COGNITIVE AND WEB PHENOTYPES OF THE WESTERN BLACK WIDOW SPIDER

by

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ABSTRACT

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Animals with brains create mental representations of their environment and their own position within it, and use these representations to make decisions. The information used to create mental representations, and how animals use representations to make decisions, are functions of an animal's evolution and ecology. Mental representations can be as simple as remembering the direction and distance home, or as detailed as a human's mental map of their own home. I used behavior assays designed to reveal the contents of mental representations to investigate how a web spider creates representation of its environment and objects within it, and uses these representations to guide decision making. Studies of how animals create internal representations have focused largely on understanding how animals with acute vision form representations based on visual information. I used western black widow spiders (*Latrodectus hesperus*) in my experiments, because web spiders have poor vision and rely largely on sensing vibrations transmitted through their webs. I first explored whether black widows form memories of where they capture prey on the web and whether they alter future web building behavior in response to past differences in prey capture location. I found that black widows did not adjust their web architecture in response to past prey capture experience. However, web architecture differed between spider sexes and spider families, and spider families differed in

their response to past prey capture experience. I next investigated whether black widows use internal representations when navigating their webs. I used assays in which I displaced spiders on their webs and observed their attempts to return home. I found that black widows used representations—in the form of path integration vectors that contain information about a spider's distance and direction from home—when navigating. I next directed my attention toward whether black widows direct their attention inward toward their internal representations. This ability is a defining feature of basic consciousness, and to my knowledge would be the first direct evidence of consciousness in an invertebrate. I designed a novel assay of inwardly directed attention by manipulating whether a spider's representation matched its currently occupied web or not and providing spiders with a salient prey cue that they would only ignore if they were distracted by the mismatch between internal representation and external web. Black widows did sometimes become distracted by such a mismatch, and thus possessed basic consciousness. The assay I used to detect consciousness in black widows is generalizable and could be used to test for consciousness in myriad animals. Finally, I explored whether black widows store specific information about their captured prey in memory. I used an assay of searching effort to determine whether black widows remember prey capture location and relative prey size. Black widows formed memories of captured prey, regardless of prey capture site. However, black widows only differed their search effort in response to changes in relative prey size when they had captured prey on the floor of their enclosure. This could indicate that either black widows only formed memories of prey size after capturing prey on the floor or that black widows only used the remembered prey size to make decisions after capturing prey on the floor, but the assay I used could not differentiate between the two

possibilities. My behavioral assays revealed that black widows create mental representations of their web and prey, and that they possess basic consciousness. Each of my experiments considered the evolution and ecology of black widows, and demonstrated that investigations of the contents of animal minds that use behavioral assays in conjunction with knowledge about the animal's ecology, evolution, and neurobiology are especially fruitful.

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

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. Black widow spiders construct webs which are extended phenotypes and have been shown to be plastic under certain conditions. 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 plasticity in response to variation in prey capture location. 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.

Introduction

Extended phenotypes are traits that are expressed outside the body of the animal, and are often a 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 of 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 on 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 in the organism that creates the extended phenotype. Physiological differences may in turn arise from factors such as genetic variation or sexual dimorphism. Those 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 times 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 1.1). We also tested for genetic variation in web architecture, and for genetic variation in the plastic response to experience of the site of prey capture (genetic variation, and genetic variation in plasticity hypotheses in Table 1.1). Finally, we tested for sexual dimorphism in web architecture, and sexual dimorphism in the plastic response to experience of the site of prey capture (sexual dimorphism, and sexual dimorphism in plasticity hypotheses in Table 1.1).

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.

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 to webs built by unrelated spiders.
"Genetic variation in plasticity" – Plasticity has a genetic component of variation.	Spider families will differ in their response to prey capture location.
"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.

Table 1.1 Hypotheses and predictions. 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

Black widow spiders create space-filling cobwebs (Fig. 1.1) (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 walks 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 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 make 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).

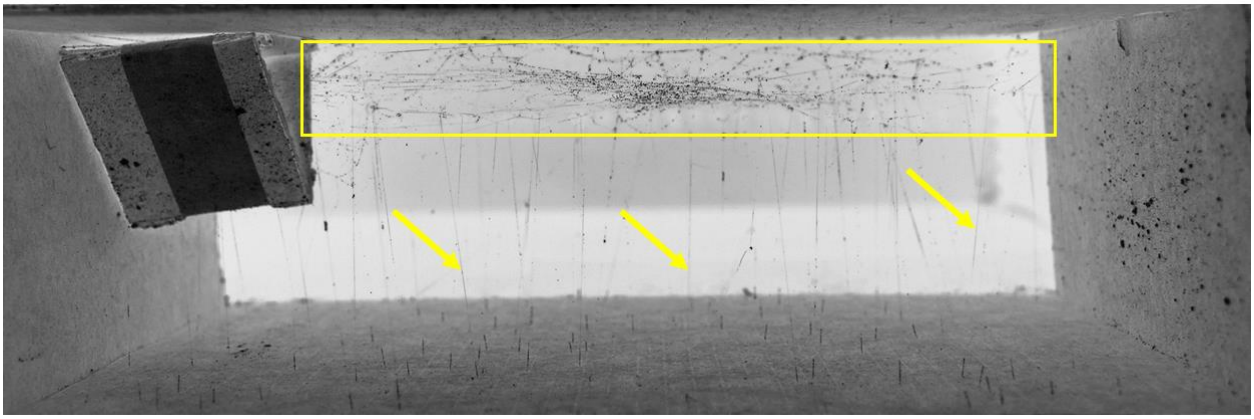

Figure 1.1: Typical black widow spider web. The web is constructed in a cardboard frame that includes a triangular prism spiders use as a retreat. The web sheet (yellow rectangle) extends from the retreat and is the major structural and defensive component of the web. Gumfooted lines (yellow arrows) descend from the sheet and are anchored to the substrate. Gumfooted lines are the primary foraging component of black widow webs.

In a prior study with adult female black widows, we varied the spiders' experience of the site of prey capture over four 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. 2019). 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 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 0.47L plastic cups, and fed them one cricket (approximately 1cm body length) every two weeks. We monitored the spiders for the production of egg cases and transferred any egg cases laid to 0.47L 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. hesperu*s 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 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 & Walsh 1998). Our experimental design thus provides only a rough approximation of whether there is a genetic variation component.

Black widow spiderlings spend approximately two 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 0.47L 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× larger). This criterion may not have eliminated the risk of including some spiderlings that had fed on a sib. However, this would contribute to within-family variation rather than force any of the causes of variation that we tested for. Further, the experience of eating a sibling in the maternal web is not similar to capturing prey that has become entangled in either the gum-footed lines or sheet of a web, and is thus unlikely to confound the prey capture experience treatment.

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 one 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 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 seconds, 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 one 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. 1.1) in which they constructed the web we used in our analyses (Quade et al. 2019). We placed these frames inside 5.7L plastic shoeboxes. We coated the interior of the shoeboxes with petroleum jelly to

ensure the spiders only attached silk threads to the cardboard frame. We allowed spiders one 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 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 1.2a) and one with a perspective looking through the sheet of the web (Fig 1.2b).

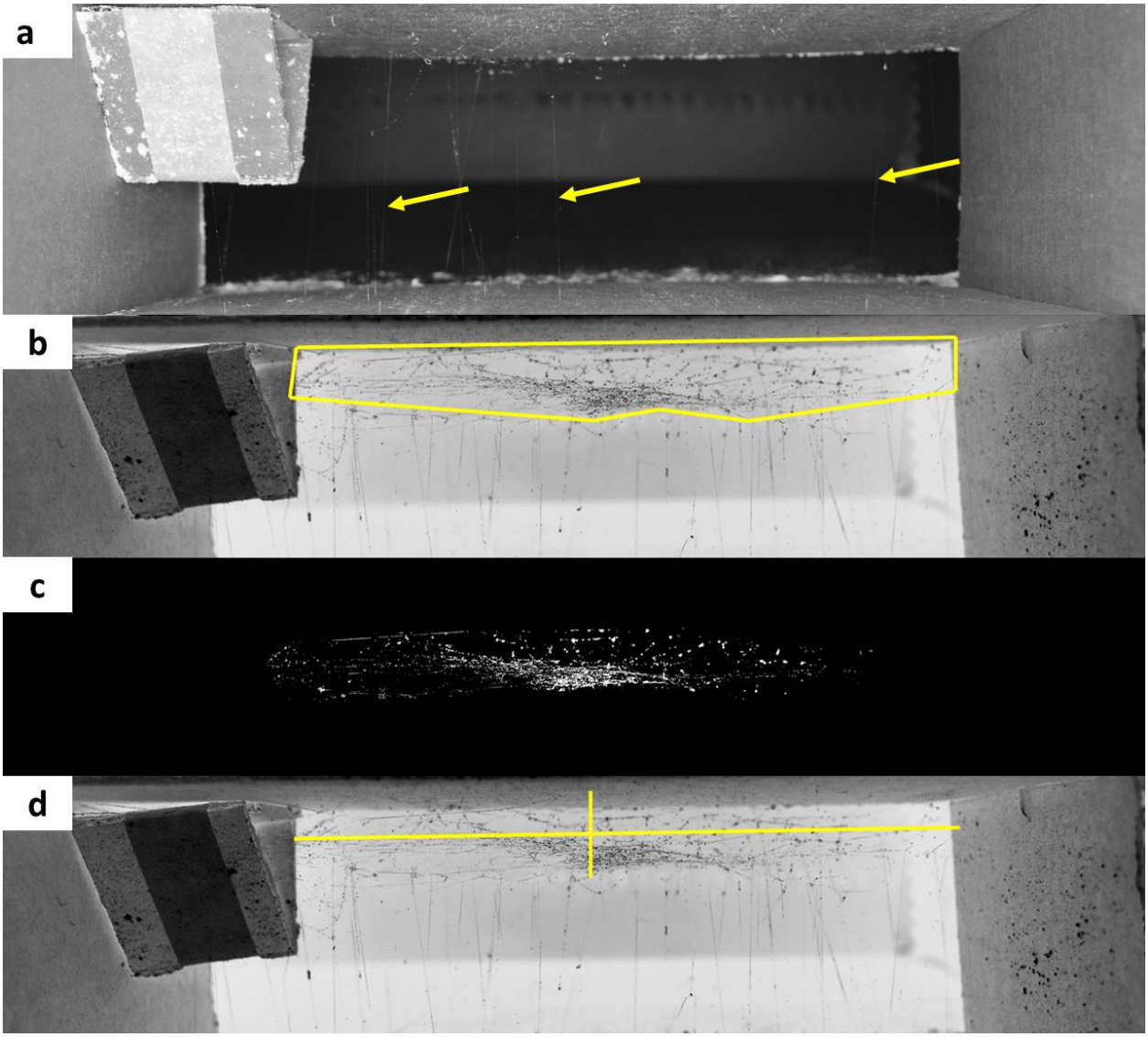

Figure 1.2: Quantification of the components of a black widow web. a: example of image used to count gumfooted lines (yellow arrows). **b:** image showing the sheet outlined using the free hand select tool in ImageJ before being cut and pasted into a new image. **c:** example of a sheet that has been cut and pasted into an image with a black background. Image shows the sheet after adjusting using the threshold function. **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.

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 1.2a). We used ImageJ (National Institute of Health, Bethesda, MD, U.S.A.) to convert each image into black and white, which enhanced the contrast between the gumfooted lines and the background of each image (Fig 1.2a). 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. (2019). We imported the images taken perpendicular to the sheet in ImageJ. We used the set scale tool to measure the ruler shown 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 1.2b). 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 1.2c). 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 is 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 between across the tallest part of the sheet from edge to edge and across the widest part of the sheet from edge to edge (Fig 1.2d). 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. 2019). 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: $r = -0.101$, $p = 0.316$, $n = 92$; sheet area with sheet shape: $r = 0.128$, $p = 0.210$, $n = 92$). 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 number of gumfooted lines, we used a model 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 x treatment term, and one without family and family x treatment terms. To estimate the significance of the family x treatment interaction, we used ANOVA to test the significance of the full model compared to the model with the family x treatment term removed. To estimate the significance of the family term, we used ANOVA to test the significance of the model with the family x treatment term removed compared to the model with the family and family x 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 2019). 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 x treatment and 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 x 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 2019). We used the effectsize package to calculate the percent variance explained for each of the fixed terms in our models (Makowski et al 2020). 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 2019). 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 any measures 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).

Results

Number of gumfooted lines

We found no effect of the experience treatments on the number of gumfooted lines the spiders produced (Table 1.2; Fig 1.3a). Spider families differed significantly in the number of gumfooted lines produced and in their response to experience treatments (Table 1.2; Fig 1.3). 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 1.2; Fig 1.3a). Male and female spiders differed in their response to feeding treatments, and this difference was of moderate effect size (Table 1.2; Fig 1.3a).

Term	$F_{dfn, dfd}$, p	Effect size (variance explained)
Treatment	$0.34_{2, 129}$, 0.626	0.005
Sex	**$217.68_{1,129}$, p < 0.01**	**0.627**
Family	**p < 0.01**	-
Treatment x Sex	**$30.89_{2,129}$, p < 0.01**	**0.324**
Treatment x Family	p < 0.01	-

Table 1.2 Number of gumfooted lines. Full model coeffecients of determination: R^2_m = 0.44, R^2_c = 0.89. Variation in black widow web architecture according to prey capture location treatment, spider sex, spider family, treatment by sex interaction treatment by family interaction. Significant terms indicated in bold.

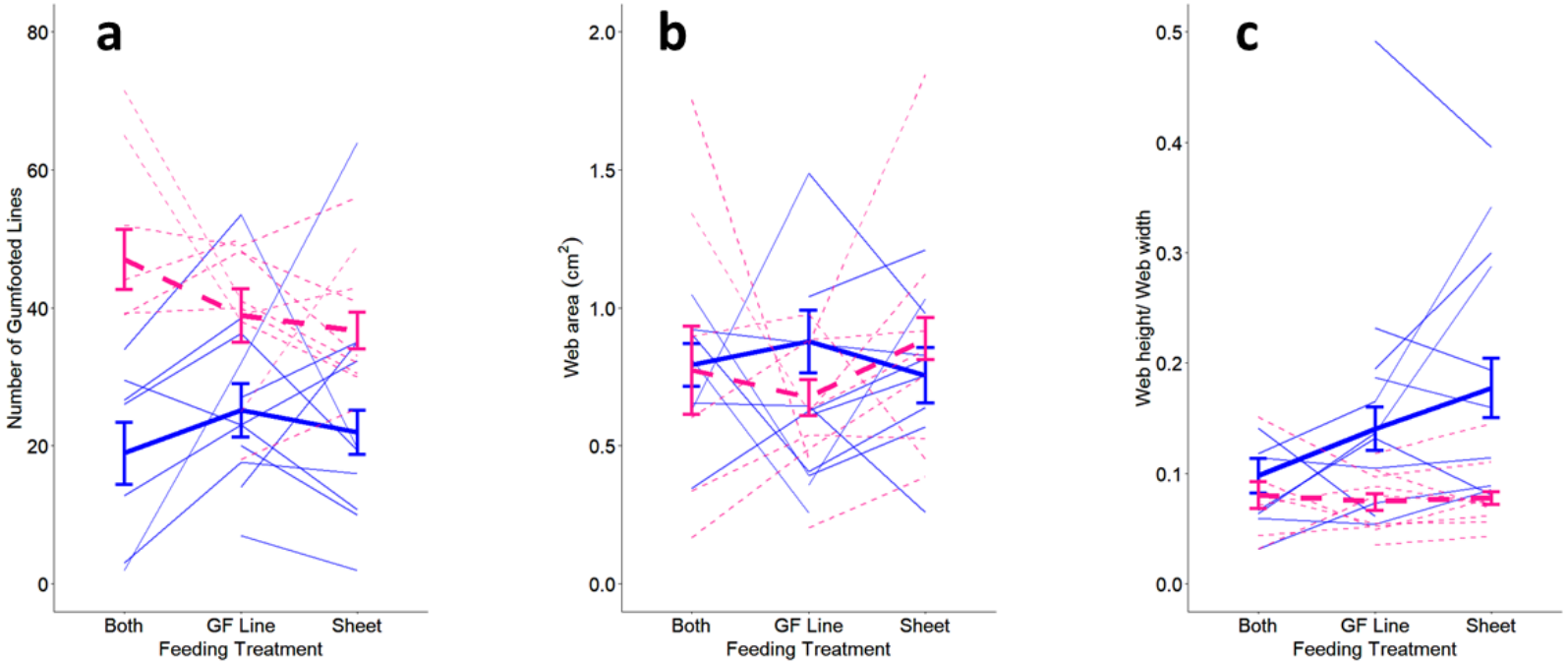

Figure 1.3: Number of gumfooted lines (a), cross-sectional area of sheet (b), and web shape (c) by black widow family and sex. Thin solid blue lines show family average of male spiders, and thin dashed pink lines show family averages of female spiders. Thick solid blue lines show averages for all male spiders, and thick dashed pink lines show averages for all female spiders.

Sheet cross-sectional area

Sheet cross-sectional area did not differ between experience treatments, families, or sexes (Table 1.3; Fig 1.3b). We also detected no interaction between treatment and family or between family and sex (Table 1.3; Fig 1.3).

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 x Sex	$0.379_{2, 115.81}$, 0.685	0.0064
Treatment x Family	p = 0.402	-

Table 1.3: Sheet cross-sectional area. Full model coeffecients of determination: R^2_m = 0.007, R^2_c = 0.085. Variation in black widow web architecture according to prey capture location treatment, spider sex, spider family, treatment by sex interaction treatment by family interaction. Significant terms indicated in bold.

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 1.4; Fig 1.3c). 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 1.4, Fig 1.3c, Fig 1.4). Male and female spiders did not differ in their response to treatment (Table 1.4, Fig 1.3c).

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 x Sex	$2.76_{2, 110.59}$, 0.679	0.024
Treatment x Family	p = 0.413	

Table 1.4: Sheet shape. Full model coeffecients of determination: R^2_m = 0.167, R^2_c = 0.507. Variation in black widow web architecture according to prey capture location treatment, spider sex, spider family, treatment by sex interaction treatment by family interaction. Significant terms indicated in bold.

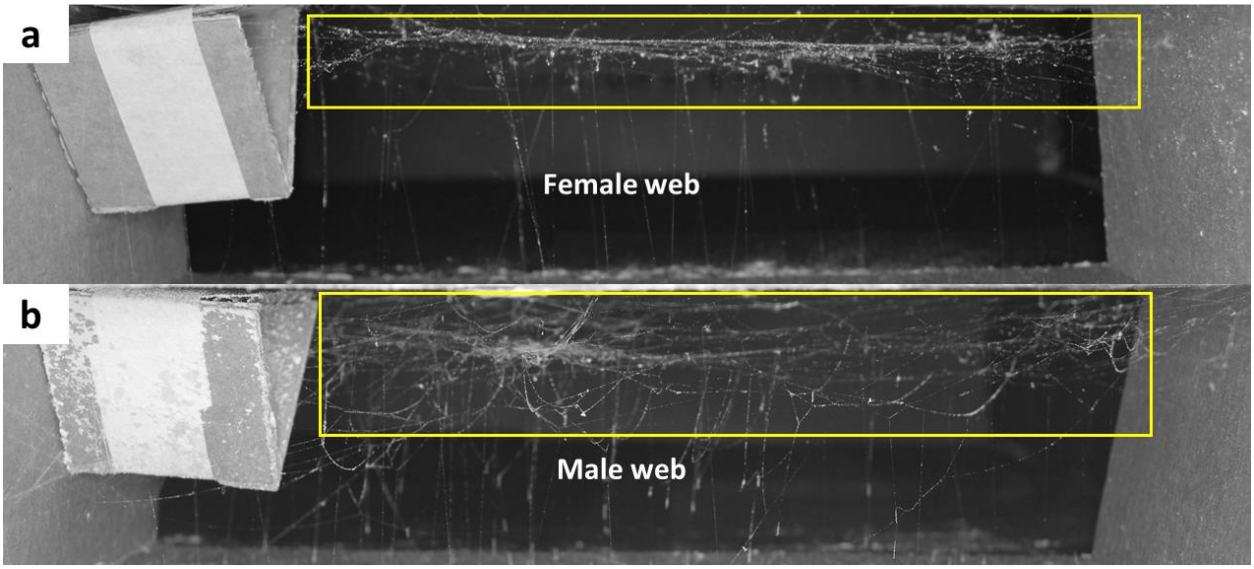

Figure 1.4: Typical male (a) and female (b) black widow webs. Yellow rectangles outline the sheet of both webs. Male spiders constructed sheets that did not differ in overall silk investment or width but were proportionately taller than the sheets constructed by females.

