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Orb-web spider color polymorphism through the eyes of multiple prey and predators

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Summary statement: We assessed the role of both prey and predator vision on the evolution and maintenance of color polymorphism and color conspicuousness of the orb-web spider *Gasteracantha cancriformis* (Araneidae).

ABSTRACT

The sensory drive theory predicts that signals, sensory systems, and signaling behavior should coevolve. Variation in the sensory systems of prey and predators may explain the diversity of color signals, such as color polymorphism. The spider *Gasteracantha cancriformis* (Araneidae) possesses several conspicuous color morphs. The aim of the present study was to assess whether the color polymorphism of *G. cancriformis* may be maintained by pressure from multiple signal receivers, such as prey and predators with distinct color vision systems. In orb-web spiders, the prey attraction hypothesis states that conspicuous colors are prey lures that increase spider foraging success via flower mimicry. However, in highly defended species, conspicuous colors could also be a warning signal to predators. We used color vision modelling to estimate chromatic and achromatic contrast of *G. cancriformis* morphs as perceived by potential prey and predator taxa. Our results revealed that individual prey and predator taxa perceive the conspicuousness of morphs differently. For instance, the red morph is perceived as quite conspicuous to lepidopteran prey and avian predators, but not by dipteran prey and hymenopteran prey and predators. Therefore, the multiple prey and multiple predator hypotheses may explain the evolution of color polymorphism in *G. cancriformis*. However, the spider's coloration did not resemble flower coloration, which suggests that the species' conspicuousness is not the result of flower mimicry. Other parameters that are not evaluated by color vision models, such as distance, shape, angle, pattern geometry, and contour, could also affect the perception of color morphs by both prey and predators and thereby influence morph survival.

INTRODUCTION

The evolution and maintenance of color polymorphism have traditionally been attributed to apostatic selection (Clarke, 1979). Assuming that predators form a search image (Tinbergen, 1960), the advantage of rarity promotes the coexistence of multiple prey types

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and stabilizes polymorphisms (Bond 2007). Nonetheless, other adaptive and non-adaptive explanations for the evolution and maintenance of color polymorphisms have been proposed (Gray and McKinnon, 2007). For instance, gene flow between populations with distinct phenotypes that are favored by natural selection could maintain polymorphism within populations (Farkas et al., 2013; Gray and McKinnon, 2007).

In the context of visual signaling, the distinct visual systems of prey and predators may play a role in the evolution and maintenance of color polymorphisms (Ruxton et al., 2004; White and Kemp, 2015). Animal communication involves the generation, emission and transmission of a signal, and processing of the signal by a receiver, in which an appropriate response is elicited (Endler 1993). Any factors that affect these steps can influence signal efficiency and, as a result, affect the direction of communication evolution (Endler 1993). Thus, the diversity of signals can be attributed to variation in the sensory systems of receivers. Considering that the same "color" may be perceived as cryptic or conspicuous by different species (Endler and Mappes 2004), each color morph of polymorphic populations may represent an adaptation to particular visual systems of prey or predator species (Endler, 1992; Ruxton et al., 2004; White and Kemp, 2015).

Many orb-web spiders exhibit conspicuous coloration. The prey attraction hypothesis states that the bright coloration of some spiders lures insects, possibly by mimicking flower coloration (e.g. Craig and Ebert, 1994; Hauber, 2002). The hypothesis has been empirically tested several times, and most studies have found support for it. The polymorphic *Nephila pilipes* (Nephilidae) present a melanic and a bright colored morph (Tso et al., 2004). The bright color patterns of this species are thought to resemble symmetric flower patterns that may attract bees, owing to the innate preference of bees for symmetry (Chiao et al., 2009). Moreover, yellow patches on the spider's body may be perceived as food resources by flowers visitors (Tso et al. 2004). Besides being attractive to pollinators, the yellow patches on the species' body also seems to attract hymenopteran predators. Therefore, it is possible that there is a trade-off between foraging success and predation risk in polymorphic populations in which some morphs are more cryptic than others (Fan et al. 2009).

The predators of orb-web spiders possess very distinct visual systems. Birds, for example, are tetrachromats, whose photoreceptors are most sensitive to ultraviolet-violet,

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blue, green, and red (Hart 2001), whereas spider hunting wasps, such as members of the Sphecidae, are trichromats, whose photoreceptors are most sensitive to ultraviolet, blue, and green (Peitsch, 1992; Briscoe and Chittka, 2001). Similarly, the insect prey of orb-web spiders also vary in their types of color vision. For example, bees are trichromats with spectral sensitivities that are similar to those of sphecid wasps (Briscoe and Chittka, 2001), whereas some lepidopterans are tetrachromats, and some dipterans possess photoreceptors with five different sensitivity peaks (Schnaitmann et al., 2013). Therefore, the maintenance of spider color polymorphism may result not only from a trade-off between prey attraction and capture success but also from selective pressure from multiple receivers (Endler, 1992; Ruxton et al., 2004; White and Kemp, 2015). Color perception depends on both the signal reflectance and observer visual system, as well as on the background reflectance spectrum and ambient light intensity (Endler 1990). Physiological models of color vision include all these factors and have been effective for objectively studying animal coloration (i.e., independent of human subjective assessment; Renoult, 2015).

