

Universidade Federal de Goiás
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O papel da interação com presas e
predadores na variação cromática de
Gasteracantha cancriformis
(Araneidae)

Orientador Felipe Malheiros Gawryszewski

Goiânia – GO

Agosto de 2017

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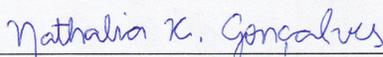
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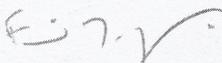
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ATA DA SESSÃO PÚBLICA DE DEFESA DE DISSERTAÇÃO DE Nº 153

Aos dezoito dias do mês de agosto do ano de dois mil e dezessete (18/08/2017), às quatorze horas (14h), no auditório do ICB V - Campus Samambaia - UFG, reuniram-se os componentes da banca examinadora: **Prof. Dr. Felipe Malheiros Gawryszewski - ICB/UFG; Prof. Dr. Fausto Nomura - ICB/UFG; Prof. Dr. Rodrigo Hirata Willemart - USP;** para, em sessão pública presidida pelo primeiro examinador citado, procederem à avaliação da defesa de dissertação intitulada: **“O papel da interação com presas e predadores na variação cromática de *Gasteracantha cancriformis* (Araneidae)”**, em nível de mestrado, área de concentração em Ecologia e Evolução, de autoria de **Nathalia Ximenes Gonçalves**, discente do Programa de Pós-Graduação em Ecologia e Evolução da Universidade Federal de Goiás. A sessão foi aberta pelo presidente, que fez a apresentação formal dos membros da banca. A palavra, a seguir, foi concedida à(o) autor(a) da dissertação que, em cerca de 40 minutos, procedeu à apresentação de seu trabalho. Terminada a apresentação, cada membro da banca arguiu à(o) examinada(o), tendo-se adotado o sistema de diálogo sequencial. Terminada a fase de arguição, procedeu-se à avaliação da dissertação. Tendo-se em vista o que consta na Resolução nº 1127 de dezembro de 2012 do Conselho de Ensino, Pesquisa, Extensão e Cultura (CEPEC), que regulamenta o Programa de Pós-Graduação em Ecologia e Evolução, a dissertação foi APROVADA, considerando-se integralmente cumprido este requisito para fins de obtenção do título de Mestre em Ecologia e Evolução pela Universidade Federal de Goiás. A conclusão do curso dar-se-á quando da entrega da versão definitiva da dissertação na secretaria do programa, com as devidas correções sugeridas pela banca examinadora, no prazo de trinta dias a contar da data da defesa. Cumpridas as formalidades de pauta, às 17 h e 15 min., encerrou-se a sessão de defesa e, para constar,

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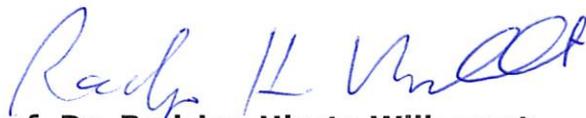
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Sumário

Agradecimentos	4
Apresentação	5
1. Capítulo	7
1.1. Resumo	8
1.2. Introdução	9
1.3. Materiais e Métodos	12
1.3.1. Color vision model	12
1.3.2. Multiple prey hypothesis	14
1.3.3. Multiple mimic models hypothesis	15
1.3.4. Multiple predator hypothesis	17
1.4. Resultados	18
1.4.1. Multiple prey hypothesis	18
1.4.2. Multiple mimic models hypothesis	18
1.4.3. Multiple predator hypothesis	19
1.5. Discussão	19
1.6. Referências.....	23
1.7. Tabelas.....	29
1.8. Figuras	31
1.9. Material suplementar	34
2. Capítulo 2	37
2.1. Resumo	38
2.2. Introdução	39
2.3. Materiais e Métodos	42
2.3.1. 3D model construction	42
2.3.2. Chromatic and achromatic contrast of spider models	42
2.3.3. Prey capture experiment	44
2.3.4. Predation experiment	46
2.4. Resultados	47
2.4.1. Conspicuousness of spider models to prey and predators	47
2.4.2. Prey capture experiment	47
2.4.3. Predation experiment	48
2.5. Discussão	48
2.6. Referências.....	52
2.7. Tabelas	59
2.8. Figuras	62
2.9. Material suplementar	67
Considerações finais	69
Referências	71

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Apresentação

O estudo da coloração animal começa com os trabalhos de Wallace, Darwin, Thayer, Poulton e Cott (Caro et al., 2017a), que realizando descrição de padrões de cores
5 contribuíram para a compreensão da função adaptativa da coloração (Caro, 2016; Caro et al., 2017a). Além deles, a área também recebeu apoio do Kettlewell e Ford, com suas ideias acerca do polimorfismo de cor (Caro et al., 2017b). Algumas das categorias propostas por Wallace são válidas até os dias atuais e podem refletir interação com potenciais parceiros sexuais, interações sociais ou com presas e predadores e
10 termorregulação (Caro, 2017; Endler & Mappes, 2017). O interesse na área tem aumentado nos últimos 10 anos, assim como as métodos tem se tornado mais diversificados (Endler & Mappes, 2017). Atualmente, as principais subáreas - produção de cor, percepção, evolução e função - avançam com o advento da espectrofotometria (Caro et al., 2017a), uso de fotografia digital (Stevens et al., 2007) e de modelos de visão
15 (Delhey et al., 2015), que complementam os trabalhos de campo.

A teoria de “sensory drive” prediz a coevolução entre sinais, sistemas sensoriais e comportamento de sinalização (Endler, 1992). Portanto, é esperado que cores e sistemas visuais coevoluam como um sistema de comunicação especializado (Osorio & Vorobyev, 2008), explicando a diversidade de colorações e de sistemas visuais que encontramos na
20 natureza. Os grupos com sistemas visuais mais conhecidos inclui aves, primatas e himenópteros. Em primatas, a evolução da tricromacia e a coloração de frutas coletadas por estes animais pode refletir tal relação coevolutiva, na qual esse tipo de visão de cores teria evoluído como uma adaptação para encontrar frutas. Posteriormente, frutas consumidas por primatas e frutas consumidas por aves passaram a apresentar diferentes
25 colorações (Osorio & Vorobyev, 2008). Já a importância da coloração na comunicação entre flores e polinizadores pode ser exemplificada pela presença de padrões UV em flores amarelas polinizadas por abelhas, que as orientam ao contato com os órgãos reprodutivos da planta, garantindo a polinização. Isso indica que existe adaptação de flores para esse grupo específico de polinizadores, considerando que além disso, flores
30 polinizadas por aves não apresentam padrões UV (Papiorek et al., 2015).

Aranhas de teia orbicular predam e são predadas por organismos com sistemas sensoriais distintos, incluindo organismos tricromatas, tetracromatas e pentacromatas (Briscoe & Chittka, 2001; Schnaitmann et al., 2013; Hart, 2001; Peitsch, 1992). Algumas famílias de aranhas apresentam padrões de coloração chamativos. Em Nephilidae e Araneidae estes padrões foram associados à atração de presas (White & Kemp, 2015). Atração de presas é frequentemente atribuída ao mimetismo da coloração floral (White et al., 2016). Algumas dessas aranhas além de apresentarem coloração conspícua também são polimórficas. Portanto, a diversidade de sinais visuais também pode ser atribuída à variação do sistema sensorial dos receptores. Nesse caso, a evolução e manutenção do polimorfismo de cor pode resultar da interação com presas e predadores com sistemas visuais distintos (Ruxton et al., 2004; White & Kemp, 2015), visto que um mesmo estímulo pode ser visto como críptico para um organismo, mas como conspícuo para outro (Endler & Mappes, 2004).