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 to 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 effected 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 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 in 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 effect was much smaller than the difference in number of gumfooted lines produced.

These results suggest that black widow web architecture is sensitive to genetic or physiological differences between spiders, but is not sensitive 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 to no 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-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 four weeks) (Thompson et al. 2019). 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; Thompson et al. 2019). 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 experience of the site of prey capture suggest 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 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 rule out that the differences we found between webs of male and female spiders exist throughout earlier 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 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. 2019). 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 are 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. Our objective was to understand how external in internal inputs can effect black widow web architecture, and we conclude that external inputs exert almost no effect on web architecture while internal inputs exert large effects. 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.

References

Bailey N (2012) Evolutionary models of extended phenotypes. *Trends in Ecology and Evolution.* **27**(10). 561-569

Barton K (2019) MuMIn: Multi-Model Inference.

Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann, Dai B, Scheipl F, Grothendieck G, Green P, Fox J (2019) lme4: Linear Mixed Models using 'Eigen' and S4

Benjamin SP, Zschokke S (2003) Webs of theridiid spiders: construction, structure, and evolution. *Biological Journal of the Linnean Society*. **78**. 293-305.

Blackledge TA, Summers AP, Hayashi CY (2005) Gumfooted lines in black widow cobwebs and the mechanical properties of spider capture silk. *Zoology*. **108**. 41-46.

Blackledge TA, Zevenbergen JM (2007) Condition-dependent web architecture in the western black widow, *Latrodectus hesperus*. *Animal Behaviour*. **73**. 855-864.

Blamires SJ (2010) Plasticity in extended phenotypes: orb web architectural responses to Variations in prey parameters. *The Journal of Experimental Biology*. **213**. 3207-3212

Blamires SJ. Chao I-C, Tso I-M (2010) Prey type, vibrations and handling interactively influence spider silk expression. *The Journal of Experimental Biology*. **213**. 3906-3910

Blamires SJ (2013) Plasticity in extended phenotypes: orb web architectural responses to variations in prey parameters. *Journal of Experimental Biology*. **213**. 3207-3212.

Blamires SJ, Martens PJ, Kasumovic MM (2018) Fitness consequences of plasticity in an extended phenotype. *Journal of Experimental Biology*. 221.

Dawkins R (1982) The extended phenotype. *Oxford University Press*, Oxford, UK

DiRienzo N, Aonuma H (2018) Plasticity in extended phenotype increases offspring defense despite individual variation in web structure and behaviour. *Animal Behaviour*. **138**. 9-17

DiRienzo N, Montiglio P-O (2016a) The contribution of developmental experience vs. condition to life history, trait variation and individual differences. *Journal of Animal Ecology*. **85**. 915-926

DiRienzo N, Montiglio P-O (2016b) Linking consistent individual differences in web structure and behavior in black widow spiders. *Behavioral Ecology*. **27**(5). 1424-1431

Enders F (1975) The influence of hunting manner on prey size, particularly in spiders with long

attack distances (Araneidae, Linyphiidae, and Salticidae). *The American Naturalist*. **109**(970). 737-763

Eberhard WG, Barantes G, Madrigal-Breners R (2008) Vestiges of an orb-weaving ancestor? The "biogenetic law" and ontogenetic changes in the webs and building behavior of the black widow spider *Latrodectus geometricus* (Araneae Theridiidae).

Flatt T (2005) The evolutionary genetics of canalization. *The Quarterly Review of Biology*. **80**(3). 287-316

Ghalambor CK, McKAy JK, Carrol SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*. **21.** 394-407

Gregoric M, Kiesbuy HC, Quinones Lebron SG, Rozman A, Agnarsson, Kuntner M (2013) Optimal foraging, not biogenetic law, predicts spider orb web allomotry. *Naturwissenschaften*. **100**. 263-268.

Hill PSM, Wells H, Shadley JR (2006) Singing from a constructed burrow: why vary the shape of the burrow mouth? *Journal of Orthoptera Research*. **15**(1). 23-19

Johnson JC, Kitchen K, MCB Andrade (2011) Family affects sibling cannibalism in the black widow spider, *Latrodectus hesperus*. *Ethology*. **116**. 770-777

Kasumovic MM, Andrade MCB (2004) Discimination of airborne pheromones by mate-searching male western black widow spiders (*Latrodectus hesperus*): species- and population-specific responses. *Canadian Journal of Zoology*. **82**. 1027-1034

Kuznetsova A, Brockhoff PB, Christensen RHB, Jensen SP (2019) lmerTest: Tests in Linear Mixed Effects Models.

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. *Sinauer Associates, Inc.*, Sunderland, MA

Makawski D, Luedecke D, Ben-Shachar MS, Kelley K, Stanley D (2020) effectsize: Indices of Effect Size and Standardized Parameters.

Mhatre M and Bertram S (2018) Tree cricket baffles are manufactured tools. *Ethology*. **124**(9). 691-693

Minter NJ, Franks NR, Robson Brown KA (2012) Morphogenesis of an extended phenotype: four-dimensional ant nest architecture. *Journal of the Royal Society Interface*. **9**(68). 586-595

Nakata K (2012) Plasticity in an extended phenotype and reversed up-down asymmetry of spider orb webs. *Animal Behaviour*. **83**. 821-826

Nussey DH, Wilson AJ, Brommer JE (2007) The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology*. **20**(3) 831-844.

Perampaladas K, Stoltz JA, Andrade MCB (2008) Mated redback spiders re-advertise receptivity months after mating. *Ethology*. **114**. 589-598.

Quade FSC, Holtzheimer J, Frohn J, Toepperwien M, Salditt T, Prpic N-M (2019) Formation and development of the male copulatory organ in the spider *Parasteatoda tepidariorum* involves a metamorphosis-like process. *Scientific Reports*. **9**.

R Core Team (2018) R: A Language and Environment for Statistical Computing

Schaedelin FC, Taborsky M (2009) Extended phenotypes as signals. *Biological Reviews*. **84**. 293-313

Schneider JM, Vollrath F (1988) The effect of prey type on the geometry of the capture web of *Araneus diadematus*. *Naturwissenshcaften*. **85**. 391-394

Sherman PM (1994) The orb-web: an energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. *Animal Behaviour*. **48**. 19-34

Snell-Rood EC (2013) An overview of the evolutionary causes and consequences of behavioura; plasticity. *Animal Behaviour*. **85**. 1004-1011

Salomon M (2011) The natural diet of a polyphagous predator, *Latrodectus hesperus* (Araneae: Theridiidae) over one year. *The Journal of Arachnology*. **39**(1). 154-160.

Stoltz JA, McNeil JN, Andrade MCB (2007) Males assess chemical signals to discriminate just-mated females from virgins in redback spiders. *Animal Behaviour*. **74**. 1669-1674.

Thompson C, Rodriguez RL, Sergi C (2020) Repeatability but no short-term plasticity in the web architecture of *Latrodectus hesperus* western black widow spiders. *Ethology*.

West-Eberhard MJ (1989) Phenotypic Plasticity and the Origin of Diversity. *Annual Review of Ecology and Systematics*. **20**. 249-278

West-Eberhard MJ (2003) Developmental plasticity and evolution. *Oxford University Press*. Oxford, UK.

West-Eberhard MJ (2005) Developmental plasticity and the origin of species differences. *PNAS.*

102. 6543-6549

Zevenbergen JM, Schneider NK, Blackledge TA (2008) Fine dining or fortress? Functional shifts in spider web architecture by the western black widow *Latrodectus hesperus*. *Animal Behaviour*. **76**. 823-829

Zuk M, Bastiaans E, Langkilde T, Swanger E (2014) The role of behaviour in the establishment of novel traits. *Animal Behaviour*. **92**. 333-344

Chapter Two: Black widow spiders use path integration on their webs

Abstract

Many animals store information about their external environment in memory and use this information to facilitate movement and decision making. The information used to navigate has been described for numerous animals, and is dependent on the ecology of the animal. Spider webs make up nearly all of a spider's physical and sensory environment. Here, we first asked whether black widow spiders form memories of their web and then asked whether the contents of these memories include a path integration vector. Black widows made navigational errors when we rotated their web in their absence and searched about the web upon realizing they made an error. Black widows were able to move on their web without retracing their steps, often taking shortcuts to a goal location without sensory cues to guide navigation. Black widows also began moving back to their retreat on a path parallel to their path out from the retreat, even after being moved on the web, and searched about the web for the retreat after not finding it initially. These results show that black widows use path integration when navigating. These results suggest that using internal representations of an animal's position within its environment, such as by using path integration, is widespread among animals with very different brain types and sensory ecologies, representing a convergent solution to common navigational problems.

Introduction

Many animals store information about their environment and the relation of their bodies to their environment in memory, and use this information to guide their behavior as they move about their surroundings (Shettleworth 2010). The information content of these memories varies widely, from the cumulative distance and direction from a reference location used in path integration, to representations of the spatial relationship between multiple landmarks (Shettleworth 2010; Webb 2019; Toledo et al. 2020). Explaining this variation in information content, and how the information is used in navigation, requires taking into account differences in sensory biology, ecology, and brain architecture across animals (Healy and Braithwaite 2000; Shettleworth 2010).

A wide variety of ambulatory invertebrates have been shown to navigate using information from memory, rather than by following immediately available sensory cues. The use of path integration is widespread, occurring for example in ants, bees, dung beetles, and spiders (Seyfarth et al. 1982; Moller and Goerner 1994; Mueller and Wehner 1988; Collett and Collett 2000; Ortega-Escobar 2002; Cheng 2006; Wehner 2009; Ortega-Escobar and Ruiz 2010; Webb 2019; Cross et al. 2020; Dacke et al. 2020; Ortega-Escobar 2020). Some invertebrates use more sophisticated forms of navigation. Honeybees, for instance, learn the location of food using information communicated by the waggle dance of another bee and use this information to guide their navigation to food patches they have not visited before (Menzel et al. 2006). And *Portia* jumping spiders perform feats of navigation in complex environments by planning indirect prey-attack routes that put the prey out of sight of the spider for prolonged intervals (Tarsitano and Jackson 1997; Cross and Jackson 2016; Cross et al. 2020).

Navigation using information stored in memory has for the most part been previously documented in ambulatory animals that possess acute vision, and rely at least partially on visual input for navigation (although *Cupiennius salei* wandering spiders do not seem to need vision when navigating, even though they possess acute vision; Seyfarth et al. 1982; Land and Barth 1992; Webb 2019). However, there is evidence that suggests that even invertebrates that navigate only in a small and well-defined area may also do so using information stored in memory. Web spiders, for example, are sit-and-wait predators with poor vision that locate prey through cues provided by web vibrations caused by the prey (Landolfa and Barth 1996; Clemente et al 2005; Foelix 2011; Eberhard 2020). These spiders might therefore be expected to primarily follow web cues and require little in the way of information stored in memory to navigate. Nevertheless, there is evidence that web spiders form memories of the position or direction of their retreat on the web (LeGuelte 1969); of distances between web components (Eberhard 1988; Eberhard & Hesselberg 2012); and of the content of their webs (e.g., captured prey and egg sacs) (Rodríguez et al 2015). Whether and how web spiders navigate using information stored in memory remains to be explored.

Here we ask whether a web spider navigates its web using information stored in memory, and whether it does so by representing its location relative to its retreat as a path integration vector. We worked with western black widow spiders (*Latrodectus hesperus*). These spiders make webs with distinct components that serve prey-capture, structural, and defensive functions: a tangle of lines and a sheet from which descend a forest of gumfooted lines anchored to the substrate, and a retreat (Blackledge and Zevenbergen 2007) (Fig. 2.1). Here we

focus on how they navigate on the sheet plane. Specifically, we analyze how the spiders return to their retreat when they are out on the sheet.

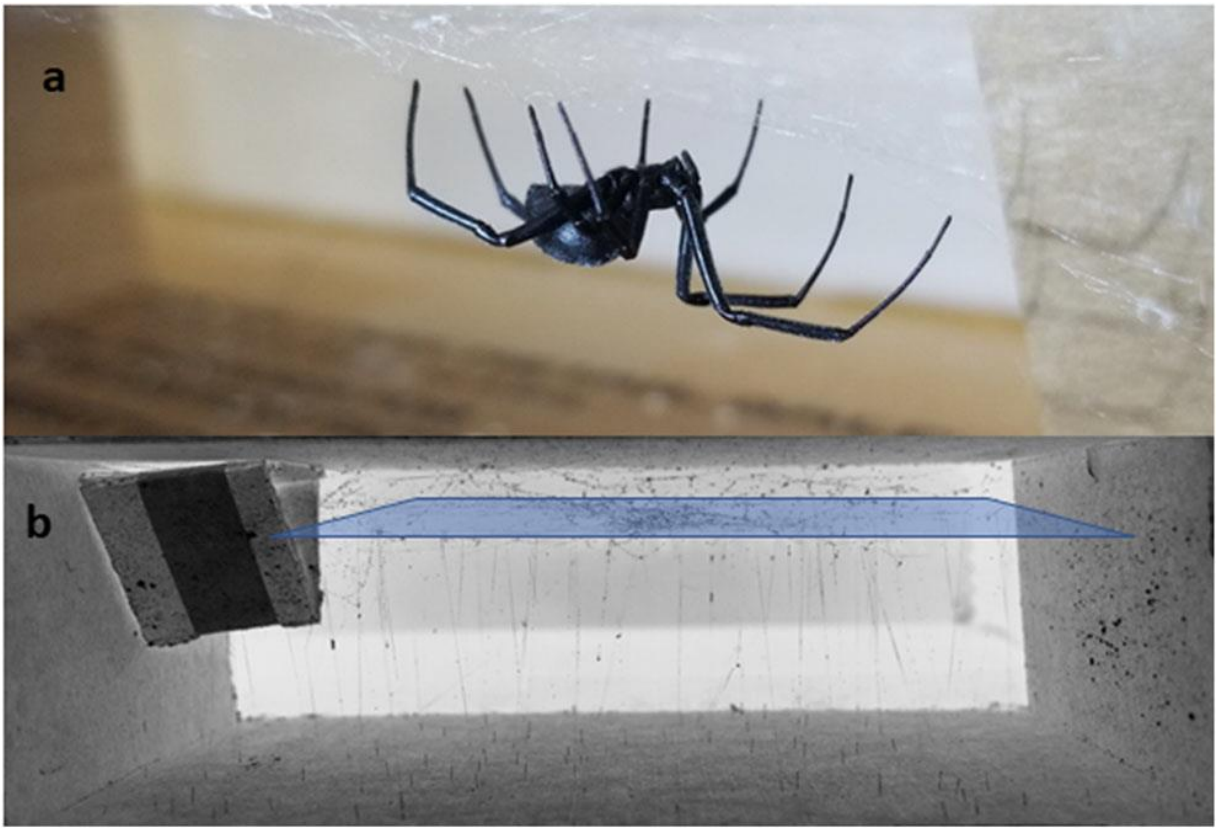

Fig. 2.1: Black widow spider and representative web with a sheet and vertical gum-footed lines. Black widow spiders construct space-filling cobwebs (Benjamin and Zschokke 2003). (**a**) A female black widow spider in a typical posture on the sheet of her web. (**b**) Negative image of a black widow web constructed in a cardboard frame. The spiders use the triangular prism we provide (left) as a retreat. The sheet extends from the retreat to the far side of the frame. The bottom of the sheet forms a two-dimensional plane (blue rectangle inset in **b**). Spiders do most of their navigation along this plane, and all of the navigation we describe in our experiments occurred on the plane formed by the web sheet. The gumfooted lines are the primary prey-capture structures of black widow webs

We first tested the broad hypothesis that black widows navigate using information stored in memory. We tested two predictions of this hypothesis. First, the spiders should make navigational errors when returning to the retreat after the web has been rotated in their absence so that there is a mismatch between their memory of their position on the web relative to the retreat and their actual position relative to the retreat. The second prediction

arises from a detail of the behavior of web spiders. When their memory-based expectation about a feature of the web does not match the actual web (e.g., when its contents are removed in their absence), they engage in prolonged searching behavior, moving around the web, plucking or tugging on its lines in an attempt to reacquire the lost item or re-orient themselves on the web (LeGuelte 1969; Rodríguez and Gamboa 2000; Rodríguez and Gloudeman 2011; Rodríguez et al. 2013; Rodríguez et al. 2015; Kilmer and Rodríguez 2018). This first hypothesis therefore makes the prediction that spiders that have made navigational errors should evince having an expectation of finding the retreat where they went to by engaging in sustained localized searching at or near the site where they would expect the retreat to be but do not find it (as opposed to immediate exploration to seek cues of the direction or position of the retreat; which would of course occur eventually when spiders have become lost).

Having found support for this hypothesis (see below), we then tested the hypothesis that the information that black widow spiders store in memory takes the form of a path integration vector (path integration hypothesis). We tested three predictions of this hypothesis. First, spiders should be able to navigate their web without retracing their steps (e.g., they should be able to take shortcuts). Second, spiders lured away from the retreat and then prompted to return should be able to head broadly in the direction of the retreat predicted by a path integration vector created on their path out from the retreat without first searching, and search upon reaching the expected location of the retreat without finding it. We did not expect spiders to head exactly in the direction of the retreat as predicted by a path integration vector, because path integration is prone to error accumulation (Collett 1996; Pavel and Cronin 2020). We expected spiders to search in a sustained localized manner upon reaching the area they

expect to find the retreat (Wehner and Srinivasan 1981; Durier and Rivault 1999; Pavel and Cronin 2020). Third, spiders lured away from the retreat and then displaced to a new location on the web before being prompted to return to the retreat should follow a path that approximates a path integration vector extending from the location they were lured to the retreat. Thus, after being displaced, spiders should follow a path that is parallel to the path integration vector, but displaced by the distance between the location spiders were lured to and the location spiders were moved to.

Methods

We collected sub-adult and adult female *L. hesperus* from urban areas of Medford, Oregon in June 2017 and June 2018 and shipped them to our lab at UWM. Once the spiders were in the laboratory, we housed them individually in plastic cups and provided them with two 1.5 cm-long crickets. During periods of normal husbandry, we provided each spider with one cricket every two weeks. When spiders were going to be used in an experiment, we fed them 10 and 3 days before experiments began to standardize satiety. We preserved voucher specimens in 95% ethanol.

In all experiments, we attempted to reduce the potential for spiders to use external cues when navigating by forcing spiders to build webs in plain cardboard frames or plastic boxes in which their sheet formed a flat plane, and to build retreats within triangular prisms that we affixed to the rigid wall of the frame or box (Fig. 2.1). This removed the possibility of spiders using up/down cues to find their retreat, and reduced the possibility that the cardboard of the retreat would contrast against the wall of the enclosure enough to be visible given web-building spiders poor eyesight (Clemente et al. 2005).

Do black widow spiders make navigational errors when their web has been rotated in their absence?(And do the spiders reveal expectations about finding the retreat by sustained localized searching when they have made such errors?)

We used an assay similar to that used by LeGuelte (1969): we briefly removed spiders from their webs and rotated the frame that housed the web while the spiders were removed, so that in our treatment group (below) the location of the retreat relative to the spider had changed by 180^{o} (i.e., if the spiders were removed with the retreat to their left, the retreat would be to their right when placed back on the web). We constructed cardboard frames modelled after those used by Blackledge and Zevenbergen (2007) (Fig. 2.1b). These frames measured 25 cm long x 7.5 cm tall x 15 cm wide, and were open on the two long sides. The frames contained a 5 x 5 x 5 cm triangular prism that we attached with tape to the top center of one short side in which spiders constructed their retreat, and ensured that the retreats of all spiders were in the same relative location within the frame and on the same horizontal plane as the web sheet. Over two weeks, spiders constructed webs that spanned the entirety of each frame (Fig. 2.1b).

To begin a trial, we lured each spider to the edge of the sheet, halfway between the retreat and the far side of the frame, by vibrating a forceps on the sheet to imitate trapped prey. When spiders approached the forceps and turned around (facing the middle of the web) to begin flicking silk, we briefly removed the spider off the web using a plastic vial and severed the spider's dragline so the spider was no longer attached to the web. We alternated which edge we lured spiders to so we had equal numbers of spiders that we lured to the edge to the right and to the left of their retreat. In the experimental treatment, we then rotated the frame

180° and then replaced the spider on the edge of its web sheet facing the middle of the web (as much as possible, we matched the direction spiders were facing relative to the sheet when we removed and replaced them), so the retreat was on the opposite side of the frame relative to where the spider had been removed from (n = 28). In two control groups, we removed the spider as above but then either did not rotate the frame (n = 11) or rotated it 360° (n = 9).

