



# Nocturnal and diurnal predator and prey interactions with crab spider color polymorphs

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## Abstract

Understanding the complex interplay of factors shaping polymorphic changes within individuals represents a longstanding conundrum in biology. Some crab spiders (Thomisidae) are examples of sit-and-wait predators that can change their body coloration. Many factors may influence crab spider color polymorphism with multiple explanations receiving various levels of support. Here we examined the daytime and nighttime activities and predator and prey interactions for two yellow-white polymorphic crab spiders, *Thomisus labefactus* and *Ebrechtella tricuspoidatus* in the field. We thereupon conducted a manipulative experiment using dummies with color morphs visibly resembling the spiders when placed on background-matched flowers. We measured the spectra reflected from the dummies and their floral backgrounds and used insect visual models to determine if they are likely to be visible to a range of insects by night and day. We found that both color morphs of each species were more active by night than by day. Our visual models revealed that the spider's bodies were unlikely to be cryptic. Together, these results suggest that the crab spiders might exploit flower colorations during the night but not during the day. They also indicated that explanations of why crab spiders utilize certain color polymorphs are context dependent and will vary with time, and whether predators, prey, or both, are present.

## Significance statement

Crab spiders are an excellent model for investigating a long-standing challenge in evolutionary biology: understanding the causes and consequences of polymorphic coloration in animals. Studies have postulated a range of explanations with some support for each. Broader studies encompassing all interactions between spiders and their predators and prey across the day and night are urgently needed. Here we combined an around-the-clock spider activity survey with field experiments and insect visual models to show that the types of interactions between spider color morphs and their predators and prey differ over the day and night. Our study suggests that outcomes of experiments examining the adaptive drivers of polymorphisms may be dependent upon the context within which the observations were made, and that examining interactions across temporal contexts is required to fully uncover the various drivers of the polymorphisms.

**Keywords** Color polymorphism · Diurnal interactions · Nocturnal interactions · Temporal context · Thomisidae

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## Introduction

A variety of genetic, biochemical, behavioral, and structural and functional polymorphisms have been described in animals, with variability potentially manifested across generations, among populations, or within individual lifetimes. Within-individual polymorphisms include switching between eusociality and solitary, and vice-versa, in bees and wasps (Kocher et al. 2018), changing from a larval to adult forms in insects (Wigglesworth 1959; Truman 2019), and gender changes among sequentially hermaphroditic fish (Todd et al. 2016). Understanding the factors that shape within-individual polymorphic changes in animals represents an ongoing conundrum in evolutionary biology (Gray and McKinnon 2007; Mitchell-Olds et al. 2007; Karpestam et al. 2016). One reason for this is because finding model populations with a specific polymorphism among individuals is extremely difficult (Sánchez-Guillén et al. 2011; Wellenreuther et al. 2014).

Color polymorphisms are one of the most widespread types of within-individual polymorphism among animals. Yet its genetic, environmental, and other determinants are not well discerned (McLean and Stuart-Fox 2014). Crab spiders (Araneae: Thomisidae) are sit-and-wait predators that do not build webs to capture their prey. Instead they extend their forelegs and wait motionlessly on a flower, leaf, or branch for prey to approach (Viera et al. 2017). Adult females of several flower-dwelling crab spiders change their body coloration from white to yellow and vice-versa (and in some rare cases purples and pinks can also be developed) depending on the color of the flowers they occupy (Théry and Casas 2002; Heiling et al. 2005). Color switching has been estimated, in selected species, to take around 10–15 days (Schmalhofer 2000; Herberstein and Gawryszewski 2013; Llandres et al. 2013). Crab spider coloration thus represents a readily observable form of within-individual polymorphism (Insausti and Casas 2008; Ajuria Ibarra and Reader 2014). Accordingly, crab spider populations are excellent models for testing the influences of a range of factors on polymorphisms in animals.

A number of studies have reported crab spiders to preferentially forage on flowers similar in color to their predominant color morph (Heiling et al. 2005; Théry et al. 2005). That is, predominantly white spiders tend to choose white flowers (but see Heiling et al. 2005), while predominantly yellow spiders tend to choose yellow flowers. It might thus be presumed that crab spiders change color to become camouflaged from predators and/or hide from prey when their body coloration becomes mismatched from the flower they happen to occupy (Morse 2007).

Contemporary studies, nonetheless, suggest that polymorphic color changes in crab spiders are more complex

than previously thought (Brechtbühl et al. 2010; Gawryszewski et al. 2012). A recent review (Brechtbühl et al. 2010) proposed four possible explanations for the existence of crab spider color polymorphism: (1) defensive crypsis, (2) offensive crypsis, (3) exploitation of flower coloration, and (4) predator and/or prey indifference, with all receiving varying levels of support from independent studies.

Defensive crypsis as an explanation for crab spider polymorphic color change suggests that predators of the spiders cannot perceive them against a flower if their body is strongly color-matched against it. This hypothesis is primarily supported by studies of several European crab spiders (Chittka 2001; Théry and Casas 2002; Théry et al. 2005; Defrize et al. 2010). The offensive crypsis explanation on the other hand proposes that flowers harboring a crab spider are avoided by potential prey, so the spiders need to color-match their bodies against the flower occupied to avoid alarming their prey. This explanation is supported by research showing Australian native bees to be attracted to, but unlikely to land on, flowers occupied by a crab spider that is not color-matched to the floral background (Heiling and Herberstein 2004; Llandres and Rodríguez-Gironés 2011).