The orb-web spider *G. cancriformis* constructs large webs and rests in the web hub during the day (Levi, 1978). Females of the species possess a hard abdomen with three pairs of spines and vary in color, with some morphs quite conspicuous to human observers (Levi, 1978; Gawryszewski and Motta, 2012). The ventral side of females are mostly black, sometimes with small bright spots. In one studied population, the dorsal side of females possessed black or reddish spines and four different color patterns: yellow, white, red, and a combination of black and white (Gawryszewski 2007; Gawryszewski and Motta, 2012). Adult females measure from 5 to 7 mm in length and 10 to 13 mm in width (Muma, 1971), whereas the males are brownish, small, and do not exhibit chromatic variation (Levi, 1978). The prey attraction hypothesis does not seem to explain the coloration of the orb-web spider *Gasteracantha cancriformis* (Araneidae), since both naturally bright morphs and yellow-painted individuals failed to capture more prey than either naturally cryptic morphs or black-painted individuals (Gawryszewski and Motta, 2012). Although evidence is still needed, Edmunds and Edmunds (1983) suggested that the conspicuous body coloration of *Gasteracantha* spiders might serve as a warning signal to predators. The aim of the present study was to investigate three hypotheses for the

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evolution and maintenance of color polymorphism, using *G. cancriformis* as a model. Two derivations from the prey attraction hypothesis include (1) the *multiple prey hypothesis*, which posits that color morphs are perceived differently by different prey taxa and that each color morph is adapted to lure a specific type of prey, and (2) the *multiple mimic model* hypothesis, which posits that the spiders attract prey *via* aggressive mimicry of flower colors and that each color morph mimics a different flower color. In addition, (3) the *multiple predator hypothesis* posits that the conspicuous colors found in spiders could serve as warning signals to predators and that color polymorphism could evolve and be maintained if each color morph is adapted to the vision of a specific predator.

MATERIALS AND METHODS

Color vision model

To estimate the perception of *G. cancriformis* color morphs by distinct predators and prey groups, we used the color vision model proposed by Chittka (1992). Although this model has been only validated with behavioral experiments on honeybees, its general form allow us to apply it for other taxa (e.g. Thery and Casas 2002). The model requires four inputs: (1) the irradiance reaching the observed object, (2) the observer photoreceptor excitation curves, (3) the background reflectance to which photoreceptors are adapted to, and (4) the reflectance curve of the observed object. First, the sensitivity factor *R* was determined for each photoreceptor, as follows:

$$R = 1/\int_{700}^{300} I_B(\lambda)S(\lambda)D(\lambda)d \qquad (1)$$

where $I_B(\lambda)$ is the spectral reflectance function of the background, $S(\lambda)$is the spectral sensitivity function of each photoreceptor, and $D(\lambda)$ is the illuminant irradiance spectrum. Secondly, the quantum flux *P* (relative amount of photon catch) is calculated, as follows:

$$P = R\int_{700}^{300} I_S(\lambda)S(\lambda)D(\lambda)d \qquad (2)$$

where $I_S(\lambda)$is the spectral reflectance function of the stimulus. Assuming that the maximum excitation of a photoreceptor is 1, the phototransduction process is determined by:

$$E = P/(P + 1) \qquad (3)$$

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Stimuli spectra are projected in specific color spaces. The coordinates of each spectrum are calculated using photoreceptor excitations, as follows (Chittka et al. 1994):

$$X1 = \sin 60^\circ \, (E_3 - E_1) \tag{4}$$

$$X2 = E_2 - 0.5\,(E_1 + E_3) \tag{5}$$

For tetrachromat organisms (Théry and Casas, 2002):

$$X1 = \frac{2\sqrt{2}}{3} \cos 30^\circ \, (E_3 - E_4) \tag{6}$$

$$X2 = E_1 - \frac{1}{3}\,(E_2 + E_3 + E_4) \tag{7}$$

$$X3 = \frac{2\sqrt{2}}{3}\,[\sin 30^\circ (E_3 + E_4) - E_2] \tag{8}$$

We extended the model of Chittka (1992) to accommodate pentachromatic organisms, as follows:

$$X1 = \frac{5}{2\sqrt{2}\sqrt{5}}\,(E_2 - E_1) \tag{9}$$

$$X2 = \frac{5\sqrt{2}}{2\sqrt{3}\sqrt{5}}\,[E_3 - \left(\frac{E_1 + E_2}{2}\right)] \tag{10}$$

$$X3 = \frac{5\sqrt{3}}{4\sqrt{5}}\,[E_4 - \left(\frac{E_1 + E_2 + E_3}{3}\right)] \tag{11}$$

$$X4 = E_4 - \left(\frac{E_1 + E_2 + E_3 + E_4}{4}\right) \tag{12}$$

Chromatic contrast between a color stimulus and background, or between two color stimuli, is calculated as the Euclidean distance (ΔS) between two points in color space, as follows:

$$S = \sqrt{\sum_{i=1}^{n} (X_{a_i} - X_{b_i})^2} \tag{13}$$

where X_i (i = 1, 2, 3,..., n) represents the coordinate in the color space.

The color spaces are chromaticity diagrams and, thus, do not estimate the achromatic contrast between objects. Nonetheless, achromatic contrasts can be important

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visual cues used by both prey and predators. In bees, achromatic contrast is more important than chromatic cues for objects that subtend a visual angle smaller than ~15°, which means that bees have to be very close to flowers in order to use their color vision for discrimination tasks (Giurfa et al., 1997). Similarly, birds use achromatic contrast in detection of small objects (Osorio et al., 1999). We estimated the achromatic contrast as the excitation (Eq. 3) of the photoreceptor responsible for achromatic discrimination in each organism (Chittka and Kevan 2005).

