

RESEARCH ARTICLE

Multifunctionality of an arthropod predator's body coloration

Hsien-Chun Liao¹ | Chen-Pan Liao^{1,2} | Sean J. Blamires^{1,3}  | I-Min Tso^{1,4} ¹Department of Life Science, Tunghai University, Taichung, Taiwan²Department of Biology, National Museum of Natural Science, Taichung, Taiwan³Evolution and Ecology Research Centre, University of New South Wales, Sydney, New South Wales⁴Center for Tropical Ecology and Biodiversity, Tunghai University, Taichung, Taiwan**Correspondence**

I-Min Tso

Email: spider@thu.edu.tw

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Abstract

1. Animal body colours can be shaped by many factors, including the need to attract mates, avoid predators and lure prey. In some contexts, these needs might compete. A number of studies have recently demonstrated that the silver, white, yellow or red bodies of spiders attract mates, lure prey or startle predators. Nevertheless, when spider bodies display different colours, little is known about the multifunctionality of the colours and whether they interact. The Australasian coin spider, *Herrenia multipuncta*, displays unconventional body coloration, with orange, black and grey regions across its body.
2. We hypothesized that its coloration serves a multifunctional role, with the dorsal orange bands on its prosoma attracting prey and its orange ventrum deterring predators. We tested our hypothesis with field and laboratory experiments using dummies and real spiders, and modelling the visibility of the various colours to different predators and prey.
3. Our field experiment showed significant prey attraction towards the orange-grey dorsal pattern during the day and night, while our laboratory experiment showed that the lizard *Japalura swinhonis* stared at spiders and hesitated before attacking spiders when the orange abdominal region was uncovered. Our various visual models confirmed our experimental results by showing that the orange and grey body parts were always visible when contrasted against their natural backgrounds.
4. Combined, our analyses provide evidence to conclude that the orange body colour of *H. multipuncta* is multifunctional, serving in both prey attraction and predator avoidance.

KEYWORDSBody colours, *Herrenia multipuncta*, pigmentation, predator avoidance, prey luring, visual models

1 | INTRODUCTION

The function of animal body colours has fascinated biologists for decades. Nevertheless, the evolutionary and ecological drivers of different coloration patterns within and between animals are largely unknown, for reasons that follow. Firstly, many ecological factors can shape body coloration, including the need to attract mates, to escape predators, to feed, and the limited availability

of the necessary nutrients and pigments to invest in specific colours (Endler, 2006; Higginson, Wert, Rowland, Speed, & Ruxton, 2012). Secondly, the various factors influencing body coloration often compete, so their effectiveness can vary across ecological circumstances (Peng, Blamires, Agnarsson, Lin, & Tso, 2013). Accordingly, factors that shape body coloration can vary considerably between and among animals and across ecological contexts (Stevens, 2007).

Among invertebrates, spiders provide brilliant examples of body coloration, with some species exhibiting shades of yellow, white, green, silver and red, among other colours (Blamires, Hou, Chen, Liao, & Tso, 2014; Blamires et al., 2012; Heiling, Chittka, Cheng, & Herberstein, 2005; Hsuing, Deheyn, Shawkey, & Blackledge, 2015a; Insausti & Casas, 2008; Peng et al., 2013). Many spider bodies constitute a mix of different colours, with considerable variety within and between species (Ajuria-Ibarra, Tapia-McClung, & Rao, 2017; Gawryszewski, Llandres, & Herberstein, 2012; Hsiung, Deheyn, et al., 2015a; Rao, Castaneda-Barbosa, Nunez-Beverido, & Diaz-Fleischer, 2015; They, 2007). The variety in body colour patterns among spiders is thought to be primarily a product of body surface features so most are unlikely to be readily changed (Hsiung, Deheyn, et al., 2015a). However, recent analyses have implicated cuticular pigments such as carotenoids, ommochromes, bilins, guanines and eumelanins as producing vibrant spider body colours that are changeable across habitats or diet (Hsuing, Blackledge, & Shawkey, 2015b; Hsuing, Justyn, Blackledge, & Shawkey, 2017; Insausti & Casas, 2008).

The evolutionary significance of different spider body colours is still a matter of conjecture. Some spiders use their body coloration to attract mates (Lin, Zhang, Liao, Hebets, & Tso, 2015), as prey lures (Blamires et al., 2014, 2012; Tso, Zhang, Tan, Peng, & Blamires, 2016; White & Kemp, 2016; Zhang, Chen, et al., 2015a), to avoid (Bonte & Maelfait, 2004; Brechbuhl, Casas, & Bacher, 2010; Bush, Yu, & Herberstein, 2008; Hoese, Law, Rao, & Herberstein, 2006), startle or confuse predators (Liu, Blamires, Liao, & Tso, 2014; Moya-Lorano, Taylor, & Fernandez-Montraveta, 2003; Zhang, Mao, et al., 2015b), for thermoregulation (Robinson & Robinson, 1978), or perhaps a complex interplay of these functions. Most of what is known about the function of spider body colours emanates from studies of single features, for example conspicuous bright stripes or spots (Blamires et al., 2012; Tso et al., 2016). We accordingly know little about how different colours function when spider bodies display a variety of colours.