O objetivo dessa dissertação foi avaliar como a interação com múltiplos receptores influencia a variação cromática em *G. cancriformis*. No capítulo 1, usamos um modelo de visão para avaliar a hipótese de múltiplos receptores (presas e predadores) na manutenção do polimorfismo. É esperado que coloração de cada morfo seja percebida distintamente por suas presas e predadores; e que os morfos de *G. cancriformis* apresentem colorações semelhantes a diferentes coloração de flore. No capítulo 2, usamos uma abordagem experimental para testar essas mesmas hipóteses. Com respeito a coloração conspícua em *G. cancriformis*, avaliamos a hipótese de atração de presas e a hipótese de aposematismo. Quanto ao polimorfismo, avaliamos se cada morfo atrai diferentes presas e/ou adverte impalatabilidade a diferentes grupos de predadores.

Capítulo 1

O polimorfismo de uma aranha de teia orbicular na visão de presas e predadores

Artigo submetido: *Evolutionary Ecology*

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

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Running title: Orb-web spider color polymorphism

Key words: *Gasteracantha cancriformis*; Araneidae; Visual communication; Aposematism; Luring; Prey attraction

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

55 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
60 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
65 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
70 explain the evolution of color polymorphism in *G. cancriformis*. 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.

75 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 and stabilizes polymorphisms (Bond 2007). Nonetheless, other adaptive and non-adaptive
80 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).

85 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
90 (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).

95 Many orb-web spiders exhibit conspicuous coloration. Although sexual selection is a common explanation for bright coloration in other taxa such as birds (Ryan, 1990), this scenario is less likely to happen in orb web spiders, because they have limited visual acuity (Foelix, 2011). *Argiope argentata* (Araneidae), for instance, seems to possess only one photoreceptor (Tiedemann, 1986). The prey attraction hypothesis states that the
100 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
105 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
110 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, 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 et al., 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.

145 The aim of the present study was to investigate three hypotheses for the 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 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
150 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.

155

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
160 model has been only validated with behavioral experiments on honeybees, its general form allow us to apply it for other taxa (e.g. They 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
165 factor R was determined for each photoreceptor, as follows:

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

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:

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

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

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

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

$$X1 = \sin 60^\circ (E_3 - E_1) \quad (4)$$

$$X2 = E_2 - 0.5 (E_1 + E_3) \quad (5)$$

For tetrachromat organisms (Théry and Casas, 2002):

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

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

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

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

175 follows:

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

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

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

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

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:

$$\Delta S = \sqrt{\sum_{i=1}^n (X_{ai} - X_{bi})^2} \quad (13)$$

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

180

The color spaces are chromaticity diagrams and, thus, do not estimate the achromatic contrast between objects. Nonetheless, achromatic contrasts can be important 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 $\sim 15^\circ$, which means that bees have to be very close to flowers in order to use their color vision

185

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, 2002). 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).

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).

210 **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). Those species are not necessarily sympatric with *G. cancriformis*. However, these insect orders are commonly intercepted by orb-webs in field experiments

(Craig and Ebert 1994; Tso et al. 2002) and represent the diversity of visual systems among insects (Briscoe and Chittka, 2001). Furthermore, we do not expect a great variation of spectral sensitivity within a taxon (Briscoe and Chittka, 2001).

220 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.

225 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 (λ_{\max} ; Eguchi et al., 1982) were used to estimate the photoreceptor curves (for details see Govardovskii et al., 2000).

230 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

235 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

240 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 \times 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

245 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. Marginal and conditional R^2 were estimated according to the

recommendations of Nakagawa and Schielzeth (2013).

As a reference point, we used a color discrimination threshold of $\Delta S = 0.11$, which
250 is the threshold value below which trained bees are unable to distinguish different flower
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
255 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

260 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
265 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. Even though we did not use data of
270 sympatric species, flowers spectral curves variation are subtle, because there is a constraint
on flower pigments blending (Chittka and Menzel, 1992; Chittka et al. 1994).

First, to evaluate color regardless of the observer, we compared hue (Eq. 13),
saturation (Eq. 14) and brightness (Eq. 15) of flowers and spiders (Anderson and Prager,
2006):

$$H_3 = \lambda_{Rmid} \quad (13)$$

275 where λ_{Rmid} is the wavelength at the middle point between the minimum and maximum
reflectances;

$$S_8 = (R_{max} - R_{min})/B_2 \quad (14)$$

where R_{\max} and R_{\min} are the maximum and minimum reflectance points; and $B_2 = \sum_{\lambda=300}^{\lambda=700} R_i / n_w$, where R_i is the reflectance corresponding to each wavelength point, and n_w is the total wavelength intervals;

$$B_3 = R_{\max} \quad (15)$$

280 where R_{\max} is the maximum reflectance.

Secondly, we calculated a matrix of chromaticity distances between average color loci of each spider color morph and each individual flowers species, and computed the percentage of these values that were below the theoretical detection threshold of 0.11.

285 **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 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
290 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
295 curves from sensitivity peaks). Again, those species are not sympatric with *G. cancriformis*, but we do not expect a great variation of photoreceptors types within hymenopterans (Peitsch et al., 1992) nor Passeriformes (Hart, 2001).

The multiple predator hypothesis predicts that different predator taxa perceive color morphs differently. To assess this prediction, we established two linear mixed
300 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 \times 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
305 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.

We estimated marginal and conditional R^2 for the models following the recommendations of Nakagawa and Schielzeth (2013).

As in the multiple prey hypothesis, we used discrimination thresholds as reference points. For the chromatic contrast, we considered color discrimination thresholds of $\Delta S = 0.11$ and $\Delta S = 0.06$ 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.

315 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 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).

330 Multiple mimic models hypothesis

We found three peaks of hue for the flowers, around 400, 500 and 600 nm, to which spider morphs are near: the black and white morph has a hue around 400 nm, the hue of yellow morphs is near 500 nm, and the red morph is around 600 nm (Fig. 2A). The saturation metric had only one peak, to which black and white, white and yellow morphs were close (Fig. 2B). The brightness metric also only presented a single peak, white, red and yellow morphs had average brightness near to this peak (Fig. 2C).

For all three prey species, only the white patch of the black and white morph had values 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*. 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. 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. For *F. addipe* these values were 0.4%, 0.2%, and 0.5% respectively.

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. 3A,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. 3A). 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 threshold of 0.11 (Fig. 3B). 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. 3C). The white morph also had the highest excitation value for *P. triangulum*, followed by the black and white, yellow, and red morphs, respectively (Fig. 3D). The model coefficients are provided in the supplementary material (Table S3 and S4).

DISCUSSION

Our statistical analyses show that the majority of *G. cancriformis* morphs have a high probability of being detected by potential prey and predators, and the degree of detectability varies according to the signal receiver. Therefore, we suggest that the multiple prey and multiple predator hypotheses are plausible explanations for the

evolution of color polymorphism in *G. cancriformis*. In addition, hue values of spiders and flowers support the flower mimicry hypothesis.