For our modeling, we used the reflectance data of *G. cancriformis* color morphs that was collected during a previous study (for reflectance curves see figure 1.8 in Gawryszewski, 2007, and figure 5 in Gawryszewski and Motta 2012). These data have already been used to estimate the visual contrast of the yellow and white morphs and the black and white morphs from the perspective of *Apis mellifera* (Gawryszewski and Motta 2012). The spiders were collected from a Brazilian savanna physiognomy, namely Cerrado *sensu stricto,* which is characterized by shrubs and trees of 3 to 8 m tall that are contorted and possess thick, fire-resistant bark, a crown cover of >30%, and additional herbaceous vegetation (Oliveira-Filho and Ratter 2002). The background reflectance was estimated from the average reflectance of leafs, leaf litter, bark, and grasses that were collected from the same area as the spiders (see figure 5 in Gawryszewski and Motta, 2012). To avoid issues with negative values and unrealistic positive values we adjusted the reflectance data by subtracting the reflectance values by the minimum value of each measurement. As illuminant spectrum, we used the International Commission on Illumination (CIE) standard illuminant of D65, which is comparable to open areas, such as the Brazilian savanna (Chittka, 1996).

Multiple prey hypothesis

Using the model described above, we estimated the chromatic and achromatic conspicuousness of the *G. cancriformis* morphs (yellow, white, red, and white parts of the black and white morph) to a suit of potential prey: *A. mellifera* (Hymenoptera, Apidae), *Drosophila melanogaster* (Diptera, Drosophilidae), and *Fabriciana adippe* (Lepidoptera, Nymphalidae). We selected these insect orders because they are commonly intercepted by orb-webs in field experiments (Craig and Ebert 1994; Tso et al. 2002) and represent

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the diversity of visual systems among insects (Briscoe and Chittka, 2001). For *A. mellifera* and *D. melanogaster*, we used photoreceptor sensitivity curves from the literature (Peitsch et al., 1992; Schnaitmann et al., 2013). It was recently shown that, together with R7-R8 photoreceptors, R1-R6 photoreceptors contribute to color vision in *D. melanogaster* (Kelber and Henze, 2013; Schnaitmann et al., 2013). Therefore, we included the R1-R6 photoreceptor curve, treating *D. melanogaster* as a pentachromat. The graphical curves were extracted directly from the figures of relevant publications using DataThief III version 1.7 (Tummers, 2006). For *Fabriciana adippe,* however, no photoreceptor sensitivity curves are available, so electrophysiological measurements of photoreceptor sensitivity peaks ($\lambda_{max;}$ Eguchi et al., 1982) were used to estimate the photoreceptor curves (for details see Govardovskii et al., 2000).

For achromatic contrast, bees only use the green photoreceptor (Giurfa et al., 1996), whereas flies only use the outer photoreceptors (R1-R6; Kelber & Henze, 2013). Because the exact mechanism used by lepidopterans for achromatic discrimination is incompletely understood, we assumed that they employ the same mechanism as in bees. The color hexagon model assumes that photoreceptors respond to half their maximum for the background they are adapted to, so that the photoreceptor excitation for background is equivalent to 0.5 units (Chittka, 1992).

The multiple prey hypothesis predicts that different prey taxa perceive color morphs differently. To assess whether each spider morph was perceived differently by prey species, we constructed two linear mixed models, one for chromatic contrast and one for achromatic contrast. Either chromatic (ΔS) or achromatic contrast were used as the dependent variable, and spider morph and prey taxon were used as the independent variables (contrast = spider morph × observer). The spider morph was defined as yellow, white, red, or black and white, and the observers were defined as hymenopteran, dipteran, or lepidopteran. Individual spiders were used as random effects. Normality and homogeneity were verified by visual inspection of quantile-quantile and residuals vs. fitted values plots. We computed all nested models and used the Akaike Information Criterion to select the best model.

As a reference point, we used a color discrimination threshold of $\Delta S = 0.11$, which is the threshold value below which trained bees are unable to distinguish different flower

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colors (Chittka, 1996). However, discrimination thresholds are variable and can change depending on the study species, learning conditions, previous experience, background coloration, whether the task involves discrimination between colors or detection against the background, and whether objects are compared sequentially or simultaneously (Avarguès-Weber & Giurfa, 2014). It should also be noted that threshold values were not behaviorally validated for other taxa. Therefore, values originated here using the Chittka model (1992) must be taken carefully.

Multiple mimic models hypothesis

To test the multiple mimic models hypothesis, we compared how flowers and spider morphs are perceived by prey. We gathered all flower reflectance data available in the Floral Reflectance Database (FReD; Arnold et al., 2010), excluding reflectance data from lower flower parts, leaves, bracts, stamens, the inner parts of bell-shaped flowers, and unknown items, as well as spectrum files that did not cover 300 to 700 nm. Most species in the database have only one reflectance spectrum, and for species with multiple reflectance spectra, we randomly selected a single spectra. We did not average the reflectance of these species because there was no information available on whether these measurements referred to different individuals or different parts of single flowers. In total, we gathered reflectance data from 859 plant species.

We calculated a matrix of chromaticity distances between average color loci of each spider color morph and each individual flowers species and plotted these values using a Kernel density estimation. If spiders are mimicking some group of flower colors, we would expect to find at least one peak near or below the theoretical detection threshold of 0.11. We also followed the same procedure for the absolute achromatic difference between individual flowers and the mean achromatic value for each color morph. If spiders and flowers are similar in the achromatic dimension, we would expect to find one peak near zero.

Multiple predator hypothesis

The methodology used to investigate the multiple predator hypothesis methodology was very similar to that used for the multiple prey hypothesis, except that we used predator

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species in our models. As predators, we considered the bird *Parus caeruleus* (Paridae) and the wasp *Philanthus triangulum* (Sphecidae), since birds and wasps are the main predators of orb-web spiders (Rayor, 1996; Foelix, 2010), are visually guided hunters, and have distinct color vision systems. For *P. caeruleus*, we used photoreceptor sensitivity curves available in the literature (Hart, 2001), and for *P. triangulum*, we used photoreceptor sensitivity peaks to estimate photoreceptor sensitivity curves (data available in Briscoe and Chittka, 2001; see Govardovskii et al. 2000 for estimation of sensitivity curves from sensitivity peaks).