The Australasian coin spiders (genus *Herrenia*, family Nephilidae) place their elongated ladder-shaped webs close to tree trunks (Kuntner, 2005). Body coloration can vary among *Herrenia* species. In *H. multipuncta*, the dorsal side of the abdomen is grey (Figure 1), which presumably enables them to occupy their webs without their body contrasting against the grey-brown tree trunk. The dorsal side of the prosoma, contrastingly, is black with a pair of small conspicuous central orange bands and a pair of larger and brighter orange bands along the flanks. The ventral side of the abdomen is almost entirely orange with a central black spot. Their ventral prosoma is predominantly black with a central orange spot (Figure 1). This orange coloration is distinct from the yellow colours of crab spider bodies or the spots and stripes in other orb-web-building spiders (Blamires et al., 2012; Chuang, Yang, & Tso, 2008), and the various shades of red that occasionally appear (e.g. Blamires et al., 2014), so is likely derived from different pigments (Hsuing et al., 2017). Some individuals have additional red spots on their book lungs (see Figure 1a). These can vary in colour among individuals, from a dull brown that

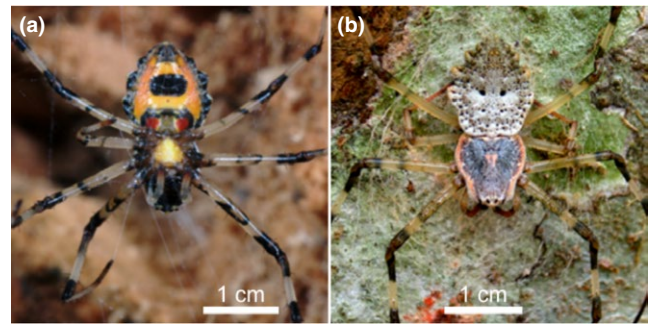


FIGURE 1 Ventral (a) and dorsal (b) views of *Herrenia multipuncta*

is indistinguishable from the background to a bright red. Coloration on the book lungs is relatively common in orb-web spiders, but their function has never been tested.

The function of different body colours, for example yellow, white, green, silver and so on, within spider species has been examined in detail (Blamires et al., 2012, 2014; Tso, Liao, Huang, & Yang, 2006; Tso et al., 2016), but bright orange is a relatively rare body colour in spiders. This may be because orange body coloration is a product of cuticle β -carotenoid deposition (Toews, Hofmeister, & Taylor, 2017), and few spiders produce cuticular carotenoids de novo or are unable to attain them from their diet (Hsuing et al., 2017). We have incidentally noticed that *H. multipuncta* positions itself in a manner that displays its dorsal orange bands when foraging and lifts its abdomen to expose its bright orange ventrum when disturbed. It has been speculated elsewhere that the same body colours can impart different signals to different receivers depending upon their size, shape, intensity and position (Cheng, Yang, Lin, Herberstein, & Tso, 2010; Pérez-Rodríguez, Jovanir, & Stevens, 2017). We accordingly hypothesized here that the orange bands on the dorsal side of the prosoma of *H. multipuncta* attract prey, while the bright orange ventral side of the abdomen startles predators. To test our hypothesis, we performed field and laboratory experiments using both real and dummy spiders exposed to different predators and prey, in conjunction with derivations of models assessing the visibility of the different body parts to invertebrate predators and prey, and a vertebrate predator.

2 | MATERIALS AND METHODS

2.1 | Field experiment

To test whether the orange bands on the dorsal side of *H. multipuncta*'s prosoma attract prey, we conducted a field experiment in a subtropical forest dominated by large lemon-scented gum, *Corymbia citriodora*, and Camphor, *Cinnamomum camphora*, trees at Lian-Hua-Chi Research Centre (LHCRC), Taiwan Forestry Research Institute, Yu-Chi, Nantou County, Taiwan (120°52'58.6"E, 23°55'8.8"N).