370 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
375 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
380 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). Contrary to our findings, a recent study using the receptor noise-limited color vision model showed that insects prey perceive coloration of *Verrucosa arenata* morphs differently, however the maintenance of color
385 polymorphism does not seem to be influenced by multiple prey. Yellow morphs of *V. arenata* have higher chromatic contrast than white morphs for Diptera and Hymenoptera. Whereas in the achromatic dimension the white morph had a higher contrast for both prey taxa (Ajuria-Ibarra et al. 2017). Color morphs may, instead be influenced by other factors such as illumination or different visual channels of relevant observers (Ajuria-
390 Ibarra et al. 2017; White and Kemp, 2016).

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
395 relevant considering the multiple prey hypothesis. 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). If we look to each prey group separately, we also have
400 different detectabilities between the chromatic and achromatic channels. However, spider
morphs presented similar levels of achromatic detectability by all the prey taxa examined,
therefore, this idea seems inconsistent with the multiple prey hypothesis for *G.*
cancriformis.

Several authors have proposed flower mimicry as a mechanism of prey attraction
405 by orb-web spiders *via* conspicuous body coloration (e.g. Craig and Ebert, 1994; Hauber,
2002). However, the hypothesis has seldom been tested. Our results showed that,
considering only color metrics, most of the *G. cancriformis* morphs have similar
coloration to flowers. However, when we modeled color perception to potential prey,
only the black and white morph had values a large percentage of flowers below to the
410 theoretical discrimination threshold. 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). 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*)
415 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
420 (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).

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
425 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

430 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
435 ingestion difficult along with the species' bright colors constitute aposematism (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.

440 Color polymorphism may seem counterintuitive in aposematic species, but it may occur in cases of anti-apostatic selection, overdominance, or equal fitness from different selection pressures (Stevens & Ruxton, 2012). If spider morphs are conspicuous to prey 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
445 avoided by prey. Not only selective pressures from prey and predators may influence color polymorphism, but also thermoregulatory effects and the effect of illumination on the signaler detectability (Rao and Mendoza-Cuenca, 2016; Rojas et al., 2014). Therefore, polymorphism may result from multiple evolutionary forces, in which some morphs signals their impalatability to predators, whereas other morphs are protected from certain
450 predators due to camouflage, meanwhile, they may benefit from thermoregulatory behavior by occupying different microhabitats.

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
455 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
460 (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.

465

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Competing interests

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Tables

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

Model	df	Δ AIC	Marginal R ²	Conditional R ²
<i>Multiple prey hypothesis</i>				
Chromatic dimension				
$\Delta S \sim \text{morph}^* \text{observer}$	17	0.0	0.87	0.96
$\Delta S \sim \text{morph} + \text{observer}$	11	23.9	0.89	0.95
$\Delta S \sim \text{observer}$	8	52.4	0.33	0.96
$\Delta S \sim \text{morph}$	9	61.5	0.50	0.51
$\Delta S \sim 1$	6	90.6	0	0.53
Achromatic dimension				
excitation $\sim \text{morph}^* \text{observer}$	17	0.0	0.82	0.99
excitation $\sim \text{morph} + \text{observer}$	11	57.6	0.77	0.86
excitation $\sim \text{morph}$	9	72.2	0.79	0.89
excitation $\sim \text{observer}$	8	84.7	0.004	0.60
excitation ~ 1	6	100.2	0	0.66
<i>Multiple predator hypothesis</i>				
Chromatic dimension				
$\Delta S \sim \text{morph}^* \text{observer}$	13	0.0	0.86	0.99
$\Delta S \sim \text{morph} + \text{observer}$	10	6.9	0.88	0.99

$\Delta S \sim \text{observer}$	7	30.6	0.29	0.99
$\Delta S \sim \text{morph}$	9	54.5	0.58	0.58
$\Delta S \sim 1$	6	74.9	0	0.63
Achromatic dimension				
excitation $\sim \text{morph}^*\text{observer}$	14	0.0	0.78	1
excitation $\sim \text{morph}+\text{observer}$	10	14.4	0.78	0.96
excitation $\sim \text{observer}$	7	21.1	0.007	0.86
excitation $\sim \text{morph}$	9	36.9	0.80	0.98
excitation ~ 1	6	43.9	0	0.92

Figures

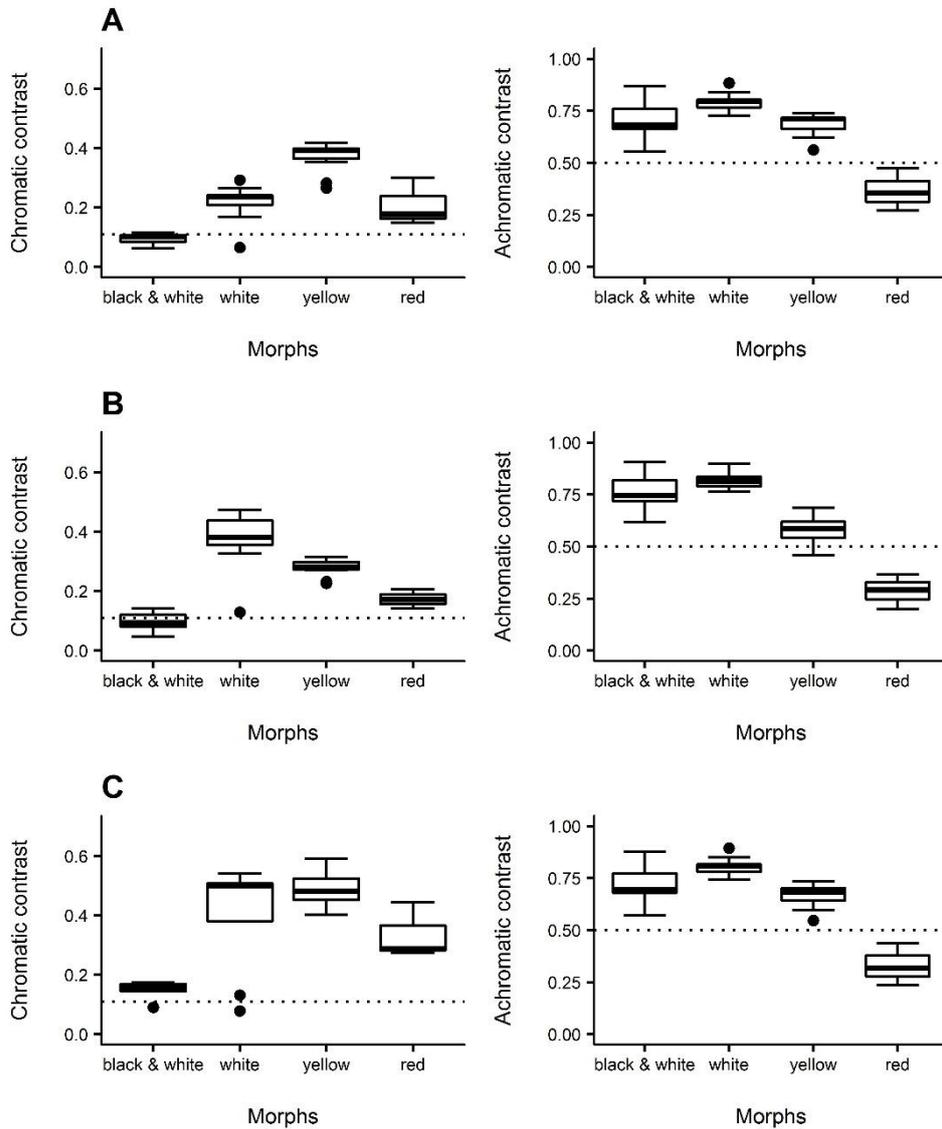
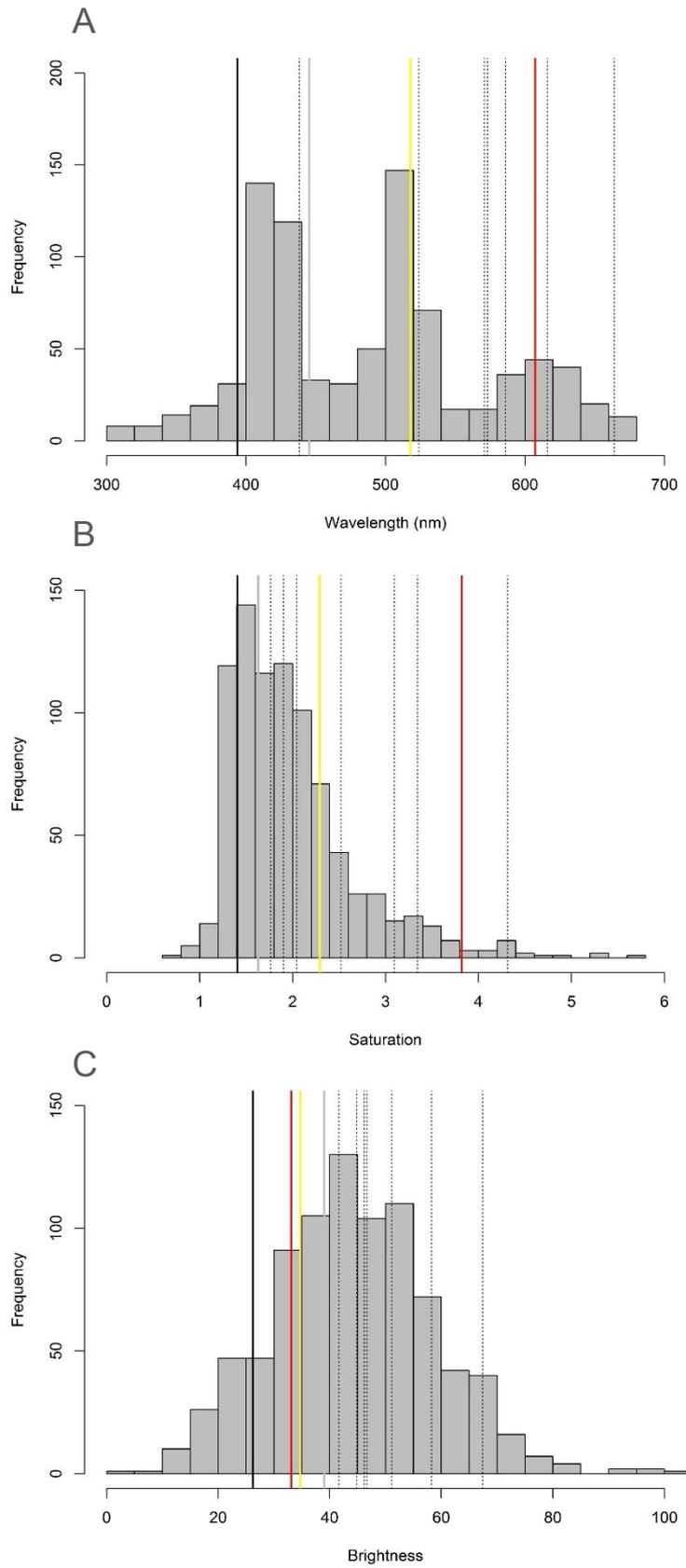