The multiple predator hypothesis predicts that different predator taxa perceive color morphs differently. To assess this prediction, we established two linear mixed models, one for chromatic contrast and one for achromatic contrast. Either chromatic (ΔS) or achromatic contrast were used as the dependent variable, and spider morph and predator taxon were used as the independent variables (contrast = spider morph × observer). The spider morph was defined as yellow, white, red, or black and white, and individual spiders were used as random effects. Normality and homogeneity were verified by visual inspection of quantile-quantile and residuals vs. fitted values plots. We computed all nested models and used the Akaike Information Criterion to select the best model.

As in the multiple prey hypothesis, we used discrimination thresholds as reference points. For the chromatic contrast, we considered color discrimination thresholds of ΔS = 0.11 and ΔS = 0.06 for the wasp (Dyer and Chittka, 2004) and bird (Théry et al., 2005), respectively. For the achromatic contrast, we considered double cones in birds (Hart, 2001), and assumed green photoreceptors for wasps, as in bees.

Visual modeling calculations were conducted using the 'colourvision' R package (Gawryszewski, 2017). Linear mixed models were performed using the 'nlme' package (Pinheiro et al., 2016), and graphs were plotted using the 'ggplot2', 'ggExtra', and 'gridExtra' packages (Wickham, 2009; Attali, 2016; Auguie, 2016; R Core Team, 2015).

RESULTS

Multiple prey hypothesis

For chromatic contrast, the model that included the interaction between spider morph and prey taxon presented the lowest AIC value (Table 1). The yellow morph presented

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the highest ΔS value for *A. mellifera* and *F. adippe* vision, whereas the white spider presented the highest ΔS value for *D. melanogaster*, followed by the yellow morph (Fig. 1). The white patch of the black and white spiders presented a ΔS value that was very close to the theoretical discrimination threshold for all prey species (Fig. 1). The red spiders presented ΔS values near the theoretical discrimination threshold for *A. mellifera* and *D. melanogaster*, but not for *F. adippe* (Fig. 1). For prey achromatic contrast, the model that included the interaction between variables presented the lowest AIC value (Table 1). For all prey groups, the white morph had the highest excitation value, followed by the black and white, yellow, and red morphs, respectively (Fig. 1). The model coefficients are provided in the supplementary material (Table S1 and S2).

Multiple mimic models hypothesis

For all three prey species, only the white patch of the black and white morph had a density peak near the chromatic theoretical discrimination threshold of 0.11 when compared to all flower reflectance spectra: 44.5% for *A. mellifera*, 16.8% for *D. melanogaster,* and 35% for *F. addipe.* (Fig. 2). For the other spider morphs only a small proportion of the Euclidean distances between flowers and morphs presented values < 0.11. For *A. mellifera* only 1.6% of yellow morphs presented values lower than 0.11, 3.4% of white morphs, and 4.8% of red morphs (Fig. 2). For *D. melanogaster* only 2.4% of yellow morphs had values lower than 0.11, 4.0 % of white spiders, and 3.0% of red morphs (Fig. 2). For *F. addipe* this values were 0.4%, 0.2%, and 0.5% respectively (Fig. 2). For all three prey species a large proportion of flowers had similar achromatic values to the yellow morph, white morph, and the white patch of the black and white morph, but not to the red morph (Fig. 3).

Multiple predator hypothesis

For the chromatic contrast, the model with interaction between variables presented the lowest AIC value (Table 1). The black and white morph presented the lowest ΔS value for both predators (Fig. 4A,B; Table S3). The yellow morph was the one with highest ΔS value for *P. caeruleus*, though white and red morphs presented similar values (Fig. 4A). For *P. triangulum*, the white spider morph presented the highest ΔS value, followed by the yellow and red morphs, and the red morph was near the theoretical discrimination

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threshold of 0.11 (Fig. 4B). For the achromatic contrast, the model that included the interaction between variables presented the lowest AIC value (Table 1), even though the values of the two predator species were very similar. For *P. caeruleus*, the white morph had the highest excitation value, followed by the yellow, black and white, and red morphs, respectively (Fig. 4C). The white morph also had the highest excitation value for *P. triangulum*, followed by the black and white, yellow, and red morphs, respectively (Fig. 4D). The model coefficients are provided in the supplementary material (Table S3 and S4).

DISCUSSION

Our statistical analyses suggested that the multiple prey and multiple predator hypotheses are plausible explanations for the evolution of color polymorphism in *G. cancriformis*, but provided poor support to the flower mimicry hypothesis. If conspicuous colors in orb-web spider attracts prey, then polymorphism may occur in luring systems by multiple mimic models that act as sensory traps for particular prey taxa. Conversely, polymorphism may occur as a result of multiple receiver biases, where each morph lures specific prey taxa that possess specific sensory, neural, or perceptual biases (White and Kemp, 2015). In *G. cancriformis*, spider morphs conspicuousness is perceived differently by prey species. The yellow and white morphs were the most conspicuous to all prey taxa. The former being more contrasting from the background for honeybee color vision, and the latter, for flies. Whereas, the red morph, although inconspicuous for honeybee and flies, showed high detectability for butterflies. Yellow spider morphs could experience higher foraging success as a result of insects preference for yellow signals. Yellow pollen and yellow flower centers, both of which are likely adapted to insect visual systems, are among the most common traits of floral color patterns (Papiorek et al., 2016; Lunau, 2000). On the other hand, red morphs are conspicuous to lepidopterans and may be effective for attracting them. Another recent study also using color vision models showed that insects prey perceive coloration of *Verrucosa arenata* morphs differently, and they may be a selective pressure on the evolution of these color patterns variation (Ajuria-Ibarra et al. 2017). Therefore, the distinct perceptions of insect taxa support the multiple prey

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hypothesis as a mechanism for the evolution of color polymorphism in stationary predators.