The exploitation of flower coloration explanation posits that crab spiders will choose to occupy flowers frequently visited by their prey and their body coloration may serve as an enhancer of the flower's exploitative color signal. It thus suggests that flowers with a crab spider present should be visited more often than spider-free flowers of the same color, and has support from studies of UV-reflecting crab spiders occupying UV-reflective flowers (Heiling et al. 2003, 2005; Llandres and Rodríguez-Gironés 2011).

The indifference hypothesis proposes that flower visitors may detect spiders but have no interest in avoiding them. For instance, bumblebees and honeybees seem to be indifferent to flowers occupied by crab spiders, even if they detect their presence (Brechtbühl et al. 2010). Crab spiders must, according to this explanation, change their coloration for other reasons (e.g., for thermoregulation or to conserve the expression of some pigments). Brechtbühl et al. (2010) speculated that the indifference hypothesis might only hold when social insects are the spider's prey, as developing an avoidance response is expected to be expensive and the loss of a worker or two probably has only marginal effects on the overall fitness of the population in question.

We envisage here that the defensive and offensive crypsis hypotheses to be the most compelling explanations for the evolution of crab spider color polymorphisms, as any coloration that facilitates their hiding from predators and/or prevents prey from fleeing is likely to be strongly selected for. The idea of spiders matching, and even enhancing, flower attractive coloration seems less feasible as there is some risk of detection by predators and/or prey if the flower's signal is imprecisely imitated or learned by predators or prey to be associated with

a spider (Yeh et al. 2015; Taylor et al. 2016). The indifference hypothesis also seems, to us at least, to not hold much traction as an explanation for the evolution and sustenance of crab spider color polymorphisms. However, it offers us a useful latent explanation for testing each of the others against.

The many compelling lines of evidence for each of the above explanations for within-individual crab spider color polymorphisms suggest that, depending on the predator, prey, spider species, and ecological context, a combination of top-down (from predators) and bottom-up (from prey) mechanisms might simultaneously shape the adoption of spider color polymorphisms (Cheng et al. 2010; Yeh et al. 2015). We note nevertheless that most of the studies of crab spider color polymorphisms to date have investigated the responses of individual prey types toward individual crab spiders under daylight illumination. Since we know that color patterns on some large orb web spider bodies, particularly those harboring whites and yellows, can attract insects toward their webs during both the day and night (Tso et al. 2007; Chuang et al. 2008; Blamires et al. 2012, 2014; Peng et al. 2020), it is reasonable to expect that yellow and white crab spider bodies, and the flowers they occupy, might be visually distinguishable by the spider's predators and/or prey during both the day and night.

Here we comprehensively surveyed the daytime and nighttime activities of two yellow and white polymorphic Taiwanese crab spiders, *Thomisus labefactus* and *Ebrechtella tricuspisidatus*, that are known to change color to match their floral background (Su et al. 2020). We followed this up with a manipulative field experiment assessing the predator and prey interactions with yellow and white color morphed dummy spiders and modelled the visibilities of each color morph to a range of insects. Crypsis is implied should we find spiders in the field more frequently on flowers that are the same color as their bodies. Our follow-up experiments and visual models were used to differentiate whether any implied visual attraction is likely to be defensive (i.e., directed toward predators) or offensive (directed toward prey). Should the experiments find prey attraction by flowers and spider bodies while our modelling suggests imprecise color-matching between the “spider” bodies and flowers, we interpret it as the spiders utilizing the attractive coloration of the flower, with the spider body enhancing that signal. We performed our surveys and experiments across both day and night and ran diurnal and nocturnal visual models to test whether crab spider body coloration is equally attractive to predators and/or prey by day and night.

## Methods

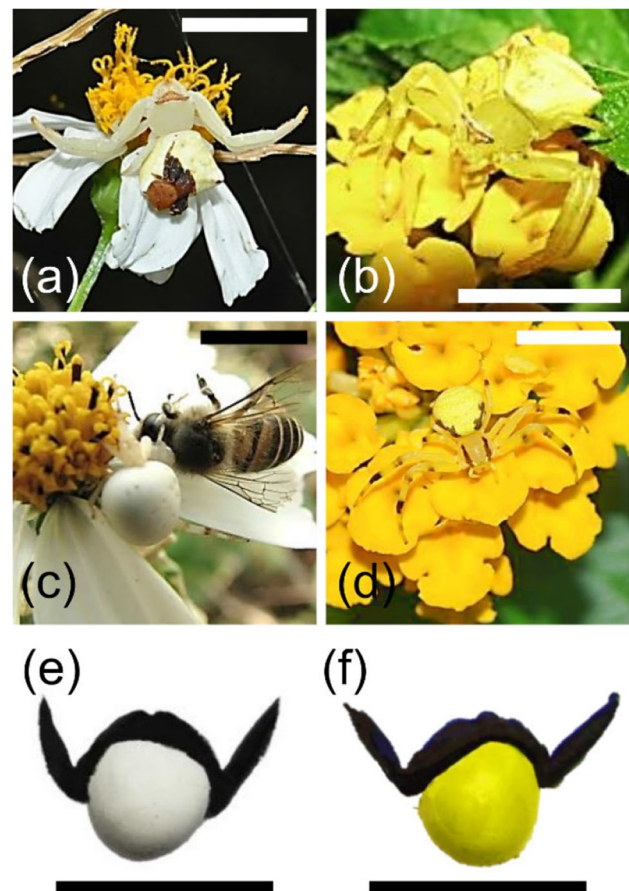
### Spiders and study site

We monitored the activities of *T. labefactus* and *E. tricuspisidatus* across two subsites within Daken Nature Park, Taichung

City, Taiwan (E : 120°47'01.79E, N : 24°10'21.97E): (i) Butterfly Ecological Education Park and (ii) Lienkeng Lane Reserve. We used these two subsites and combined the data to represent the entire area as the Park itself was too large to sample in its entirety (see Royle and Nichols 2003). These two crab spider species can readily change their body coloration between yellow and white (Fig. 1a–e) and, while not yet experimentally verified, they are thought to do it primarily to color-match the flowers they happen to occupy (Su et al. 2020).