Fig. 1. Chromatic (left) and achromatic (right) contrasts of four *Gasteracantha cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) when viewed against a Brazilian savanna background by prey with distinct visual systems. (A) *Apis mellifera* (Hymenoptera). (B) *Drosophila 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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Fig. 2. Frequency of color properties of flowers (N = 859): (A) hue, (B) saturation, (C) brightness. Average values of each *Gasteracantha cancriformis* morph are represented

with solid colored lines: black and white morph (black line; N = 6), white morph (gray line, N = 10), yellow morph (yellow line, N = 13), red morph (red line, N = 3). Flowers
480 from the Brazilian Savanna (N = 7) are represented with dotted lines.

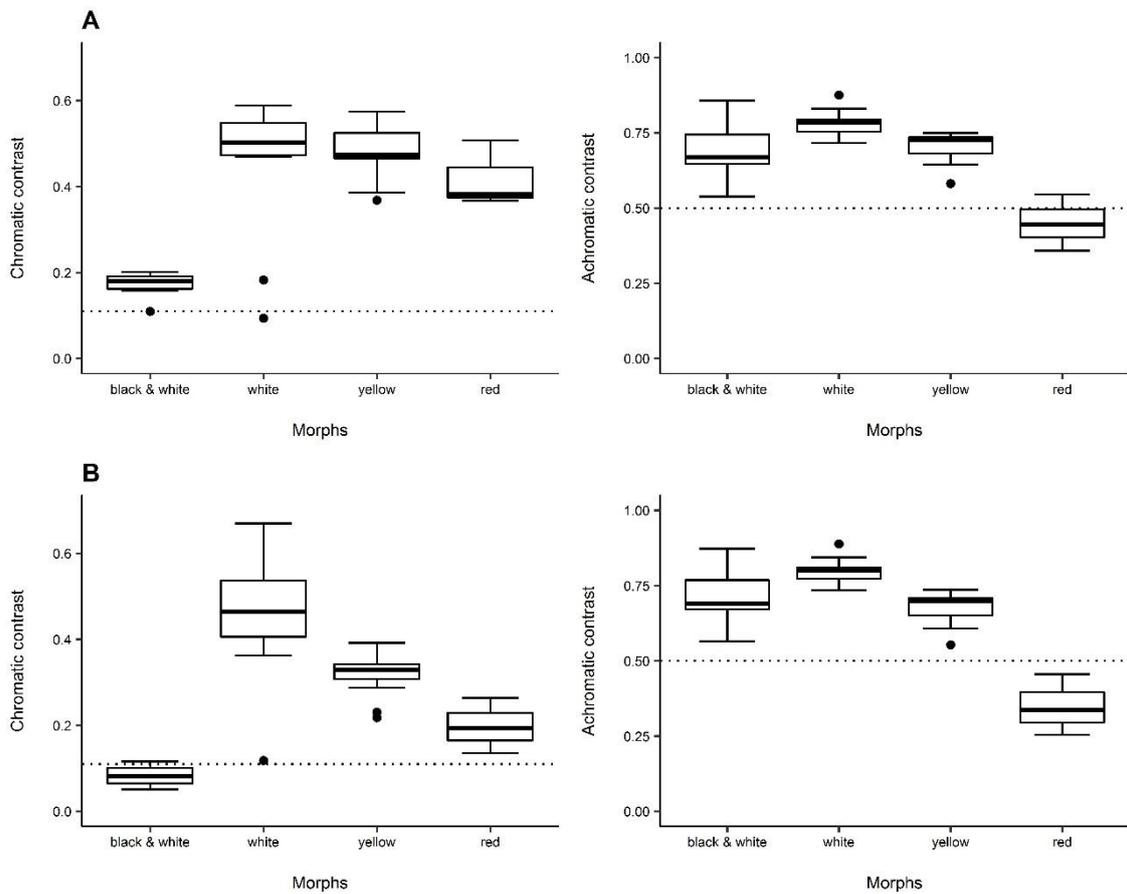


Fig. 3. Chromatic (left) and achromatic (right) contrasts of four *Gasteracantha cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) when viewed against a Brazilian savanna background by predators with distinct visual systems. (A) *Parus caeruleus* (Passeriformes). (B) *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).