For the achromatic dimension, the statistical analyses also suggested an interaction between spider morph and prey taxon. However, there was very little difference between the morphs when viewed by different prey. Therefore, the differences in achromatic contrast between prey perception may be statistically significant but not biologically relevant. A recently proposed scenario for the evolution of color polymorphism is that different morphs exploit different visual channels in prey. The yellow morphs of *G. fornicata* would benefit from stimulating the dipteran chromatic channel, whereas white morphs would benefit from stimulating the achromatic channel (White and Kemp, 2016). However, this is inconsistent with *G. cancriformis* because the spider morphs presented similar levels of achromatic detectability by all the prey taxa examined.

Several authors have proposed flower mimicry as a mechanism of prey attraction by orb-web spiders *via* conspicuous body coloration (e.g. Craig and Ebert, 1994; Hauber, 2002). However, the hypothesis has seldom been tested. Our density graphs show that the color of most of the *G. cancriformis* morphs was not similar to any group of flowers, which suggests that the coloration of the *G. cancriformis* did not result from flower mimicry. Conversely, a study of various orb-web spider species that also used color vision models found that, as perceived by dipterans and hymenopterans, the colors of spiders are very similar to those of flowers (White et al., 2016). It is tempting to use correlational data to infer causal relationships, however, this finding may only reflect the diversity of flower colors and spider colors. Color vision modelling of the prey of the orchid mantis (*Hymenopus coronatus*) suggested that pollinators are unable to distinguish the colors of the mantis and flowers, and a field experiment showed that the mantis actually attracts more pollinators than flowers (O'Hanlon et al., 2013). However, the modification of non-color mantis traits suggested that other types of flower mimicry, such as symmetry, contrasting color patterns, and morphological similarities to flower petals, had no effect on prey attraction (O'Hanlon, 2014). Therefore, mantis coloration is effective in attracting prey, but may not result from flower mimicry. Instead, it may result from sensory exploitation (O'Hanlon, 2014).

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The results of the present study also support the multiple predator hypothesis for the maintenance of color polymorphism in *G. cancriformis*, as the spider morphs conspicuousness was perceived differently by predator species in both the chromatic and achromatic dimensions. The red morph could be targeting bird predators but would appear relatively inconspicuous to a hymenopteran predator. In contrast, the white and yellow morphs are highly detectable by both predator taxa. The colors of two of the four *G. cancriformis* morphs (yellow and red) are typical of aposematic species (Endler and Mappes, 2004). Conspicuous coloration is especially advantageous when it increases the mismatch with the background and facilitates predator learning (Endler and Greenwood, 1988). Spiders of the genus *Gasteracantha* possess spines and a hard abdomen. Moreover, the hunting wasp *Sceliphron laetum* avoids provisioning initial instars with *Gasteracantha* spiders (Elgar and Jebb, 1999). Morphological and behavioral defenses that make ingestion difficult may also constitute an aposematic signal, along with the species' bright colors, which enhance predator memory and learning (Endler and Greenwood, 1988; Ruxton et al., 2004). Though aposematism is not commonly reported in spiders (Oxford and Gillespie, 1998), Brandley et al. (2016) conducted an experiment with black widow models and found that models with red markings were more likely to be avoided by birds than all black models. If spider morphs are conspicuous to prey but do not resemble flowers, it is possible that the conspicuousness of *G. cancriformis* reflects a trade-off between an antipredatory strategy and foraging success, in which common spiders morphs would be avoided by prey.

Thermoregulation is another possible explanation for color polymorphism in orb-web spiders. In *Verrucosa arenata* (Araneidae), yellow and white morphs exhibited different thermal responses when exposed to artificial heat. The white morphs absorbed less visible radiation and shorter wavelengths than the yellow morphs, which could protect the spiders' internal tissue from the harmful effects of UV radiation (Rao and Mendoza-Cuenca, 2016). However, absorbing pigments are likely to offer protection against UV damage (Gawryszewski et al., 2015), which is analogous to the melanin found in the human epidermis (Brenner and Hearing, 2008) and commercial sunscreen lotions (Antoniou et al., 2008).

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We only considered chromatic and achromatic discrimination, but color pattern geometry, shape, contour, size, angle, texture, and distance of visual detection (Troscianko et al., 2009) may also influence the behavior of both prey and predators toward spiders since different species use distinct visual cues for stimuli detection and recognition (Théry and Gomez, 2010). Furthermore, color vision models do not include other perceptual mechanisms, such as cognition, color categorization, past experiences, or memory imprecision (Renoult et al., 2015), even though these factors may affect detectability and, consequently, influence the survival rate of morphs in different ways (Théry and Gomez, 2010). Additionally, non-adaptive explanations, such as overdominance and allele equilibrium in absence of selection, are often ignored when studying polymorphisms in an ecological perspective. Finally, predation experiments and field experiments that evaluate the prey taxa caught by the different spider morphs are paramount to validate and complement the findings of the present study.

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Competing interests

No competing interests declared.

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Tables

Table 1. Delta Akaike Information Criterion (AIC) of Linear Mixed Models of the chromatic and achromatic contrasts of prey and predators.

