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RESEARCH ARTICLE

Relationship between colouration and body condition in a crab spider that lures pollinators

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SUMMARY

Sit-and-wait predators have evolved several traits that increase the probability of encountering prey, including lures that attract prey. Although most crab spiders (Thomisidae) are known by their ability to change colour in order to match the background, a few use a different strategy. They are UV-reflective, creating a colour contrast against UV-absorbing flowers that is attractive for pollinators. The nature of the relationship between colour contrast and foraging success is unknown, as is how spiders trade off the potential costs and benefits of strong colour contrast. Therefore, this study investigated the relationship between spider colouration, foraging success and background colouration in a crab spider species known to lure pollinators *via* UV reflectance (*Thomisus spectabilis*). Field data revealed that spider body condition – a proxy of past foraging success – is positively related to overall colour contrast. We experimentally tested the effect of satiation and background colour on spider colour change. Throughout the experiment, spiders changed their colour contrast regardless of their food intake, suggesting that colour contrast and the UV component contributing to overall contrast are not caused by spider condition. Although spiders responded to different backgrounds by subtly changing their body colour, this did not result in colour matching. We believe that the observed variation in colour contrast and hence conspicuousness in the field, coupled with the spiders' reaction to our manipulation, could be the result of plasticity in response to prey.

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Key words: colour signal, Thomisidae, *Thomisus*, UV, ultraviolet, predator–prey.

INTRODUCTION

Even though sit-and-wait predators do not actively search for their food, several strategies have evolved that may increase their probability of capturing prey. Examples include the selection of profitable patches (Janetos, 2004; Metcalfe et al., 1997; Scharf and Ovadia, 2006), displaying cryptic and disruptive colouration to avoid detection by prey (Cott, 1957), and the building of traps (Scharf and Ovadia, 2006; Shear, 2004). In addition to these somewhat passive strategies, many sit-and-wait predators employ tactics that actively attract their prey. Prey attraction evolved in many different taxa and often exploits prey signals used in sexual interactions or when searching for food. For instance, the bolas spider *Mastophora dizzydeani* releases a chemical that mimics the pheromones of female moths, which attracts male moths of the species *Spodoptera frugiperda* to its sticky ball trap (Eberhard, 1977; Eberhard, 1980). Exploiting a non-sexual response, the common death adder, *Acanthophis antarcticus*, captures lizards by waving a conspicuous worm-like caudal lure that incites a predatory response in their prey (Nelson et al., 2010).

Several species of crab spiders (Thomisidae) are sit-and-wait predators that exploit the interaction between plants and insects by sitting on flowers to ambush pollinating insects (Morse, 2007). Their colouration usually resembles the colour of the flowers they are sitting on (Chittka, 2001; Morse, 2007; Thery, 2005; Thery and Casas, 2002). Furthermore, crab spiders, such as the European *Misumena vatia* and *Thomisus onustus*, can adjust their body colour to match the flower colour background, which makes them less detectable by prey such as honeybees (Gabritschevsky, 1927; Packard, 1905; Thery and Casas, 2002; Thery, 2005; Thery, 2007).

However, camouflage is not the only strategy used by crab spiders. Some crab spiders seem to be highly conspicuous to their prey, rather than blend into the background. These spiders are UV-reflective (they appear white to humans), which, when viewed through honeybee eyes, creates a strong contrast against the UV-absorbing flower (Heiling et al., 2005). Instead of deterring prey by increasing their visibility, these spiders are attractive to pollinators (Heiling et al., 2003), a similar strategy used by some orb-web spiders (Herberstein and Wignall, 2011). When honeybees had to choose between flowers with and without the contrasting UV-reflective Australian crab spider *T. spectabilis*, they were more likely to land on flowers occupied by their predators (Heiling et al., 2003). When the same experiment was performed with the non-UV-reflective European *M. vatia*, the honeybees chose flowers at random irrespective of whether they were occupied by a spider (Herberstein et al., 2009). Moreover, when the level of UV reflected by *T. spectabilis* was experimentally eliminated, honeybees actively avoided flowers occupied by crab spiders (Heiling et al., 2005).

Despite the apparent benefit of UV reflectance for Australian crab spiders, there is a high level of intra- and inter-individual variation

in the amount of UV reflected and hence colour contrast by spiders (Llandres et al., 2011). The overall aim of this study was to explain how some of this variation might be generated. First, we aimed to quantify the variation in colour contrast in field-captured spiders and relate this to their recent foraging success. Based on the use of colour to attract prey, we predicted that individuals that reflect more UV light and, consequently, formed a stronger colour contrast against a UV-absorbing flower background would capture more prey and, as a result, would be in a better condition than individuals that reflected less UV light. Such data may indicate the strength of a relationship between two variables, but they do not indicate causality. Our second aim was to use a manipulative experiment to understand the causality of colour contrast in these crab spiders. Only manipulating the feeding level of spiders can reveal whether it is satiation that causes colour contrast or *vice versa*. *Thomisus spectabilis* can attract pollinators based on the strength of its colour contrast (*via* UV reflectance), and thus we expect food-limited spiders to increase contrast and attract more prey. However, if colour contrast is the result rather than the cause of recent foraging success, only food-satiated spiders should be able to increase contrast. If greater visibility, however, entails costs, such as greater risks of predation, we expect satiated spiders to reduce the contrast. In addition to adjusting their colour in response to their satiation level, we also expect crab spiders to adjust their colouration according to the background they are sitting on, as this will affect their visibility to approaching prey.