Immediately after replacing each spider on the web, we administered a puff of air by squeezing the bulb of an aspirator to prompt them to return to the retreat. In the 0° and 360° rotation treatments we were able to place the spiders approximately on the same spot from which we had removed them. In the 180° rotation treatment we were not able to do this, as removing and replacing spiders from the edge of the sheet meant that in the 180° rotation we replaced spiders on the opposite edge of the sheet from where we removed them. The gumfooted lines of each web prevented us from removing/replacing spiders at the center of the sheet (perhaps the ideal) without damaging the web and introducing potential sensory cues. We placed these spiders at approximately the same distance to the retreat from which we removed them. This difference in the site of replacement of the spiders on the web may have introduced a confound into this experiment, whereby spiders in the 0° and 360° controls may have been more likely to be placed near their (now severed) draglines than spiders in the 180° treatment. However, the spiders in the 0° and 360° controls would have needed to search briefly to locate those draglines (or other cues); we therefore checked whether they did so or started moving to the retreat right away.

After replacing the spiders on the web and puffing them with the aspirator, we noted the direction in which the spider moved. We also noted whether spiders searched before

initiating their return and after reaching either the retreat or another end of the cardboard frame where there was no retreat. In this experiment, our view of the spiders was from the side and allowed for us to record whether a spider moved toward or away from the retreat when attempting to return to it. All spiders that moved when puffed moved toward or away from the retreat (i.e., no spiders moved directly across the web to the opposite edge of the sheet). Thus, all data for this experiment are binary measurements taken from real time observations of spider behavior.

We excluded spiders that did not move when puffed (n = 4); these spiders did not move from the area where we replaced them on the web and instead adopted a "crouch" posture in which all legs were drawn close to the body and the body pressed tightly against the web sheet. We used logistic regression to test for differences in likelihood of moving toward or away from the retreat between the 0^{o}, 360^{o}, and 180^{o} rotation treatments. We also used logistic regression to test for differences in likelihood of searching between spiders that moved toward the expected or toward the actual retreat location in the 180^{o} rotation treatment.

Do black widow spiders use path integration?

To test the first prediction of the path integration hypothesis (that black widows can use shortcuts to return to the retreat), we placed spiders in plastic boxes that were 40 cm long by 33 cm wide by 17 cm tall. The boxes contained a 2.5 x 2.5 x 2.5 cm hollow cardboard triangular "prism" approximately 5 cm from the top of the box in the center of the long side, which the spiders used as a retreat. We gave the spiders two weeks to construct a web.

To begin a trial, we lured spiders from their retreat using rubber-tipped forceps that we vibrated on the web at the edge of the sheet furthest from the retreat to simulate prey. We

attempted to lure the spiders out from their retreat in a circuitous path. Once spiders were within approximately 2.5 cm of the forceps and edge of the sheet, we removed the forceps from the web and administered a puff of air by forcefully squeezing the bulb of an aspirator to make spiders move back to their retreat.

We filmed each trial and converted each video into a series of jpeg images captured every 10th frame with DVDVideoSoft (Digital Wave Ltd, United Kingdom). We then used ImageJ to stack and focus each image to produce a single image with a visible trace of each spider's movement during each trial (Fig. 2.2). We used the stacked images to compare the route spiders took from the retreat to the forceps and the return route after being puffed with air.

Although we attempted to lure spiders out from their retreat in a circuitous path, we only succeeded in some cases (n = 15); other spiders took straight paths out (n = 8) (Fig. 2.2). We report all results, but the focus of our test was on the spiders that took circuitous routes out from the retreat (spiders that took straight paths out could have been using path integration to return to the retreat, but their return paths would be the same if they followed cues left behind on their path out, making these spiders uninformative regarding path integration).

We used logistic regression to test whether spiders that took circuitous paths out from the retreat were more likely to take a different path back to the retreat than spiders that took a straight path out from the retreat. We also used a binomial test to investigate whether spiders that took a circuitous route out from the retreat and did not retrace their paths on the return route were more likely to take a shorter route on the way back to the retreat. We also used a

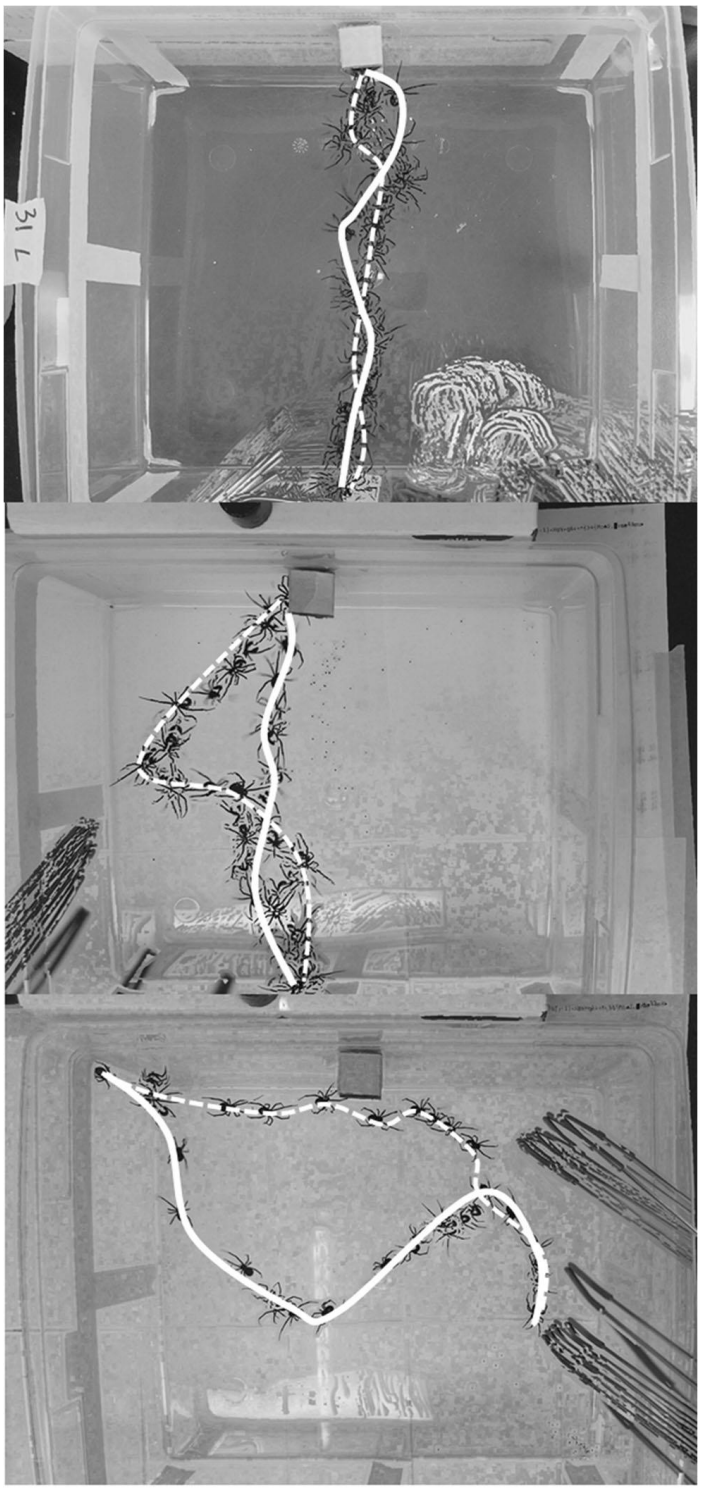

Fig. 2.2: Examples of paths followed by black widow spiders from and back to the retreat. Paths out from retreat (dashed lines) and back in to retreat (solid lines) of spiders in the first experiment testing the path integration hypothesis. Some spiders retraced their paths when returning to the retreat (top image); some spiders took more direct, shorter paths back in to the retreat (middle image); and some spiders took different, but not direct, paths back in to the retreat (bottom image)

paired t-test to investigate whether the paths back to the retreat of spiders that took circuitous paths away from the retreat were shorter than the paths out.

To test the second prediction of the path integration hypothesis (that black widows should be able to head in the direction of the retreat predicted by a path integration vector created on their path out from the retreat without first searching, and search upon reaching the expected location of the retreat without finding it), we used 40 x 33 x 17 cm plastic boxes as in the experiment above, except we partially coated the interior of the boxes with petroleum jelly so that the spiders could not attach silk to part of the box. This resulted in spiders constructing webs that had sheets that extended approximately two-thirds of the way across the box from the retreat and left the third of the box furthest from the retreat open, allowing us room to conduct the experimental manipulation (below). Spiders constructed full webs in these frames within one week.

In each trial in this experiment, we lured spiders to one of the corners of the edge of the sheet furthest from the retreat, then subjected them to one of two treatments or a control before disturbing them by puffing them with air at a rate of approximately one puff per second until they either found the retreat or adopted a crouching posture somewhere on the web. We attempted to puff air at an angle perpendicular to the edge of the sheet. (We tested for correlation between the angle of the first puff and the angle spiders moved immediately after the first puff, and found no overall correlation; $r = -0.12$, $p = 0.67$. However, we also checked for individuals that moved perpendicularly to the angle of puffed air; see below.) In the two treatments, we briefly lifted each spider from the web, severed its dragline, and replaced the spider on the edge of the sheet of the web either at the same ($n = 4$) or a different location ($n =$

4) from where we removed it (the latter being directly in front of the retreat and approximately 15 cm from the location we removed spiders from) before puffing them. The sample size in these treatments is small because this manipulation was difficult and we only succeeded in these few cases. In the control group (n = 18) where we did not lift the spiders from the web at all, we began to puff them with air as soon as they reached the corner of the web we lured them to. Thus, the data from all individuals in this experiment, whether in a treatment or control group, served to test that the spiders should be able to head in the direction of the retreat predicted by a path integration vector created on their path out from the retreat without first searching, and search upon reaching the expected location of the retreat without finding it (follow a path approximately the same direction and length as the solid arrows in Fig 2.3). Further, the spiders in the displacement treatment test whether they follow a path approximately parallel to the one that would bring them to the retreat had they not been displaced.

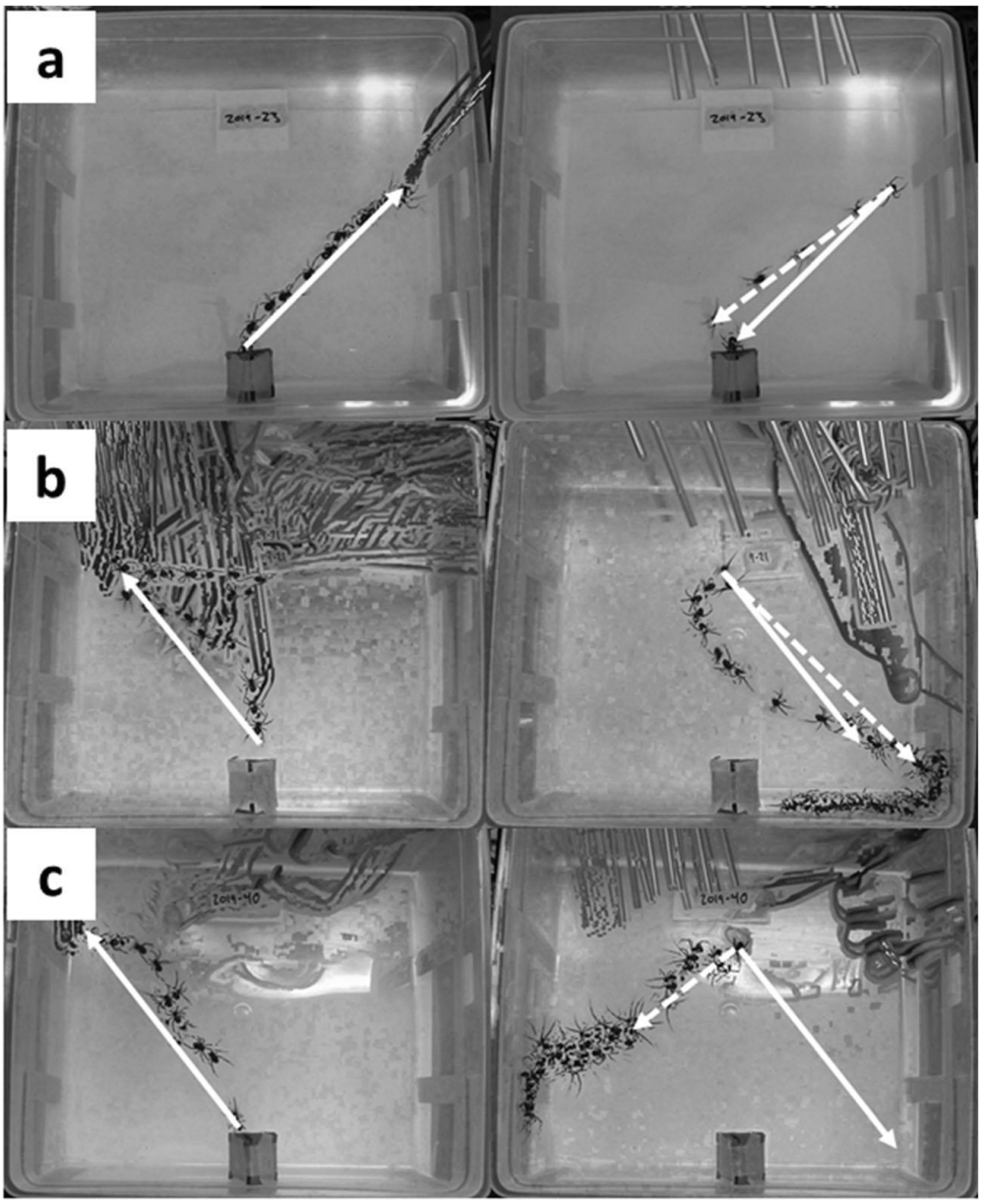

Fig. 2.3: Examples of predicted and observed paths of black widow spiders in our test of the path integration hypothesis. Paths taken by spiders on their path from the retreat to the lure (left images) and back to the retreat (right images). Solid arrows in left images show the direct path from the retreat to the location spiders were lured. Solid arrows in right images show the predicted path of each spider if they navigated using path integration. The solid arrows in right images are the same length and opposite direction as the solid arrows in left images and are therefore equivalent to a path integration vector for each spider. The dashed arrows in right images show the actual path spiders took from the start of their return path (beginning of arrows) until the location they began to search (head of arrows). (**a**) An unmoved control spider that followed a path that closely matched the predicted path to return to the retreat. (**b**) A moved spider that followed a path that closely matched the predicted path to return to the retreat. (**c**) A moved spider that followed a path did not closely match the predicted path to return to the retreat

We video recorded all trials and converted each video into a series of stacked jpegs captured every 10th frame to allow us to observe the paths spiders took away from and back to the retreat. From these images, we obtained the following data: whether spiders searched before fleeing to the retreat when puffed with air; whether they moved in the direction of the retreat predicted by a path integration vector; whether they searched after arriving at the expected position of the retreat; whether they found the retreat; and whether they ceased searching after finding the retreat. We used two criteria for whether spiders moved in the predicted direction of the retreat. First, we used ImageJ to measure the angles spiders moved on their return path toward the retreat relative to the edge of the sheet, and the angle of the most direct path back to the retreat from where we lured the spiders to, i.e. the angle of the path integration vector, (also relative to the edge of the sheet). We tested for a correlation between these angles. Second, considering that one would not necessarily require the spider to follow *exactly* the same angle back, we also used a more relaxed criterion: whether spiders initially moved in a direction that was within 30 degrees on either side of the direction of the retreat predicted by a path integration vector (i.e., within 30 degrees of solid arrows in Fig 2.3) (the actual range of angle differences for these spiders was 1.9 to 23.8 degrees, with an average difference in angle of 11.7 degrees).

Because we were only able to obtain small sample sizes in crucial treatments for this experiment (n = 4 spiders lifted from the web and replaced at the same location on the sheet; another n = 4 lifted from the web and replaced at a different location), we focused our analysis on qualitative descriptions of the behavior and the correlation between predicted and observed path angles of spiders returning to their retreats.

Results

Black widow spiders make navigational errors when their web has been rotated in their absence (and search locally when they have made such errors)

We briefly removed spiders from their web, rotated their web by 0°, 180°, or 360° around the vertical axis, replaced the spiders on their web, and prompted them to return to the retreat by puffing them with an aspirator. None of the 48 spiders in this experiment searched before starting to attempt to return to the retreat. Spiders in the 180° rotation treatment were significantly more likely to move toward the original location of the retreat (away from its current location) than spiders in the 0° or 360° rotation treatments ($\chi^2_2 = 11.86$, $p = 0.0027$) (Fig 2.4a). Interestingly, no spider moved in any other direction (e.g., orthogonal to the retreat-opposite wall line as would be expected if spiders were merely fleeing directly away from the puffed air). Further, in the 180° rotation treatment, spiders that moved away from the current location of the retreat were significantly more likely to search upon reaching the end of the cardboard frame (where the mental model would have generated an expectation for the position of the retreat) ($\chi^2_2 = 10.01$, $p = 0.0016$) (Fig. 2.4b). No spiders searched before reaching the end of the cardboard frame. Spiders that searched after fleeing in the wrong direction initially searched in a localized manner, near where the retreat would have been had they fled the correct direction. These spiders eventually expanded their search area until they found the actual location of the retreat. The few spiders that searched after fleeing in the actual direction of the retreat searched near the retreat before entering the retreat and remaining there.

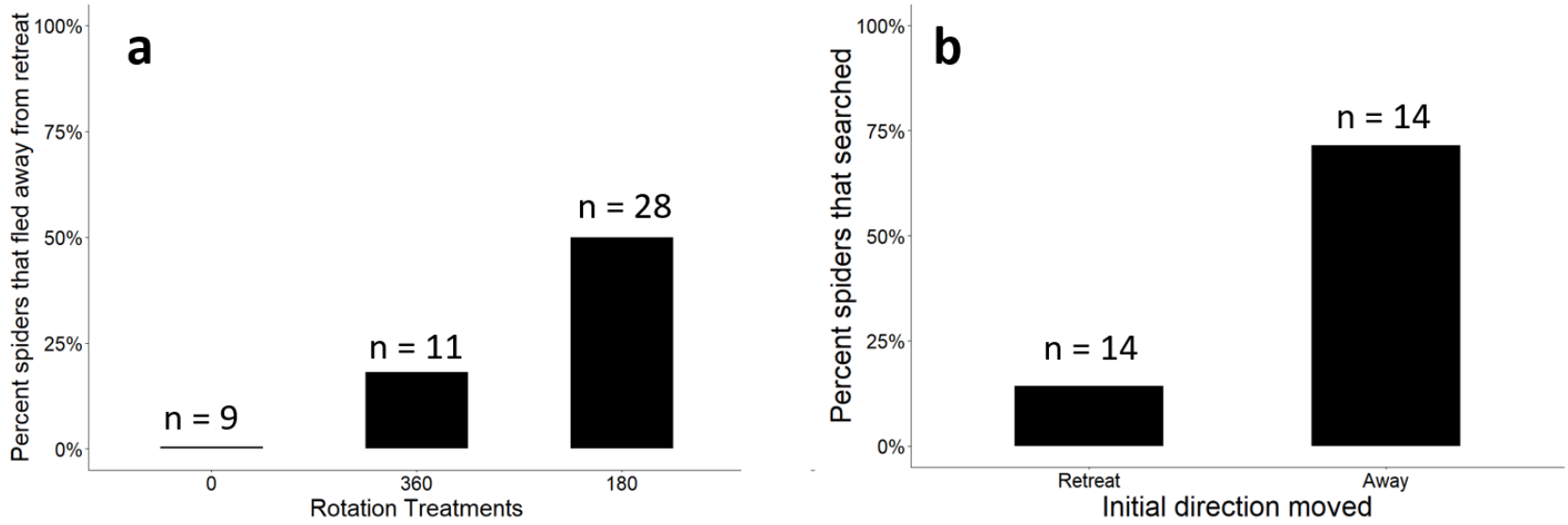

Fig. 2.4: Test of the ability of black widow spiders to form memories of their web. (**a**) Percent spiders that fled away from the retreat across box rotation treatments. (**b**) Percent spiders that searched after fleeing away from or to the retreat (180^{o} rotation treatment)

Black widow spiders take shortcuts

We attempted to lure black widows out from their retreat in a circuitous path by simulating a prey item on their web with a forceps, and then disturbed them with a puff of air from an aspirator to induce them to return to the retreat. We were able to get 15 spiders to take circuitous routes out from the retreat, but 8 spiders took straight routes out (Fig. 2.2).