Supplementary information

Tables

490 Table S1. Results of Linear Mixed Model comparing chromatic contrast of *Gasteracantha cancriformis* morphs viewed by different groups of prey.

	Value	SE	df	t	p
Intercept	0.0947	0.0175	56	5.4185	< 0.0001
Red morph	0.1136	0.0436	28	2.6019	0.0146
White morph	0.1221	0.0365	28	3.3455	0.0023
Yellow morph	0.2773	0.0221	28	12.5034	< 0.0001
Observer <i>F. adippe</i>	0.0540	0.0072	56	7.4013	< 0.0001
Observer <i>D. melanogaster</i>	0.0020	0.0072	56	0.2712	0.7872
Red morph & observer <i>F. adippe</i>	0.0736	0.0462	56	1.5913	0.1172
White morph & observer <i>F. adippe</i>	0.1334	0.0421	56	3.1700	0.0024
Yellow morph & observer <i>F. adippe</i>	0.0662	0.0130	56	5.1003	< 0.0001
Red morph & observer <i>D. melanogaster</i>	-0.0375	0.0462	56	-0.8121	0.4202
White morph & observer <i>D. melanogaster</i>	0.1530	0.0421	56	3.6366	0.0006
Yellow morph & observer <i>D. melanogaster</i>	-0.0936	0.0130	56	-7.2091	< 0.0001

Table S2. Results of Linear Mixed Model (log transformed) comparing achromatic contrast of *Gasteracantha cancriformis* morphs viewed by different groups of prey.

	Value	SE	df	t	p
Intercept	-0.3605	0.0491	56	-7.3419	<0.0001
Red morph	-0.67	0.0869	28	-7.7143	<0.0001
White morph	0.1291	0.0620	28	2.0817	0.0466
Yellow morph	-0.0224	0.0598	28	-0.3742	0.7110
Observer <i>F. adippe</i>	0.0799	0.0074	56	10.7462	<0.0001
Observer <i>D. melanogaster</i>	0.0205	0.0074	56	2.7551	0.0079
Red morph & observer <i>F. adippe</i>	-0.3309	0.0281	56	-11.7844	<0.0001
White morph & observer <i>F. adippe</i>	-0.0513	0.0080	56	-6.4438	<0.0001
Yellow morph & observer <i>F. adippe</i>	-0.2531	0.0133	56	-19.0999	<0.0001
Red morph & observer <i>D. melanogaster</i>	-0.1307	0.0281	56	-4.6552	<0.0001
White morph & observer <i>D. melanogaster</i>	-0.0042	0.0080	56	-0.5228	0.6032
Yellow morph & observer <i>D. melanogaster</i>	-0.0466	0.0133	56	-3.5162	0.0009

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Table S3. Results of Linear Mixed Model comparing chromatic contrast of *Gasteracantha cancriformis* morphs viewed by different groups of predators.

	Value	SE	df	t	p
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Intercept	0.1704	0.0220	28	7.7355	< 0.0001
Red morph	0.2484	0.0413	28	6.0153	< 0.0001
White morph	0.2761	0.0510	28	5.4093	< 0.0001
Yellow morph	0.3163	0.0267	28	11.8502	< 0.0001
Observer <i>P. triangulum</i>	-0.0873	0.0077	28	-11.394	< 0.0001
Red morph & observer <i>P. triangulum</i>	-0.1333	0.0260	28	-5.1233	< 0.0001
White morph & observer <i>P. triangulum</i>	0.0964	0.0613	28	1.5726	0.1270
Yellow morph & observer <i>P. triangulum</i>	-0.0794	0.0096	28	-8.2352	< 0.0001

500 Table S4. Results of Linear Mixed Model (log transformed) comparing achromatic contrast of *Gasteracantha cancriformis* morphs viewed by different groups of predators.

	Value	SE	df	t	p
Intercept	-0.3809	0.0428	28	-8.898	< 0.0001
Red morph	-0.4321	0.0741	28	-5.8287	< 0.0001
White morph	0.1362	0.0541	28	2.5159	0.0179
Yellow morph	0.0246	0.0517	28	0.4763	0.6376
Observer <i>P. triangulum</i>	0.0333	0.0047	28	7.1148	< 0.0001
Red & observer <i>P. triangulum</i>	-0.3015	0.0484	28	-6.2265	< 0.0001
White & observer <i>P. triangulum</i>	-0.0124	0.0048	28	-2.5765	0.0155
Yellow & observer <i>P. triangulum</i>	-0.0742	0.007	28	-10.601	< 0.0001

Coloração conspícua em aranha
polimórfica: evidência de evitação de
predadores, mas não de atração de
presas

Conspicuous colors in a polymorphic orb-web spider: evidence of predator avoidance, but not of prey attraction.

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Keywords: *Gasteracantha cancriformis*, Araneidae, color conspicuousness, visual lure, warning coloration

Abstract

Conspicuous colors in animals may be explained by mate attraction, predator avoidance and prey attraction. The latter is specially studied in sit-and-wait organisms, regarded as a strategy that increases foraging success through luring. Predators lure prey by mimicking food or other resources of potential interest. Alternatively, some animals avoid predation by visually signaling unpalatability to predators. Moreover, evolution of a visual signal is affected by properties of the environment, receiver processing mechanisms, and the behavior of senders and receivers. Variation in one of these factors may explain the evolution of color polymorphism of conspicuous species. Here we aimed to investigate the role of multiple receivers on the evolution and maintenance of color conspicuousness and color polymorphism in *Gasteracantha cancriformis* (Araneidae). We tested four hypotheses: (1) conspicuous coloration in *G. cancriformis* is prey lure strategy; (2) conspicuous coloration in this spider is a warning signal; (3) each morph of *G. cancriformis* lures specific prey taxa; (4) each morphs of *G. cancriformis* warns specific predator taxa. In sum, we expected that each morph would result from the adaptation to specific sensory and perceptual systems of prey and/or predator taxa. To assess the role of prey, we conducted a prey capture experiment in the field, in which webs were assigned to four treatments: yellow, red, and black models, or no model. For each treatment, we counted the number of prey trapped on the webs and the calculated webs

damaged area. To assess the role of predators on the evolution of conspicuous color patterns and polymorphism, we conducted a second field experiment, allocating yellow, red and black spider models in nylon threads along the vegetation, and observed the number of attack markings for each of them. Our results did not corroborate the prey attraction hypothesis nor the prey specific adaptation of color polymorphism. On the predation experiment, we found that black spider models presented more markings, which indicates that yellow and red models were less preferred, possibly suggesting that spider coloration may play a role on predator avoidance. Our results, however, do not corroborate the hypothesis that multiple predators influence polymorphism evolution. Color polymorphism in this species is possibly a multi-functional attribute, where some morphs benefits from aposematism, whereas others may alternative fitness advantages. Non-adaptive explanation should also be considered in future experiments of the evolution and maintenance of color polymorphisms.