### Temporal activity survey

To monitor the activities of the two species of crab spiders during the day and night, we conducted a survey at our sites every 2 h over a 72-h period. We conducted the surveys by walking along the public paths at each site. Nighttime surveys were conducted using small headlamps. Each spider seen was captured and its body length measured on site



**Fig. 1** White and yellow crab spider species *Thomisus labefactus* (a, b) and *Ebrechtella tricuspisidatus* (c, d) color morphs; and two types of dummies (e, f) used in this study. Width of black/white band indicates 1 cm

using digital callipers. We marked the flowers on which spiders were found using individually labelled scotch tape onto which we recorded the flower's color, the spider's color, and its body length. We considered all monitoring conducted between 7:00 and 17:00 to be daytime sampling and all monitoring conducted between 19:00 and 05:00 to be nighttime sampling.

### Field manipulation experiment

We followed our surveys up over the 3 days that proceeded it with a manipulative experiment, at the same sites as the temporal activity survey, using spider-like dummies. Our experiment investigated whether white and yellow spider color morphs are likely to attract predators and/or prey when they are color-matched to their floral background. Accordingly, we constructed dummies that visually resembled, to a human, *E. tricuspidatus* white and yellow color morphs using a white resin clay (KID, Taipei, Taiwan), yellow (No. 4502 Zitrongelb lemon yellow acryl paint, LUKAS, Düsseldorf, Germany, and No. 427 Permanent yellow light water color, ShinHan Art Materials Inc., Seoul, Korea) and black (No. 28 black poster color, Pentel Co., Ltd., Taipei, Taiwan) paints, and an adhesive (white pulp adhesive, Magic Doh, Taipei, Taiwan). The dummy's forelegs and prosoma were painted black (see Fig. 1e, f) because our color contrasts measurements (see the “[Measuring reflectance spectra of dummies and flowers](#)” section for methodological details) found an extremely low and flat reflectance spectra for the spider legs and prosoma. We therefore expected them to better resemble black paint to any viewing insect than any of the other colors of paint we had available (see Supplemental Information, Fig. S1 and Fig. S2).

Our comparisons of the reflectance spectra from the white and yellow clay and paint and corresponding spider bodies found that the chromatic and achromatic contrasts of the dummy bodies resembled but did not exactly match those of the corresponding spider bodies when viewed by the model insects (Fig. S2). Reasons for this might include the spiders starting to change their color prior to us measuring their spectra, perhaps as a consequence of handling and/or changing of their environment. Given this, we refrained from making any further direct comparisons between the spider's body and dummy coloration. Moreover, our objective of the survey was to ascertain whether spiders in the field selected flowers that color-matched their bodies, while the dummy experiment was done exclusively to experimentally differentiate between cryptic and other potential explanations for the polymorphism, so it was not absolutely necessary to attain a precise color-match between the spiders and dummies.

Our experiment involved two treatment groups ( $n = 30$  per color morph): “dummy present” or “dummy absent.” For the “dummy present” treatment group, we fixed a

dummy to a flower that was similar in color to its size (diameter = 0.35 mm) using black insect pins. Being black, thus spectrally flat and close to zero reflectance throughout, the pins did not have any influence on the visibility of the dummies to any of the spider's predators or prey. To create the “dummy absent” treatment group, we fixed a dummy to the ground beside a flower but out of sight of flying predators or prey. This was done to control for any odor given off by the clay or paint used near the flower. Since the objective of the survey was to determine what, if any, kind of crypsis was utilized, we did not consider it pertinent to place any dummies on flowers whose colors differed from that of their body. We placed infrared video cameras (CX700 HDD, Sony Asia Pacific, Taipei, Taiwan) ~1–2 m perpendicular from each experimental flower and monitored them for prey and predator visits during daytime (i.e., between 08:00 and 12:00) and nighttime (i.e., between 20:00 and 00:00) monitoring sessions. Only when we recorded more than 2 h of video footage was a particular monitoring session included in our subsequent analyses. Dummies were preferred for these experiments over dead or preserved spider bodies, as the dummies could be postured in the exact same way as a foraging live spider (i.e., on a flower with forelegs extended). Furthermore, the chemicals used to preserve dead specimens will change their color and/or add an odor (Rowland 1979).

The taxonomic order of all insects seen interacting with the dummies was recorded, whenever feasible. To compare predator and prey attraction rates across color morphs and treatments, we recorded only those insects with behaviors that indicated they were attracted to a dummy or flower, or both. Accordingly, we recorded all instances of insect scanning behaviors (i.e., the characteristic side-to-side movements indicating an insect is visually evaluating a dummy/flower; Dafni and Kevan 1996; Yeh et al. 2015) observed. We also recorded any instances of insects landing on flowers. These behaviors were easily distinguished from other behaviors such as random flying or hovering near the flowers. All insects that were identified to be scanning dummies/flowers were considered predators, as they were primarily wasps. All insects identified as landing on flowers were considered prey and were primarily bees and flies by day, and moths by night.