Thirteen of 15 spiders that took circuitous routes out took a different path back to the retreat (Fig. 2.5a, gray lines); the other 2 spiders retraced their steps (Fig. 2.5a, blue lines). By contrast, only 1 of 8 spiders that took straight routes out took a different (circuitous) path back (Fig 2.5b, blue line), all others retracing their steps (Fig 2.5b, gray lines) ($\chi^2_1 = 12.98$; $p = 0.0003$). Spiders that took circuitous routes out from the retreat were significantly more likely to take more direct (shorter) paths back to the retreat (two-tailed binomial test, p-value = 0.035; Fig 5a). Those return paths were on average 22% shorter than the paths out (paired-t test: $t_{14} = 2.26$; $p = 0.04$; Fig 2.5a).

Black widow spiders flee in the expected direction of the retreat

We lured spiders away from the retreat, lifted some to a different area of the web, and then puffed them with air. We noted whether the black widows searched immediately after being puffed before attempting to return to the retreat, whether they initially moved in the direction of the retreat predicted by use of a path integration vector, and whether they searched after getting near the predicted location of the retreat (see Fig. 2.3a-c for examples of spiders that initially did or did not move in the predicted direction of the retreat after we puffed them with air).

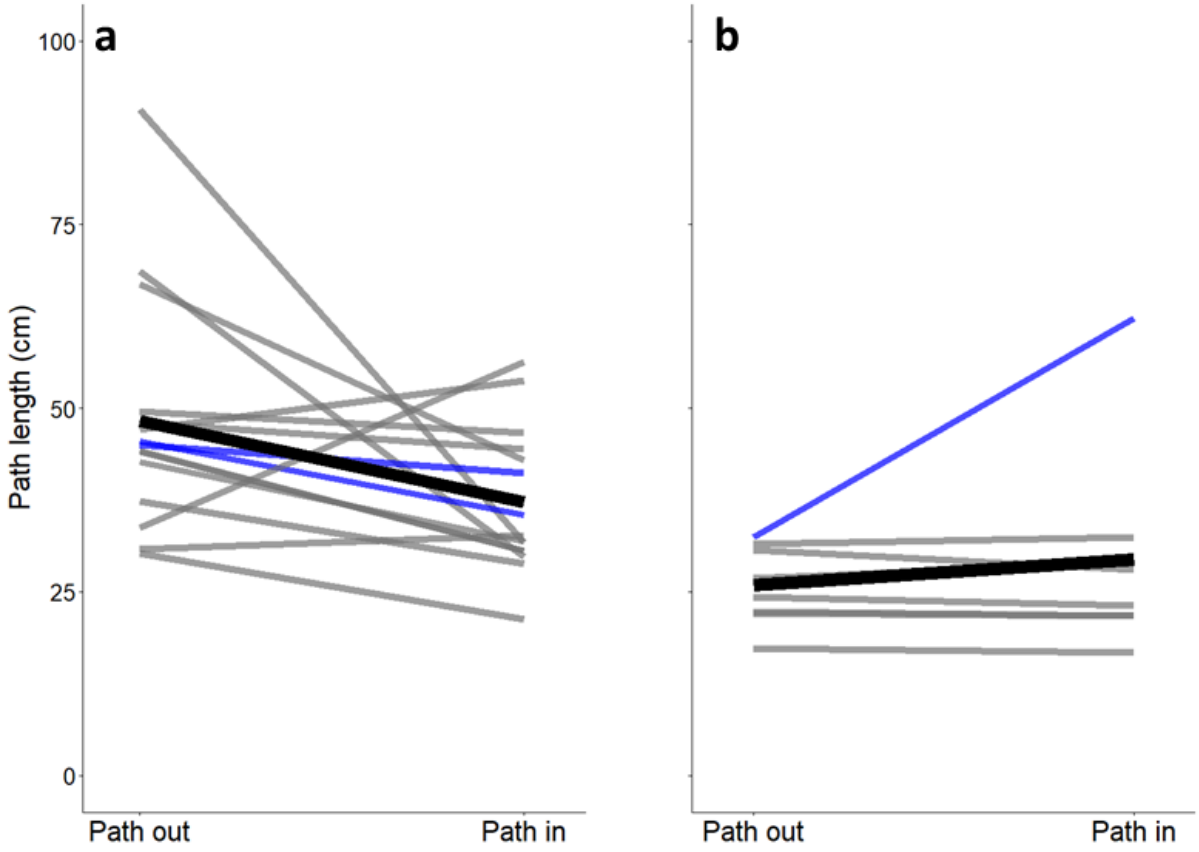

Fig. 2.5: Test of the ability of black widow spiders to take short cuts. Path lengths of spiders that took circuitous **(a)** or straight **(b)** paths out from the retreat. (**a**) The paths of spiders that retraced their paths are shown in grey and the spider that took a longer path back is shown in blue. (**b**) The paths of spiders that took different paths are shown in grey and the paths that overlapped are shown in blue. The black lines show the average path length of spiders in each panel.

None of the 26 spiders in this experiment searched before beginning their return to the retreat. Seventeen of the 18 control spiders initially moved in the direction of the retreat (one-tailed binomial test, $p < 0.0001$). Nine of those 17 spiders found the retreat immediately without searching; the remaining 8 began to search after reaching the expected location of the retreat and not finding it.

All four spiders that we lifted from the web and replaced in the same spot initially moved in the predicted direction (i.e., within 30 degrees on either side of the direction predicted by a path integration vector). One of those four spiders found the retreat

immediately; the remaining three found it after searching. Three of the 4 spiders that we moved to a different location initially moved in the predicted direction. All three of these spiders began to search after reaching the expected location of the retreat. Only 1 of those 3 spiders found the retreat after searching; the other two adopted a crouching posture in the corner of the web. The one spider that was displaced and did not initially move in the predicted direction took a path that was approximately perpendicular (still not toward the retreat) to the predicted path and adopted a crouching posture in the corner of the web after reaching the edge of the box. Thus, across all treatments, 14 out of 14 spiders that got to the expected position of the retreat without finding it there searched. By contrast, none of the ten spiders that immediately found the retreat searched. The spiders that searched initially did so near the expected location of the retreat (search times ranged from 1-89 seconds, average 19 seconds) and expanded their search location until they found the retreat (except for the two spiders that did not find the retreat after being displaced, which adopted a crouching posture after expanding their search area).

Across all spiders, the angle of the paths out from the retreat was significantly correlated with the angle of the paths back to the retreat, with a medium effect size ($r = 0.45$, $p = 0.022$, $n = 26$) (Fig. 2.6). However, two spiders did not seem to be using path integration nor sensory cues to find the retreat. One spider moved perpendicularly to the puffed air (suggesting this may have been due to the angle of puffing); the other moved in a direction orthogonal to the predicted direction of the retreat (suggesting overcompensation for the displacement) (Fig. 2.6, black and blue asterisks, respectively). Excluding these two spiders yields a correlation with a large effect size ($r = 0.64$, $p = 0.001$).

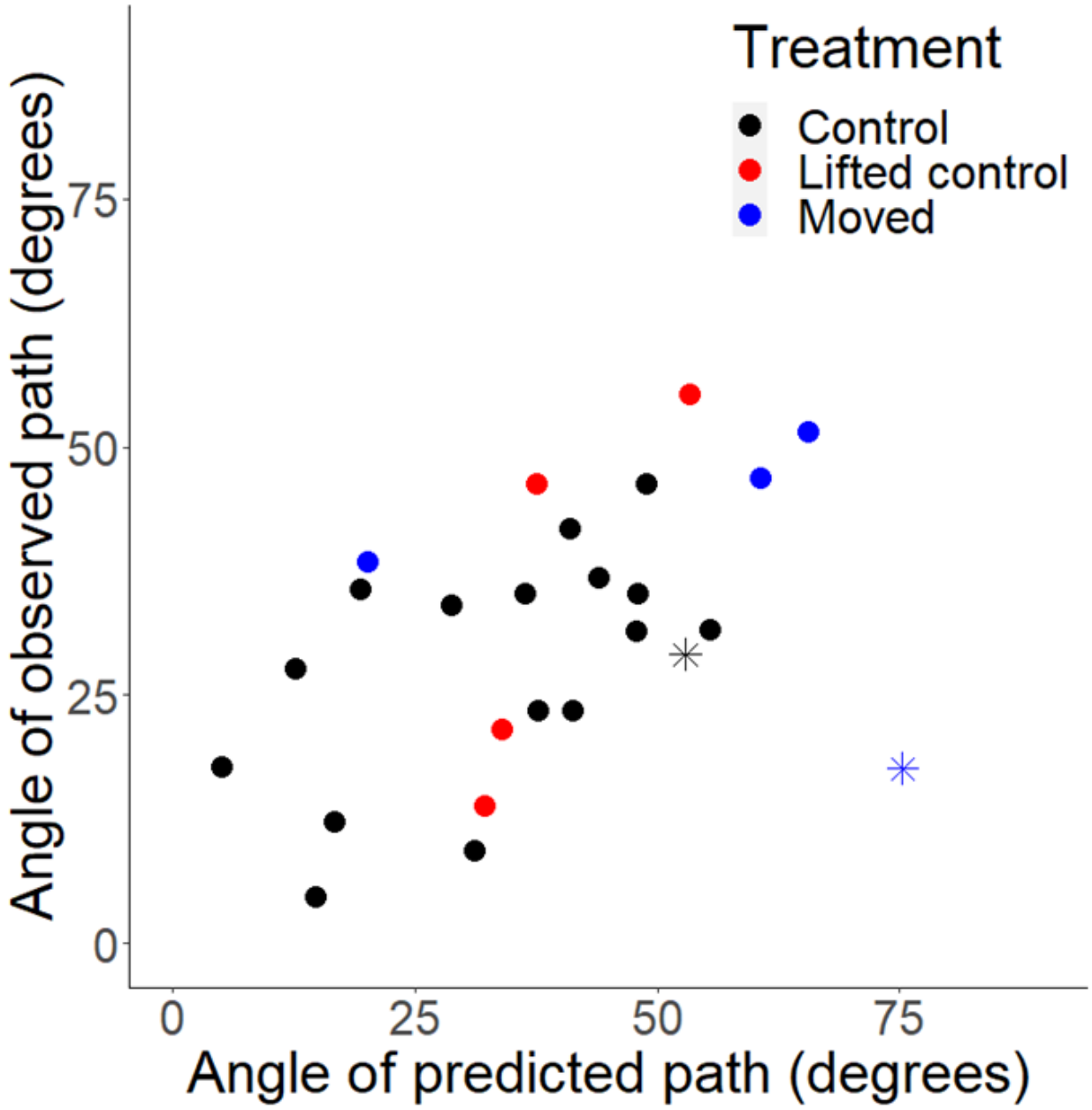

Fig. 2.6: Correlation between angles of predicted black widow spider paths to the retreat and observed paths to the retreat. Treatment shown by point color.

Discussion

We provide evidence that black widow spiders, an invertebrate with a non-ambulatory lifestyle that senses the world mainly through substrate vibrations, use path integration to navigate their webs. The spiders made navigational errors when the contents of their memory did not

match the web; searched in a localized way when their expectation of arriving at the retreat was violated; were able to return to the retreat with short cuts, without searching about for cues nor retracing their steps; and when experimentally displaced tended to move in a direction consistent with following a path integration vector, and searched in a localized manner upon reaching the predicted location of the retreat and not finding it.

These findings are interesting given the sensorium of black widows (Landolfa and Barth 1996; Clemente et al. 2005; Foelix 2011). It is highly unlikely that black widows use visual information when creating their path integration vectors or navigating to their retreats, because web-building spiders lack acute vision, black widows are active only at night, and spiders in our experiments searched in close proximity to the retreat (where tactile searching would be least necessary if the spiders were using visual input to locate the retreat). This offers an interesting contrast with other invertebrates that navigate with path integration, because even among animals that use proprioceptive information (such as number of steps walked or the direction of wind) to determine the distance or direction of a goal to navigate toward, visual input is also important for orienting during the trip (Ortega-Escobar 2002; Mueller and Wehner 2007; Wehner 2009; Ortega-Escobar and Ruiz 2010).

It is also interesting that black widows use path integration to navigate, because webs likely provide enough structural cues to guide navigation as to make the use of path integration unnecessary (Benjamin and Zschokke 2003). In each of our experiments we forced spiders to create webs in which the retreat and plane of the sheet were at the same height and the sheet began immediately at the entrance of the retreat. In natural *Latrodectus* webs, spiders construct retreats at a location peripheral to the sheet of the web, and often at a different

height than the sheet (Benjamin and Zschokke 2003). The entrances to retreats are small relative to the rest of the web, so this peripheral location results in a funnel shaped area of web leading from the sheet to the retreat (Benjamin and Zschokke 2003). This web structure could provide black widows with information about whether their location on the web is above or below the entrance to the retreat, as well as a web structure that could guide navigation to the retreat by following the narrowing web to the retreat entrance.

Although spiders navigated using path integration, it is also clear that the spiders used external cues present on the web (as is broadly the case in web spiders; Foelix 2011; Eberhard 2020). In each of our experiments, a number of the spiders initially navigated using only path integration and made the predicted errors in finding their retreat after we had displaced them on the web. However, these spiders eventually began searching the web for the retreat, which suggests that there are sensory cues in the web the spiders are able to follow. This searching behavior after moving to the expected location of the retreat is also a classic feature of navigating by path integration, as path integration is error prone and animals often must search for a goal location after using path integration to move near the goal (Wehner and Srinivasan 1981; Durier and Rivault 1999; Pavel and Cronin 2020). It is also possible that black widows can choose whether to navigate using path integration or by following cues present on the web, and this could explain the even split in our first experiment of spiders that initially fled toward or away from the retreat.

In our present study we provide evidence of the use of path integration by black widow spiders. Further investigation of the specific information web spiders use for path integration and how they supplement path integration with direct sensory cues from the web would be

fruitful. It is obvious why some invertebrates would need path integration for successful navigation, such as desert ants that navigate between food sources and their nest in environments that may not provide direct sensory cues to guide navigation (Mueller and Wehner 1988; Wehner 2009). It is not obvious why web spiders should need path integration, given the availability of direct cues on the web. Thus, our findings indicate that animals differing widely in brain architecture, sensorium, and umwelt, share the ability to create and use representations of their position relative to their surroundings in memory. These shared capabilities across vertebrates and invertebrates likely represent convergence, as the last common ancestor of all these groups likely had a simple neural system (Feinberg and Mallat 2016). These capabilities are therefore likely to be a relatively basic feature that many brain types can express that they evolved as a common solution to the problems presented by navigating a complex world.

References

Benjamin SP, Zschokke S (2003) Webs of theridiid spiders: construction, structure and evolution. Biol J Linn Soc Lond. 78: 293-305

Blackledge TA, Zevenbergen JA (2007) Condition-dependent spider web architecture in the western black widow *Latrodectus hesperus*. Anim Behav. 73: 855-864

Cheng K (2006) Arthropod Navigation: Ants, Bees, Crabs, Spiders Finding Their Way. In The Oxford Handbook of Comparative Cognition. Oxford University Press.

Clemente CJ, McMaster KA, Fox L, Meldrum L, Main BY, Stewart T (2005) Visual acuity of the sheet-web building spider *Badumna insignis* (Araneae, Desidae). J Arachnol. 33: 726-734

Collett TS (1996) Insect navigation en route to the goal: multiple strategies for the use of landmarks. J Exp Biol. 199: 227-235

Collett TS, Collett M (2000) Path integration in insects. Curr Opin Neurobiol. 10: 757-762

Cross FR, Jackson RR (2016) Execution of planned detours by spider-eating predators. J Exp Anal Behav. 105: 194-210

Cross FR, Carvell GE, Jackson RR, Grace RC (2020) Arthropod intelligence? The case for Portia. Front Psychol. 11

Dacke M, el Jundi B, Gagnon Y, Yilmaz A, Byrne M, Baird E (2020) A dung beetle that path integrates without the use of landmarks. Anim Cogn. 23: 1161-1175

Durier V, Rivault C (1999) Path integration in cockroach larvae, Blatella germanica (L.) (insect: Dictyoptera): Direction and distance estimation. Anim Learn and Behav. 27: 108-118

Eberhard WG (1988) Memory of distances and directions moved as cues during temporaray spiral construction in the spider Leucauge mariana (Araneae: Araneidae). J. Insect Behav. 1: 51-58

Eberhard WG, Hesselberg T (2012) Cues that spiders (Araneae: Araneidae, Tetragnathidae) use to build orbs: lapses in attention to one set of cues because of dissonance with others? Ethology. 118: 610-620

Eberhard WG (2020) Spider webs. Behavior, function, and evolution. University of Chicago Press.

Feinberg TE, Mallatt JM (2016) The ancient origins of consciousness: How the brain created

experience. The MIT Press

Foelix R (2011) Biology of Spiders. Oxford University Press.

Healy S, Braithwaite V (2000) Cognitive ecology: a field of substance? TREE. 2000; 15: 22-25

Kilmer JT, Havens ZS, Rodríguez RL (2018) Ontogenetic approach reveals how cognitive capability and motivation shape prey-searching behavior in *Pholcus phalangiodes* cellar spiders. Ethology. 124: 657-666

Land MF, Barth FG (1992) The Quality of vision in the Ctenid spider *Cupiennius salei*. J Exp Biol. 164: 227-242

Landolfa MA, Barth FG (1996) Vibrations in the orb web of the spider *Nephila clavipes*: cues for discrimination and orientation. J Comp Physiol A. 179: 493-508

LeGuelte L (1969) Learning in Spiders. Am Zool. 9: 145-152

Menzel R, De Marco RJ, Greggers U (2006) Spatial memory, navigation and dance behavior in *Apis mellifera*. J Comp Physiol A.

Moller p, Goerner P (1994) Homing by path integration in the spider *Agelena labyrinthica* Clerck. J Comp Physiol A. 174: 221-229

Mueller M, Wehner R (1988) Path integration in desert ants, *Cataglyphis fortis*. Proc Natl Acad Sci USA. 85: 5287-5290

Mueller M, Wehner R (2007) Wind and sky as compass cues in desert ant navigation. Naturwissenschaften. 94: 589-594

Ortega-Escobar J (2002) Evidence that the wolf-spider *Lycosa tarantula* (Araeae, Lycosidae) needs visual input for path integration. J Arachnology. 30: 481-486

Ortega-Escobar J, Ruiz MA (2010) Visual odometry in the wolf spider *Lycosa tarantula* (Araeae: Lycosidae). J Exp Biol. 217: 395-401

Ortega-Escobar J (2020) Homing in the arachnid taxa Araneae and Amblypygi. Anim Cogn. 23: 1189-1204

Pavel RN, Cronin TW (2020) Path integration error and adaptable search behaviors in a mantis shrimp. J Exp Biol. 223

Rodríguez RL, Gamboa E (2000) Memory of captured prey in three web spiders (Araneae:

Araneidae, Linyphiidae, Tetragnathidae). Anim Cogn. 3: 91-97

Rodríguez RL, Gloudeman MD (2011) Estimating repeatability of memories of captured prey formed by *Frontinella communis* spiders (Araneae: Linyphiidae). Anim Cogn. 14: 675-682

Rodríguez RL, Kolodziej RC, Hoebel G (2013) Memory of prey larders in golden orb-web spiders, *Nephila clavipes* (Araneae: Nephilidae). Behav. 150: 1345-1356

Rodríguez RL, Briceno RD, Briceno-Aguilar E, Hoebel G (2015) *Nephila clavipes* spiders (Araneae: Nephilidae) keep track of captured prey counts: testing for a sense of numerosity in an orb-weaver. Anim Cogn. 18: 307-314

Seyfarth EA, Hergenroeder R, Ebbes H, Barth FG (1982) Idiothetic orientation of a wandering spider: compensation of detours and estimates of goal distance. Behav Ecol Sociobiol. 11: 139-148

Shettleworth SJ (2010) Cognition, Evolution, and Behavior. Oxford University Press.

Tarsitano MS, Jackson RR (1997) Araneophagic jumping spiders discriminate between detour routes that do and do not lead to prey. Anim Behav. 53: 257-266

Toledo S, Shohami D, Schiffner I, Lourie E, Orcham Y, Bartan Y, Nathan R (2020) Cognitive map-based navigation in wild bats revealed by a new high-throughput tracking system. Science. 369: 199-192

Webb B (2019) The internal maps of insects. J Exp Biol. 222: 1-13

Wehner R (2009) The architecture of the desert ant's navigational toolkit (Hymenoptera: Formicidae). Myrmecological News. 12: 85-96

Wehner R, Srinivasan MV (1981) Searching behavior of desert ants, genus Cataglyphis (Formicidae, Hymenoptera). J Comp Physiol 142: 315-338

Chapter Three: Distraction by internally directed attention reveals basic consciousness in animals.