Here we addressed these questions by analysing the relationship between the colouration of the spider *T. spectabilis* and their condition using field data from two years and by testing in the laboratory how this predator responded to different feeding regimes and background colours. We calculated how spiders are perceived by the visual system of the crab spider's prey, the honeybee *Apis mellifera*, and used data from reflectance spectra independent of any visual model.

MATERIALS AND METHODS

Reflectance spectra measurements

To measure the reflectance spectra of organisms and objects, we used an optical fibre probe (Ocean Optics Inc., Dunedin, FL, USA) connected to a spectrometer (USB2000, Ocean Optics Inc.) and a light source (PX-2 light source, Ocean Optics Inc.). The probe was positioned at 45 deg above the samples. The reference spectrum was taken using the WS-1 Diffuse Reflectance Standard (Ocean Optics Inc.; >98% reflectance from 250 to 1500 nm). The dark spectrum was taken from the black velvet used as background to the measurements. We took five spectral measurements from each organism and object.

Calculation of honeybee photoreceptor excitation, chromatic and achromatic contrasts and mid-point wavelength

We evaluated how the spiders (*Thomisus spectabilis* Doleschall 1859), flowers and objects are perceived by potential prey, the honeybee *Apis mellifera* Linnaeus 1758, by calculating photoreceptor excitations and colour contrasts using the colour hexagon model (Chittka, 1992; Chittka, 1996). First, the relative quantum catch of each bee photoreceptor, P, was calculated by:

$$P = R\int_{300}^{700} I_S(\lambda)S(\lambda)D(\lambda)\mathrm{d}\lambda\ , \quad (1)$$

where $I_S(\lambda)$ is the reflectance calculated from the spiders and flowers, $S(\lambda)$ is the spectral sensitivity function of each bee photoreceptor, $D(\lambda)$ is the illuminant spectrum CIE D65 [daylight illumination as defined by the International Commission on Illumination (Chittka and Kevan 2005)], and R is the sensitivity factor, calculated by:

$$R = \frac{1}{\int_{300}^{700} I_B(\lambda)S(\lambda)D(\lambda)\mathrm{d}\lambda}\ , \quad (2)$$

where $I_B(\lambda)$ is the reflectance of the environmental background. For the environmental background, we used the leaf spectrum provided in the literature (Chittka and Kevan 2005).

The excitation of each bee photoreceptor – E_{UV}, E_{Blue} and E_{Green} – was calculated from the relative quantum catch of the photoreceptors, P:

$$E = \frac{P}{P+1}\ . \quad (3)$$

E_{UV}, E_{Blue} and E_{Green} for each spider and flower were calculated using the mean of the excitation values calculated from the five reflectance spectra taken for each spider and flower.

These values were used to calculate coordinates in the bee colour hexagon (Chittka, 1996; Chittka et al., 1992):

$$x = \sin 60°(E_{Green} - E_{UV})\,, \quad (4)$$

$$y = E_{Blue} - 0.5(E_{Green} + E_{UV})\,. \quad (5)$$

Then, the colour contrast was calculated by the Euclidian distance between the spiders and the flower in the colour hexagon:

$$\Delta St = \sqrt{(x_{spider} - x_{flower})^2 + (y_{spider} - y_{flower})^2}\ , \quad (6)$$

where x and y are the coordinates of the hexagon calculated by Eqns 4 and 5, respectively. We also calculated the colour contrast of spiders in relation to the origin of the colour hexagon (x=0 and y=0) by the following equation:

$$\Delta S_{origin} = \sqrt{x_{spider}^2 + y_{spider}^2}\ . \quad (7)$$

In the origin of the colour space lie colours similar to the background that photoreceptors are assumed to be adapted to. In the case of the honeybee colour space, the background is a leaf. Spectra that evenly stimulate all photoreceptors, such as a flat line between 300 and 700 nm, also lie in the centre of the colour space. Bees use only the green photoreceptor to evaluate the achromatic contrast (Giurfa et al., 1997). Therefore, we also calculated the achromatic contrast of spiders against the flower by subtracting the flower's E_{Green} from the spider's E_{Green}.

In addition to using a visual model to evaluate colouration of spiders from the perspective of bees, we calculated the mid-point wavelength of spider reflectance spectra. Midpoint wavelength was calculated by finding the wavelength with the reflectance value equivalent to the middle point between the maximum and minimum reflectance (R_{50}):

$$R_{50} = \frac{R_{max} - R_{min}}{2}\ , \quad (8)$$

where R_{max} is the maximum reflectance and R_{min} is the minimum reflectance. Calculation of the mid-point wavelength using this method is especially useful for detecting shifts in the position of a sigmoidal curve, as in the case of crab spider spectra. This calculation complements the calculation of photoreceptors excitation by providing a parameter of spider reflectance independent of any visual model.

Variation in spider colouration

We collected female *Thomisus spectabilis* Doleschall, 1859 (Thomisidae) spiders sitting on white daisies *Bidens alba* var. *radiata* (Asteraceae) at Airlie Beach, Queensland, Australia, in May 2009 (N=42). We also used previously published data (reflectance spectra) from *T. spectabilis* spiders collected from the same site and flowers in April 2008 (N=67) (Llandres et al., 2011). In the laboratory we weighed the spiders and measured the tibia–patella length of their first leg. Spiders whose tibia–patella length did not exceed 2.00 mm were not considered in the analyses because they are too small for an accurate colour measurement. We collected 13 white daisies in 2009 and used data from eight white daisies collected in 2008 (Llandres et al., 2011) from the same sites where we collected spiders. We measured the light reflectance of the dorsal side of the spider abdomen and the flowers using the methodology described above. We compared the spider colour (mid-point wavelength, colour contrast and achromatic contrast against the flower), mass and leg length, and the flower colour (honeybee photoreceptor excitation values) between years using a *t*-test. Because there was no difference in flower colour between years (supplementary material Table S1), we pooled the colour data of flowers collected in 2008 and 2009 to generate a flower model against which to calculate chromatic and achromatic contrast between spiders and flowers.