To begin the experiment, we randomly chose 24 large trees (>2 m in height) in the study site on which *H. multipuncta* had been

recorded in a previous survey. On each of the trees, we placed one of the following four spider dummies, which were made from a combination of grey and black coloured paper and orange acrylic paint to approximate the size, shape and coloration of real *H. multipuncta*, in random order using silicon glue: (a) no colour dummies, where neither the orange bands nor the grey abdomen was present; (b) grey dummies, where the grey abdomens only were present, the prosoma being entirely black; (c) orange dummies, where the orange prosomal bands only were present, and the abdomen was black; and (d) orange-grey dummies, where both the orange prosomal bands and grey abdomens were present (see Figure 2). Six each of the four types of dummies were used. All dummies were glued on to the tree trunks using an acrylic adhesive ~2 m off the ground, which we estimated to be the height at which *H. multipuncta* was most commonly found.

We placed 24 Sony CX700 HDD video cameras with infrared night viewing scopes ~1–2 m perpendicular from each tree holding a dummy and recorded all animal interactions with the dummies between 800 and 1,200 hr (daytime monitoring) and 2,000 to 000 hr (night-time monitoring). The 1,118.2 hr of video footage (537.6 hr of daytime monitoring and 580.6 hr of night-time monitoring) across the four treatments was viewed in the laboratory at Tunghai University, Taichung, Taiwan, and all animals that directly interacted with a dummy (i.e. responded to it by moving directly towards it) were identified to taxonomic order. When an insect was seen flying directly towards a dummy without deviating from its path, we recorded it as a “prey luring event.” When an animal was seen launching an attack towards the dummy, it was recorded as a “predation event.” We then calculated the “prey attraction rate” as the number of prey luring events per hour of video footage and the “predator attraction rate” as the number of predation events per hour of video footage.

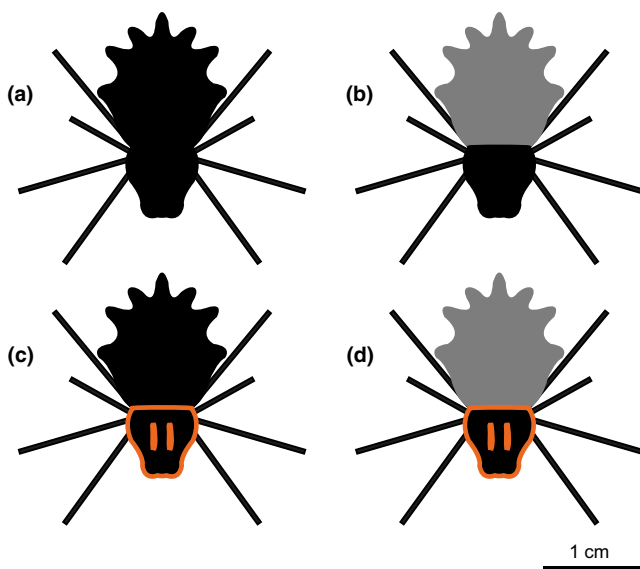


FIGURE 2 Dummy spiders used in field experiments showing the: (a) no colour, (b) grey, (c) orange and (d) orange-grey treatments

2.2 | Laboratory experiment

During our field experiment, we observed that individual *H. multipuncta* always performed a “push-up” style movement whenever a lizard approached it (see Supporting Information) Video S1. This movement seemed to be done to reveal the spider's bright orange abdominal ventrum. We thus tested whether the bright orange abdominal ventrum of *H. multipuncta* functions to startle predators by conducting a laboratory experiment where we exposed female *H. multipuncta* to tree lizards, *Japalura swinhonis*.

We collected 50 female *H. multipuncta* from Fonghuanggu Bird and Ecology Park, Nantou County, Taiwan, and brought them to the Department of Life Science, Tunghai University, and placed them in 40 × 100 × 100 cm wooden enclosures with front and back removable transparent fibreglass lids. Chestnut tree bark was provided internally for the spiders to build their webs on. Once the spiders had built webs, they were sustained on a diet of three mealworms every 2 days over 1 week. After this acclimation period, the spiders were assigned to one of two treatments ($n = 25$ in each): manipulated or control. To spiders in the manipulated treatment, we painted the orange region of the ventral abdomen black using a non-toxic acrylic paint that had been previously used to manipulate spider body colours (Tso et al., 2016). To spiders in the control treatment, we added the same amount of black paint to the black spot on the spider's ventral abdomen. This did not alter the coloration of the ventral side of the abdomen, but the black paint added to the spider body controlled for the paint applied in the manipulated treatment. The paint was applied the same way by the same person (H.C.L.) every time. To apply the paint, we anaesthetized the spiders using CO₂ and commenced the experiment 24 hr later, thereby allowing full recovery. We did not manipulate the red spots on the book lungs as they were not always clearly present and not possible to manipulate without harming the spider.