Abstract

Whether animals, especially invertebrates, are conscious is currently debated. One of the greatest challenges in determining whether an animal is conscious is that most animals cannot report on their conscious states, if they have any. In this paper, we describe an assay for determining whether animals possess basic consciousness—subjective experience. We use distraction from an external cue by attention to an object in the mind as the key indicator of consciousness. We argue that only subjective experience is both necessary and sufficient to cause distraction by inwardly directed attention. We use our assay to test for consciousness in black widow spiders. Black widows become distracted by directing their attention internally when their web does not match their expectations. Black widows also noticeably switch attention from internally to externally directed. Variants of this assay are broadly applicable to test for basic consciousness in animals.

Introduction

At its most basic, consciousness is the phenomenon by which "there is something it is like to be" a particular entity with a private perspective (Nagel 1974; Barron and Klein 2016). That is to say, basic consciousness is defined by the presence of subjective experience, which arises when brains construct, and direct attention towards, internal representations of an individual's environment and the individual's position within it (Webb and Graziano 2015; Barron and Klein 2016; Feinberg and Mallatt 2016).

Many researchers now agree that consciousness is not restricted to humans, but there is debate about how widespread it may be among other species. Some authors suggest that consciousness may be limited to mammals and birds, arguing that the particular type of top-down feedback that occurs between the mammalian cortex and thalamus, or between the avian pallium and thalamus (termed “reentrant signaling”), is necessary for the formation of consciousness (Butler et al. 2005; Edelman et al. 2005; Butler et al. 2008; Cabanac et al. 2009). Other authors suggest, however, that further vertebrate groups and perhaps even some invertebrates have sufficiently complex hierarchical processing and reciprocal signaling to also support consciousness even if they lack a cortex or pallium (Strausfeld 2011; Barron and Klein 2016; Feinberg and Mallatt 2016; Haberkern and Jayaraman 2016; Chittka and Wilson 2016; Gazzaniga 2018). For instance, comparative neuroanatomical studies indicate that insects and spiders, if not all arthropods, have evolved brain structures that, in forming representations of the animal within its environment, function similarly to the structures that in vertebrate brains create subjective experience (Barron and Klein 2016; Feinberg and Mallatt 2016; Gazzaniga 2018). A further suggestion that the brains of arthropods may produce the internal

representations that are necessary for consciousness comes from simulations of human cortical neurons, which demonstrate that relatively few neurons (1000s to 10 000s) suffice to create representations of objects and make predictions about those objects (Hawkins and Ahmad 2016; Hawkins et al. 2017). The number of neurons in insect and spider brains ranges from approx. 5000 to nearly one million except for very small species (Babu 1975; Miklos 1998; Eberhard & Wcislo 2011, Menzel 2012). Experimental evidence also shows at least some invertebrates create internal representations—bumblebees recognize objects across multiple sensory modalities and *Portia* jumping spiders store internal representations of the number of prey items in a scene, for example (Cross and Jackson 2017; Solvi et al. 2020).

Thus, at least some insects and spiders may have sufficient numbers of neurons to form the representations required for basic consciousness. The creation of such representations is necessary, but not sufficient, to produce conscious experience. It could be possible for animals to store representations in memory and use them to guide behaviour without directing attention internally to the representation. This would generate no inner subjective experience. It is the direction of attention inward to the representation that is both necessary and sufficient to create subjective experience (Hawkins and Blakeslee 2004; Webb and Graziano 2015; Barron and Klein 2016; Feinberg and Mallatt 2016). Note that the content of the representation does not matter for conscious experience—consciousness can arise from attention directed toward even relatively simple representations.

Investigations of animal consciousness have largely focused on neural correlates of consciousness (neuroanatomy and neurofunction that is correlated with the contents of a particular conscious experience), or on identifying brain anatomy and function in animals that

are thought to be analogous to the neural structures and processes necessary for the production of consciousness in mammals (Edelman et al. 2005; Edelman and Seth 2009; Barron and Klein 2016; Feinberg and Mallatt 2016; Nieder et al. 2020; Payne et al. 2021). However, we do not yet understand whether a given brain activity produces a conscious experience, is a prerequisite for consciousness, or a byproduct of it (de Graaf et al. 2012; Krakauer et al. 2017). Further, the search for neural correlates of consciousness cannot be applied to many animals, as it requires the ability to report or indicate subjective states in some way—nor would we expect animals that possess only basic to consciousness to have the capacity for this sort of self-reporting (Edelman and Seth 2009; Koch et al. 2016). Consequently, progress will require assays based on observable behaviours that could only be the result of conscious experience in animals (cf. Krakauer 2017). Such behavioural assays would be powerful tools for the exploration of the variety of animals and brain architectures that can sustain consciousness.

We suggest that the dependence of conscious experience on attention to internal representations (Hawkins and Blakeslee 2004; Webb and Graziano 2015; Barron and Klein 2016; Feinberg and Mallatt 2016) provides the basis for a powerful behavioural test for basic consciousness, or subjective experience. This is because a key feature of conscious experience is that what is perceived is a brain-generated representation, not the direct sensory information—in fact, sensory inputs are heavily influenced by brain-generated, top-down feedbacks (Hawkins and Blakeslee 2004; Edelman et al. 2005; Webb and Graziano 2015; Hawkins 2021). Further, such internal representations are not up-to-date, but instead "fill in" many if not most details from memory and processing heuristics (Hawkins and Blakeslee 2004). If attention is directed inward, a conscious being perceives only a brain generated

representation, and can fail to notice otherwise obvious sensory cues. We have all experienced such times of distraction. Recall, for instance, a time you were lost in thought—perhaps mulling over the best way to phrase an especially difficult sentence—and you failed to notice a colleague or family member call your name. You were distracted by attending to your internal representation of a difficult sentence—to be distracted by an object in the mind, one must be attending to that object and experiencing the attention subjectively (Webb and Graziano 2015). Neural processing without subjective experience could involve becoming distracted by external stimuli. But only subjective experience could yield being distracted by internally directed attention.

The rationale that internally directed attention to mental representations should distract conscious animals from the current state of their environment provides the basis for a powerful behavioural test: seeking evidence of distraction due to attention to out-of-date internal representations. Animals that are conscious at a basic level should exhibit behaviour that reveals such distraction, followed by an eventual updating of the representation. This updating should in turn be evidenced by a concordant switch in attention to a sensory cue in the external environment, as when the representation of the door in the above example is updated by directing attention to the new location of the keyhole. The above requires a qualifier ("should at least sometimes be distracted by subjective attention"). This qualifier follows from human experience. We do not all have out of date models all the time, but most have out of date models some of the time. Consequently, the main prediction of the basic consciousness hypothesis is qualitative: at least some individuals in a given species or population should be distracted by attention to an object in their mind.

The assay we propose, and its underlying logic, are broadly applicable to animals with a broad range of natural histories and behaviours. Here we use it to test the hypothesis that black widow spiders have basic consciousness.

In previous work, we have shown that black widow spiders make navigational decisions using internal representations of their location on the web relative to their home (formed through path integration) (Sergi et al. 2021). This is a widespread form of internal representation of the individual in relation to its environment in animals in general and arthropods in particular (Webb 2019; Eberhard 2020; Grob et al. 2021). Here we ask whether black widow spiders subjectively experience attention to those internal representations.

In the application of our assay, we take full advantage of the biology and behaviour of black widow spiders. First, the variability of spider web architecture allows us to create mismatches between a spider's internal representation of its web and the web that it currently occupies (Methods). Second, we use the non-directional searching behaviour that is common among web spiders when they encounter a mismatch between their memory of the web and the actual web (e.g., when the web has been rotated in their absence, or when they lose prey they have captured; Movie S1) (LeGuelte 1969; Rodriguez et al. 2015; Sergi et al. 2021). This searching behaviour allows us to assess whether and when a spider has noticed a mismatch between memory and actual web. Third, black widow prey detection behaviour allows us to detect when a spider switches attention to a current prey item on its web. When a prey is snared by their web, spiders turn in the direction of the prey (sometimes with startling precision and speed), and they visibly use their legs to sense prey-generated vibrations on the web (Nakata 2010; Briceño et al. 2011; Foelix 2011; Nakata 2013). Black widows adopt a

distinctive “listening posture” with the first pair of legs extended wide when they direct their attention towards web vibrations (Movie S1). This behaviour would provide a clear visual indication of when a spider has switched from directing its attention internally to its (potentially out of date) representation to updating from current stimuli, should the spiders possess basic consciousness.

The hypothesis that black widow spiders have the subjective experience of attending to their internal representation of their web makes the following prediction: The spiders should be distracted from attending to a pressing proximate cue from the web (prey) by a mismatch between their internal representation and the current external environment; i.e., upon noting such a mismatch the spiders should search non-directionally rather than respond to the prey; and eventually, noticeably switch their attention from this mismatch to the pressing cue after some interval of distraction. The prey cues provided are salient to a hungry spider, and to ignore them would require a spider to direct its attention elsewhere. To be distracted by a perceived mismatch between a representation of the web and the actual occupied web requires that the spider subjectively experience that attention—that it be distracted by attending to an object in the mind.

By contrast, if the spiders do not have basic consciousness, they should attend immediately to the pressing prey cue and not first search about the web.

Methods

We collected sub-adult and adult female black widows from urban areas of Medford, Oregon in June 2017 and June 2018. Prior to beginning our experiments, we housed the spiders in plastic deli cups and provided each spider with two 1.5 cm-long crickets. During periods of normal

husbandry, we provided each spider with one 1.5 cm-long cricket every two weeks. However, when spiders were going to be used in an experiment, we fed these spiders 10 days and three days before experiments began to standardize satiety across spiders. We preserved voucher specimens in 95% ethanol. We tested each spider only once.

Are black widow spiders distracted by attention to their internal representation of the web?

We created mismatches between a spider's internal representation of its web and its current web by moving each spider from one web she had constructed to another web she had constructed. We also attempted to create mismatches of different magnitude: the two webs varied in how much their layout differed, as detailed below. We attempted to create different degrees of mismatch, because we were interested in whether spiders would be more likely to become distracted when the layout of their two webs was more different. However, this manipulation would not necessarily change the degree of perceived mismatch, if small-scale differences in the web between where a spider was removed and replaced are what cause the perceived mismatch. Our hypothesis that black widows have basic consciousness thus depends on whether spiders become distracted after being removed from the web, and not on whether they are more likely to be distracted when experiencing a greater degree of mismatch.

We constructed cardboard frames that were 28 cm long x 14 cm wide x 10 cm high and had a 5 x 5 x 5 cm prism retreat on the top center of one short side. Half of the frames had an open interior, and the other half had a 10 x 10 cm wall that partially divided the frame halfway between the retreat entrance and the far end of the frame (Fig 3.11a). These frames were open on top and bottom to allow us unobstructed viewing of the spiders on their web (Fig 3.1a, and

Movie S1, S2). We cut holes in the frame behind the spiders' retreat to allow us to remove the spiders from the frame without altering the web. We used those holes to move spiders from one web to another, by coaxing them to the end of the retreat and then scooping them from the web using a plastic specimen vial.

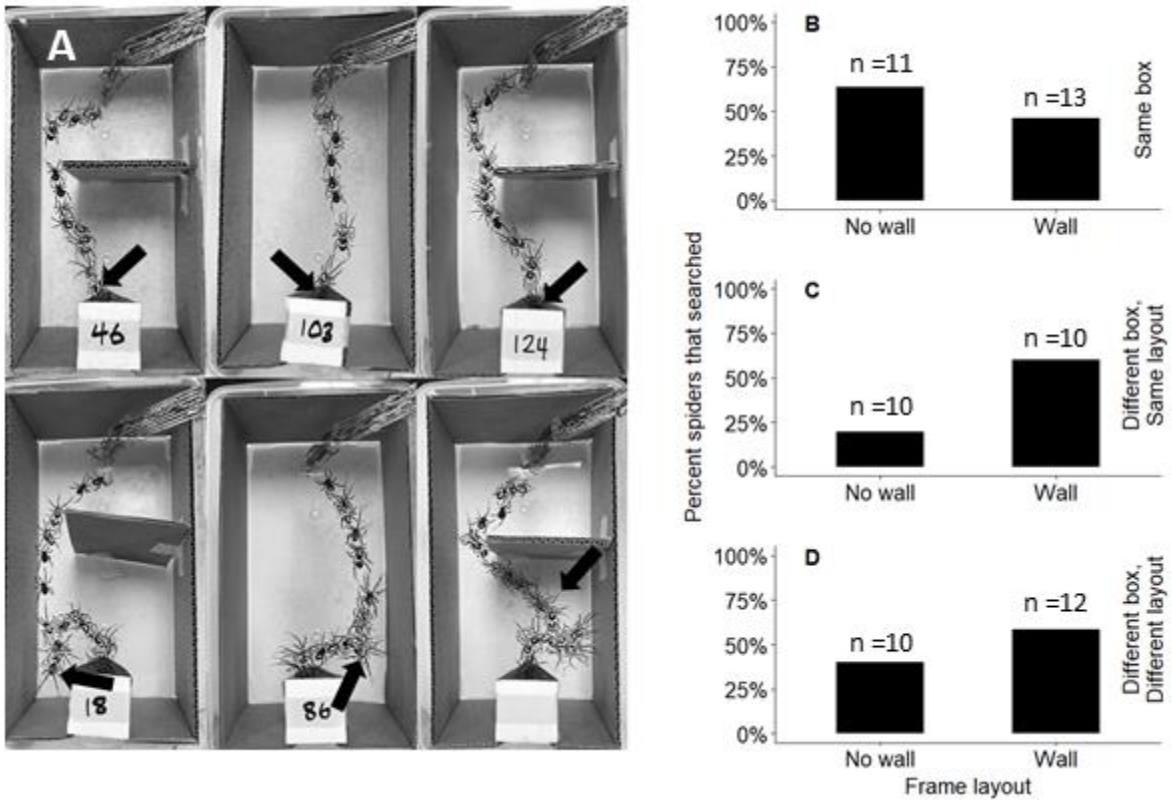

Fig. 3.1. Behaviour of spiders offered a cricket in webs that matched/did not match their mental model of their web. (A) Examples of the searching/prey-approach behaviour of the spiders. Top row: some spiders immediately attended to and moved to attack the cricket. Bottom row: other spiders first searched before switching their attention to the cricket. Each image shows stacked images of spiders navigating in experiment three. Arrows indicate the individual image in the stack in which the spiders adopted the listening posture, after which all spiders moved toward the cricket without further searching. **(B-D) Percentage of spiders that searched before approaching the cricket across levels of mismatch between their mental model of the web and the actual web.** The levels of mismatch were as follows: (B) Spiders tested with no mismatch that were removed and replaced in the same frame, with or without a wall present. (C) Spiders tested with relatively low mismatch that were removed and replaced in a different box with an identical layout. (D) Spiders tested with high mismatch that were removed and replaced in a different box with a different layout.

The frames we used resulted in spiders constructing webs that spanned the entire interior of the frame. The spiders also constructed webs that had a flat plane that made up the uppermost portion of the web, and on which they navigated. The only differences between webs constructed by the same spider were differences due to different frame layouts (see below) or due to fine-scale differences in web architecture. Thus, we controlled for external factors (such as up-down cues that would be present on webs that are not flat)

We allowed each spider to construct one web on two frames over a span of two weeks (1 week/frame), and we assigned them at random to one of four web change combinations: a frame with no wall on both weeks; a frame with a wall on both weeks; a frame with no wall on week one and a frame with a wall on week two; or a frame with a wall on week one and a frame with no wall on week two.

To complete the implementation of the treatments and initiate a trial, we moved each spider from her second frame to her first frame, through the back of the web's retreats. Note that all spiders were moved only between webs that they themselves built. We also created a control group in which we removed spiders from their retreat as above and replaced them right away in the same retreat (and offered a cricket, as above), so that they were tested in the same web they had just been removed from.

Thus, this experiment included three levels of difference between the webs spiders had occupied immediately prior, and the webs they were moved to for trials (Fig 3.1b-d): there were spiders moved between frames of different layouts (which we consider a high level of difference); spiders moved between frames of the same layout but with slightly different webs (a low level of difference, as the fine-scale structure of black widow webs varies between webs

constructed by the same individual (Enders 1975; Benjamin and Zschokke 2003)); and spiders removed and placed back on the same exact web but that nevertheless experienced being removed and replaced. The high level of difference between frames with no wall and frames with a wall was due to the shape of the web spiders constructed in each type of frames. In frames with no wall, spiders built webs that spanned the entire frame resulting in a rectangular web when viewed from above. In frames with a wall, spiders built webs that spanned the open area of the frame resulting in a web that was roughly "C" shaped when viewed from above (Fig. 1A). Black widows move about and explore their environment as they construct webs (Benjamin and Zschokke 2003), so their internal representations of the overall structure of the webs might differ between webs built in frames with and without a wall. Web-building spiders can also sense both distance and direction of vibrations transmitted across their webs, so the sensory environments of webs constructed in the different frames also differed (Mortimer et al. 2019).

Immediately after placing spiders in the trial web, we offered them a cricket by vibrating a cricket on the top of the web at the far end of the cardboard frame. We offered crickets to spiders in the furthest location of the web from spiders. We allowed spiders two minutes to leave the retreat. For spiders that left the retreat within two minutes, we continued to vibrate the cricket in each trial until the spider had approached the cricket and begun to flick silk at the cricket (n = 66 out of 87 trials), or until the spider ceased searching and remained motionless for two minutes. We excluded spiders that exited the retreat and either escaped from the box or ceased all behaviour without approaching the cricket (21 out of 87 trials).

As an extra, unmanipulated control we observed feeding trials of spiders in boxes identical to those described above, matching hunger levels to experimental groups (n = 14). We filmed all trials. We conducted all video analyses blind to the treatments. We watched the videos and noted whether spiders went directly from the retreat to the cricket (Movie S2), or searched before navigating to the cricket (Movie S1). We also noted whether spiders that first searched then adopted the listening posture as they switched from searching to approaching the cricket (Movie S1). Among spiders that searched, we measured the time spent searching, the distance moved while searching, and the number of times spiders changed direction while searching.

Is searching by black widow spiders explained by disturbance?

To test that the disturbance of our manipulations alone does not cause spiders to become distracted from attending to crickets, we allowed spiders two weeks to construct webs in cardboard frames identical to the no-wall frames used in our first experiment. We randomly assigned spiders to one of two disturbance treatments. In the first, lower disturbance treatment, we began by puffing spiders, wherever they were located on the web, with air from an aspirator. We administered puffs continuously at approximately one puff per second until the spider adopted the crouch posture for three consecutive puffs. We then offered spiders a cricket on the most distant area of the web from where the spider crouched. We continued to vibrate the cricket on the web until the spider approached and flicked silk at the cricket or until the spider had remained inactive for two consecutive minutes. In the second, higher disturbance treatment, we first offered spiders. We stole the crickets from spiders as soon as the spiders began to flick silk on the cricket. After we stole the crickets from spiders, we puffed

spiders with air as in the lower disturbance treatment. Once spiders adopted the crouch posture for three consecutive puffs, we then offered them another cricket.

We filmed each trial and used the videos to observe spider behaviour. We recorded whether each spider approached the cricket after being puffed. For spiders that did approach the cricket, we also recorded the length of time it took from the last puff until the spider began to flick silk on the cricket and whether spiders searched after leaving the crouch posture but before attending to the cricket. We assessed whether any spiders searched before attending to the cricket.

Is searching by black widows explained by hunger?

To test whether hunger influenced whether spiders attended to the cricket or to the mismatch between mental model and web, we estimated nutritional state by comparing the relative abdomen sizes of spiders. Spider abdomens are flexible and expand as spiders consume more prey, and abdomen size is correlated with nutritional state (Gunnarsson 1988). Spider sternums are inflexible parts of the exoskeleton located on the ventral side of the cephalothorax. We therefore compared the abdomen width/sternum width ratio to estimate relative hunger for each spider that approached the cricket. We measured sternum and abdomen width from the images that we used to create our stacked images in ImageJ. For this analysis, we included only spiders for which we could find images that were clear enough to distinguish the edges of the sternum and abdomen and in which the spider was oriented so that its sternum was horizontal (26 spiders that searched and 25 that did not search).

To test whether hunger differed between spiders that attended to the cricket immediately and spiders that were first distracted, we used a two-sample t-test to test whether

the average abdomen/sternum ratio differed between spiders that searched and those that attended immediately to the cricket.

