynch and Walsh 1998).

Data availability

All data generated or analyzed during this study are included in this published article's supplementary information files.

Results

Number of gumfooted lines

We found no overall effect of the experience treatments on the number of gumfooted lines the spiders produced (Table 2; Fig. 3a). Spider families differed significantly in the number of gumfooted lines produced and in their response to experience treatments (Table 2; Fig. 3a). Male and female spiders had a significant difference of large effect size in their overall production of gumfooted lines, with males producing fewer gumfooted lines than females (Table 2; Fig. 3a). Male and female spiders differed in their

Table 2 Variation in the number of gumfooted lines in black widow webs according to prey capture location treatment, spider sex, spider family, treatment by sex interaction, and treatment by family interaction. Significant terms are indicated in bold. Full model coefficients of determination. $R^2_m = 0.44$, $R^2_c = 0.89$

Term	$F_{dfn, dfd}, p$	Effect size (variance explained)
Treatment	0.34 _{2, 129} , 0.626	0.005
Sex	217.681, 129, $p < 0.01$	0.627
Family	$p < 0.01$	-
Treatment \times sex	30.89_{2, 129}, $p < 0.01$	0.324
Treatment \times family	$p < 0.01$	-

response to the experience treatments, and this difference was of moderate effect size (Table 2; Fig. 3a).

Sheet cross-sectional area

Sheet cross-sectional area did not differ between experience treatments, families, or sexes (Table 3; Fig. 3b). We also detected no interaction between treatment and family or between family and sex (Table 3; Fig. 3).

Sheet shape

The shape of web sheets (sheet height/sheet width) did not differ between experience treatments. Spider family had a significant effect on web shape, but there was no interaction between treatment and family (Table 4; Fig. 3c). Male and female spiders had a small effect size difference in web shape, with male spiders making proportionally taller (sheet height/sheet width) webs than female spiders (Table 4, Fig. 3c, and Fig. 4). Male and female spiders did not differ in their response to treatment (Table 4, Fig. 3c).

Discussion

We analyzed variation in black widow web architecture in terms of plasticity in response to long-term differences in the experience of the site of prey capture and compared it with our previous study to understand how different time scales of environmental variation affect web architecture plasticity. We also tested for genetic variation in web architecture, genetic variation in the plastic response to prey capture experience, sex differences in web architecture, and sex differences in plasticity. We found that each internal input we tested affected at least one web component, but the external input had no effect independent of the internal inputs. Specifically, spider families differed in the number of gumfooted lines they produced and in the shape of sheets they produced. Spider

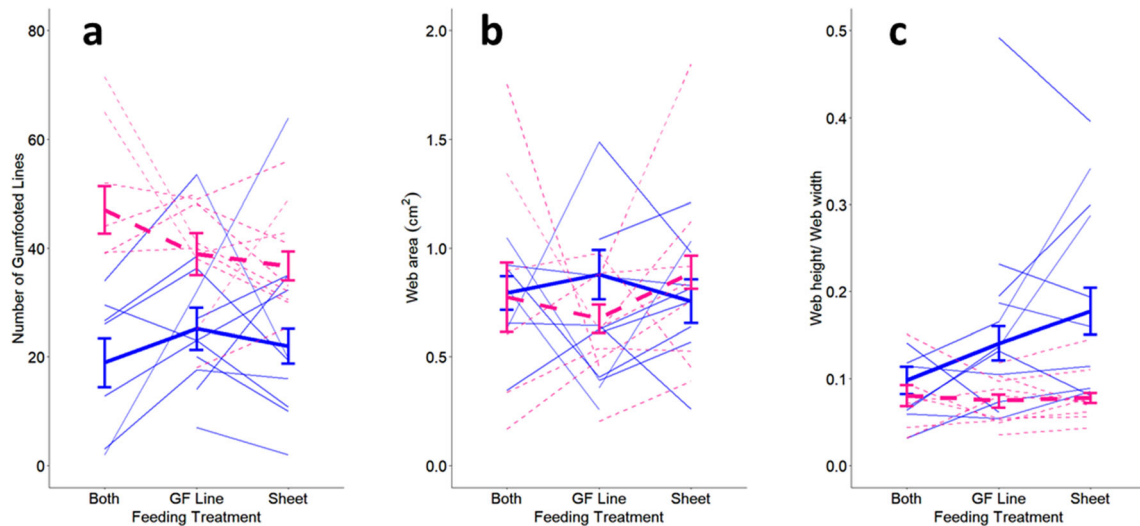


Fig. 3 Variation in number of gumfooted lines (a), cross-sectional area of sheet (b), and web shape (c) according to black widow family and sex and experience treatment. Thin solid blue lines show family average of male