### Measuring reflectance spectra of dummies and flowers

To ascribe defensive or offensive crypsis or the exploitation of attractive flower coloration to one or both color morphs, it was necessary to determine the visibility of the dummies against their floral-matched background when viewed by different insects (White et al. 2015; Olsson et al. 2018). We thus used a reflectance probe (Ocean Optics QR-400–7-UV–VIS) connected to a pulsed deuterium-tungsten



halogen light source (DT1000, Ocean Optics, Inc.) and USB4000 spectrometer (Ocean Optics, Inc., Dunedin, FL, USA) in the laboratory at Tunghai University, Taichung, Taiwan, to measure the reflectance spectra of randomly selected white and yellow color morphs of each species of spiders from the two subsites ( $n = 30$  for each species; 15 from each site), as well as a sample of 15 yellow (*Lantana camara*) and 15 white (*Biden pilosa*) flower petals from the two subsites, along with all of the materials used to make the white and yellow spider dummies. The spiders were anesthetized using CO<sub>2</sub> and spectra were ascertained by scanning a 2 mm<sup>2</sup> area of randomly selected subsections of the dummy's "abdominal" dorsum using the spectrometer. The relative angle of the objects to the collection probe was 90°. The integration time was set to vary with the measurements depending on the reflection rate of the highest peaks (fixed to an intensity of 60,000) and the spectra generated was averaged to 1.0. The spectrometer was calibrated to its minimum (0% reflectance) and maximum (100% reflectance) settings using Labsphere certified white and black reflectance standards. For additional technical details of our machinery and procedures, see Blamires et al. (2014), Yeh et al. (2015), and Tso et al. (2016). For details pertaining to the setup of the reflectance probe and photorefectance measurements, see Blamires et al. (2020).

We used the reflectance spectra derived above and shown in Fig. S2 to develop five insect neuroethological color vision models to quantify whether the yellow and white dummies were visible to potential predators and prey when occupying yellow or white flowers, respectively, by day and/or night. The insects chosen for the visual modelling were the (i) Japanese yellow swallowtail butterfly (*Papilio xuthus*; Koshitaka et al. 2008); (ii) fruit fly (*Drosophila*; Yamaguchi et al. 2010); (iii) honeybee (*Apis mellifera*; Hempel de Ibarra et al. 2014); and (iv) tiger moth (*Arctia plantaginis*; Henze et al. 2018) (Table 1). These insects were chosen for visual modelling because they represent relatively common potential prey for *T. labefactus* and *E. tricuspidatus* at our

study site. The model derived for honeybees was also used to represent potential prey (i.e., wasps), as was done elsewhere in similar studies (e.g., Yeh et al. 2015). The procedures used follow those described by Warrant and Nilsson 1998, Vorobyev et al. (1998), Johnsen et al. (2006), and Liao et al. (2019) (see the Supplemental Methods (see the Supplemental Methods within the online Supplemental Information for a description of the entire modelling processes, including the calculations and assumptions made about the photoreceptor sensitivities for each model insect).

## Statistical analysis

For the temporal activity survey, we calculated detection probabilities as the metric to estimate the occupancy rate of the white and yellow spider of each species (Welsh et al. 2013). We used this metric because the area we sampled over was extremely large, so the chance of repeatedly encountering individually marked spiders of each species and color was low (Royle and Nichols 2003). Additionally, we sampled different numbers of yellow and white flowers over the sampling period, and the amount of time spent sampling during the day and night was unequal. We used mixed-effect log-linear models with binomial error distributions to compare the detection probabilities between species across the day and night. The model included one fixed factor, which combined spider species (*T. labefactus* or *E. tricuspidatus*), spider color (white or yellow), flower color (white or yellow), and observation time (daytime or nighttime). We also included two random factors: (1) the date of the daytime/nighttime survey and (2) a combination of spider species, spider color, and flower color, nested within day or night. The first random factor accounted for the interaction between sampling date and daytime and nighttime sampling, and was recorded as follows: "1st daytime," "1st nighttime," "2nd daytime," "2nd nighttime," and so on for the duration of the survey. The second random factor accounted for

**Table 1** Absorbance spectra parameters used in color contrast calculations. Number in parentheses is the ratio of receptor number  $n_i$  as used in Eq. (4)

Model	Chromatic absorbance spectra				Achromatic absorbance spectra	Reference
	$i=1$	$i=2$	$i=3$	$i=4$	$i=a$	
Diurnal butterfly ( <i>Papilio xuthus</i> )	UV (0.11)	NB (0.11)	DG (0.22)	R (0.56)	R (0.56)	Koshitaka et al. 2008
Diurnal fruit fly ( <i>Drosophila</i> )	R7p (0.16)	R7y (0.16)	R8p (0.34)	R8y (0.34)	R1-6 (0.1)	Yamaguchi et al. 2010
Diurnal honey bee ( <i>Apis mellifera</i> )	UVS (0.17)	SWS (0.08)	MWS (0.75)	—	MWS (0.75)	Hempel de Ibarra et al. 2014
Diurnal tiger moth ( <i>Arctia plantaginis</i> )	UV (0.11)	Blue (0.11)	Green (0.78)	—	Green (0.78)	Henze et al. 2018
Nocturnal hawkmoth ( <i>Deilephila elpenor</i> )	UVS (0.11)	SWS (0.11)	MWS (0.78)	—	MWS along (0.78)	Johnsen et al. 2006

the interactions between spider species, spider color, and flower color, within the first random factor. For instance, “species A-white morph-white Flower-1<sup>st</sup> Daytime” and so on. Including these random factors allowed us to account for any intrinsic variation across time periods (due to weather, temperature, wind speed, or other factors), and to cope with any pseudo-replication due to repeated measurements. After fitting our model, we performed comparisons among all levels of the fixed factors and adjusted their *p*-values using a Benjamini–Hochberg procedure to control for a false discovery rate.