Statistical Analysis

Fine scale differences in web structure could be sufficient for black widows to become distracted by the mismatch, in which case we would see some spiders in each treatment become distracted. Alternatively, large scale differences in web structure might be required for black widows to become distracted by the mismatch, in which case only spiders moved between frames with different layouts would become distracted by the mismatch. Thus, our assay does not require that black widows become distracted more often, or remain distracted for longer, when given a greater level of mismatch. Rather, any spider that directs its attention toward an internal model would ignore the pressing cricket cue, regardless of the level of mismatch between mental model and current web. Consequently, the main prediction of the basic consciousness hypothesis is qualitative: at least some spiders should be distracted from attending to the prey by attention to their internal representation.

We assessed the normality of each response variable in our statistical models below using Shapiro-Wilks tests, and assessed heterogeneity of variance using Levene's tests. We log transformed response variables when appropriate, and when that did not achieve homogeneity of variance we used Welch ANOVAs that allow for heterogeneity of variance.

We were also interested in whether we would be able to manipulate which spiders would be distracted by attention to their internal representation. We used logistic regression to test whether spiders given different levels of mismatch differed in their likelihood of searching before attending to the cricket cue. In this model, likelihood of searching was the dependent

variable, and level of frame layout change (including the no-change controls in which spiders were replaced in the same web), presence of wall, and the interaction between them were the predictors. We also used logistic regression with a Poisson distribution to test whether spiders that first searched differed between levels of mismatch in the number of times spiders changed direction while travelling. In this model, number of direction changes was the dependent variable and level of frame layout change, presence of wall, and the interaction between them were the predictors. We then used a standard least squares model to test whether spiders that first searched differed between levels of mismatch in the amount of time they spent searching before switching their attention to the cricket cue. In this model, log time spent searching was the dependent variable, and level of frame layout change, presence of wall, and the interaction between them were the predictors. We also tested whether spiders that first searched differed between levels of mismatch in the log distance moved while travelling. In this models, distance moved was the dependent variables, and level of frame layout change, presence of wall, and the interaction between them were the predictors. We also noted whether spiders noticeably switched their attention to the cricket cue by adopting the listening posture.

To test whether hunger influenced a spider's likelihood of searching, we used Welch ANOVA to test for a difference in mean abdomen width / sternum width ratio between spiders that did and did not search.

To test whether disturbance could explain spiders not attending to crickets, we used logistic regression to test whether more and less disturbed spiders differed in their likelihood of attacking the cricket after being puffed with air. We also used linear regression to test whether

more or less disturbed spiders differed in the amount of time it took for them to approach the cricket after being puffed.

We conducted all statistical analyses using JMP v. 15.2.1. (SAS Institute Inc., Cary, NC) and used the ggplot2 package (Wickham 2016) in R (R Core Team 2020) to create figures.

Results

We conducted an experiment in which we had spiders construct webs in two separate web frames over two consecutive weeks (Methods). We then moved spiders from their second web and placed them back on their first, and offered them a cricket by vibrating it in the web. We recorded the spiders' behaviour with a video camera, and in analysis noted whether they searched about the web and eventually switched to directing their attention toward the cricket. To further ask whether the magnitude of the difference between mental representation and actual web influences the likelihood of distraction, we attempted to create treatments presenting different levels of mismatch. In "low mismatch" treatments spiders built webs in two identical frames and in "high mismatch" treatments spiders built webs in two frames that differed in their internal structure (see Movies S1, S2; Supplemental Material). We also created a control in which spiders were removed from and replaced on the same web.

Of 66 spiders tested with some level of mismatch in the layout of the web, 32 searched for some interval (7-161 seconds) before approaching the cricket, and 34 approached the cricket directly (Fig 3.1; Movie S1; Movie S2). Thus, about half of the spiders were distracted from attending to the pressing cue of a cricket on their web, for up to almost 3 min.

Searching spiders moved around the web, waving their first pair of legs in circular movements, and using those legs to pull on the web’s threads (Movie S1). The movement of

spiders during searching meant that the spiders turned away from the proffered cricket, changing the direction in which they were facing at least once in all cases (mean = 3 direction changes, range = 1 – 11 direction changes).

All 32 spiders that searched eventually switched their attention to the cricket. Switches in attention were readily apparent, as spiders adopted the listening posture when they began to attend to the cricket cue, and this listening posture was distinct from any movement spiders made during bouts of searching (Movie S1). Spiders then promptly reached the cricket without any additional searching, and started flicking silk at it: spiders reached the cricket within 1-21 seconds of adopting the listening posture, with 9 of the 32 spiders turning and adopting the listening posture once more before approaching the cricket.

We were unable to manipulate whether spiders would be initially distracted from the prey. Across all levels of mismatch (across panels b-d, Fig 3.1) we found no difference in the likelihood of searching (layout change: $\chi^2_1 = 1.14$, p=0.56; presence of wall in frame: $\chi^2_1 = 0.74$, p=0.39; layout change x presence of wall interaction: $\chi^2_1 = 3.98$, p=0.14) (Fig 3.1b-d). Among spiders that searched, we also did not detect a difference in search times across all levels of mismatch (treatments corresponding to Fig. 1B-D; layout change: $F_{2,26} = 0.34$, p=0.72; presence of wall: $F_{1,26} = 3.89$, p=0.06; layout change x presence of wall interaction: $F_{2,26} = 2.80$, p=0.08). We also found no difference in the distance that spiders moved while searching (layout change: $F_{2,26} = 0.98$, p=0.39; presence of wall: $F_{1,26} = 0.70$, p=0.41; layout change x presence of wall interaction: $F_{2,26} = 0.55$, p=0.58). Neither was there a detectable difference in the number of times spiders changed direction while searching (layout change: $\chi^2_1 = 1.84$, p=0.40; presence of wall: $\chi^1_1 = 1.06$, p=0.30; layout change x presence of wall interaction: $\chi^2_1 = 2.26$, p=0.32).

Not only were the above effects not significant, they were also small and unlikely to be biologically meaningful. For example, the mean difference in the likelihood of searching between spiders with webs that had a wall or not was only 12% (Fig. 3.1b-d). And the mean difference in their search times was of 6 sec (20.3 vs 14.2 sec, respectively) (effect size at the bottom of the "medium" category: r = 0.36).

Variation in the salience of the proximate cue (prey) to different spiders could influence the likelihood of distraction by attention to internal representation or speed of updating the representation. We therefore also tested the hypothesis that hunger explains whether spiders were initially distracted from attending to the prey. This hypothesis predicts that less satiated spiders will be less likely to be initially distracted before eventually switching to attacking the prey. However, relative abdomen size did not differ between spiders that did (n = 26) and did not (n = 25) search (Welch ANOVA: $F_{1,24} = 0.71$; $p = 0.40$) in the subset of spiders for which we were able to obtain measurements from the videos of trials (n = 51). Thus, hunger did not play a role in whether black widow spiders were initially distracted by the mismatch between their mental models and web.

It might be argued that searching, rather than indicating a spider noting a mismatch between its representation of the web and the actual web, could be prompted by the disturbance of our manipulation. Prior work shows that similar manipulations, on their own, do not induce searching, and that when actually disturbed black widow spiders either attempt to flee the web or adopt a crouched position and freeze (Sergi et al. 2021). Further, searching is not an attempt by spiders to flee from a disturbance, as searching spiders change direction multiple times and often pause briefly during bouts of searching. Nevertheless, we sought to

confirm that black widow searching behaviour indicates a mismatch between their mental model and the current web, and is not prompted by disturbances associated with our trial procedures. The hypothesis that disturbance induces searching predicts that spiders that are given a disturbance will search about the web. To test this prediction, we conducted an additional experiment in which we either only disturbed spiders with forceful puffs of air (n = 9) or first offered and stole a cricket from them before disturbing them with puffed air (n = 8) (Methods). In this experiment, none of 17 the spiders searched, and 15 approached the cricket directly (7 of the 9 offered cricket directly and all 8 that were first puffed), beginning to move toward it 0-90 seconds (mean = 9 seconds) after the cricket first touched the spider's web, and reaching it 3-107 seconds (mean = 19 seconds) after the cricket first touched the web. The 2 spiders that did not approach the cricket adopted the crouched position for the duration of the experiment. The amount of time from being puffed to getting the cricket did not differ between spiders that were offered a cricket before being puffed and those that were only puffed ($F_{1,15}$ = 0.003, p=0.95). These results indicate black widows do not search in response to disturbance. We also observed feeding trials of spiders that were completely undisturbed in frames identical to those in our main experiment (n = 14). None of these spiders searched before attacking the cricket, which indicates black widows do not need to search to follow prey cues and that black widows do not search as part of normal prey capture behaviour.

Discussion

We propose and showcase an assay to test for basic consciousness (inner, subjective experience) in animals using distraction by internally directed attention. We argue that if an animal can become distracted from a real-time cue by attending to its internal representation

of its environment (by attending to an object in its mind), the animal must possess basic consciousness. Using this assay, we find evidence of basic consciousness in black widow spiders: distracted by attention to their inner representation of their web, 50% of the spiders ignored the pressing, real-time cue of a prey on their web, eventually and visibly coming to update their representation to include the prey.

Our findings with black widow spiders suggest that a broad range of other invertebrates may possess basic consciousness, in the sense of forming and subjectively attending to internal representations of their body in relation to their surroundings. We suggest that the framework of testing for distraction due to attention to an object in the mind could be broadly applied to test whether any animals that have been shown to form mental models subjectively attend to these models. Such tests are likely to be highly illuminating, especially if combined with neuroanatomical and neurofunctional studies to reveal how such subjective attention is generated (Krakauer et al. 2017).

We were unable to manipulate how likely spiders were to become distracted by varying the degree of mismatch between their internal representation and their current webs, but our observation that some spiders ignored a prey cue while searching about the web before switching their attention to the cricket shows that these spiders were initially distracted from attending to the prey cue. The distraction was caused by attention to an object in the mind—a perceived mismatch between mental representation and actual web—because we controlled for potential external factors that could lead to distraction (see Methods), and only distraction could lead an otherwise motivated spider to ignore an obvious prey cue.

Hunger also did not appear to play a role in whether spiders initially attended to their internal representations, as abdomen size did not predict a spider's likelihood of becoming distracted. Thus, although we have evidence of subjectively-experienced attention to internal representations of the spiders' webs, we do not understand variation in whether spiders are distracted from real-time cues by such attention. We note, however, that we would also not expect all humans to always be distracted from real-time cues either.

Some authors consider that consciousness evolves as a solution to the challenge of processing the vast amounts of input that arise when animals evolve vision (Feinberg and Mallatt 2013; Feinberg and Mallatt 2016). However, web-building spiders have poor vision, likely since the emergence of their lineage in the Cambrian (Clemente et al 2005; Strausfeld et al. 2006). Instead, we suggest that consciousness may evolve as a brain's way to solve perceptual and decision-making problems, regardless of primary sensory modality. Even among animals that do not sense primarily through vision, sensory environments are astonishingly complex, and could have provided the necessary selective forces for the evolution of consciousness.

Because the last common ancestor of arthropods and vertebrates had a simple neural system that likely did not form representations of its body within its environment (Feinberg and Mallatt 2016), the presence of consciousness in these groups likely represents evolutionary convergence, as may also be the case for mammals and birds (Edelman et al 2005; Damasio 2018). This, in turn, suggests that consciousness may arise in animals as a common solution to the challenges of adaptive perception and decision-making, making its phylogenetic distribution likely to be more widespread than currently anticipated (Wallace and Wallace 2009; Barron and

Klein 2016; Feinberg and Mallatt 2016). (But see Strausfeld and Hirth (2013) for evidence of common descent).

The neuroanatomical argument for consciousness in some invertebrates is powerful (Barron and Klein 2016; Feinberg and Mallatt 2016), and we provide a method for confirming consciousness in many animals suspected of being conscious. Our assay could provide a more direct test of consciousness in animals that will then allow a more focused search for the neuroanatomical and neurofunctional components that produce consciousness.

References

Babu KS (1975) Post embryonic development of the central nervous system of the spider Argiope aurantia (Lucas). J of Morpho 3:325-342

Barron AS, Klein C (2016) What insects can tell us about the origins of consciousness. Proc Nat. Acad Sci USA. 113:4900-4908

Benjamin SP, Zschokke S (2003) Webs of theridiid spiders: construction, structure and evolution. Biol J of the Linn Soc. 78:293-305

Briceño RD, Eberhard WG. (2011) The hub as a launching platform: rapid movements of the spider Leucauge mariana (Araneae: Tetragnathidae) as it turns to attack prey. J of Arach. 39:102-112

Butler AB, Manger PR, Lindalh BIB, Arhem P. (2005). Evolution of the neural basis of consciousness: a bird-mammal comparison. BioEssays. 27:923-936

Butler AB. (2008). Evolution of brains, cognition, and consciousness. Brain Res Bull. 75:442-449

Cabanac M, Cabanac AJ, Parent A (2009) The emergence of consciousness in phylogeny. Behav Brain Res. 198:267-272

Chittka L, Wilson C (2018) Inside the mind of a bee is a hive of sensory activity. Aeon.

Clemente CJ, McMaster KA, Fox L, Meldrum L, Main BY, Stewart T (2005) Visual acuity of the sheet-web building spider Badumna insignis (Aranea, Desidae). J Arach. 33:726-734

Cross F, Jackson R (2017) Representation of different exact numbers of prey by a spider-eating predator. Inter Foc. 7

Damasio A (2018) The strange order of things. New York: Pantheon Books.

de Graaf TA, Hsieh PJ, Sack AT (2012) The 'correlates' in neural correlates of consciousness. Neuro and Biobehav. Rev. 36:191-197

Eberhard WG (2020) Spider Webs: Behaviour, function, and evolution. Chicago, IL: University of Chicago Press

Edelman DB, Baars BJ, Seth AK (2005) Identifying hallmarks of consciousness in non-mammalian species. Consc and Cogn. 14:169-187

Edelman DE, Seth AK (2009) Animal consciousness: A synthetic approach. Trend in Neuro. 32:476-484.

Enders F (1975) The Influence of Hunting Manner on Prey Size, Particularly in Spiders with Long Attack Distances (Araneidae, Linyphiidae, and Salticidae). Am Nat 109:737-763

Feinberg TE, Mallatt J (2013) The evolutionary and genetic origins of consciousness in the Cambrian Period over 500 million years ago. Front in Psych 4

Feinberg TE, Mallatt J (2016) The Ancient Origins of Consciousness. Cambridge MA: The MIT Press

Foelix R (2011). Biology of spiders. Oxford UK: Oxford University Press

Gazzaniga MS (2018) The Consciousness Instinct: Unraveling the Mystery of How the Brain Makes the Mind. New York: Farrar, Straus and Giroux

Grob R, el Jundi B, Fleischmann PN (2021) Towards a common terminology for arthropod spatial orientation. Etho Eco and Evo. 33:338-358

Gunnarsson B (1988) Body size and survival: implications for an overwintering spider. Oikos. 52:274-282

Haberkern H, Jayaraman V (2016) Studying small brains to understand the building blocks of cognition. Curr Op in Neurobio. 37:59-65

Hawkins J (2021) A Thousand Brains. New York: Basic Books.

Hawkins J, Ahmad S (2016) Why neurons have thousands of synapses, a theory of sequence memory in neocortex. Front In Neuro Circ. 10

Hawkins J, Ahmad S, Cui Y (2019) A theory of how columns in the neocortex enable learning the structure of the world. J Neuro Circ. 11

Hawkins J, Blakeslee S (2004) On Intelligence. New York: Time Books. Henry Holt and Company, LLC

Krakauer JW, Ghazanfar AA, Gomez-Marin A, MacIver MA, Poeppel D (2017) Neuroscience needs behaviour: Correcting a reductionist bias. Neuron. 93:480-490.

Koch C, Massimini M, Boly M, Tononi G (2016) Neural correlates of consciousness: progress and problems. Nat Rev: Neurosci. 17:307-321

LeGuelte L (1969) Learning in Spiders. Am Zool. 9:145-152

Menzel R (2012) The honeybee as a model for understanding the basis of cognition. Nat Rev Neurosci. 13:758-768

Miklos GLG (1998) The evolution and modification of brains and sensory systems. Daedalus. 127:197-216

Mortimer B, Soler A, Wilkins L, Vollrath F (2019) Decoding the location information in the orb web vibrations of Araneus diadematus and Zygiella x-notata. J of the Royal Soc Int. 15

Nagel T (1974) What is it like to be a bat? The Phil Rev. 83:435-450

Nakata K (2010) Attention focusing in a sit-and-wait forager: a spider controls its prey-detection ability in different web sectors by adjusting thread tension. Proc of the Royal Soc of London B Bio Sci. 277:29–33

Nakata K (2013) Spatial learning affects thread tension control in orbweb spiders. Bio Let. 9:1–4

Nieder A, Wagner L, Rinnert P (2020) A neural correlate of sensory consciousness in a corvid bird. Science 369:1626-1629

Payne HL, Lynch GF, Aronov D (2021) Neural representations of space in the hippocampus of a food-caching bird. Science. 373:343-348

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rodriguez RL, Briceno RD, Briceno-Aguilar E, Hoebel G (2015) Nephila clavipes spiders (Araneae: Nephilidae) keep track of captured prey counts: testing for a sense of numerosity in an orb-weaver. Anim Cog. 18:307-314

Sergi, C.M., Antonopoulos, T., and Rodríguez, R.L. (2021). Black widows use path integration on their webs. Behav. Eco. and Socio. 75

Solvi C, Al-Khudhairy SG, Chittka L (2020) Bumble bees display cross-modal object recognition between visual and tactile senses. Science. 367:910-912

Strausfeld NJ (2011) Arthropod Brains: Evolution, Functional Elegance, and Historical Significance. Cambridge, MA: Harvard University Press

Strausfeld NJ, Strausfeld CM, Loesel R, Rowell D, Stowe S (2006) Arthropod phylogeny: onychophoran brain organization suggests an archaic relationship with the

chelicerate stem lineage. Proc of the Royal Soc B. 273:1857-1866.

Strausfeld NJ, Hirth F (2013) Deep Homology of Arthropod Central Complex and Vertebrate Basal Ganglia. Science. 340:157-161

Wallace RG, Wallace R (2009) Evolutionary radiation and the spectrum of consciousness. Cons and Cog. 18:160-167

Webb B (2019) The internal maps of insects. J of Exp Bio. 222

Webb TW, Graziano MSA (2015) The attention schema theory: a mechanistic account of subjective awareness. Front in Psych. 6

Wickham H (2016) Ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.

Chapter Four: Black widow spiders (*Latrodectus hesperus*) remember prey capture location and size, but only alter behavior for prey caught at particular sites.

Abstract

Animals form memories and use them to guide future behaviors. The information stored in memory is selected to include only details that results in adaptive decision making. Understanding the contents of animal memories can provide insight into an animal's ecology and evolution. In this paper, we use an assay of searching behavior to reveal the contents of black widow spiders memory. We provided prey in two major components of black widow webs, and then stole the prey to elicit searching behavior. We used search effort, in terms of likelihood of searching and the number of bouts of searching, to determine whether spiders form memories of their prey, and whether their memories include any specific features of their prey. Black widows were significantly more likely to search after experiencing prey theft, which demonstrates the spiders form memories of their prey. Black widows were also more likely to search for relatively larger prey, but this effect depended on the site of prey capture within the web (they searched only for prey snared at the web's main capture site). This indicates that black widows also form memories of the relative size of their prey and its capture location. Further, their natural history helps interpret when these details are stored or used, and when not. Our results underscore the importance of behavioral observations for understanding the contents of animal memories.

Introduction

Animals make memories about their environment and their movements whithin it, and use the information in these memories to guide subsequent decision making (Shettleworth 2010). Food caching animals, for example, possess extraordinary spatial memory that is adaptive in the context of locating stored food (Smith and Reichman 1984; Sherry and Duff 1996). The information that an animal stores in memory is a function of the sensory biology and ecology of the animal (Healy and Braithwaite 2000; Pravosudov and Clayton 2002; Dukas 2004; Shettleworth 2010; Pravosudov and Roth 2013). Further, memory formation and retention is energetically expensive, and thus the information stored in memory is curated adaptively (Mery and Kawecki 2005; Placais and Preat 2006; Shettleworth 2010; Tello-Ramos et al. 2019). Selection thus favors the formation of memories that lead to adaptive behavior when the memory is later recalled (Shettleworth 2010). The information stored in memory can also vary between closely related species (Kamil et al. 1994; Platt et al. 1996; Shettleworth 2010), which means that studying what information an animal stores in memory can give useful insights into the relationship between ecology and the evolution of cognitive abilities.