535 **Introduction**

Predator avoidance (Ruxton, Sherratt, & Speed, 2004), prey attraction (White & Kemp, 2015), and mate attraction (Ryan, 1990) are typical explanations for conspicuous colors in animals. Conspicuous signals in prey may indicate the presence of secondary defenses that are worth avoiding by predators, such as noxiousness, morphological attributes (claws, spines) or behavioral responses (Endler & Greenwood, 1988; Ruxton et al., 2004). Conspicuous patterns may also increase the foraging efficiency if these patterns are deceptive signals (e.g. aggressive mimicry, Jackson & Cross, 2013). Color-based prey lures have been investigated in various taxa, which includes Diptera, Mantodea, Decapoda, Araneae, Lophiiformes, Siphonophorae (White & Kemp, 2015). In the Australian crab spider *Thomisus spectabilis*, flowers occupied by a spider attracted more pollinators than empty flowers, suggesting that some signaling strategy was luring insects (Heiling, Herberstein, & Chittka, 2003). Some human-white spiders have a UV-white reflectance which creates a high color contrast against the flower background. This contrast deceives honeybees, possibly due to the bees preference for contrasting markings on flowers (Heiling, Chittka, Cheng, & Herberstein, 2005; Heiling et al., 2003). Conspicuous coloration may also be under sexual selection. For instance, spiders of the

genus *Maratus* are sexually dimorphic, females are cryptic, whereas males have conspicuous abdomens, which they raise on courtship displays (Girard & Endler, 2014).

Conspicuous patterns in orb-web spiders have been regarded as a prey lure
555 through floral coloration mimicry (Craig & Ebert, 1994; Hauber, 2002). The
polymorphism in some colorful orb-web spiders is usually explained by a trade-off
between prey attraction and predator avoidance. Conspicuous typical morphs of *Nephila*
pilipes (Tetragnathidae), for instance, presents a higher prey capture success than melanic
morphs (Tso, Tai, Ku, Kuo, & Yang, 2002). In addition, yellow cardboard models of
560 *Nephila pilipes* showed higher prey capture rates than models made of black paper.
However, yellow paper models also attracted more insect predators in comparison to
black paper models, reflecting a trade-off between foraging success and predation risk
(Fan, Yang, & Tso, 2009). In orb-web spiders, sexual selection most likely did not play a
role on the evolution of color patterns because males and females have poor visual acuity
565 and limited color vision (Foelix, 2011).

However, the visibility of a color signal is dependent not merely on its reflectance
spectrum, but also on the ambient light intensity, background reflectance spectrum,
transmission properties of the air, and the observer visual system and neural processing
(Endler, 1990). Variation on one of these factors may be an additional explanation to the
570 evolution and maintenance of color polymorphisms (Endler, 1992; White & Kemp, 2015).
If emitted color signals are perceived differently by specific prey or predators, the
polymorphism may be maintained by multiple receivers selective pressure (White &
Kemp, 2015). For instance, the same individual may be perceived as cryptic by one
species and conspicuous to another (Endler & Mappes, 2004). In this scenario, each
575 morph would be the result of adaptation to particular sensory system of groups of prey
and/or predators (Endler, 1992; Ruxton et al., 2004; White & Kemp, 2015).

Orb-web spiders prey upon and are predated by groups with very distinct visual
systems. From the prey side, hymenopterans are trichromatic (Briscoe & Chittka, 2001),
lepidopterans are tetrachromats (Briscoe & Chittka, 2001), and some dipterans are
580 pentachromats (Schnaitmann, Garbers, Wachtler, & Tanimoto, 2013). From the predators
side, birds are tetrachromats, with spectral sensitivity showing peaks on UV-Violet, blue,
green and red (Hart, 2001), whereas predatory wasps (Hymenoptera) are trichromats,

with sensitivity peaks on UV, blue and green (Peitsch et al., 1992). Therefore, conspicuous color patterns and color polymorphism in these spiders may result from the interaction
585 with multiple visually oriented prey and/or predators (Endler, 1992; Ruxton et al., 2004; White & Kemp, 2015).

The orb-web spider *Gasteracantha cancriformis* (Araneidae) is widely distributed in the Americas. Adult females measure from 10 to 13 mm in width and 5 to 7 mm in length, and adult males measure from 2 to 3 mm (Levi, 1978; Muma, 1971). They are
590 diurnal and construct large webs with tufts (Levi, 1978; Muma, 1971). Females of this species present the abdominal dorsum with several color patterns in the same population, some of which appears quite conspicuous to the human vision (Gawryszewski & Motta, 2012; Levi, 1978). The morphs frequency was constant over two years at the same study site, the yellow ones being the most abundant, followed by white, black & white, and red
595 (Gawryszewski, 2007). Conversely, males are brownish and not polymorphic (Levi, 1978). Unlike other orb-web spiders, experimental evidence suggest that conspicuous colors in *Gasteracantha cancriformis* do not increase its foraging success (Gawryszewski & Motta, 2012). Alternatively, conspicuous colors in this species may be a warning signal against predators (Edmunds & Edmunds, 1983; Gawryszewski & Motta, 2012; Pékar, 2014)
600 because spiders of the genus *Gasteracantha* have sharp spines on and a hard abdomen (Edmunds & Edmunds, 1983). Furthermore, the mud-dauber wasps (*Sceliphron laetum*) avoid providing initial instars of their nest with species of *Gasteracantha*, possibly due to the spines and difficult digestion of a hard integument (Elgar & Jebb, 1999). As in other organisms, *G. cancriformis* prey upon several different insect orders, and is potentially
605 predated by both avian and wasp species. Therefore, the color polymorphism in this species may be, at least in part, maintained by selective forces arising from prey and predators with distinct visual systems.

Therefore, in this study we experimentally tested four hypotheses concerning the conspicuous color patterns and color polymorphism evolution and maintenance in
610 *Gasteracantha cancriformis*. Firstly, we tested again the prey lure hypothesis for *Gasteracantha cancriformis*, but with spiders models and additional color morphs. This hypothesis predicts that conspicuous spiders have an increased foraging success in comparison to duller individuals. Secondly, we tested whether conspicuous color patterns

are aposematic signals, in this case we expected that predators avoid conspicuous morphs.

615 Thirdly, we tested whether color polymorphism results from adaptation to multiple prey, consequently, each color morph would be adapted to attract specific prey taxa. Finally, we evaluated whether color polymorphism results from adaptation to multiple predators, consequently, each morph should be avoided by different predators.

620 **Methods**

3D model construction

The experiments were conducted using 3D models of the spider *Gasteracantha cancriformis*. The initial model was constructed on the free software 123D Catch (version 1.1, Autodesk, San Francisco, California, USA), which creates a virtual scan of any object
625 from photographs. Approximately 80 photographs were taken from the dorsal and ventral surfaces of a spider female individual. Afterwards, the resulting scan was imported to MeshMixer (version 2.9.2, Autodesk, San Francisco, California, USA), where it was edited to correct irregularities and set to the scale of 12.00 x 8.51 x 3.65 mm. We printed models using acrylonitrile butadiene styrene (ABS) plastic in a 3D printer (version UP mini,
630 Tiertime, Beijing, China). The models were then dyed red, yellow or black using atoxic water based acrylic paint (color reference numbers: 350, 340 and 320; Acrilex, São Paulo, Brazil).

Chromatic and achromatic contrast of spider models

635 To compare how *Gasteracantha cancriformis* models are perceived by prey and predator taxa, we used Chittka (1992) color vision model, which requires four inputs: irradiance, background reflectance, object reflectance, and photoreceptors curves of observer. For the field irradiance, we considered the CIE standard illuminant D65 (International Commission on Illumination), that is adequate to the Brazilian savanna
640 (Chittka, 1996). We used background reflectance data from a previous study, which considered the average of items collected from the Brazilian savanna (see Fig. 5 in Gawryszewski & Motta, 2012). We measured the reflectance of paints (red, N = 3; yellow, N = 3; black, N = 3) using a Praying Mantis Lambda 1050WB Spectrometer (PerkinElmer, Waltham, Massachusetts, United States; for methods see Montgomerie, 2006).