Our aim with these analyses was to understand which component of the overall colour of spiders contributes to colour contrast values and variation within. However, it is unadvisable to separate individual E values (e.g. E_{UV} and E_{Blue}) from the overall colour contrast, as the hymenopteran visual system does not process these components separately (Chittka and Wells 2004; Dyer et al., 2011). We can, however, analyse the chromatic contrast (generated by the combination of E_{UV}, E_{Blue} and E_{Green}) separately from the achromatic contrast; these contrasts are indeed processed independently by the hymenopteran visual system.

Relationship between spider colouration and spider body condition

We used residuals of the linear regression ln(mass) × ln(tibia–patella leg length) as an index of spider condition (Jakob et al., 1996). To test the relationship between spider condition and spider colouration, we generated models that could explain their relationship and selected models that best fitted the data. The year that spiders were collected was also included in models as an explanatory variable. We opted to analyse each colour parameter in separate regressions because of the collinearity of these variables.

In our model selection process we included polynomial models because exploratory analyses suggested a curvilinear relationship between spider condition and spider colour. In total, we fitted 11 linear regression models for each colour parameter, including interactions with the year that spiders were collected (2008 or 2009). In these models, spider condition was entered as the dependent variable and the colour parameter and year were entered as the explanatory variables. For each model we calculated Akaike's information criterion (AIC) and the Bayesian information criterion (BIC), and for each colour parameter we selected models with the lowest values. Both indices are calculated similarly, but BIC punishes the inclusion of new variables more heavily (Zuur et al., 2009). We also inspected the statistical significance of model coefficients for models that generated the lowest AIC and BIC values. Before fitting models and calculating higher-order polynomial variables, we centred the continuous explanatory variable – i.e. colour parameters – otherwise the slope estimation of lower-order variables becomes unreliable (Schielzeth, 2010). We visually validated models, looking for deviation from normality of residuals, heterogeneity and violation of independence (Zuur et al., 2009). Models were fitted and validated using R (R Development Core Team, 2008). BIC was calculated using the R package nlme (Pinheiro et al., 2008).

Effect of spider condition and background colouration on spider colouration

To understand how *T. spectabilis* varied their colour in response to feeding regimes, background colour and the interaction of these factors, we submitted females to two treatments in a factorial design for 30 days. Spiders were randomly placed in containers with two different colour backgrounds: UV-bright, white to human eyes, and UV-dull, yellow to human eyes. The spiders in each of the colour treatments were further subjected to one of two feeding regimes: high prey and low prey. At the beginning of the experiment, treatments had the following sample size: low feeding regime and white UV-bright containers (N=10), high feeding regime and white UV-bright containers (N=10), low feeding regime and yellow UV-dull containers (N=9) and high feeding regime and yellow UV-dull containers (N=9). However, from the second to the third measurement we lost three spiders in the high feeding/white UV-bright containers, two in the high feeding/yellow UV-dull containers and one in the low feeding/yellow UV-dull container (because of death of spiders, exclusion of spiders that laid eggs and moulted and spiders that went missing).

The low feeding regime consisted of one housefly (*Musca domestica*) 15 days after the beginning of the experiment, whereas the high feeding regime consisted of two houseflies per week during the first 2 weeks and eight houseflies per week during the last 2 weeks. The containers were made of coloured cardboard (11×11×11 cm). We calculated the E_{UV}, E_{Blue} and E_{Green} that each container colour generated on *A. mellifera* vision using the methodology described earlier (N=5 for white UV-bright and N=5 for yellow UV-dull containers). The top of the containers was covered with plastic cling wrap (Glad Wrap®), which allows the transmission of all wavelengths between 300 and 700 nm. The reflectance spectra of the colour containers are shown in supplementary material Fig. S1. The experiment was conducted in a glasshouse with controlled temperature (night: 16°C; day: 25°C; 12 h:12 h light:dark cycle) and perspex panels, which allowed the passage of all wavelengths between 300 and 700 nm (Heiling and Herberstein, 2004).

We weighed spiders, measured their first leg tibia–patella length and collected reflectance spectra of their abdomen at the start of the experiment, 15 days into the experiment and at the end of the experiment. As for the field data, we used the residuals of the linear regression log(mass) × log(tibia–patella leg length) as an index of spider condition. We calculated spider mid-point wavelength, photoreceptor excitation values and colour contrast to the origin in the *A. mellifera* hexagon colour space using the methodology described earlier. We decided to use the colour contrast to the origin of the colour space and use E_{Green} instead of the chromatic and achromatic contrasts because the chromatic and achromatic would necessarily be different between groups (at the start of the experiment spiders had similar colours but the backgrounds were different). The colour contrast to the origin and E_{Green} provide a way to estimate the change in the bee colour space that is comparable between spiders in both colour treatment groups.