We collected 50 *J. swinhonis* by hand immediately prior to the commencement of the following experiment from a Formosan Acacia forest within the grounds of Tunghai University, Taichung City, Taiwan (120°35'40.42"E, 24°10'52.63"N). We placed one lizard within each of the enclosures containing a control or manipulated spider and recorded the spider's behaviour when interacting with the lizard using digital video cameras placed ~1 m perpendicular to the transparent lid of each enclosure. According to this design, each spider encountered one lizard. We started filming the interactions ~10 min after the lizard was introduced to allow the lizards to initially explore and familiarize itself with the enclosure. A typical spider–lizard interaction proceeded as follows: (a) the introduced lizard moving around in the cage and eventually finding the spider on the bark and approaching it. (b) The spider performing “push-ups.” (c) The lizard staring at the violently shaking spider for several seconds before either attacking or moving away from it. (d) The spider resuming its normal posture and the lizard reapproaching it. We therefore defined the interval between initiation of “push-ups” by the spider and lizard attack/retreat as the “lizard attack time.” The number of “push-ups” the spider did was divided by the lizard attack time

and was considered the “push-up rate.” The time interval between *J. swinhonis* leaving a spider and coming back a second time was considered the “latency to attack.”

2.3 | Predator and prey visual models

The above experiments were done on the assumptions that (a) the different coloured body parts of *H. multipuncta* are visible to its predators and prey and distinguishable from background colours by day and night, and (b) the coloration of the dummies used in the field experiment is indistinguishable from the coloration of the spiders to its predators and prey by day and night. We thus tested each of these assumptions as follows prior to performing our experiments.

We collected five female *H. multipuncta* and 30 samples of tree bark from our field site. We then anaesthetized the spiders using CO₂ and measured reflectance spectra (300–700 nm) of the orange on their prosoma and opisthosoma, the black regions of their prosoma and the grey regions of their dorsal abdomen, as well as the tree bark, using a spectrometer (USB4000-UV-VIS, Ocean Optics, Inc., Dunedin, Florida, U.S.A.). The spectrometer was connected to a laptop running the program OceanView 1.6.3 (Ocean Optics, Inc.). One read fibre was connected to the spectrometer and six illumination fibres connected to a deuterium–tungsten halogen light source (DT-1000, Ocean Optics, Inc.) placed 5 mm vertically above the anaesthetized spiders. Labsphere certified white and black reflectance standards were used to calibrate the spectrometer to 100% and 0% reflectance, respectively. The area captured for all body parts, the tree bark, and the white and black standards, was 2 mm², and the integration time was 150 ms. Multiple measurements from each of the body colours were taken and averaged to account for any sampling error (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013). All of the measurements were made in a dark room. We repeated these measurements for the grey and black coloured paper and orange paint used to make the dummies.

The reflectance spectra generated were used to construct a series of diurnal and nocturnal animal vision models. Observations conducted prior to performing the field experiment found honeybees to represent the primary diurnal prey type of *H. multipuncta*, while a variety of moths represented the primary nocturnal prey type. We also observed wasps to interact with *H. multipuncta* in the field as predators. We therefore estimated the relative diurnal achromatic and chromatic contrasts of hymenopteran UV, blue and green photoreceptors using the colour hexagon model of Chittka (1992). Although the model was derived based on knowledge of honeybee photoreceptor physiology (Chittka, 1992), it can be applied to estimate the photoreceptor sensitivities on exposure to specific colour stimuli of other hymenopterans owing to the similarities in the photoreceptor spectral sensitivities across the group (Briscoe & Chittka, 2001). Since moths represent the primary nocturnal prey type for *H. multipuncta*, we also constructed a neuroethological hawkmoth nocturnal visual model (Johnsen et al., 2006). Dipterans represented another common prey for the spiders, and we observed that lizards predominantly attack *H. multipuncta* in the field. We

therefore constructed an additional *Drosophila melanogaster* visual model (Yamaguchi, Desplan, & Heisenberg, 2010) and a generalized lizard visual model (Fleishman, Leal, & Sheehan, 2006; Fleishman, Ogas, Steinberg, & Leal, 2016).

Details pertaining to the above visual models, including all calculations, are provided in the Supporting Information.

2.4 | Statistical analyses

For the field experiment, two negative binomial regression models were used to fit the prey attraction rate and predator attraction rates separately. For each model, the independent variables included prosoma colour (i.e. orange present or absent), abdomen colour (i.e. grey present or absent), monitoring time (i.e. daytime or night-time) and all possible interactions. Natural log of the period of monitoring (in hours of footage) was included as an offset term since the amount of footage taken for each dummy was unequal. We eliminated all redundant interaction terms by using a backward elimination process until a minimal corrected Akaike information criterion (AICc) was met or no interaction terms remained. We merged factors participating in any interaction to estimate the effects and perform pairwise multiple comparisons among levels of merging factors.