645 Photoreceptor sensitivity data of prey and predators were obtained from the literature:
Apis mellifera (Hymenoptera; Peitsch et al., 1992), *Drosophila melanogaster* (Diptera;
Schnaitmann et al., 2013), *Fabriciana adippe* (Lepidoptera; Eguchi, Watanabe, Hariyama,
& Yamamoto, 1982), *Parus caeruleus* (Passeriformes; Hart, 2001), *Philanthus triangulum*
(Hymenoptera; Briscoe & Chittka, 2001). We followed the same set of calculations as in
650 Ximenes & Gawryszewski (2017; chapter 1 of this dissertation) to estimate chromatic and
achromatic contrast of paints used to create spider models. Achromatic and achromatic
contrasts are measures of the degree of spider model conspicuousness when viewed by
potential prey and predators.

We assumed, for insects, the discriminability threshold of 0.11 predicted for
655 honeybees (Dyer & Chittka, 2004) and 0.06 for bird (Théry, Debut, Gomez, & Casas,
2005) as a reference point to estimate whether spider models can be discriminated from
the background. If there is similarity among these stimuli, we expected to find values of
chromatic contrast near, or lower than, 0.11 for insects and 0.06 for birds. For the
achromatic dimension, we considered the value of 0.5 units, which, by definition, is the
660 photoreceptor excitation for background (Chittka, 1992). If models and background are
similar, we expected to find achromatic contrast values values near 0.5. It should be noted
that the chromatic threshold value for insects was based specifically on honeybee visual
system, and among different insect taxa this value may vary. Discrimination thresholds
may also be influenced by background reflectance, past experience, and learning
665 conditions of the observer (Avarguès-Weber & Giurfa, 2014).

In addition, we also used chromatic and achromatic contrast of *G. cancriformis*
morphs estimated previously in Ximenes & Gawryszewski (2017; chapter 1 of this
dissertation), to compare paints to real spider colors, as perceived differently by prey and
predators. To achieve this, we calculated the chromatic distance between the average
670 value of color morphs and each paint measurement. We did not calculate the chromatic
distance of black paint against the background because, by definition, a black spectrum
does not carry chromatic information, and may generate spurious values by color vision
models (Gawryszewski, 2017). We also extracted and plotted hue (Eq. 1), saturation (Eq.
3) and brightness (Eq. 4) of spiders and paints in order to compare color metrics regardless
675 of the signal receiver (Anderson & Prager, 2006):

$$H_3 = \lambda_{Rmid} \quad (1)$$

where λ_{Rmid} is the wavelength at the middle point between the maximum and minimum reflectance points;

$$B_2 = \sum_{\lambda 300}^{\lambda 700} R_i / n_w \quad (2)$$

where R_i is the reflectance at each wavelength, and n_w is the total of wavelength intervals;

$$S_8 = (R_{max} - R_{min}) / B_2 \quad (3)$$

680 where R_{max} and R_{min} are the maximum and minimum reflectances;

$$B_3 = R_{max} \quad (4)$$

These metrics were obtained using the same spectral curves previously mentioned and calculations were conducted using the R package pavo (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013).

685 *Prey capture experiment*

The study was conducted over 15 days in June and July 2015 in an area of Cerrado *sensu stricto* physiognomy located at the Universidade de Brasília's experimental site 'Fazenda Água Limpa' (-15.945°S, -47.940°W), Brasília, Brazil. This physiognomy is an open area with a crown cover of >30%, it is composed by herbaceous vegetation, shrubs
690 and trees of 3 to 8 m tall, which are contorted, thick, and fire-resistant (Oliveira-Filho and Ratter 2002). Spider webs were usually found on average at 1.09 meters height.

Everyday eight webs were randomly marked and then assigned to one of four experimental groups. The spiders were removed from webs and replaced with a yellow spider model (N=30), a red spider model (N=30), a black spider model (control; N=30),
695 or the web was left without any model (control; N=30), with the aid of tweezers. The experimental group was assigned randomly, following the constrain rule of equal sample size per group per day (blocked per day). *Gasteracantha cancriformis* spiders usually slowly flee through one of the web support threads or they fall from the web. Therefore, neither spiders removal nor models placement damaged the web.

700 We monitored webs visually once every three hours, from ca. 10 a.m. till at ca. 4 p.m. We registered the number and Order of insects trapped by the web. Furthermore,

the webs were photographed to measure the proportion of damaged areas relative to the capture area. The proportion of damaged areas indicates, indirectly, prey that intercepted the web (Tso, 1996). We analyzed the photographs using ImageJ (version 1.48, Schneider, Rasband, & Eliceiri, 2012). We circumvented the outer spiral thread and the inner spiral thread, and then computed the area between them using the ‘analyze’ tool to get the capture area from the images, for each observation phase. Eleven individuals were deleted from the web damage analysis due to camera malfunctioning in one of the observation phases (yellow = 3, red = 2, black = 3, empty web = 3). Sixteen individuals were not included because of poor contrast between the background and webs (yellow = 5, red = 4, black = 4, empty web = 5), two because there was no scale to measure the photos (empty web = 2).

The number and type of prey capture was analyzed using a Linear Mixed Model (LMM) with log transformed response data: $\log(\text{capture}) \sim \text{morph} * \text{taxon}$. ‘Capture’ refers to the number of prey that were trapped by the web (insects of the first phase subtracted from insects observed in the last phase), ‘morph’ refers to the color of the model, and taxon to the prey taxon intercepted by webs. The ID of spider entered as a random factor. The variable taxon was grouped as ‘Diptera’ and ‘other taxa’, due to the low number of prey caught from other taxa than Diptera. We also tested ‘day’ (when each treatment was conducted) as a random effect, but the model with ‘day’ as random effect had a higher AIC value than the model without it ($\text{AIC}_{\text{day}} = 1025.581$, $\text{AIC}_{\text{norrandomeffect}} = 1021.581$).

The prey capture was also estimated considering the proportion of damaged area relative to the total capture area of each web. The analyses followed a General Linear Model (GLM) with square-root transformed data: $\sqrt{\text{final damage}} \sim \sqrt{\text{initial damage}} * \text{morph}$. ‘Final damage’ refers to the proportion of damaged areas in relation to the total web capture area in the last observation phase during the day. ‘Initial damages’ refers to the proportion of damaged areas found just before starting the experiment. Day did not enter as random effect because the AIC value indicated that the model without random effect was a better fit to the data ($\text{AIC}_{\text{day}} = -112.3499$, $\text{AIC}_{\text{norrandomeffect}} = -114.3499$). We visually inspected residuals in quantile-quantile plot and residuals vs. fitted and residuals vs. independent graphs. We used the Akaike Information Criteria to select the best model.

Predation experiment

For this experiment, we added a layer of white non-toxic modeling clay
735 ('Abelhinhas', color reference number: 519; Acrilex, São Paulo, Brazil) to the dorsal
surface of the spider model, and then dyed models with atoxic water-based acrylic paint,
as in the previous experiment. The plasticity of modeling clay allows for distinction
between vertebrate and invertebrate predator marks (e.g., a wasp mandible vs. a birds
peck; Low, Sam, McArthur, Posa, & Hochuli, 2014). Furthermore, spider models made
740 of modeling clay were already successfully used in spider predation experiment (Gonzaga
& Vasconcellos-Neto, 2005). We fixed models in a transparent nylon thread tied between
two trees (Mean \pm standard deviation: 0.655 ± 0.142 m thread length), at approximately
85 cm in height (Mean \pm standard deviation: 0.842 ± 0.148 m). To prevent ant access via
nylon threads, we covered thread extremities with industrial grease.