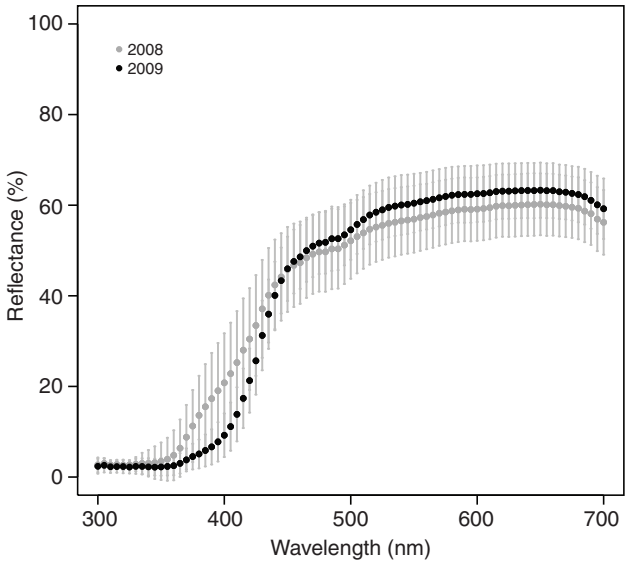

Fig. 1. Mean reflectance spectra of the crab spiders (*Thomisus spectabilis*) collected in 2008 (N=67) and 2009 (N=42). Error bars represent standard deviations.

To test the effectiveness of our feeding treatment, we first tested its effect on spider condition during the experiment. We then tested the effect of feeding treatment, background colouration and the interaction of these variables on the spiders' mid-point wavelength, E_{Green} and the colour contrast to the origin of honeybee colour space. Because of the collinearity of these variables we decided to analyse each colour parameter in separate regressions. We analysed data using a linear mixed model to take into account the unbalanced sample size and the repeated measures.

Dependent variables of the models were spider condition, mid-point wavelength, colour contrast to the origin and E_{Green}. Colour contrast and E_{Green} were Box–Cox transformed before model fitting. We first fitted, using the restricted maximum likelihood (REML), a full model with all fixed factors, interactions and a random intercept and slope for each spider during the measurements (Zuur et al., 2009). Full models started with the following set of fixed effects: (1) value of the dependent variable before the experiment, (2) day of dependent variables measurement (categorical with two values: 15 days after the beginning of the experiment and at the end of the experiment), (3) feeding treatment (categorical: low and high), (4) colour treatment (categorical: white UV-bright and yellow UV-dull) and (5) the interaction between feeding and colour treatments. In the random effects we included: (1) spider individuals (random intercept) and (2) day of dependent variables measurement (random slope). To select the model with the best random term, we compared the full model with a model with the same fixed effects but with only the intercept in the random effects. We selected the best model based on the likelihood ratio. We then proceeded to find the optimal fixed effect structure. We fitted a series of nested models by removing, one by one, model fixed effects, but fitting models using maximum likelihood (ML) instead of REML. We then compared models using AIC and BIC and inspected t-statistics of model's coefficients. We validated models looking for deviation from normality of residuals, heterogeneity and violation of independence (Zuur et al., 2009). Models were fitted and validated using R (R Development Core Team, 2008). Linear mixed models were fitted using the R package nlme (Pinheiro et al., 2008).

RESULTS

Variation of spider colouration between years

The reflectance spectrum of field-collected *T. spectabilis* was different between years. In 2008, the spiders reflected more light between 350 and 450 nm than in 2009 (Fig. 1). As a consequence, the mid-point wavelength of spiders and spider colouration as perceived by the honeybee was also different between years (Table 1). The mid-point wavelength of spiders in 2008 was on average 13 nm lower than in 2009. The colour contrast of the spiders against the flower background was 0.09 units higher in 2008 than 2009 (Table 1). Similarly, E_{UV} was 0.10 units higher in 2008 than in 2009. In contrast, E_{Blue} and E_{Green} had very similar values in both years, although the average difference of only 0.02 units in E_{Blue} was statistically significant at the 5% level (Table 1). E_{Blue} and E_{Green} of spiders and flowers had very similar values; however, E_{UV} were higher for spiders than for flowers (Table 1, supplementary material Table S1). Even though spiders in 2009 were slightly heavier and had longer leg lengths than in 2008, these differences were not statistically significant (Table 1).

Relationship between spider colouration and spider body condition

Variation in leg length explained more than 85% of the variation in mass ($F_{1,107}$=672.950, P<0.001). Residuals of this regression, used as an index of spider condition, suggested that the condition of the spiders was more variable in 2008 than in 2009, but the difference was not statistically significant (Levene's test: $F_{1,107}$=1.87, P=0.17).

Table 1. Mid-point wavelength, chromatic and achromatic contrast, and excitation values for each of the honeybee's photoreceptors (E_{UV}, E_{Blue} and E_{Green}) generated by the reflectance spectra of the crab spider *Thomisus spectabilis*, as well as mass and tibia–patella leg length from spiders collected in 2008 and 2009

	2008 (N=67)	2009 (N=42)	t	P
Mid-point wavelength (nm)	418.2±18.1	431.3±7.6	4.45	<0.01
Chromatic contrast	0.21±0.07	0.12±0.06	7.48	<0.01
Achromatic contrast	0.02±0.02	0.03±0.02	1.42	0.16
E_{UV}	0.66±0.09	0.56±0.08	5.92	<0.01
E_{Blue}	0.83±0.04	0.81±0.03	2.04	0.04
E_{Green}	0.80±0.02	0.81±0.02	1.42	0.16
Mass (g)	0.115±0.100	0.134±0.086	0.98	0.33
Leg length (mm)	3.65±0.98	3.81±0.96	0.81	0.42

Values are means ± s.d., and were compared using a t-test (d.f.=107).
Achromatic contrast and E_{Green} have the same statistical results because achromatic contrast is calculated from spider E_{Green} and flower E_{Green}, which is constant.