For the manipulation experiment, we used a mixed-effect Poisson model to fit prey attraction rates and predator scanning rates of the dummies. The fixed effects were dummy type (yellow, white, or absent) and flower color. Random intercepts included were (1) the observed date and time when fitting present/absent data and (2) the spider/dummy identity when fitting prey attraction rates and predator scan rates. Natural log-transformed observation time (hours) was also included as an offset term in the Poisson models. We used linear models to fit the color contrasts for the various comparisons. All of the models were fitted using Markov chain Monte Carlo (MCMC) simulations using the R package “brms” (ver. 2.12.0, Bürkner 2017). We assigned informative priors (Student-*t* with *df*=7) for main effect and flat priors (5-scaled Student-*t* with *df*=3) for the intercept and standard deviation of random effects and residuals. Only if the highest density interval (HDI) of a posterior distribution completely fell outside the region of practical equivalence (ROPE =  $1.0 \pm 0.1$  event rate) was it considered as significant (Kruschke and Liddell 2018). We compared the prey compositions among dummy and flowers of different color using a permutational  $\chi^2$  homogeneity test and adjusted the *p*-values of pairwise comparisons using the Benjamini–Hochberg method to control the false discovery rate. We used generalized linear models with Poisson

error distributions to compare the diurnal and nocturnal prey attraction rates and diurnal and nocturnal predator scanning rates across dummy color morphs and treatments.

## Results

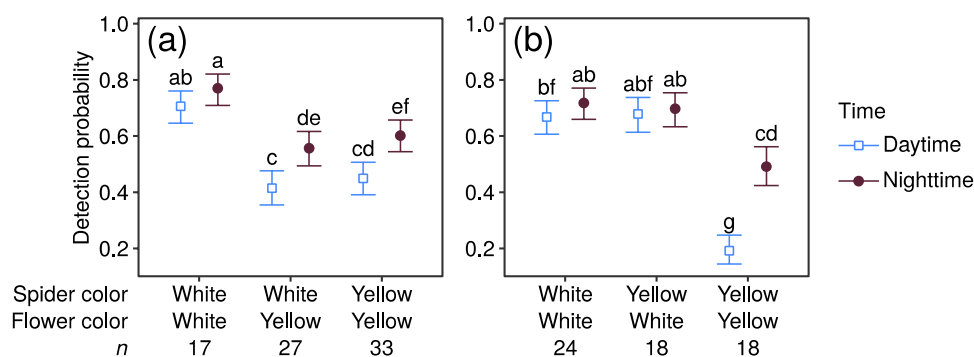
### Temporal activity survey

We found the detection probabilities for both species to be greater during nighttime surveys than during daytime surveys. Furthermore, the detection probabilities of the two species of crab spiders differed when on different colored flowers. Contrastingly, spiders of either color morph were more likely detected on yellow flowers during the nighttime than during the daytime (Fig. 2, see Table S1 of the Supplemental Information for raw statistics). We considered this result to indicate that, contrary to our initial expectations, crypsis was not the reason that either of the spider’s change color in the field. The following combinations were too few (<5) to include in our analyses: (i) yellow *E. tricuspidatus* on white flowers and (ii) white *T. labefactus* on yellow flowers.

### Field manipulative experiment

We recorded a total 731.2 h of video footage across the daytime (347.5 h: 93.5 h for the white flowers/dummy present, 83.5 h for the white flowers/dummy absent, 86.3 h for the yellow flowers/dummy present, 84.2 h for the yellow flowers/dummy absent treatments) and nighttime (383.7 h: 103.7 h for the white flowers/dummy present, 90.8 h for the white flowers/dummy absent, 101.8 h for the yellow flowers/dummy present, 87.3 h for the yellow flowers/dummy absent treatments).

Wasps were the predominant predator identified, which were observed scanning dummies during the day. Butterflies,



**Fig. 2** Spider detection probabilities for *E. tricuspidatus* (a) and *T. labefactus* (b) color morphs when inhabiting different colored flowers. Circles/triangles and whiskers indicate the posterior means and the 95% highest density intervals, respectively. Letters above whisk-

ers indicate the ranks of multiple comparisons. *n* indicates the number of spiders monitored. Observations were conducted every 2 h over 72 h

moths, honeybees, and flies were the primary prey identified by day and night. During the day, the composition of the prey that were attracted to the yellow flowers were significantly different to those attracted to white flowers, with more butterflies, fewer bees, and fewer flies (Supplemental Information, Fig. S3a). Contrastingly, the insects attracted to the various dummy/flower colors were relatively similar at night, when more than 90% of the prey attracted were moths (Fig. S3b).