Web spiders offer interesting opportunities for studying the formation of memories. These spiders build webs with diverse structures in manifold environments, and capture an array of arthropod prey (Blackledge et al. 2009; Foelix 2011; Eberhard 2020). Web spiders form memories of their web and its contents and use these memories to guide decision making. Some remember the overall layout of their web and their position in it (LeGuelte 1969; Sergi et al. 2021). Some remember the location in the web where they captured prey, and alter their webs to make them better able to capture prey in the web location where past prey captures

have occurred (Nakata 2012). Many web-building spiders also remember the contents of their web, such as the presence of prey, the relative size of captured prey, and even the relative number of captured prey (Rodríguez and Gamboa 2000; Rodríguez and Gloudeman 2011; Rodríguez et al. 2013; Rodríguez et al. 2015; Kilmer and Rodríguez 2018, 2019).

Most of the evidence regarding spider memories of their web and its contents comes from species that build orb webs, which are relatively simple in the sense that they are two-dimensional structures. There are, however, many spiders that build more complex, three-dimensional webs (Eberhard 2020). Such webs may offer a greater number of features that may afford greater possible detail for memory. However, this may also make it a more challenging for a spider to adaptively represent the relevant aspects necessary to aid navigation and decision making.

In this study, we analyze the content of memories of captured prey in black widow spiders, *Latrodectus hesperus* (Theridiidae). Black widow spiders build three-dimensional cobwebs with distinct components (Fig. 4.1), and they capture diverse arthropod prey, including both terrestrial and flying insects that vary widely in size (Benjamin and Schokke 2003; Blackledge and Zevenbergen 2007; Salomon 2011). Black widows primarily capture terrestrial prey which become stuck to the sticky-ended gumfooted lines of their webs (Fig. 4.1) (Benjamin and Schokke 2003; Salomon 2011). However, they also capture flying prey on the sheet of their web and, although few in number, these prey are larger on average than the terrestrial prey, and might therefore be of relatively high value and importance (Salomon 2011). We therefore asked whether the memories black widow spiders form of their prey include information about prey size and the location at which the prey was captured.

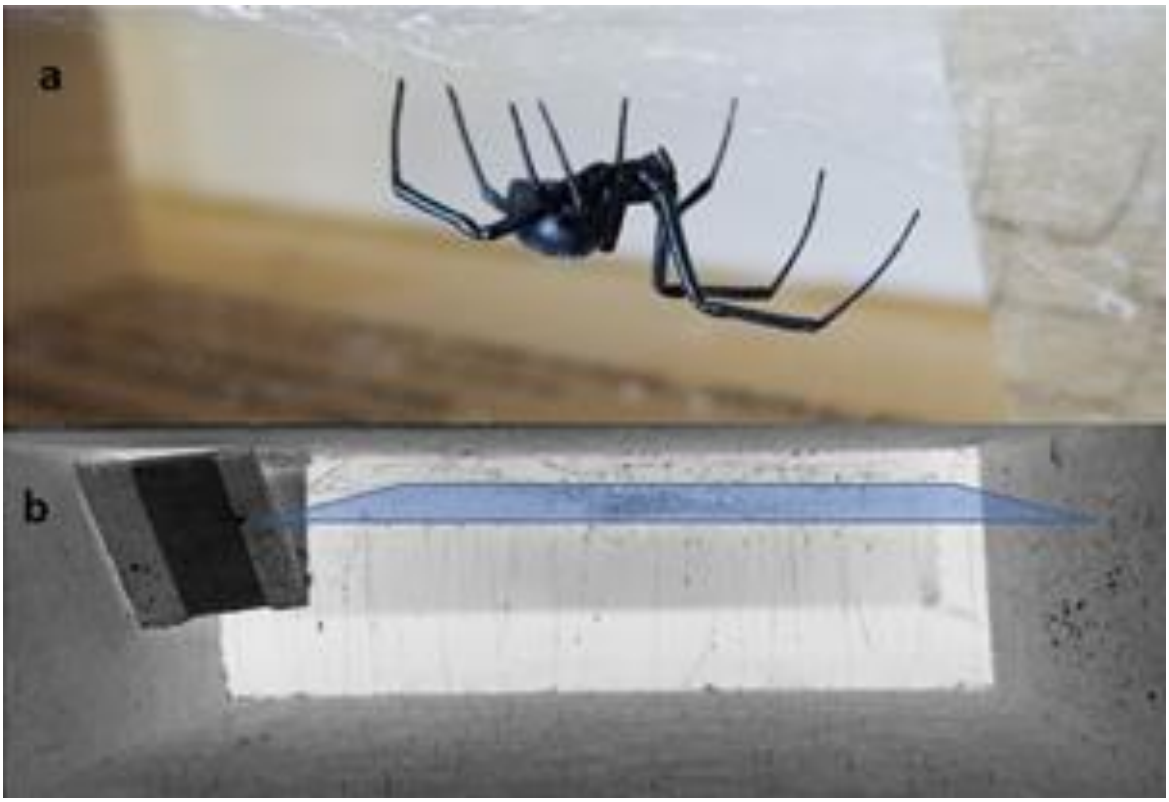

Fig. 4.1: Black widow spider and representative web with a sheet and vertical gum-footed lines. Black widow spiders construct space-filling cobwebs (Benjamin and Zschokke 2003). (**a**) A female black widow spider in a typical posture on the sheet of her web. (**b**) Negative image of a black widow web constructed in a cardboard frame. The spiders use the triangular prism we provide (left) as a retreat. The sheet extends from the retreat to the far side of the frame. The bottom of the sheet forms a two-dimensional plane (blue rectangle inset in **b**). Spiders do most of their navigation along this plane, and all of the navigation we describe in our experiments occurred on the plane formed by the web sheet. The gumfooted lines are the primary prey-capture structures of black widow webs. Reproduced with permission from Sergi et al. 2021

We used an assay of memory afforded by the natural history and behavior of web spiders (LeGuelte 1969; Rodríguez and Gamboa 2000; Rodríguez and Gloudeman 2011; Rodríguez et al. 2013; Rodríguez et al. 2015; Kilmer and Rodríguez 2018, 2019). Whenever a prey item is snared in a spider's web, the spider quickly approaches it, every once in a while briefly orientating by tugging on the web to correct its trajectory (Foelix 2011). However, a different kind of searching can also occur in the absence of a "triggering" stimulus. This searching is prolonged and non-directional (see below), with the spider walking around the web and pulling or plucking on web

threads to gain vibrational information (e.g., movie S1), and occurs whenever the layout of the web is changed in the spider's absence (so the spider seeks to re-orient itself) (LeGuelte 1969; Sergi et al. 2021), or when an prey item in the web is removed (so the spiders seeks to re-acquire it) (Rodríguez and Gamboa 2000; Opell 2001; Rodríguez and Gloudeman 2011; Rodríguez et al. 2013; Rodríguez et al. 2015; Kilmer and Rodríguez 2018, 2019). Consequently, this searching indicates the presence of a memory about the aspect of the web that has been changed. Further, variation in the likelihood of searching and in searching effort can be used to uncover the particular features represented in memory, and whether some features are more important than others (Rodríguez and Gamboa 2000; Rodríguez and Gloudeman 2011; Rodríguez et al. 2013; Rodríguez et al. 2015).

We tested two hypotheses about black widow memory. First, we tested the general hypothesis that black widows form some memory of the prey they capture in their webs. This hypothesis predicts that black widows will search for prey that they have captured and subsequently lost.

We then tested the hypothesis that black widows form memories of some specific features of their prey. This hypothesis predicts that black widows will exhibit different searching behavior when features of their captured prey differ. To test this second hypothesis, we tested two more specific hypotheses. First, we tested the hypothesis that black widows form memories of the size of their prey. This hypothesis predicts that black widows will be more likely to search and/or have higher search efforts for larger prey (assuming larger prey are preferrable). Second, we tested the hypothesis that black widows form memories of where in the web they captured prey (i.e., sheet versus gum footed lines; Fig. 1). This hypothesis predicts

that black widows will be more likely to search and/or focus their search efforts on the area of the web where the prey was captured and lost.

It might be argued that, rather than being indicative of memory, searching is caused by a disturbance on the web or to the disturbance caused when stealing prey. We therefore also tested whether black widow spiders search in response to disturbances similar to theft of prey and whether they engage in searching without any disturbance at all.

Methods

Spider husbandry

We worked with adult female black widow spiders collected from Medford, Oregon in June 2018 and May 2019. We shipped collected spiders to our lab at UWM and maintained them under our laboratory husbandry protocols until we used them in experimental trials. We kept spiders in 473 ml plastic food storage containers that contained a diagonal piece of bamboo garden stake. We fed all spiders one approximately 1.5cm-long cricket (*Acheta domesticus*) one week after being placed in their enclosures and then once every two weeks until spiders were used in an experimental trial. We began all trials three days after feeding spiders to standardize satiety across all spiders.

Experimental set-up

We used 40 x 33 x 17 cm plastic boxes for all trials. We applied strips of masking tape to the interior of the boxes to allow spiders to climb and adhere silk to the sides of the boxes. We applied tape horizontally approximately 2 cm below the top edge of the box, and along one long side and one short side of the boxes. We also applied vertical strips of tape that extended from the horizontal tape to the floor at approximately 10 cm intervals. We applied petroleum

jelly to the interior floor and walls of the region of the box without tape so that spiders could not adhere silk to one-half of the box. Spiders were thus able to construct webs that spanned half of the box and left the other half unobstructed by silk, which allowed us to complete the experimental manipulations without damaging spiders' webs. Spiders built retreats in the corner of the box where the long and short sides meet, against the horizontal tape along the top of the box. The overall structure of the spiders' webs was a triangular sheet that extended along a plain formed by the horizontal tape, with gumfooted lines that descended from the sheet and attached to the bottom of the box, and a retreat in the corner of the web furthest from the edge of the sheet.

Experimental and control procedures

To begin each trial, we placed a spider in an experimental enclosure. We then allowed spiders one week to construct a web. After the web building period, we examined each enclosure to determine whether the spider had built a web with a horizontal sheet that spanned the entire area of the box that had tape around the top. We excluded spiders that did not build webs or built webs in only part of the box. We also checked whether spiders had constructed any gumfooted lines (Fig 4.1).

We randomly assigned spiders to one of our controls (full undisturbed feeding, make hole in web, or sham theft of prey) or treatment (provide cricket in gumfooted line, then steal it; or cricket in sheet, then steal it;) conditions (Table 1). If a spider was assigned to receive a cricket on a gumfooted line, but had not made a gumfooted line, we switched that spider's treatment with the next spider in our random assignment that had made at least one gumfooted line. In each treatment in which we offered a cricket, we used a forceps to hold the

cricket against the web and vibrated it to simulate trapped prey (Table 4.1). In each treatment in which we stole the cricket, we allowed spiders to wrap crickets in silk and used a forceps to steal crickets from spiders when they began to move toward their retreats while carrying crickets.

To begin each trial, we placed the experimental enclosures in our filming location, with one video camera placed directly above the enclosure looking down (as in Supplementary video 1), and one placed near the corner of the box furthest from the spider's retreat looking diagonally down, so the view was as close as possible to looking through the web from front to back (as in Supplementary video 1). We filmed all trials using both cameras so that we could observe spider behavior in three dimensions. We filmed all trials for 60 minutes after completing the experimental manipulation (offer cricket, steal cricket, or make hole) (Table 1), except for our "Full feed" treatment in which we filmed spiders until they had finished eating the cricket (Table 4.1).

Control or Treatment	Treatment name	Description	n
Control	Full feed	Offered cricket. Allowed spiders to consume entire cricket.	10
	Make hole	Cut hole in web sheet with scissors	9
	Sham theft	Offered cricket in sheet. Stole cricket and immediately gave back to spider.	10
Treatment	Sheet	Offered cricket in sheet. Stole cricket.	20
	Gumfooted line	Offered cricket in gumfooted line. Stole cricket.	19

Table 4.1: Control and treatment groups we used to analyze memory of captured prey in black widow spiders. We assigned spiders to one of three control groups or one of two treatment groups to assess whether searching behavior varied according to whether a spider experienced prey capture and subsequent prey theft, and whether searching behavior varied according to site of prey capture.

In all trials in which we gave a cricket to a spider, we weighed the cricket before offering it to the spider and again at the conclusion of the trial. After each trial was done, we also weighed each spider. We calculated the mass of each spider by subtracting the difference in cricket mass before and after each trial from the mass of the spider after the trial.

Video analysis

We used the videos from each trial to collect behavior data. We used the event logging software Alice2 (J. T. Kilmer, unpublished) to record the times at which spiders exhibited specific behaviors: time to start searching, the total time spent searching, the number of bouts of searching, the length of the between search bout intervals, the total time spent searching the floor of the enclosure, and the number of trips each spider made to the floor of the enclosure. We considered one bout of searching to end when spiders returned to their retreat or remained motionless on the web for three minutes or longer. We also measured handling time, which we defined as the amount of time from when spiders first began to flick silk at the offered cricket to when we stole the cricket.

Statistical analysis

Spiders that searched did so in bouts lasting several minutes (see below). We therefore analyzed variation in the following response variables: whether each spider searched, the number of search bouts each spider engaged in, whether each spider searched on the floor, and the number of times each spider searched on the floor. We chose to focus on the number of search bouts, rather than summed search times across bouts because these measures were correlated (number of search bouts and search times: $r = 0.33$, $p = 0.02$; number of trips to the

floor and time on the floor: $r = 0.79$, $p < 0.001$), and we considered that the numbers of bouts give a better indication of decisions by the spiders to extend search efforts.

To analyze variation in whether spiders searched, we used logistic regression with a term for treatment or control group and a term for site of prey capture nested within treatment.

We then analyzed the behavior of the spiders in the treatment groups (likelihood of searching, number of search bouts, number of search bouts on the floor), with logistic regression (binomial and Poisson distribution) with the following explanatory terms: location of prey capture (gumfooted line or sheet), handling time, the ratio of the mass of the cricket mass and mass of the spider (hereafter "relative prey size"), and cricket location x relative prey size interaction.

In all initial models, we also included the time to start searching (number of seconds from the end of an experimental manipulation to the beginning of the first search bout) as a covariate, but we removed this from our final models because it was never significant ($p \geq 0.06$ in all models). We also did not include the cricket location x handling time and relative prey size x handling time interactions in the final models, because our hypotheses did not make predictions about these interactions, they were never significant ($p \geq 0.1$ in all models), and including them reduced our power to detect effects of the variables for which we predicted effects.

Results

Spiders in the treatment groups were 4-5 times more likely to search than spiders in any of the control groups (Tables 4.2, 4.3, Fig. 4.2). No other explanatory variable had a significant effect

on the likelihood of searching (site of prey capture, relative prey size, handling time, site of prey capture x relative prey size interaction; Tables 4.2, 4.3).

		spiders in treatment groups	spiders in control groups
prevalence of searching		38 of 39 (97%)	8 of 30 (27%)
for spiders that searched	search time	10 min (0.5-22 min)	3.5 min (0.25-1)
	search bouts	2 (1-5)	1.2 (1-2)
	bout duration	6 min (0.25-19 min)	4 min (0.5-15 min)
prevalence of searching on floor		18 of 39 (46%)	3 of 30 (10%)
spiders that searched on the floor	Time on floor	3 min (0.1-12.5min)	0.33 min (0.25-0.5 min)
	Trips to floor	4.5 (1-18)	1.7 (1-2)
	Trip duration	0.5 min (0.05-9.5 min)	0.12 min (0.05-0.5 min)

Table 4.2: Description of search effort. Spiders in treatment groups (center column) were more likely to search (top rows) than spiders in the control groups (right column). Spiders exerted more search effort on the floor after experiencing theft of relatively larger prey (bottom rows).

For spiders in the treatment groups that searched, search effort was not significantly related to any of the explanatory variables (site of prey capture, relative prey size, handling time, cricket location x cricket/spider ratio interaction) (Tables 4.2, 4.3; Figure 4.3). However, spiders were more likely to search the floor for larger prey, and the site of prey capture × relative prey size interaction was marginally not significant; no other term was significant (Tables 4.2, 4.3; Figure 4.4). And spiders increased search effort on the floor for larger prey, but only if that prey was captured in a gumfooted line (Tables 4.2, 4.3; Figure 4.5).

Response	Predictor	Statistic	p-value
Likelihood of searching (all spiders)	Treatment or Control	$\chi^2_1 = 22.95$	<0.001
	Site of Prey Capture	$\chi^2_1 = 1.76$	0.41
Likelihood of searching (treatment groups only)	Site of prey capture	$\chi^2_1 = 0.28$	0.60
	Relative prey size	$\chi^2_1 = 1.35$	0.99
	Handling time	$\chi^2_1 = 0.02$	0.88
	Site of prey capture x relative prey size	$\chi^2_1 = 8.5e\text{-}9$	0.99
Bouts of searching	Site of prey capture	z = 0.088	0.93
	Relative prey size	z = 1.26	0.21
	Handling time	z = 0.56	0.58
	Site of prey capture x relative prey size	z = 0.48	0.63
Likelihood of searching on floor	Site of prey capture	z = 1.52	0.13
	Relative prey size	z = 2.01	**0.045**
	Handling time	z = 0.44	0.66
	Site of prey capture x relative prey size	z = 1.92	0.055
Number of trips to floor	Site of prey capture	z = 1.04	0.3
	Relative prey size	z = 3.71	**<0.001**
	Handling time	z = 1.26	0.21
	Site of prey capture x relative prey size	z = 2.09	**0.036**

Table 4.3: Variation in black widow spider likelihood of searching according to treatment or control condition, site of prey capture, relative prey size, handling time, and site of prey capture x relative prey size interaction; and variation in number of search bouts, likelihood of searching on the floor, and number of times to the floor, according to site of prey capture, relative prey size, handling time, and site of prey capture x relative prey size interaction. Significant terms shown in bold.