Table 2. Akaike's information criterion (ΔAIC) and Bayesian information criterion (ΔBIC) values of models fitted for the relationship between spider condition (y) and spider colour parameters [x; achromatic contrast (AC), chromatic contrast (ΔS) and mid-point wavelength (MP)]

	ΔAIC			ΔBIC		
Model	AC	ΔS	MP	AC	ΔS	MP
$y = 1$	9.7	24.5	10.2	1.4	16.4	2.1
$y = 1 + x$	9.2	25.2	11.0	3.7	19.8	5.6
$y = 1 + year$	5.6	20.4	6.1	0	15.0	0.7
$y = 1 + x + year$	5.9	11.0	2.7	3.1	8.3	0
$y = 1 + x + (x \times year)$	2.8	5.2	3.5	2.6	5.1	3.5
$y = 1 + x + x^2$	10.8	10.3	7.7	7.9	7.6	5.0
$y = 1 + x + x^2 + year$	7.5	0	0	7.2	0	0
$y = 1 + x + x^2 + year + (x \times year)$	2.1	1.8	2.0	4.7	4.5	4.7
$y = 1 + x + x^2 + x^3$	6.4	12.0	9.6	6.2	12.1	9.6
$y = 1 + x + x^2 + x^3 + year$	3.3	1.6	2.0	5.8	4.3	4.7
$y = 1 + x + x^2 + x^3 + year + (x \times year)$	0	3.5	4.0	5.2	8.9	9.3

Values shown are the difference in relation to the best model. Model coefficients were omitted. N=109 spiders.

Model selection for the relationship between spider condition and colouration depended on the colour parameter evaluated (Table 2, Fig. 2). For colour contrast, AIC and BIC identified a second-order polynomial as the best model (Table 2). All coefficients in this model were statistically significant (supplementary material Table S2). High values of colour contrast yielded the highest condition index values. Simpler models with only first-order colour contrast and year terms also produced significant coefficients and indicated a positive relationship between condition and colour contrast (e.g. condition index ~ colour contrast + year; colour contrast coefficient, mean ± s.e.m.=1.495±0.438, t_1=3.414, P<0.001). For mid-point wavelength, the lowest value of AIC was also a second-order polynomial (Fig. 2). The shape of this curve was similar to that of colour contrast (Fig. 2), but mirrored, because in this system colour contrast and mid-point wavelength are negatively correlated. The model predicts that small values of mid-point wavelength produce the highest values of condition. For BIC, however, there were two models with the lowest values. One was the same model selected using AIC. The second was a linear relationship with no interaction term and a negative coefficient for mid-point wavelength. Both models have in common that they point to the smallest values of mid-point wavelength generating the highest values of condition index. For the achromatic contrast, AIC and BIC pointed to different models (Table 2): a third-order polynomial had the lowest AIC value, but a model with just year as an explanatory variable presented the lowest BIC values. Therefore, the polynomial regression should be treated with care, especially because in this case the third-order polynomial coefficient presented a P-value very close to the established statistical significance level (supplementary material Table S2). Models for all colour parameters evaluated pointed to year as an important variable (2009 having a higher mean spider condition index value than 2008; Table 2).

Effect of spider condition and background colouration on spider colouration

The best random effect configuration for all models included only the parameter 'spider identity' (intercept). The best-fit model for the effect of treatments on the condition index of spiders did not include background colour or the interaction between background colour and feeding treatment. Only the feeding treatment was included in the model (Table 3, supplementary material Table S3). The mean (±s.d.) condition index of spiders in the low feeding treatment changed from –0.01±0.14 to –0.06±0.10 during the 30 day treatment whereas the condition index of spiders in the high feeding treatment changed from –0.03±0.12 to 0.14±0.14 at the end of the experiment. Coefficient statistics show that the effect of feeding treatment was highly significant (supplementary material Table S3). White UV-bright containers generated an E_{UV} of 0.91±0.00, an E_{Blue} of 0.90±0.00 and an E_{Green} of 0.86±0.00, whereas yellow UV-dull containers generated an E_{UV} of 0.47±0.13, an E_{Blue} of 0.39±0.08 and an E_{Green} of 0.78±0.01.

Overall, during the experiment all spiders increased their UV reflectance (supplementary material Fig. S1), significantly increased E_{Green} and decreased their mid-point wavelength and chromatic contrast in relation to the origin of the bee colour space, regardless of the treatment applied (Table 4, Fig. 3). There was evidence supporting a small effect of background colouration on the spiders' colouration. This variable was present in the best model indicated by both AIC and BIC for the colour contrast to the origin (Table 3). Moreover, background colouration coefficient had a P-value smaller than the established statistical significance level (Table 4). Models indicated that spiders in yellow UV-dull containers produced a slightly higher colour contrast to the origin than spiders in white UV-bright containers (Table 4, Fig. 3).

There was either a very weak effect or no evidence for an effect of feeding treatment on spider colouration, because feeding treatment was not present in any of the models indicated by BIC (Table 3). Although some of the lowest AIC models did include feeding treatment, their AIC values were very similar to those of models that did not include feeding treatment (Table 3, difference <2), indicating that the inclusion of this variable did not substantially improve models. Moreover, feeding treatment coefficient had a P-value higher than the established statistical significance level (Table 4).

Similar to the feeding treatment, there was no evidence of an interaction between colour background and feeding treatment on spider colouration. The lowest AIC model for mid-point wavelength did include the interaction term, but the AIC value was very similar to that of models that did not include interaction (Table 3). In addition, the t-test indicated that the interaction term could be excluded from the model (Table 4). When this was done, both feeding regime and background colouration became non-significant at the 5% level (feeding treatment: t_1=0.945, P=0.351; colour treatment: t_1=1.387, P=0.175).