Model	df	ΔAIC
Multiple prey hypothesis		
Chromatic dimension		
ΔS ~ morph*observer	**17**	**0.0**
ΔS ~ morph+observer	11	23.9
ΔS ~ observer	8	52.4
ΔS ~ morph	9	61.5
ΔS ~ 1	6	90.6
Achromatic dimension		
excitation ~ morph*observer	**17**	**0.0**
excitation ~ morph+observer	11	57.6
excitation ~ morph	9	72.2

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excitation ~ observer	8	84.7
excitation ~ 1	6	100.2
Multiple predator hypothesis		
Chromatic dimension		
ΔS ~ morph*observer	**13**	**0.0**
ΔS ~ morph+observer	10	6.9
ΔS ~ observer	7	30.6
ΔS ~ morph	9	54.5
ΔS ~ 1	6	74.9
Achromatic dimension		
excitation ~ morph*observer	**14**	**0.0**
excitation ~ morph+observer	10	14.4
excitation ~ observer	7	21.1
excitation ~ morph	9	36.9
excitation ~ 1	6	43.9

Figures

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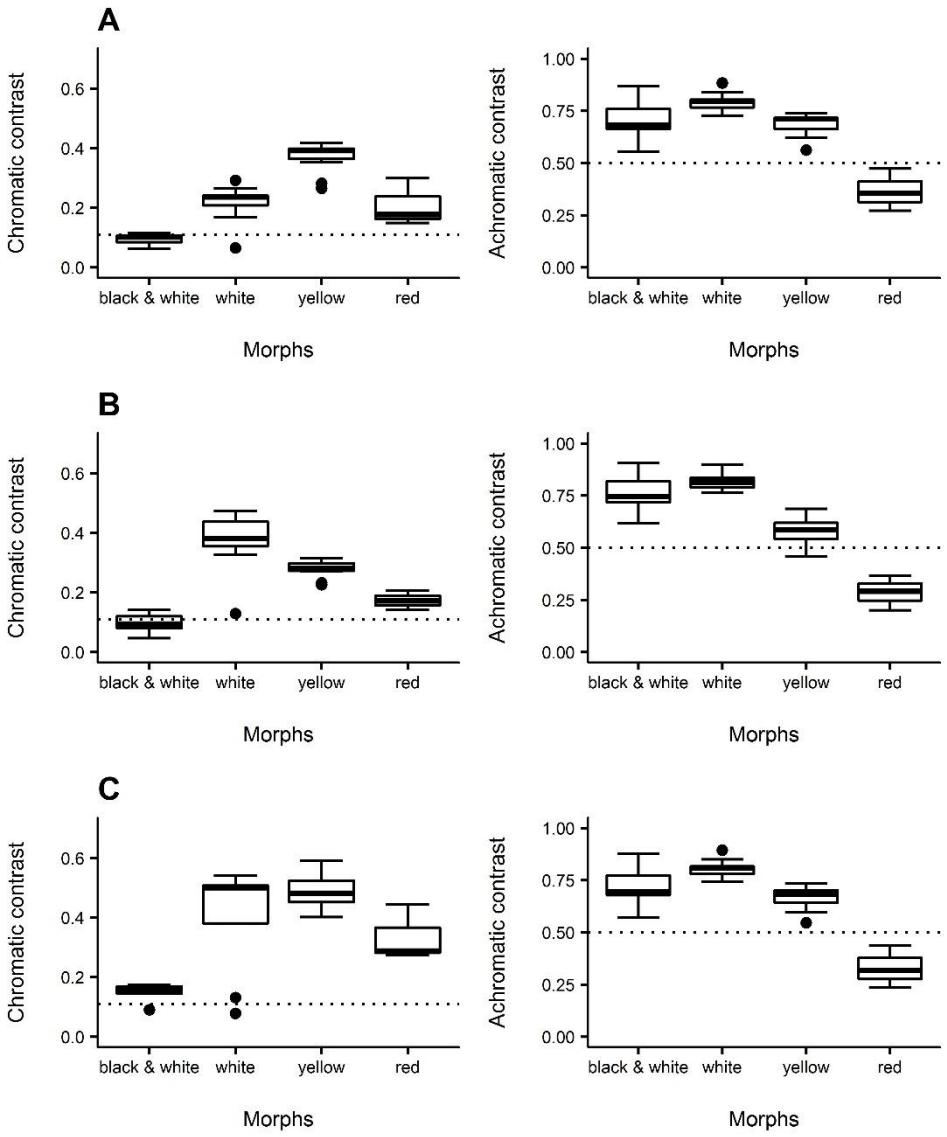

Fig. 1. Chromatic (left) and achromatic (right) contrasts of four *Gasteracantha cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) when viewed against a Brazilian savanna background by prey with distinct visual systems. (A) *Apis mellifera* (Hymenoptera). (B) *Drosophila melanogaster* (Diptera). (C) *Fabriciana adippe* (Lepidoptera). Dotted vertical lines represent the discrimination thresholds for chromatic contrast (0.11) and photoreceptor excitation for background in achromatic contrast (0.5).