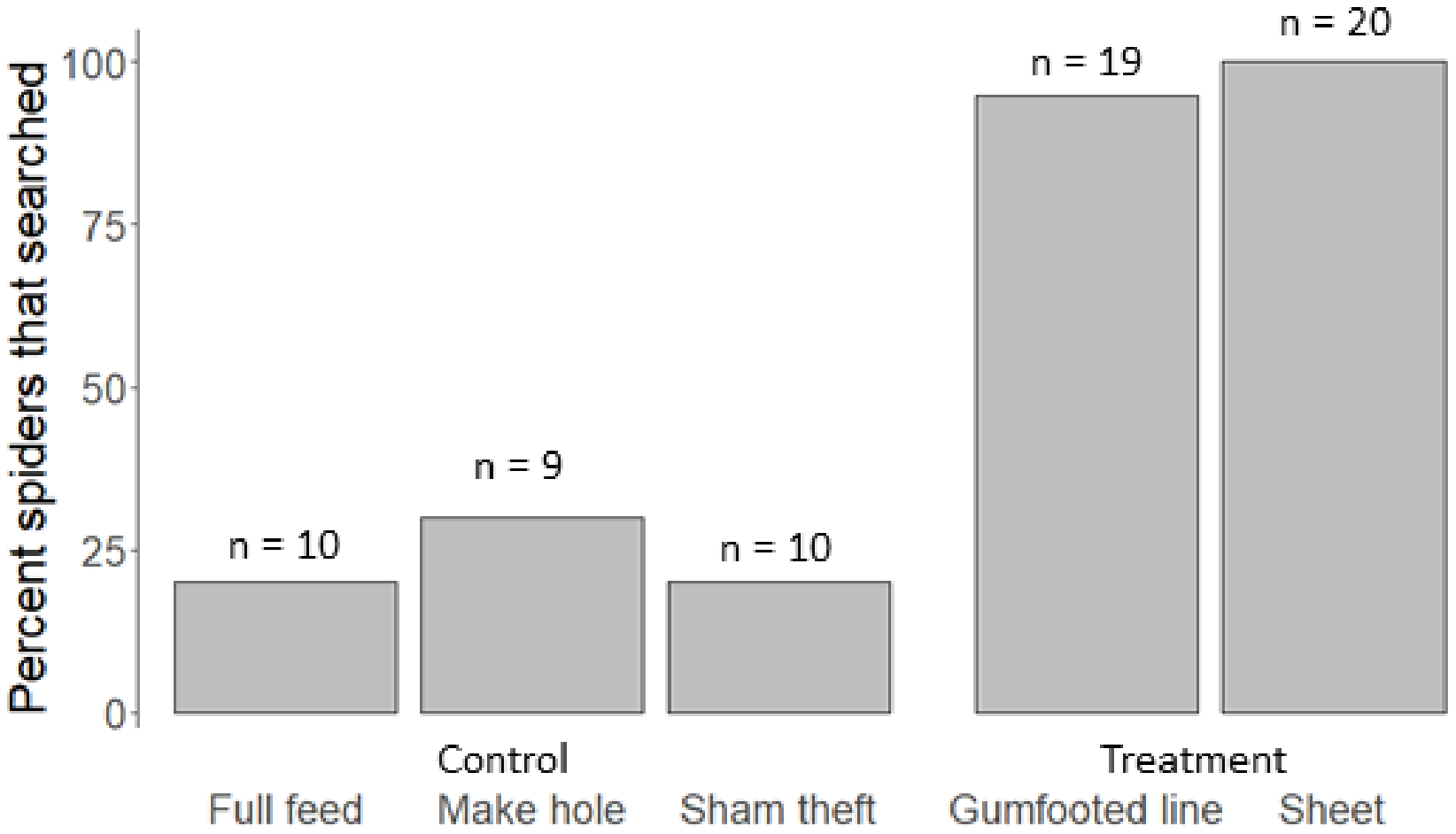

Fig. 4.2: Percent spiders that searched. Spiders were more likely to search about their webs after experiencing prey theft (right two bars) than after experiencing control conditions (left three bars)

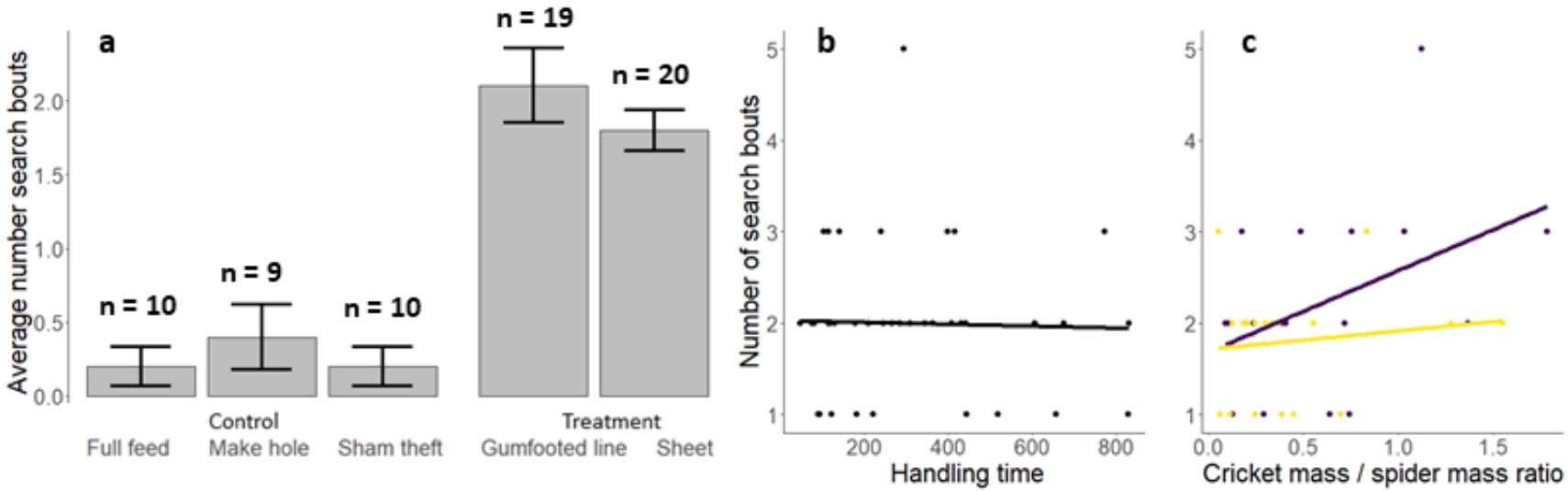

Fig. 4.3: Number of searching bouts. a) Average number (+/- standard error) of search bouts for spiders in control groups (left three bars) and treatment groups (right two bars). Sample sizes shown above bars. **b**) Number of search bouts by handling time for spiders in treatment groups. **c**) Number of search bouts by relative prey size for spider in treatment groups. Black points are spiders that captured crickets in gumfooted lines and yellow points are spiders that captured crickets on the web sheet. Lines show slope of relationship for spiders in the gumfooted line (black) and sheet (yellow treatments)

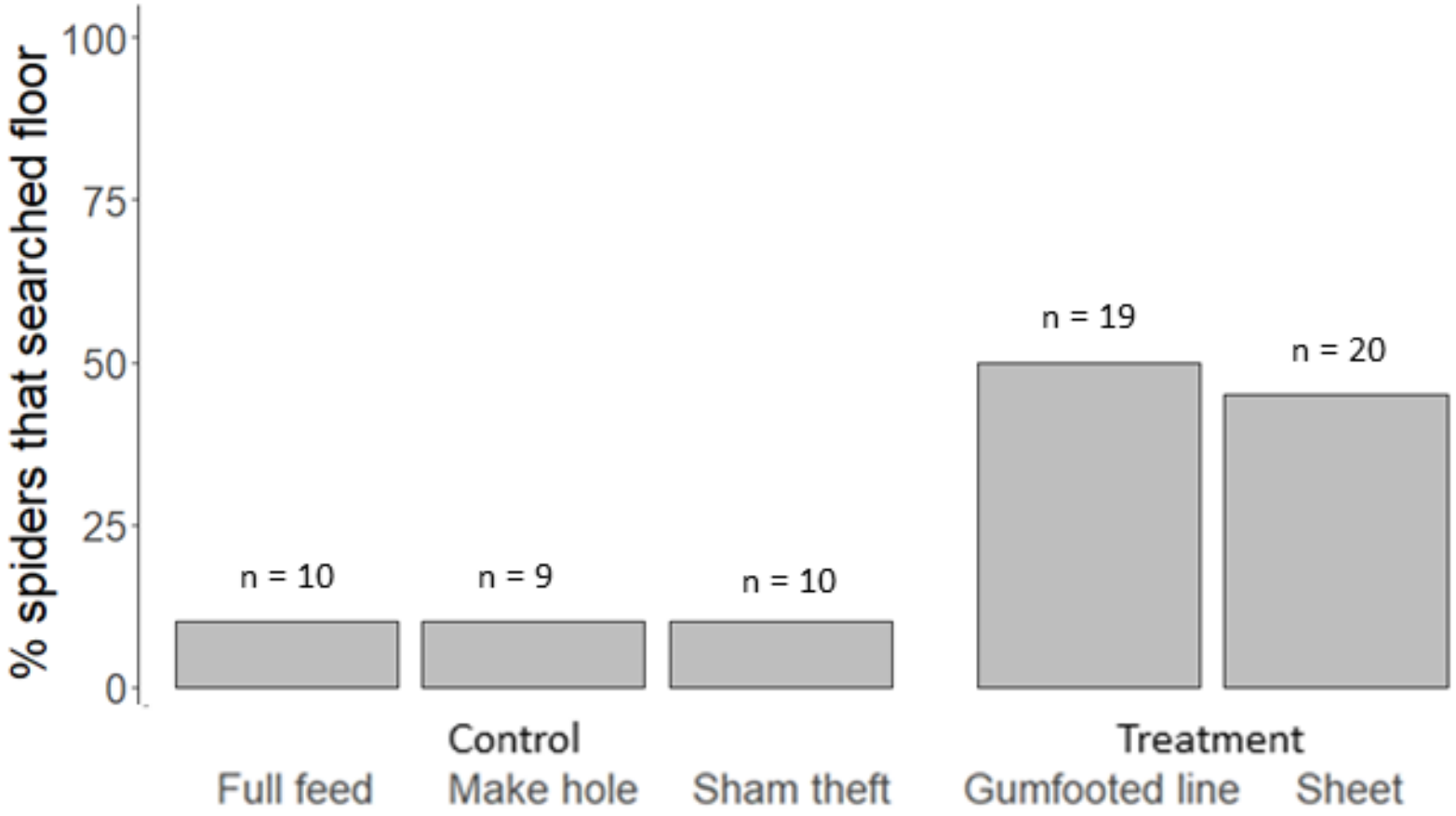

Fig. 4.4: Percent spiders that searched floor of enclosure. Spiders were more likely to search the floor of their enclosure after experiencing prey theft (right two bars) than after experiencing control conditions (left three bars)

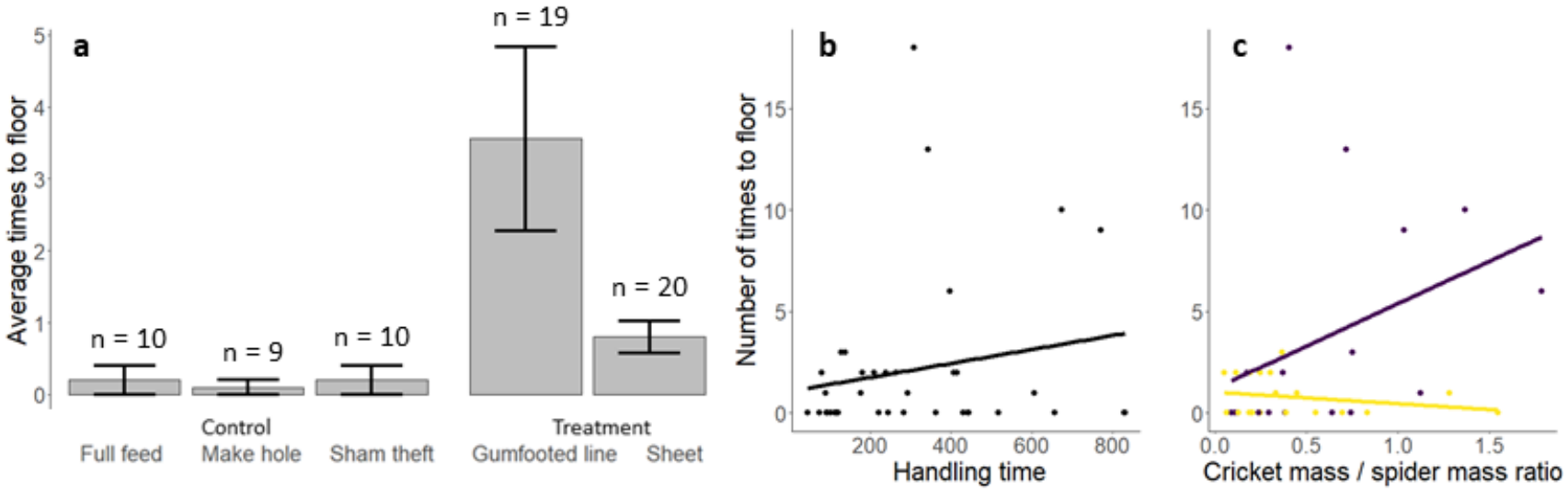

Fig. 4.5: Number of trips to floor of enclosure. a) Average (+/- standard error) number of trips to floor for spiders in control groups (left three bars) and treatment groups (right two bars). Spiders that captured crickets in gumfooted lines made more trips to the floor than spiders in all other groups. **b**) Number of trips to floor by handling time for spiders in treatment groups. **c**) Number of trips to floor by relative prey size for spiders in treatment groups. Black points are spiders that captured crickets in gumfooted lines and yellow points are spiders that captured crickets on the web sheet. Lines show slope of relationship for spiders in the gumfooted line (black) and sheet (yellow treatments). Spiders that captured crickets on a gumfooted line made more trips to the floor after capture and theft of larger prey. Spiders that captured crickets in the web sheet did not make more trips to the floor after capture and theft of larger prey.

Discussion

We tested the hypothesis that black widow spiders form memories of prey they have captured in their webs. We found that black widows do form memories of captured prey, and are much more likely to search about their web after experiencing the theft of a prey item. We also tested the hypothesis that black widows remember certain features of their prey. We also found that black widows were more likely to search on the floor, and expended more search effort there, when they had captured and lost relatively larger prey.

Our findings are in agreement with other studies that show web spiders search for lost prey, revealing memories of the presence of the prey (Rodriguez and Gamboa 2000; Rodriguez and Gloudeman 2011; Kilmer and Rodriguez 2018). However, our finding that spiders only adjusted their trips to the floor in response to relative prey size when they had captured the prey on in a gumfooted line is novel. We suggest this reflects the ecology of black widow spiders—namely that they capture primarily terrestrial prey on their gum-footled lines (Salomon 2011). Numerous species of web spiders have been shown to search longer for larger or more numerous prey (Rodriguez and Gamboa 2000; Rodriguez and Gloudeman 2011; Rodriguez et al. 2015; Kilmer and Rodriguez 2019), but other features of memories of stolen prey are not well documented.

The variation in effort searching the floor according to the interaction between site of prey capture and relative prey size suggests the spiders are adapted to store and use information about relative prey size only when the prey came from their main capture site. However, our results have two potential explanations. 1) Black widows may form memories of prey size and capture location each time they capture prey, but only use the memory of relative

prey size to guide behavior when the prey was captured in a gumfooted line. 2) Black widows only form memories of relative prey size when the prey was captured in a gumfooted line. Our experimental design does not allow us to differentiate these two possibilities.

However, this result offers an interesting insight into black widow ecology. In the field, black widows capture both terrestrial and flying prey (Salomon 2011), and some of the flying prey are quite large relative to black widows (e.g. lepidopterans, certain hymenopterans) (Solomon 2011). Flying prey would presumably become entangled in the sheet, in much the same way we offered crickets to spiders in the sheet in our experiment. However, black widows did not alter their searching behavior in response to differences in relative prey size after capturing prey in the sheet, which suggests that these flying prey capture events could be relatively unimportant in ecological and evolutionary terms.

We used simple experimental manipulations and observations of behavior to reveal the contents of a web spider's memory. Thus, our results underscore the importance of behavioral observations when examining the contents of animal memories. Our experiment and observations of behavior also provided insight into the ecology of the spider, and therefore also underscore the importance of the study of memory contents for understanding animal ecology.

References

Benjamin SP, Zschokke S (2003) Webs of theridiid spiders: construction, structure, and evolution. *Biological Journal of the Linnean Society*. **78**. 293-305.

Blackledge TA, Scharff N, Coddington JA, Szuets T, Wenzel JW, Hayashi CY (2009) Reconstructing web evolution and spider diversification in the molecular era. *PNAS*. **106**. 5229-5234.

Blackledge TA, Zevenbergen JM (2007) Condition-dependent web architecture in the western black widow, *Latrodectus hesperus*. *Animal Behaviour*. **73**. 855-864.

Dukas R (2004) Evolutionary biology of animal congition. *Annual Review of Ecology, Evolution, and Systematics*. **35**. 347-374.

Eberhard WG (2020) Spider webs. Behavior, function, and evolution. University of Chicago Press.

Foelix R (2011) Biology of Spiders. Oxford University Press.

Healy S, Braithwaite V (2000) Cognitive ecology: a field of substance? TREE. 2000; 15: 22-25

Kamil AC, Balda RP, Olson DJ (1994) Performance of four seed-caching corvid species in the radial arm maze analog. *Journal of Comparative Psychology*. **108**. 385-393

Kilmer JT, Havens ZS, Rodríguez RL (2018) Ontogenetic approach reveals how cognitive capability and motivation shape prey-searching behavior in *Pholcus phalangiodes* cellar spiders. Ethology. 124: 657-666

Kilmer JT, Rodriguez RL (2019) Miniature spiders (with miniature brains) forget sooner. *Animal Behaviour*. **153**. 25-32

LeGuelte L (1969) Learning in Spiders. Am Zool. 9: 145-152

Nakata K (2012) Plasticity in an extended phenotype and reversed up-down asymmetry of spider orb webs. *Animal Behaviour*. **83**. 821-826

Mery F, Kawecki TJ (2002) Experimental evolution of learning ability in fruit flies. *PNAS*. **99**. 14272-14279

Opell BD (2001) Egg sac recognition by female *Miagrammopes animotus* (Araneae, Uloboridae). *The Journal of Arachnology*. **29**. 244-248

Placais PY, Preat T (2013) To favor survival under food shortage, the brain disables costly memory. *Science*. **339**. 440-442.

Platt ML, Brannon EM, Briese TL, French JA (1996) Differences in feeding ecology predict differences in performance between golden lion tamarins (*Leontopithecus rosalia*) and Wied's marmosets (*Callithrix kuhli*) on spatial and visual memory tasks. *Animal Learning and Behavior*. **24**. 384-393

Pravosudov VV, Clayton NS (2002) A test of the adaptive specialization hypothesis: population differences in caching, memory, and the hippocampus in black-capped chickadees (*Poecile atricapilla*). *Behavioral Neuroscience*. **116**. 515-522

Pravosudov VV, Roth II TC (2013) Cognitive ecology of food hoarding: the evolution of spatial memory and the hippocampus. *Annual Review of Ecology, Evolution, and Systematics*. **44**. 173-193

Rodríguez RL, Gamboa E (2000) Memory of captured prey in three web spiders (Araneae: Araneidae, Linyphiidae, Tetragnathidae). Anim Cogn. 3: 91-97

Rodríguez RL, Gloudeman MD (2011) Estimating repeatability of memories of captured prey formed by *Frontinella communis* spiders (Araneae: Linyphiidae). Anim Cogn. 14: 675-682

Rodríguez RL, Kolodziej RC, Hoebel G (2013) Memory of prey larders in golden orb-web spiders, *Nephila clavipes* (Araneae: Nephilidae). Behav. 150: 1345-1356

Rodríguez RL, Briceno RD, Briceno-Aguilar E, Hoebel G (2015) *Nephila clavipes* spiders (Araneae: Nephilidae) keep track of captured prey counts: testing for a sense of numerosity in an orb-weaver. Anim Cogn. 18: 307-314

Salomon M (2011) The natural diet of a polyphagous predator, *Latrodectus hesperus* (Araneae: Theridiidae) over one year. *The Journal of Arachnology*. **39**(1). 154-160.

Sergi, C.M., Antonopoulos, T., and Rodríguez, R.L. (2021). Black widows use path integration on their webs. *Behavioral Ecology and Sociobiology*. 75

Sherry DF, Duff SJ (1996) Behavioural and neural bases of orientation in food-storing birds. *The Journal of Experimental Biology*. **199**. 165-172

Shettleworth SJ (2010) Cognition, Evolution, and Behavior. Oxford University Press.

Smith CC, Reichman OJ (1984) The evolution of food caching by birds and mammals. *Annual Reviews in Ecology and Systematics*. **15**. 329-351

Tello-Ramos MC, Branch CL, Kozlovsky DY, Pitera AM, Pravosudov VV (2019) Spatial

memory and cognitive flexibility trade-offs: to be or not to be flexible, that is the question. *Animal Behaviour*. **147**. 129-136

Curriculum vitae

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Publications:

- **Sergi C**, Thompson C, Montgomery B, and Rodríguez R. 2020. 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. *Behavioral Ecology and Sociobiology*. (74)
- **Sergi C**, Antonopoulos T, and Rodríguez R. In revision, *Behavioral Ecology and Sociobiology*. Black widow spiders use path integration to navigate their webs.
- Thompson C, **Sergi C**, and Rodríguez R. 2019. Repeatability but no short-term plasticity in the web architecture of *Latrodectus hesperus* western black widow spiders (Araneae: Theridiidae). *Ethology* (00) 1-7.
- **Sergi C** and Rodríguez R. In review. Distraction by internally directed attention reveals basic consciousness in animals. *Biology and Philosophy*
- **Sergi C**, Schlais A, Marshall M, and Rodríguez R. Submitted. Black widow spiders (*Latrodectus hesperus*) remember prey capture location and size, but only alter behavior for prey caught at particular sites. *Behavioral Ecology and Sociobiology*

Professional Presentations:

- **Sergi C**. Animals are smarter than you think.
  Biological Sciences Colloquium. Milwaukee, WI. March 2022
- **Sergi C** and Rodríguez R. Causes of Variation in black widow web architecture.
  Behaviour 2019. Chicago, IL. July 2019
- **Sergi C and Rodríguez R**. Predictive mental models in black widow spiders.
  Biological Sciences Colloquium. Milwaulee, WI. April 2019
- **Sergi C** and Rodríguez R. Predictive mental models in black widow spiders.
  International Society of Behavioral Ecology Conference. Minneapolis, MN. August 2018

- **Sergi C** and Rodríguez R. Predictive mental models in black widow spiders. *Animal Behavior Society Conference*. Milwaukee, WI. August 2018
- **Sergi C** and Blouin M. Cost to *Biomphalaria glabrata* of resisting infection by *Schistosoma mansoni*. UW-Milwaukee Biological Sciences Research Symposium. Milwaukee, WI. April 2017

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- Ruth I. Walker Award, Awarded Spring 2020
- Best graduate EEB talk, UW-Milwaukee Biological Sciences Symposium, Spring 2017 and Spring 2019
- James D Anthony Memorial Scholarship, Awarded Spring 2018 and Spring 2019

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