DISCUSSION

We found temporal and individual differences in spider reflectance, especially in the UV region of the spectrum (Fig. 1), with the average

Table 3. ΔAIC and ΔBIC values of linear mixed models fitted for the effects of feeding regime and background colouration on spider condition and spider colouration [E_{Green}, chromatic contrast to the origin (ΔS_{origin}) and mid-point wavelength (MP)]

	ΔAIC				ΔBIC			
Model fixed effects	Condition	E_{Green}	ΔS_{origin}	MP	Condition	E_{Green}	ΔS_{origin}	MP
Initial + Day	21.7	0	3.0	0.9	19.4	0	0.8	0
Initial + Day + Background	23.6	1.4	0	0.9	23.6	3.6	0	2.2
Initial + Day + Feeding	0	0.9	3.9	1.9	0	3.2	3.9	3.3
Initial + Day + Feeding + Background	1.8	2.3	0.5	1.9	4.0	6.8	2.8	5.5
Initial + Day + Feeding + Background + Feeding × Background	1.7	4.2	1.0	0	6.2	11.0	5.5	5.9

Values shown are the difference in relation to the best model (lowest value). Models also include as parameters the initial value of the explanatory variable (Initial) and the day that measurements were taken (15 days into the experiment and at the end of the experiment). Although feeding regime and interaction between feeding background are included in some of the models with the lowest AIC, model statistics do not give support to their effects (see Table 4 and Results). E_{Green} and ΔS_{origin} were Box–Cox transformed before fitting models. Models were fitted using maximum likelihood. N=38 spiders. Models included spider identity (intercept) in random effects.

mid-point wavelength shifted to the right from 2008 to 2009 (Table 1). As a consequence, when modelled into the honeybee vision, the spiders varied in the overall colour contrast against the UV-dull flowers, probably because of differences in the excitation of the honeybee UV photoreceptor. On average, the spiders were more UV-reflective and created a greater colour contrast in 2008 than in 2009. Colour contrast can be seen as a gradient of difficulty in the discrimination between two colours. The lower the value, the more difficult the task. In behavioural experiments, honeybees have been shown to discriminate targets that differ by as little as 0.06 units in their colour space (Dyer and Chittka, 2004). Furthermore, the lower the colour contrast, the longer honeybees take to learn to discriminate between two targets and the longer the bees take to make a choice between targets (Dyer and Chittka, 2004). Moreover, the ability to discriminate between two different colours is likely to be lower under natural foraging conditions, where honeybees are subjected to distracting factors (Spaethe et al., 2006). In 2009, 10 spiders fell below 0.06 units of colour contrast, but none did so in 2008. In addition, 17 spiders had a colour contrast above 0.25 in 2008, but none did in 2009. Thus, overall our results suggest that from a honeybee perspective, in 2009 more spiders were adopting a strategy of low conspicuousness, whereas in 2008 more spiders were adopting a strategy of higher colour contrast and thus greater visibility (Table 1).

When we analysed the relationship between colour and body condition, we found that condition had a quadratic relationship with overall colour contrast (Table 2, Fig. 2). In addition, the models suggested either a quadratic or linear relationship between mid-point wavelength and condition of spiders (Table 2, Fig. 2). These results, together with those of previous experiments (Bhaskara et al., 2009; Heiling et al., 2005; Heiling et al., 2003; Herberstein et al., 2009), suggest that greater conspicuousness, most likely achieved by a higher E_{UV} contrast, is advantageous for these spiders. This advantage is likely to be the result of the fact that honeybees are attracted to UV-reflective spiders (Heiling et al., 2005; Heiling et al., 2003; Herberstein et al., 2009) and, therefore, the foraging success of highly UV-reflective spiders would be greater than that of UV-dull spiders. Moreover, the fact that colour contrast and condition have a quadratic relationship suggests that the benefit comes after a certain degree of colour contrast has been achieved (Fig. 2). Contrary to the other variables evaluated, achromatic contrast did not show a clear relationship with spider condition. Model selection predicts that the inclusion of achromatic contrast does not substantially increase its quality (Table 2), but the possibility of an actual effect cannot be ruled out. Our data show that *T. spectabilis* collected from the field show little variability in E_{Blue} and E_{Green} compared with E_{UV} (see s.d. in Table 1). In a hypothetical scenario where the achromatic contrast was more variable – creating a higher contrast against the background – it is likely that this would influence spider conspicuousness and hence their foraging success.