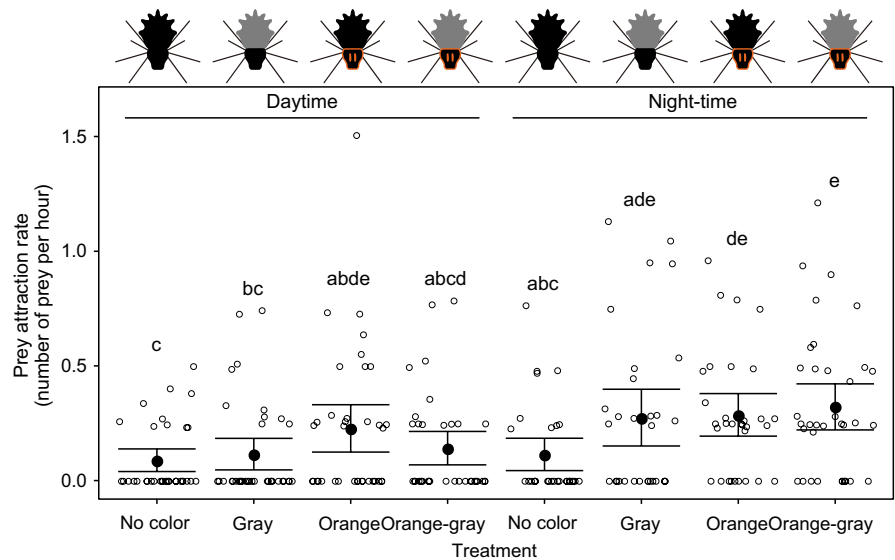
To compare the prey composition among treatments, dummies and monitoring time, we used a likelihood ratio test of homogeneity (G test) and then performed pairwise multiple comparisons using Fisher's exact tests to determine whether any interactions were significant. *P*-values of the multiple comparisons were adjusted by using the Benjamini–Hochberg method to control for the false-positive rate. For the laboratory experiment, we used Fisher's exact test to compare the probabilities and Wilcoxon rank sum tests to compare the push-up rate, staring time and latency to attack of lizards between the control (i.e. orange present) and experimental (i.e. orange absent) treatments.

We used one-sample *t* tests to test whether the various spider body parts and the corresponding coloured paper and paints used to create the dummies exceeded the chromatic discrimination threshold value for honeybee photoreceptors under differential conditioning (0.04) and absolute conditioning (0.1), respectively (Dyer, 2005). The fruit fly and lizard chromatic/achromatic contrast JNDs were also tested against the theoretical discrimination threshold (1.0). Unfortunately, no colour discrimination threshold values for honeybee achromatic contrast, hawkmoth chromatic distances or hawkmoth achromatic contrast have been determined. We tested the achromatic discrimination contrasts for honeybees and hawkmoths when viewing each of the spider body parts, paper and paints, against thresholds of 1.0 and 0, which represented “white bodies” and “black bodies,” respectively. These comparisons thus were used to determine whether the contrasts differed from a hypothetical spectra that they were each assumed to represent. The hawkmoth chromatic distance values of the different body parts were further compared against each other by one-way ANOVAs and least squared difference post hoc comparisons of means.

TABLE 1 The results of best-fit negative binomial regression model evaluating the effects of orange/grey colour and monitoring time (day/night) on number of prey lured by spider dummies. A goodness-of-fit test showed the model reasonably fit the data ($\chi^2 = 268.49$, $df = 264$, $p = 0.367$)

Factor	Estimate \pm SE	95% CIs	Z	p
Intercept	-2.423 \pm 0.249	-2.936, -1.955	-9.37	<0.001
Prosoma orange	0.923 \pm 0.259	0.426, 1.446	3.57	<0.001
Abdomen grey	0.286 \pm 0.331	-0.360, 0.943	0.86	0.387
Prosoma orange \times Abdomen grey	-0.740 \pm 0.340	-1.415, -0.078	-2.18	0.030
Time	0.237 \pm 0.241	-0.236, 0.712	0.98	0.326
Abdomen grey \times Time	0.566 \pm 0.332	-0.084, 1.222	1.70	0.089

FIGURE 3 Comparisons of prey attraction rates among four different dummy types recorded from daytime and night-time. Solid black circles and whiskers indicate the average and 95% confidence intervals, respectively. One empty circle indicates result of one dummy. No common lowercase letter between two groups indicates a significant difference. (See electronic Supporting Information Table S3 for p -values)



3 | RESULTS

3.1 | Field experiment

During the daytime, the “prey luring” events predominantly involved dipterans (52%–75%), while the night-time, the “prey luring” events predominantly (50%–88%) involved lepidopterans, primarily tussock moths and hummingbird hawkmoths (Supporting Information Table S1, Figure S1). Since the interactions between the spider’s prosomal and abdominal colours and time significantly affected the prey compositions ($\chi^2 = 9.267$, $df = 3$, $p = 0.026$), we performed multiple comparisons among eight scenarios (see Supporting Information Table S1). The results of these comparisons showed that the composition of insects lured to each treatment during the daytime were all similar to each other, but they significantly different to those lured to the night-time treatments, with the exception of the “no colour” night-time treatment (Supporting Information Figure S1). We also observed all “predatory events” to involve either wasps or tree lizards.