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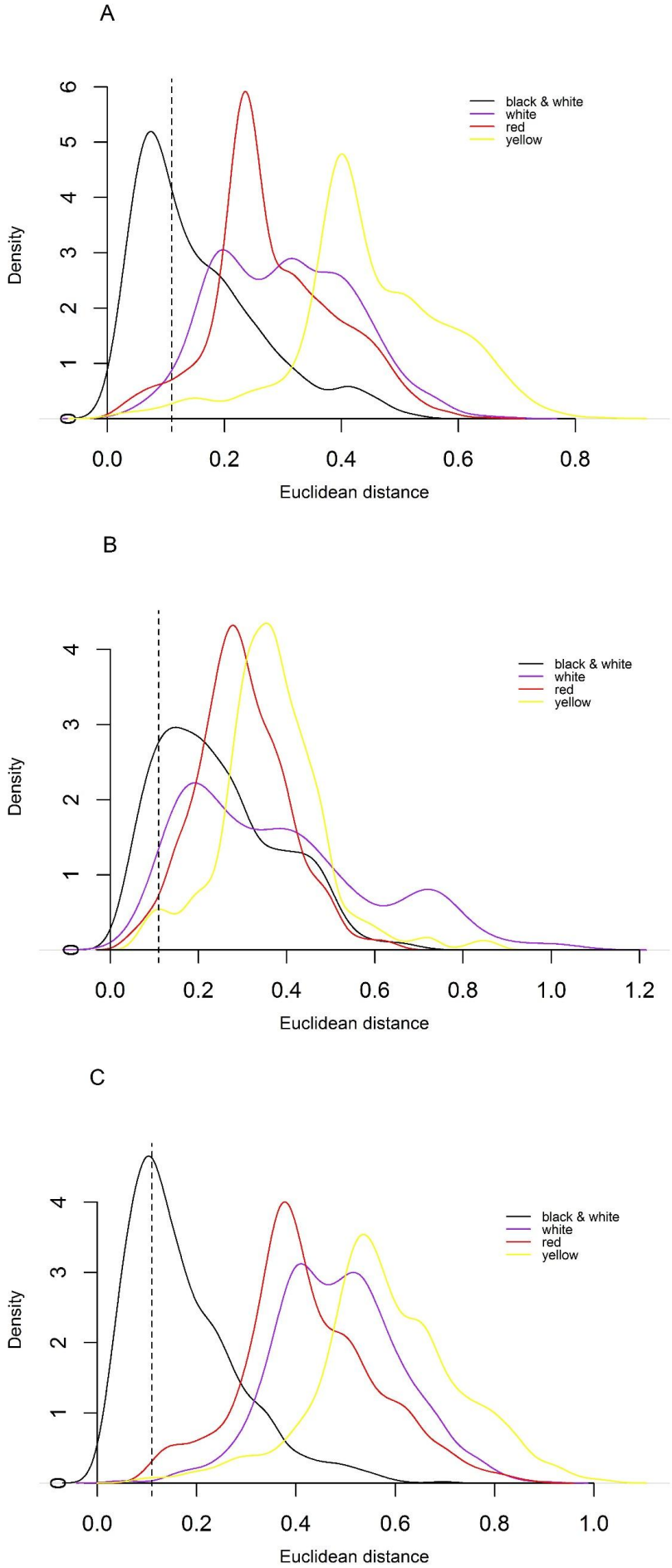

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Fig. 2. Kernel density estimation of the Euclidean distance between four *Gasteracantha cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) and flower reflectance (N=859). Estimations were based on the photoreceptors of (A) *Apis mellifera* (Hymenoptera), (B) *Drosophila melanogaster* (Diptera), and (C) *Fabriciana adippe* (Lepidoptera). Dotted vertical line represents the crude approximation of the color discrimination threshold.

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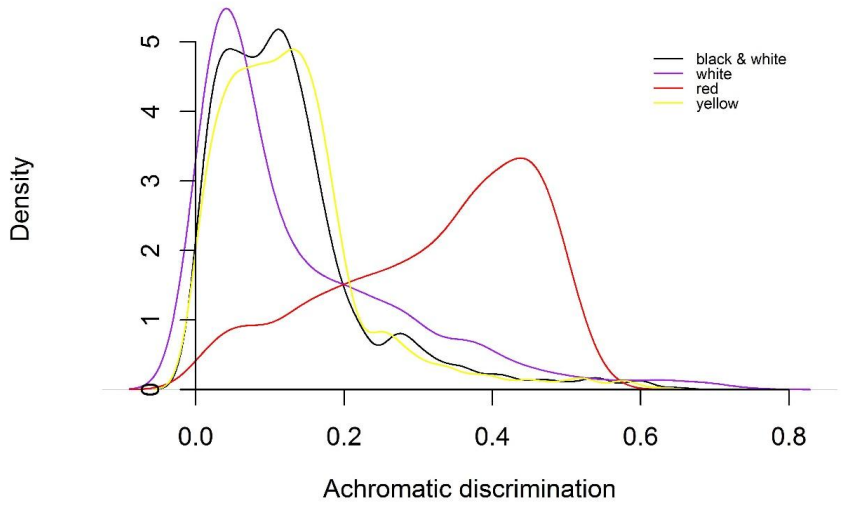
A



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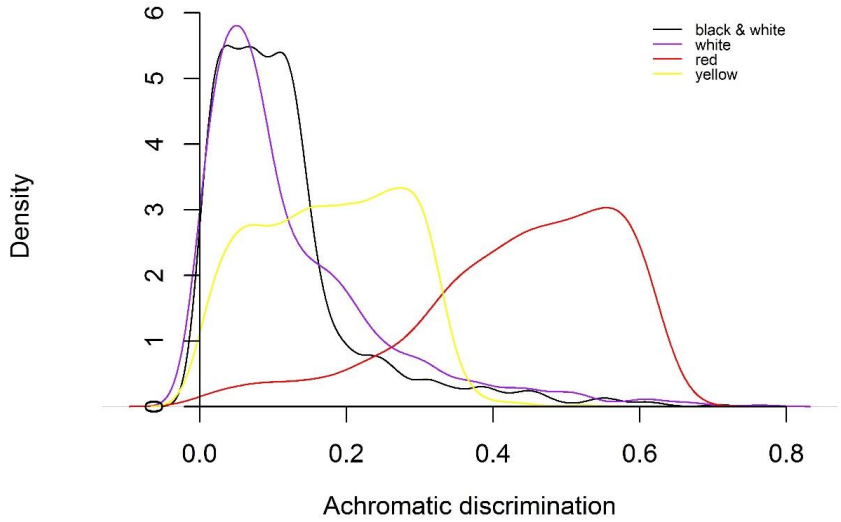