If there were a clear benefit to adopt a high-UV-reflective, highly contrasting strategy, why would some crab spiders adopt the potentially less efficient foraging strategy of low conspicuousness? Prey often change their foraging behaviour when predators are present, usually with the consequence of reducing their food intake. Common anti-predatory responses include a reduction in foraging time and the selection of less risky foraging patches (Lima, 1998). However, predator pressure can also directly affect prey colouration. For instance, the fiddler crab *Uca vomeris* can change its carapace from a bright to a dull colour over the course of a few minutes (Hemmi et al., 2006). Colonies of fiddler crabs that are highly exposed to bird predators have, on average, a duller colouration than less exposed colonies and there is evidence that individuals reduce their conspicuousness if the danger of predation is experimentally increased (Hemmi et al., 2006). Similar to fiddler crabs, crab spiders can change their body colour over several days (Gabritschevsky, 1927; Schmalhofer, 2000; Thery, 2007). Therefore, the difference in spider colouration between years quantified in our study may indicate that spiders are adjusting their body colouration in response to variation in predation pressure. Insects, such as wasps and birds, the most likely predators of spiders, are able to perceive UV light (Briscoe and Chittka, 2001; Foelix, 1996; Hart, 2001), and thus highly contrasting UV-bright spiders may suffer a higher risk of predation because of an increased conspicuousness, whereas less contrasting UV-dull spiders may reduce the probability of detection of predators by matching the UV-dull flower. Nonetheless, information on the actual predation rates of crab spiders is very limited (Morse, 2007) and further studies are needed to test those ideas. It is also noteworthy that the overall condition in 2009 was greater than that in 2008 (Table 3, Fig. 2). This suggests that, in terms of foraging success, 2009 was a more productive year than 2008. Therefore, 2009 spiders could be avoiding a riskier strategy of high conspicuousness because a more conservative low colour contrast strategy was yielding similar foraging success compared with highly contrasting spiders in 2008. As an example, the model for the relationship between condition and colour contrasts predicts that a 0.10 colour contrast in 2009 produces a condition similar to a 0.30 colour contrast in 2008 (Fig. 2).

Alternatively, but not mutually exclusive to the previous hypotheses, spiders could be adjusting their colouration in

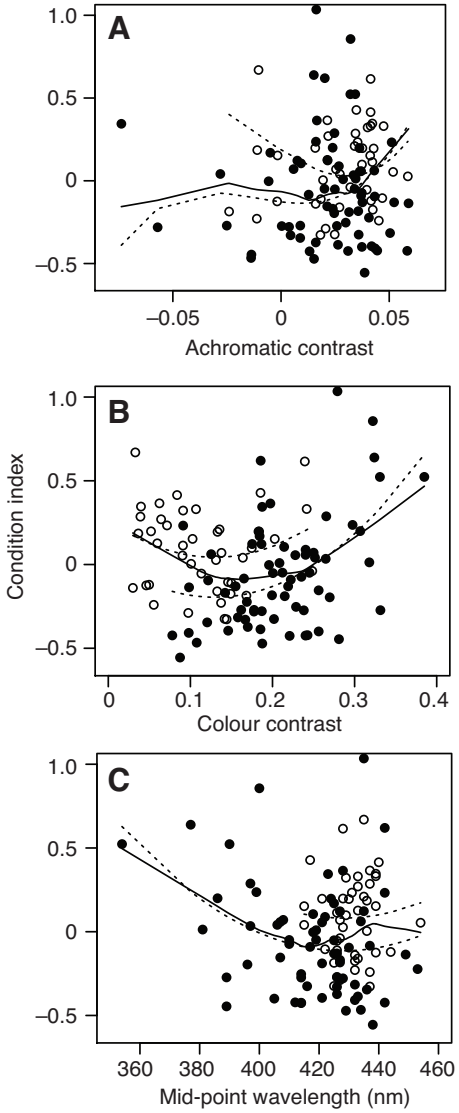

Fig. 2. Relationship between spider condition index and calculated excitation values for (A) achromatic contrast, (B) colour contrast and (C) mid-point wavelength generated by crab spider colouration. Spider condition was estimated from the residuals of the regression ln(mass) × ln(first leg tibia–patella length). White circles represent spiders collected in 2009 (*N*=42) and black circles represent spiders collected in 2008 (*N*=67). Dashed lines show models with the lowest Akaike's information criterion for each parameter evaluated (Table 3) and solid lines represent a Lowess curve (locally massed scatterplot smoothing) for data from both years. There was strong support for the colour contrast model. For mid-point wavelength, there was support for either a quadratic or a linear relationship. There was weak support for the achromatic contrast model. Graphs show raw data, but *x*-variables were centered for model fitting. Model coefficient statistics are given in supplementary material Table S2.

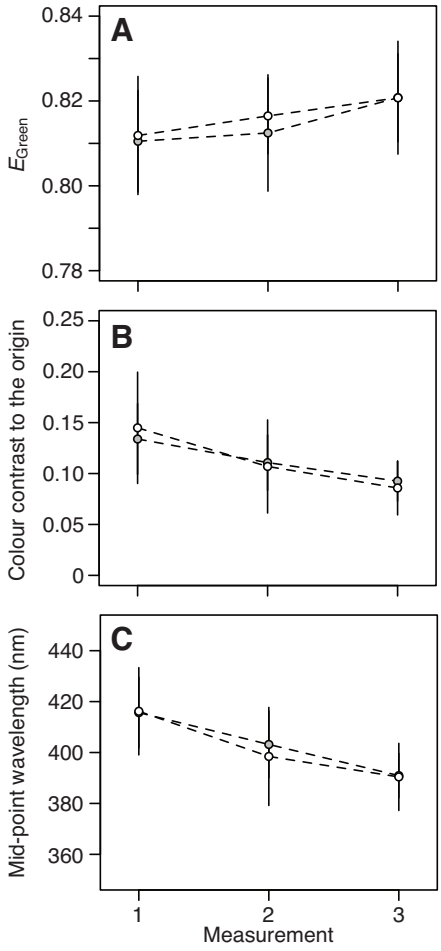

Fig. 3. Change in (A) E_{Green}, (B) colour contrast to the origin and (C) mid-point wavelength of crab spiders subjected to two colour backgrounds (white circles: white UV-bright, *N*=20; grey circles: yellow UV-dull, *N*=18). Graphs show means ± s.d. calculated from raw data for measurements before (1), during (2) and after (3) the experiment was conducted. Data were analysed using linear mixed models. There was a clear, although small, effect of background colouration on the change of colour contrast to the origin, but not for the other parameters. Spiders were also subjected to two feeding regimes during the same experiment, but there was no clear effect of this variable on spider colouration.