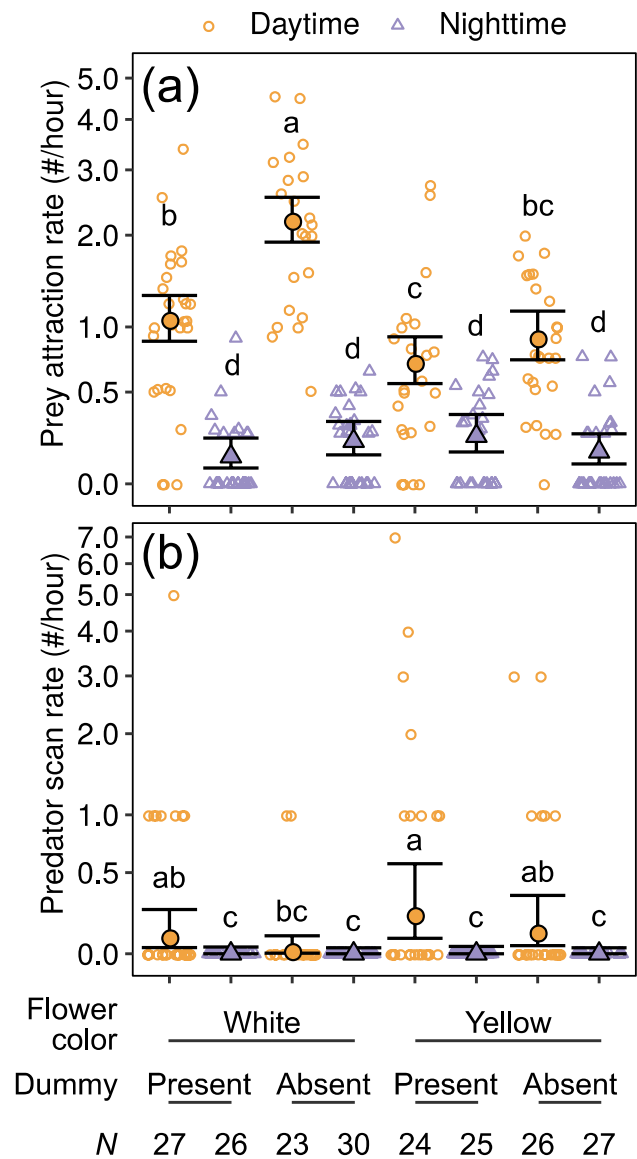
During the day, the prey attraction rates for the “dummy absent” treatment were greater than those of the “dummy present” treatment, with butterflies, bees, and flies (dipterans) representing the insects predominantly attracted. The white flowers were more frequently visited by prey than were yellow flowers at this time. The presence of dummies had an effect of reducing the prey attraction rates for both white and yellow flowers (Fig. 3a, Table S2a). At night, by contrast, there were no significant differences in prey attraction rates between the white and yellow flowers nor between the “dummy absent” and “dummy present” treatments. Flower scanning rates by predators were much sparser during the night compared to during the day, with yellow flowers appearing to be preferred over white ones. The scanning rates of flowers containing a dummy were nevertheless not significantly different from those without a dummy across the color morphs at night (Fig. 3b, Table S2b).

Our visual modelling (Fig. 4) found that all of the diurnal prey (i.e., butterflies, flies, honeybees, and moths) and predators (i.e., bee/wasps) for whom visual models were performed could distinguish between the dummies and their color-matched floral backgrounds (i.e., chromatic and achromatic JND > 1 for all models) with the exception of butterflies viewing white dummies during the day. We additionally found nocturnal moths to be able to distinguish the yellow and white dummies from yellow and white floral backgrounds respectively (chromatic and achromatic JND > 1, Fig. 4). These findings lend further support to our conclusion that crypsis cannot explain why the spider’s change color in the field.

### Discussion

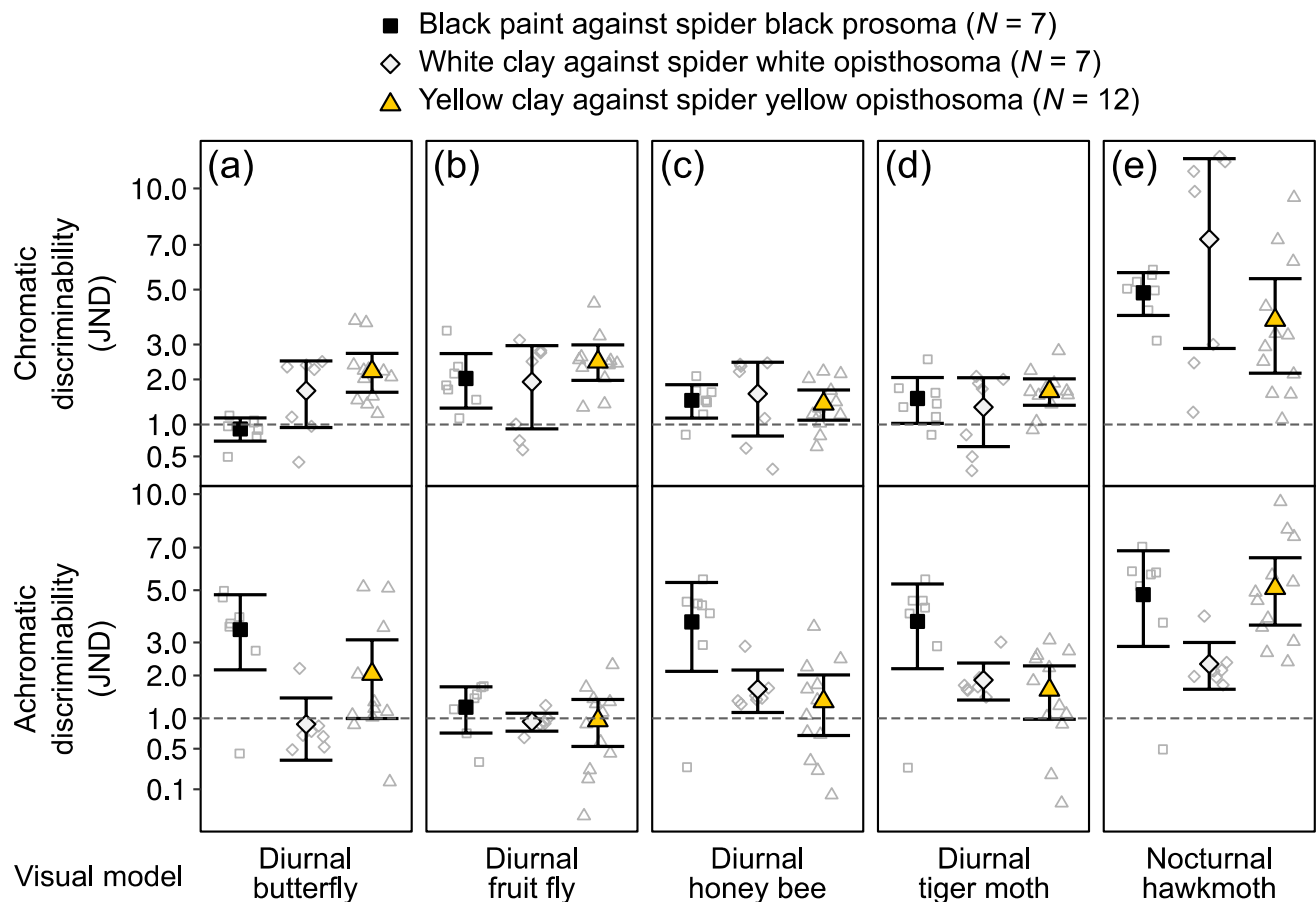
Despite many recent in-depth investigations, our understanding of the factors shaping crab spider color polymorphisms, or any animal polymorphism for that matter (Mitchell-Olds et al. 2007), remains relatively poor (Brechtbühl et al. 2010). This is probably because the visual abilities of, and interactions with, their predators and prey during the day and night across color morphs had not been thoroughly investigated, prior to this study.