After sequentially eliminated two redundant interaction terms (prosoma \times abdomen \times time, $\Delta AICc = 2.2$, $p = 0.910$; prosoma \times time, $\Delta AICc = 2.0$, $p = 0.798$) for the model fitting prey attraction rate, two interactions remained in the best-fit model (Table 1). The subsequent pairwise multiple comparisons among the

eight scenarios showed that the “orange” treatment attracted significantly more insects than the “no colour” treatment and the “grey” treatment during the daytime ($p = 0.040$). The “orange” and “orange-grey” treatments attracted significantly more insects than the “no colour” treatment during the night-time (orange vs. no colour, $p = 0.043$; orange-grey vs. no colour, $p = 0.042$; Figure 3; Supporting Information Table S2). By controlling for prosoma colour, we found that only when the dummies had grey abdomens was the number of night-time prey luring events significantly greater than those in daytime (Figure 3; Supporting Information Table S2). From these results, we surmised that (a) since the orange bands on the dorsal side of the spider’s prosoma lured ~ 1.8 times more insects than the average for all other daytime scenarios, their presence functions to attract diurnal prey, (b) the presences of any combination of the grey and/or orange (i.e. orange-grey, grey and orange) on the dorsal side of the spider’s body lured 3.2–3.7 times more insects than the average for the no colour night-time scenarios, so serves to attract nocturnal prey and (c) the higher night-time prey attraction rates compared to the daytime are a consequence of the dorsal abdominal grey patches attracting more prey.

We sequentially eliminated all of the interaction terms in the model estimating the factors influencing predatory events (prosoma \times abdomen \times time, $\Delta AICc = 2.0$, $p = 0.757$; abdomen \times time,

TABLE 2 The results of best-fit negative binomial regression model evaluating the effects of orange/grey colour and monitoring time (day/night) on number of predator lured by spider dummies. A goodness-of-fit test showed the model reasonably fit the data ($\chi^2 = 75.512$, $df = 266$, $p = 0.999$)

Factor	Estimate \pm SE	95% CIs	Z	p
Intercept	-1.486 \pm 0.420	-2.313, -0.648	-3.53	<0.001
Prosoma orange	-0.122 \pm 0.481	-1.074, 0.825	-0.25	0.800
Abdomen grey	-0.431 \pm 0.484	-1.398, 0.512	-0.89	0.373
Time	-1.491 \pm 0.562	-2.693, -0.449	-2.65	0.008

$\Delta AICc = 4.1$, $p = 0.980$; prosoma \times abdomen, $\Delta AICc = 2.0$, $p = 0.728$; prosoma \times time, $\Delta AICc = 1.8$, $p = 0.606$), and the best-fit model showed that monitoring time significantly influenced predator attraction rate; on average, the predator attraction rate in the day-time was 4.4 times more numerous than in the night-time (Table 2; Supporting Information Figure S2).

3.2 | Laboratory experiment

We observed that 44% of all spiders across treatments exhibited a “push-up” behaviour when exposed to lizards. Moreover, the “push-up” rates were not different between spiders in the two treatments (Figure 4a). All of the lizards in the control treatment stared at spiders upon noticing them in the enclosure. The durations of lizards staring

at control spiders were also longer than those for manipulated spiders (Figure 4b). The latency to attack by lizards exposed to control spiders was again significantly longer than the latency to attack of lizards exposed to manipulated spiders (Figure 4c). Nevertheless, the probabilities of the spider being attacked were similar between two treatments (i.e. 8 of 11 in spiders in the control treatment were attacked, and 5 of 7 spiders in the manipulated treatment were attacked; $p = 1.000$).

3.3 | Predator and prey visual models

The reflectance spectra for each of *H. multipuncta*'s body regions examined, as well as the materials used for making dummy spiders, are shown in the Supporting Information (Supporting Information Figure S3). The prosomal dorsal black and abdominal dorsal grey regions of *H. multipuncta*'s body exceeded chromatic discrimination thresholds, under both a differential and absolute conditioning criteria, when viewed by honeybees and fruit fly against a tree bark background by day. These body parts were likewise discriminable by hawkmoths using chromatic channels at night (see Supporting Information Table S4a).