accordance with the behaviour of the most common prey. Different species of pollinators respond differently to the presence of crab spiders (Brechbühl et al., 2009; Brechbühl et al., 2010) and some prey species are attracted to the high UV contrast (Heiling and Herberstein, 2004; Heiling et al., 2003; Llandres et al., 2011). In this scenario, one would expect that in 2008 the most common prey were those attracted to conspicuous UV-bright spiders, but the most common prey in 2009 were not. For example, honeybees are attracted and land more frequently on flowers harbouring UV-bright spiders (Herberstein et al., 2009), but Australian native bees are less likely to land on such flowers (Heiling and Herberstein, 2004; Llandres et al., 2011). If, in 2008, the most common prey were honeybees, the best strategy would be to adopt a high UV-reflectance strategy, whereas if in 2009 the most common prey were native bees, the most efficient strategy would be to reduce conspicuousness.

In our experiment on the effect of background colouration and food intake on spider colouration, we found that the spiders have the ability to change colour independently of food intake. This is supported by two pieces of evidence. First, spiders in all treatments decreased their colour contrast to the origin throughout the experiment (Fig. 3). Second, there was no statistically significant effect of feeding treatment on spider colouration. At the end of the experiment, the spiders in both feeding treatments had remarkably different body conditions (supplementary material Table S3) but almost the same colour contrast and mid-point wavelength (Table 4), suggesting that it is not body condition that causes the difference

Table 4. Linear mixed models with the lowest AIC values (Table 3) for the experiment testing the effects of feeding regime and background colouration on spider colouration

Coefficients for fixed effects	d.f.	Estimate ± s.e.m.	*t*	*P*
Lowest AIC E_{Green} model				
Intercept	36	–0.0553±0.0041	–13.606	<0.001
Initial E_{Green}	36	0.200±0.034	5.910	<0.001
Day of measurement [at the end]	31	$7.67\pm2.00 \times 10^{-4}$	3.844	<0.001
Lowest AIC chromatic contrast to the origin model (ΔS_{origin})				
Intercept	35	1.492±0.669	2.232	0.032
Initial ΔS_{origin}	35	4.302±0.739	5.818	<0.001
Day of measurement [at the end]	31	–0.223±0.030	–7.331	<0.001
Background [yellow]	35	0.173±0.078	2.213	0.034
Lowest AIC mid-point wavelength model				
Intercept	33	142.446±53.251	2.675	0.012
Initial mid-point wavelength	33	0.602±0.126	4.769	<0.001
Day of measurement [at the end]	31	–10.165±1.368	–7.433	<0.001
Feed treatment [low]	33	10.556±5.272	2.002	0.054
Background [yellow]	33	12.457±5.328	2.338	0.027
Feed treatment [low] × Background [yellow]	33	–14.095±7.509	–1.877	0.069

Coefficients of categorical values are calculated by setting one category to zero and comparing it with the others, which is shown between square brackets. E_{Green} and ΔS_{origin} were Box–Cox transformed before model fitting. Models were fitted using restricted maximum likelihood. Random term for all models: Spider identity (intercept). *N*=38 spiders.

in colouration, but that higher colour contrast results in greater foraging success and hence greater body condition. Second, the lack of feeding treatment effect, particularly with the yellow background, suggests that satiated spiders do not opt for a potentially less risky strategy of low conspicuousness. However, in our experiment, spiders were isolated from predators; therefore, the overall change in colour contrast, regardless of the feeding treatment, could have been at least partly influenced by the absence of predator cues. Without predator cues, spider may have adopted a high contrast strategy in order to increase prey capture.

Although the effect was small, spiders did respond differently to background colouration (Table 4, Fig. 3). Spiders on the white UV-bright backgrounds tended to produce a lower colour contrast to the origin than spiders on the yellow UV-dull background. Despite this difference, spiders on the yellow UV-dull background did not match the background, i.e. they did not change their reflectance to match the background reflectance (supplementary material Fig. S1). We do not know, however, whether the housefly diet we fed spiders and the artificial backgrounds affected their ability to change colours. The ommochrome pigments related to the yellow colouration in crab spiders are the same class of pigment found in fly eyes (Insausti and Casas, 2008). Indeed, crab spiders fed with red-eyed *Drosophila melanogaster* (ommochrome rich) changed to a slightly brighter yellow colour than crab spiders fed white-eyed flies (Thery, 2007). Nonetheless, the houseflies in our experiment were red-eyed, and other species of crab spiders have changed colour even against artificial backgrounds (Gabritschevsky, 1927; Packard, 1905; Thery, 2007). In contrast to our study, crab spiders *Misumena vatia* did change their colouration to match the colouration of white and yellow natural and artificial backgrounds (Gabritschevsky, 1927; Packard, 1905; Thery, 2007). However, *M. vatia* is apparently not UV-reflective and does not lure pollinators. Thus one can predict that the best strategy for this species is to always reduce conspicuousness, whereas for *T. spectabilis* it could be advantageous to increase conspicuousness in certain circumstances to lure prey *via* UV-reflectance.

In conclusion, our results show that the colour contrast created by UV-reflectance in Australian crab spiders is beneficial for spiders most likely because of an increase in their foraging success. It also reveals the nature of the relationship between spider body condition and body colouration, showing that there is a positive relationship between colour contrast and condition. Also, contrary to other crab spiders, UV-reflective *Thomisus spectabilis* did not change their colour in order to match the background. Their response to background colouration and satiation may additionally depend on the composition of the prey population.

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