spiders, and thin dashed pink lines show family averages of female spiders. Thick solid blue lines show averages for all male spiders, and thick dashed pink lines show averages for all female spiders

families also differed in response to prey capture treatments. We also found that male and female spiders differed in their overall production of gumfooted lines, and their production of gumfooted lines differed in response to prey capture treatments. Across all treatments, male spiders made only half as many gumfooted lines as female spiders. Male spiders also produced proportionally taller webs than female spiders, but the size of this difference was much smaller than the difference in number of gumfooted lines produced.

These results suggest that black widow web architecture is sensitive to genetic and physiological differences between spiders, and that those variables influence the plastic response to differences in the external environment. Our results agree with our previous study that tested for an effect of short-term differences in the experience of the site of prey capture with adult females and found little plasticity in web architecture in response to these differences (Thompson et al. 2020). Black widows experience capturing terrestrial prey and flying prey that likely becomes entangled in the sheet (Salomon 2011), but our results

suggest that black widows have not been selected to respond to even extreme differences in past prey capture experience. An alternative explanation might be that plasticity in web architecture was limited by the spiders’ silk supply. We consider this unlikely, as there was no evidence of a trade-off in silk investment between the web components we measured (the correlation between the number of gumfooted lines and sheet area was small and non-significant; see “Methods”). The spiders were also not likely limited by the amount of silk they could produce, as all spiders were fed regularly.

Interestingly, orb-weaving spiders have been shown to alter web architecture in response to past prey capture experience (Schneider and Vollrath 1998; Blamires 2010; Blamires et al. 2010; Nakata 2012; Blamires et al. 2018). The cobwebs of spiders in the family Theridiidae, which includes black widow spiders, are derived from ancestral orb webs (Eberhard et al. 2008). Thus, perhaps the changes in web architecture from orb web to cobweb have been accompanied by a weakening of plasticity in response to experience of the site of prey capture. Unlike orb-

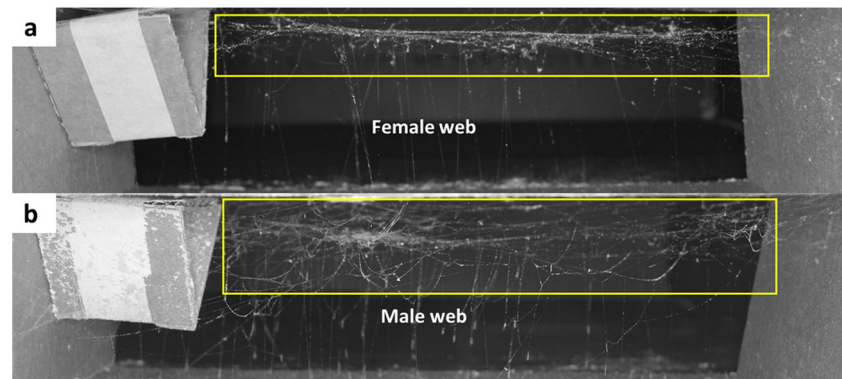
Table 3 Variation in sheet cross-sectional area in black widow webs according to prey capture location treatment, spider sex, spider family, treatment by sex interaction, and treatment by family interaction. Significant terms are indicated in bold. Full model coefficients of determination. $R^2_m = 0.007$, $R^2_c = 0.085$

Term	$F_{dfn, dfd}, p$	Effect size (variance explained)
Treatment	0.066 _{2, 111.82} , 0.937	0.0011
Sex	0.075 _{1, 116.32} , 0.785	0.0007
Family	$p = 0.843$	-
Treatment × sex	0.379 _{2, 115.81} , 0.685	0.0064
Treatment × family	$p = 0.402$	-

Table 4 Variation in sheet shape in black widow webs according to prey capture location treatment, spider sex, spider family, treatment by sex interaction, and treatment by family interaction. Significant terms indicated in bold. Full model coefficients of determination. $R^2_m = 0.167$, $R^2_c = 0.507$