Our achromatic contrast spectra comparisons for the hawkmoth model showed that the orange regions of *H. multipuncta*'s body were discriminable against their black and grey body regions when contrasted with a tree bark background via achromatic and chromatic channels (Supporting Information Table S4a). Furthermore, our calculations showed that *H. multipuncta*'s dorsal body colorations were discriminable by honeybees and fruit flies using achromatic channels (Supporting Information Table S4b) against black and white bodies, but their orange bands were indiscriminable against a black body when viewed through the honeybee's achromatic channel.

The colour contrasts of the orange, black and grey regions of the various dummies appeared discriminable against tree trunk but indiscriminable to those of *H. multipuncta*'s orange, black and grey body regions when viewed against a tree trunk by absolutely conditioned honeybees and fruit fly's chromatic channel (see Supporting Information Table S4c,d). All of the different papers used for constructing the dummies were also discriminable by hawkmoths using achromatic channels (Supporting Information Table S4d).

According to the lizard visual model, the black and orange dorsal regions on the spider's prosoma and the tree trunk background were significantly discriminable from each other when viewed by lizards (Supporting Information Table S4a,b). Nonetheless, when the orange

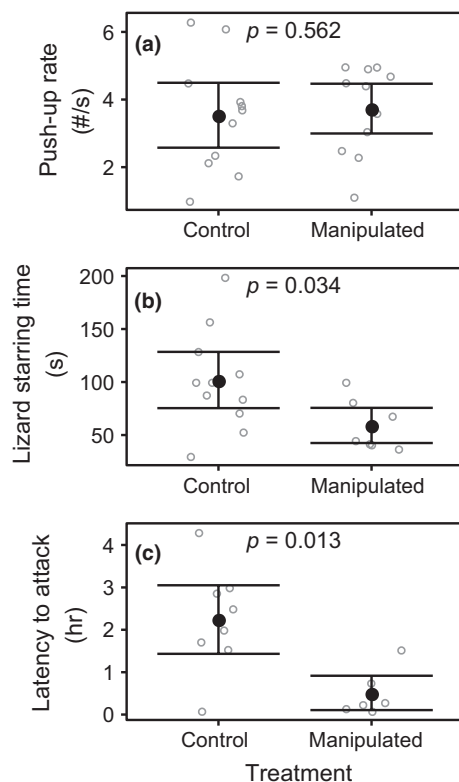


FIGURE 4 The push-up rates of *Herennia multipuncta* (a) and the staring time (b) and latency to attack (c) of lizards responding to *H. multipuncta* in control (orange present) and manipulated (orange absent) treatments. Whiskers indicate the 95% confidence intervals, and the open circles indicate the empirical data

region was covered in black paint, they were indistinguishable by lizards from the rest of the spider's body (Supporting Information Table S4c).

4 | DISCUSSION

While it has been assumed that whenever a range of colours exist across an animal's body, they perform different functions, experimental testing of the functions with robust visual modelling on an appropriate system had never been performed prior to this study. We performed a combination of field and laboratory manipulative experiments and modelled bee, moth, fly and lizard visual sensitivities to establish a multifunctional role for the various dorsal and ventral body colours of the coin spider *Herennia multipuncta*. Our field experiment tested the functionality of *H. multipuncta*'s dorsal body coloration using dummies containing orange and grey dorsal colours that resembled those of real spiders. We found that the spider's grey abdomen was discriminable and attractive to lepidopterans in the night and, to a lesser extent, the day. The dorsal orange prosomal bands attracted more diurnal insects when the other dorsal parts (e.g. the abdomen) were black. The composition of nocturnal and diurnal insects attracted to the dummies differed, with their grey abdomens attracting more insects during the night than the day. While predation events were greater at night, the dorsal coloration of the dummies did not influence predator attraction rates during the day or night. Our laboratory experiment found that the "push-up" rates of *H. multipuncta* when exposed to lizards were similar among our manipulated (i.e. when the orange region was painted black) and control (i.e. when the orange region remained exposed) treatments. However, the lizards behaved differently upon encountering spiders from each treatment. These included shorter durations of staring at a shaking spider and shorter latencies to attack when facing manipulated spiders.

Our various visual models affirmed our experimental results, suggesting that the orange regions on the dorsal prosoma of *H. multipuncta* functions to attract prey, while the bright orange ventral region functions to deter/startle predators. These results support the conclusion that the various dorsal and ventral colour patterns found on *H. multipuncta* represent a multifunctional signal. We do not know whether or not the colours perform functions additional to prey attraction and predator deterrence, such as thermoregulation (Hadley, Savill, & Schultz, 1992; Robinson & Robinson, 1978), but it is certainly plausible.