We comprehensively surveyed herein the daytime and nighttime activities of two species of yellow and white



**Fig. 3** Prey attraction rates (a) and predator scan rates (b) for the dummy present/absent treatments when located on yellow or white flowers. Solid circles/triangles and whiskers indicate the posterior means and the 95% highest density intervals, respectively. Letters above whiskers indicate the ranks of the multiple comparisons

polymorphic crab spiders from Taiwan and found that the spiders did not seem to choose to occupy flowers that were the same color as their bodies across different times of day. We did not expect this to be the case as previous surveys have suggested that the spiders occupy flowers matched to their bodies (Su et al. 2020). We, nevertheless, rarely observed a yellow *E. tricuspidatus* on a white flower, or a white *T. labefactus* on a yellow flower. These observations may have come about because of the logistic difficulties we faced in surveying the area comprehensively. We nonetheless found that white *E. tricuspidatus* occupied



**Fig. 4** Chromatic and achromatic discriminability (posterior mean  $\pm$  95% highest density interval) of colors on dummies against corresponding colors on spiders when viewed by butterfly (a), fruit fly (b), honey bee (c), and tiger moth (d) during the daytime and by

hawkmoth at nighttime (e). The dashed line represents the theoretical discrimination threshold value (JND=1). 95% HDI completely greater than the threshold indicates significantly distinguishable

yellow flowers as frequently as yellow *E. tricuspoidatus*, and yellow *T. labefactus* occupied white flowers as frequently as white *T. labefactus*. We, according to our criterion set out in the “Introduction,” concluded that crypsis, whether it be defensive and/or offensive, cannot explain why either *T. labefactus* or *E. tricuspoidatus* changed color in the field. Our findings compel us to speculate that these spiders must exploit certain flowers, regardless of their color, because they are either attractive to their prey or unattractive to their predators, or both.

Since we observed that placing a spider dummy onto either a yellow or white flower by day had the effect of reducing visitation rates of the spider’s prey to flowers, and that our visual models predicted that butterflies, flies, bees, and moths could distinguish between the dummies and their floral backgrounds, it seems that the “spider’s” bodies may act as a deterrent to prey during the day. The presence of a spider dummy, nevertheless, did not affect the prey attraction toward flowers of any color at night. At night, moths could visibly distinguish the “spiders” from their floral

background, even if the “spiders” occupied a flower that was the same color as its body. Our results thus support Llandres and Rodríguez-Gironés (2011) in concluding that factors other than crypsis better explain polymorphic coloration in the crab spiders. We expect the “exploitation of flower coloration” explains the crab spider’s dimorphic coloration by night, while the explanation of “predator and/or prey indifference” fits observations made during the day.

We considered our most profound finding to be the substantially different outcomes for our daytime and nighttime experiments and models. Both color morphs of each species were more active at night than during the day, particularly the yellow morph *T. labefactus*. The white morphs of the two species differed in their flower preferences by day and night with the white *E. tricuspoidatus* occupying flowers that color-matched their bodies at all times, while white *T. labefactus* was content to occupy any flower. As stated above, these observations are inconsistent with defensive or offensive crypsis explaining the existence of crab spider color polymorphisms. Our photorefectance measurements



from the dummies and subsequent modelling of insect visual sensitivities revealed that the dummies were distinguishable by most of the main predators or prey encountered when their bodies were color-matched to the floral background during the day.