Term	$F_{dfn, dfd}, p$	Effect size (variance explained)
Treatment	2.01 _{2, 110.51} , 0.139	0.033
Sex	20.26_{1, 114.54}, < 0.01	0.171
Family	$p < 0.01$	
Treatment × sex	2.76 _{2, 110.59} , 0.679	0.024
Treatment × family	$p = 0.413$	

Fig. 4 Typical female (a) and male (b) black widow webs. Yellow rectangles outline the sheet of both webs. Male spiders constructed sheets that did not differ in overall silk investment or width but were proportionately taller than the sheets constructed by females



weaving spiders which build a new web each day (Sherman 1994), black widow spiders occupy the same web for extended periods (Kasumovic and Andrade 2004). This might still allow black widows to modify their webs gradually in response to differences in prey capture location. In our prior study, spiders did not do so, however (over a span of 4 weeks) (Thompson et al. 2020). Even in an experiment in which black widows were placed on webs built by different individuals (hence with different architectures), the spiders did not modify the newly occupied web (DiRienzo and Aonuma 2018). Nevertheless, black widows do adjust the architecture of their web according to their body condition and level of satiation (Blackledge and Zevenbergen 2007; Zevenbergen et al. 2008). We therefore interpret our findings as indicating that black widow spiders have not been selected to sustain their (putatively ancestral) ability to adjust their web according to variation in the site of prey capture. Nevertheless, the presence of family and sex differences in web architecture and in the plastic response of web architecture to the experience of the site of prey capture suggests that these traits can evolve. This might include experience-based plasticity in web architecture, were this variable to become an important feature in the spiders' natural history. Further experiments with other theridiids will be required to draw stronger conclusions in this regard.

The differences we found between male and female webs suggest that male spiders construct webs that emphasize defense at the expense of foraging ability as they mature. However, we only quantified web architecture when male spiders were one molt from maturity, so we cannot determine whether those differences may arise earlier in ontogeny. Other studies investigating changes in black widow web architecture in contexts where defensive alterations were expected, such as the protection by females of egg cases, have found that black widows make fewer gumfooted lines and alterations to the sheet when building webs that are optimized for defense (Blackledge and Zevenbergen 2007; DiRienzo and Montiglio 2016b; DiRienzo and Aonuma 2018). Although we gave all spiders in our experiment prey that was size matched to the spiders' bodies, it is possible that at the penultimate, instar male spiders were sufficiently nutritionally provisioned to reach maturity without future feedings. It would be interesting to conduct a field experiment to determine whether

penultimate male black widows make similar architectural changes to their webs under natural conditions.

Our findings suggest that internal inputs are far more important for determining black widow web architecture than inputs from the external environment, and our results agree with previous studies that suggest that physiological inputs exert a much greater effect on web architecture than variation in the external environment (Blackledge and Zevenbergen 2007; Zevenbergen et al. 2008; DiRienzo and Aonuma 2018; Thompson et al. 2020). Other authors report effects of physiological changes, such as satiation or the production of an egg case, on web architecture (particularly reduction in number of gumfooted lines) that is of similar magnitude to the difference we found between sexes (Blackledge and Zevenbergen 2007; DiRienzo and Aonuma 2018). It appears that physiological differences consistently cause changes between webs optimized for foraging and webs optimized for defense. It would therefore be interesting to experimentally produce environmental conditions black widows perceive as safe versus dangerous and test whether variation in these conditions elicit the type of web plasticity (adjusting the web between foraging and defense emphases) that has been found in black widows in response to changes in body condition or the production of egg cases (Blackledge and Zevenbergen 2007; DiRienzo and Aonuma 2018).

In our present study, we asked how an extended phenotype varies in response to environmental variation at short and long time scales and estimated variation due to internal inputs. We found no apparently adaptive variation in the extended phenotype in response to environmental variation at either time scale. However, we found that black widow web architecture was strongly dependent on internal inputs, with web architecture varying between sexes and families. We finish by suggesting that to understand the evolution of extended phenotypes, it is important to consider how extended phenotypes respond to external and internal inputs. It is also important to understand whether the responses to these inputs differ at different time scales. From our current study, it is clear that the time scale of the input, as well as whether the input is external or internal, can affect the expression of extended phenotypes.

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