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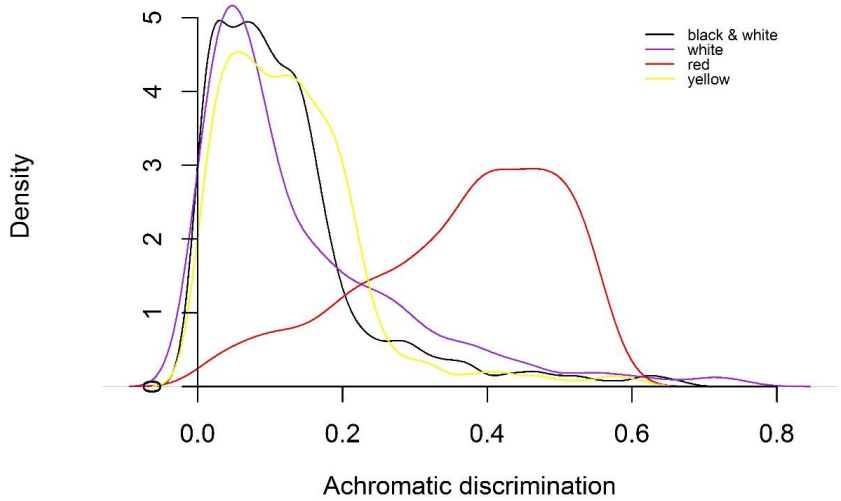

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Fig. 3. Kernel density estimation of achromatic contrast between four *Gasteracantha cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) and flower reflectance (N=859). Estimations were based on the photoreceptors of (A) *Apis mellifera* (Hymenoptera), (B) *Drosophila melanogaster* (Diptera), and (C) *Fabriciana adippe* (Lepidoptera). Dotted vertical line represents photoreceptor excitation for the background.

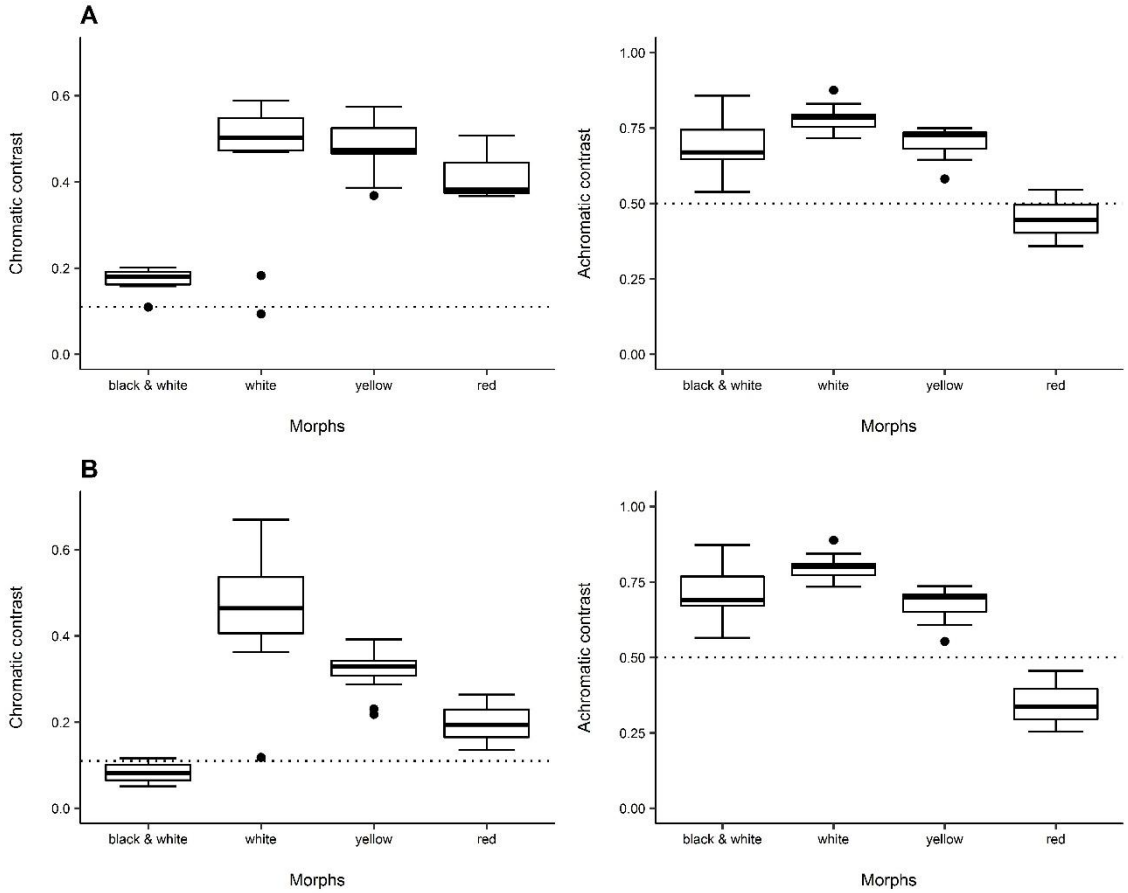

Fig. 4. Chromatic (left) and achromatic (right) contrasts of four *Gasteracantha cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) when viewed against a Brazilian savanna background by predators with distinct visual systems. (A) *Parus caeruleus* (Passeriformes). (B) *Philanthus Triangulum* (Hymenoptera). Dotted vertical lines represent the discrimination thresholds for chromatic contrast (0.06) and photoreceptor excitation for background in achromatic contrast (0.5).