We concede that issues with our methodologies, including the difficulty associated with sampling the area and the underlying assumptions made when deriving visual models from reflectance spectra (both of which we address below), give us reason to be cautious about making any definitive statements about the true causes and effects of polymorphic color change in the crab spiders studied here. Our results, nevertheless, unequivocally indicate that the utilization of the different color polymorphs is highly context dependent.

We used a large, mountainous, urban nature park for our field survey. Because of the size and ruggedness of the park, the area was divided into two subsites, which itself may be problematic for making reliable occupancy estimates of animals (Royle and Nichols 2003; Welsh et al. 2013; Albano et al. 2015). We estimated “detection probabilities” as the metric of spider occupancy rates (Welsh et al. 2013) over the area and across species and color morphs. This metric can be useful when the area is difficult to sample, the animals are rare, or when the probability of sampling animals with given features are unknown over time and/or space (McKenzie et al. 2002), such was the case here with the night and day encounter rates of the yellow or white crab spider morphs, as well as the flower they occupied. The accuracy of estimating detection probabilities, nevertheless, depends on us reliably re-identifying all of the marked individuals (McKenzie et al. 2002; Royle and Nichols 2003). We marked the flowers as opposed to the spider to prevent interference with the spiders, as this itself can induce crab spiders to change color (Herberstein and Gawryszewski 2013). However, we cannot be sure that no individuals were misidentified as present when they were absent, or absent when they were indeed present, on any given survey. Nor can we be sure that all spiders present within the subsites were always observed. We also do not know how factors such as migration and death rates from the populations might have influenced our detection probability estimates.

Assumptions made in deriving our visual models included one that the Weber fraction estimate was approximately equal to 0.5 across all of the insects for which models were performed (Vorobyev et al. 1998). We nonetheless cannot be certain, without verification, that this holds across all species of insect by both day and night (Olsson et al. 2018; Perna et al. 2019). Moreover, the receptor noise parameter has only been estimated for a few primates, a fish, some birds, and honeybees (Perna et al. 2019). Thus, the JND values that we calculated for flies, butterflies, and moths are arbitrary and the subsequent color contrast thresholds ascertained may not be applicable across situations. Additionally, the spider

dummies we created did not exactly color-match the crab spiders they were made to represent (at least not the crab spiders we capture to measure spectra on), so we cannot directly apply all of the results attained for the dummies to real crab spiders.

While these issues mean that ascribing true causes and effects of polymorphic color change in the crab spiders studied here is problematic, our data does lend itself to some definitive conclusions about the contexts and implications of using yellow and white color morphs by crab spiders. Firstly, as explained above, our field survey suggested that the spiders do not seem to be color-matching their bodies with their floral background. This accordingly rules out crypsis as an explanation for polymorphic color changes in either species. Secondly, our experiment suggested that there is a degree of variation in the attractiveness, relative to that of the background, of the different spider body colors to prey and predators depending on the time of day and the viewing insects.

Such context dependency of predator and prey interactions probably explains why different explanations of how and why crab spiders utilize their different color morphs have gained some degree of support among different studies (see Heiling et al. 2005; Herberstein et al. 2009; Brechbühl et al. 2010; Defrize et al. 2010; Llandres et al. 2011). It also suggests that that the various explanations are not mutually exclusive. Indeed, they are likely to be interactive across contexts. Moreover, their influences on crab spider polymorphisms are not likely to be static but dynamic over time and space.

Most studies examining crab spider polymorphisms to date have investigated the responses of single prey types toward different spider morphs under daylight illumination. Studies examining interactions with predators have nevertheless predominantly modelled the interactions of crab spiders with birds, such as blue tits (Théry and Casas 2002; Heiling et al. 2005; Théry et al. 2005; Herberstein et al. 2009; Defrize et al. 2010). We found that the yellow color morphs of *T. labefactus* and *E. tricuspidatus* were more active at night than their white color morphs by night or day. Our subsequent experiment using spider dummies found that each morph likely interacted with different prey across both night and day (see Fig. S2). Accordingly, the individual color preferences and behavioral biases of different insects appear to influence the types of interactions each of the color morphs will have with their predators and prey by day and night.

Moths were the predominant prey encountered at night. Moths are much larger prey than bees or flies (the predominant prey encountered in the daytime), so capturing and consuming them, even if rare, is much more profitable for the spiders than capturing and consuming bees or flies (Blamires et al. 2014; Willemart and Lacava 2017). We accordingly expect that nocturnal interactions with prey were more influential over the use of color polymorphisms

in the two species of crab spider examined than were any diurnal interactions. This expectation nonetheless requires further empirical support.

Understanding the evolutionary and environmental drivers of individual animal polymorphisms represents a persistent challenge for evolutionary biologists. We have shown here that by examining the predator and prey interactions of yellow and white color morphs of two species of crab spiders during the day and night, the influences driving crab spider polymorphisms are dynamic and vary across temporal contexts. Accordingly, we expect that any outcomes of experiments examining the adaptive mechanisms driving certain polymorphisms are likely to be dependent upon the contexts under which the various observations were made (Karpestam et al. 2016). We thus recommend that ongoing studies testing hypotheses about the adaptive value of animal polymorphisms take both top-down and bottom-up interactions into account across spatial and temporal contexts.

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**Author contribution** Y.-C.L. participated in designing the study, performed the experiments, and carried out data analysis. C.-P.L. and S.J.B. participated in designing the study, performed data analysis, and drafted the manuscript. I.-M.T. designed the study, provided resources, and helped with the manuscript. All authors gave final approval for publication.

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**Data availability** Original data is deposited as supplementary material associated with this manuscript.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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