Several studies have now shown that the body colours of various spider inhabiting aerial orb webs function to lure prey towards the web (Blamires et al., 2012, 2014; Bush et al., 2008; Chuang et al., 2008; Peng et al., 2013; White, Dalrymple, Herberstein, & Kemp, 2017). Nonetheless, the function of body coloration in spiders inhabiting tree trunk environments has not been investigated. Nor has a dual prey attraction and predator avoidance function for the same suite of spider body colours in any spider. Moreover, while different colours in different regions of spider bodies have been assumed to serve multiple functions, few studies have ever tested

these functions (White et al., 2017). Our experiments showed that the various body colours of the tree trunk dwelling spider, *H. multipuncta*, serve multiple functions including attracting prey and startling predators. Further studies examining a wider range of spider body colours and patterns across species are, nonetheless, needed to get an idea of how widespread multifunctional body coloration is among spiders.

We found here that *H. multipuncta* utilizes its orange body colours to lure prey and startle predators. While studies have found different spiders use their various body colours to lure prey and/or deter predators in different contexts (Blamires et al., 2014), the use of orange coloration for multiple purposes has, until now, never previously been reported. Some examples of other spiders with bodies containing orange regions include the dwarf spider *Ceratitis minutus*, early instar *Araneus marmoreus*, and certain morphs of the spiny spider *Austracantha minax*. It would be intriguing to determine whether the orange body colours of these spiders also have multiple functionalities.

Orange body colours are commonly associated with the deposition of carotenoid pigments (Toews et al., 2017). Carotenoid pigmentation within spider cuticle is nonetheless considered rare as they cannot be derived de novo, so must be extracted from dietary sources. This likely explains why their presence is considered an honest indicator of vitality in many animals. The most commonly reported spider body colours (i.e. silver, white) are a product of cuticular surface features (Hsiung, Blackledge, et al., 2015b), so can be attained without dietary acquisition. Our field observations suggest that *H. multipuncta* faces multiple types of predators and prey within a confined space. While this suggests inhabiting tree trunks carries a high degree of risk, the ability of *H. multipuncta* to switch from primarily dipteran prey to lepidopteran prey between day and night might provide it with sufficient dietary sources of carotenoids, therefore the means to invest in orange body colours.

Unique ecological niches can drive extreme adaptations (Kelley, Fitzpatrick, & Merilaita, 2013; Losos & Ricklefs, 2009). Certainly, invertebrates that occupy tree trunks face unique challenges (Harmer & Herberstein, 2009; Harmer, Kokko, Herberstein, & Madin, 2012). For instance, body coloration variability in tree trunk occupying peppered moths is a classic example of a tree trunk inhabiting animal that underwent rapid evolutionary change (Rudge, 2005). One extreme evolutionary adaptation attributed to orb-web spiders that inhabit tree trunks, such as *Clitaetra* spp. and *Herennia* spp., is the building of webs with extreme elongation (i.e. the web capture area extends much further below the hub than above it), probably as a consequence of severe spatial constraints (Harmer & Herberstein, 2009; Harmer et al., 2012). Moreover, there is variability in the forms of web elongation among these spiders, and this is considered to be driven by a range of constraints (Kuntner, 2005). Rapid evolution and/or high phenotypic plasticity thus seem relatively common among tree trunk invertebrates. Perhaps the multifunctional body coloration of *H. multipuncta* represents a unique evolutionary trait associated with the specific challenges faced when occupying tree trunks.

5 | CONCLUSION

We tested the multifunctionality of the dorsal and ventral body colours of the spider *Herennia multipuncta* by field and laboratory experiments using real and dummy spiders exposed to different predators and prey, along with models to assess the visibility of the various body colours to predators and prey. We confirmed our hypothesis that colours on the dorsal side of the spider body attract prey, while the bright orange ventrum startles predators. We thus concluded that the dorsal and ventral colour patterns of *H. multipuncta* represent a form of multifunctional body coloration. While previous studies have shown that various body colours in diurnal and nocturnal spiders can lure prey, we found that the orange prosomal cuticular bands of *H. multipuncta* attract prey during the day, and its prosomal orange cuticular bands and grey abdomen attract prey at night. Our modelling showed that the dorsal orange patch was visible to predators and prey, explaining why its presence increased prey attraction during the daytime and why it was more noticed by lizards. We expect that our work will inspire research to further expand our knowledge of animal coloration and its functionality.

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AUTHORS' CONTRIBUTIONS

H.-C.L. and I.-M.T. developed the conceptual framework and devised the analytical approach. H.-C.L. performed the experiments and fieldwork. H.-C.L. and C.-P.L. performed data analyses and visual model simulations. S.J.B. and C.-P.L. wrote the manuscript. I.-M.T. contributed reagents/analytical tools.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.33s566q> (Liao, Liao, Blamires, Tso, & Galván, 2019).

ORCID

Sean J. Blamires  <https://orcid.org/0000-0001-5953-3723>

I-Min Tso  <https://orcid.org/0000-0002-7296-5595>

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