745 We distributed 598 models in two experimental sites of Cerrado *sensu stricto*
physiognomy, 20 transects in each, equally divided into two areas at the Universidade de
Brasília's experimental site 'Fazenda Água Limpa' (FAL; -15.945° S, -47.940° W), and in two
areas in the National Park of Brasília (PNB; -15.753° S, -47.947° W). For each point a
model was randomly assigned: yellow (FAL, N = 100; PNB, N = 100), red (FAL, N = 100;
750 PNB, N = 99) or black (FAL, N = 100; PNB, N = 99), distant from each other in at least
three meters. These models were exposed over one month and visited twice for
registration of predators' mark attacks. We excluded 14 models from the analysis, which
were cases of broken nylon thread (red: N = 1; yellow: N = 3; black: N = 2) and models
that we could not recover (red: N = 3; yellow: N = 3; black: N = 2).

755 We analyzed data using a Generalized Linear Model (GLM) with binomial
distribution and logit link, considering the presence or absence of attack marks for the
three treatments (yellow, red and black) and location (FAL or PNB): mark (0,1) \sim
treatment*location. The predator taxon was not included in the model because only few
markings were from birds (black, N = 1; yellow, N = 3). We used Akaike Information
760 Criteria to select the best model. For graphical validation we followed the we computed
the average of residuals organized in groups of ten, previously ordered by fitted values.
The new residuals values were used to plot residuals vs. fitted values, frequency of

residuals, residuals vs. location, and residuals vs. morph (Zuur, Ieno, Walker, Saveliev, & Smith, 2009).

765

Results

Conspicuousness of spider models to prey and predators

Black acrylic paints and dark patches of *G. cancriformis* had similar spectral reflectance curves. Yellow and red morphs, although did not have reflectances similar to
770 paints, showed close inflection points (Fig. 1). Spider morphs and paints are also similar in color properties, except for black paint and spider hues, and yellow paint and spider brightness (Fig. S1).

Using the color vision model, the Euclidean distances between paint and morph were only equal or less than 0.11 for *A. mellifera*. The other two prey taxa had Euclidean
775 distances higher than the color discrimination threshold (Table S1). From the predators' side, only the Euclidean distance between the red morph and paint was less than 0.11 for *P. triangulum*, and yellow for *P. caeruleus* (Table S1). In the achromatic dimension, all paints and spider morphs were distinct (Table S1).

Although visual models indicate that the coloration of models and spiders can be
780 distinguished by most of potential observers, spider models are conspicuous compared to background items of the Brazilian Savanna (Figs. 2,3). For prey chromatic vision, the yellow paint is the most conspicuous, except for *F. adippe* (Fig. 2). Whereas in the achromatic dimension all paints had values different from 0.5, except for the red in *A. mellifera* vision (Fig. 2). Considering predators vision, the yellow paint was more
785 conspicuous to *P. triangulum* (Fig. 3A), and the red paint was more conspicuous to *P. caeruleus* (Fig. 3B). For the achromatic dimension all paints had values different from 0.5, except for the red paint in *P. caeruleus* vision (Fig. 3). Although paints and spiders can be distinguished by potential prey and predators according to the visual modeling, paints are conspicuous for most of observers, and are likely to be perceived differently by them.

790

Prey capture experiment

Throughout the experiment, a total of 937 insects were captured by webs. Diptera was the most common taxon (85%), followed by Coleoptera (4%), Hemiptera (0,7%), and

Hymenoptera (0,2%). Ten percent could not identified. The lowest AIC model included
795 only the prey taxon variable (Fig. 4, Tables 1,2). Neither spider models, nor the interaction
between spider models and prey taxa added significant explanatory power to the variation
on the number of prey intercepted by webs (Fig. 4, Table 1). On the other hand, web
area damaged appear to be influenced by the treatment, as the best model includes the
morph variable (Table 1). Empty webs showed slightly more web area damaged than
800 webs with any of the color models (Model estimates: empty web: 0.7856, yellow: 0.7245,
black: 0.7256, red: 0.7376; Fig. 5, Table 3).

Predation experiment

We have found distinct types of impressions at the modeling clay region of the
805 model. A few were a single ‘U’-shaped or ‘V’-shaped mark left by birds (N = 4), other
types of markings were more superficial and varied on patterning. We considered these
types as impressions left by arthropods, as suggested in Low et al. (2014). Black models
showed more attack marks than yellow and red models, both at FAL and PNB (Fig. 6).
The best generalized linear model considered the number of attacks according to the
810 spider morph and location (Table 4). However, this model only differs in AIC in 1.8 from
the model that considers location alone, suggesting that the contribution of ‘morph’ to the
model is low. Nonetheless, the estimated difference in the probability of finding a mark
between color morphs was high. At FAL, the model estimates a 34% probability of finding
a mark in black models, and of 24% for yellow and red models. At PBN, the model
815 estimates a 23% probability of finding a mark in black models, and of 15% in red and
yellow morphs.

Discussion

Most studies on orb-web spiders suggest that the conspicuous body colors attracts
820 prey, increasing spiders foraging success (e.g. Craig & Ebert, 1994; Hauber, 2002). As in
a previous study (Gawryszewski & Motta, 2012), our results did not corroborate the prey
attraction hypothesis for *Gasteracantha cancriformis*, nor the multiple prey hypothesis for
the polymorphism maintenance. Our results suggested either no effect of spider color

morphs, or a negative effect of spider presence, regardless of color, on the prey capture
825 success of webs.

Insect past experience and web traits such as pattern and color are used by insects
to avoid predators (Craig, 1994). Craig (1994) showed that bees of species *Trigona*
fluviventris could perceive webs of *Nephila* and *Argiope* and learn to avoid them. She
associated discrimination of webs by bees to chromatic information considering that
830 brighter webs were more frequently avoided. Morphs of *Gasteracantha cancriformis* are
conspicuous to some prey taxa (theoretical evidence from Gawryszewski & Motta, 2012
and from chapter 1 of this dissertation), therefore reflectance cues of spider body may be
used by insects to avoid being captured. If insects avoided conspicuous morphs, we would
expect that webs with black painted models and webs without models intercepted more
835 prey than webs with yellow models. However, we found that empty webs presented
higher damage than webs with a model, possibly suggesting that insects perceive spiders,
regardless of color information. In bees, chromatic cues are used only when the object
subtend a visual angle $> 15^\circ$. For instance, honeybees could not detect the color of a 2
cm wide flower if their distance to the flower was less than 8 cm (Giurfa, Vorobyev, &
840 Brandt, 1997). Alternatively, prey might be using the achromatic information to avoid
Gasteracantha webs. Furthermore, olfactory information from the paint we used could
potentially be used by prey to avoid webs with spiders model. However, in a previous
experiment there was no difference in capture rates between yellow painted and naturally
yellow spiders (Gawryszewski & Motta 2012).

845 Other studies also found similar results to ours. These studies propose that, instead
of prey attraction, spider coloration may provide camouflage to prey (Nakata &
Shigemiyu, 2015; Václav & Prokop, 2006; Vanderhoff, Byers, & Hanna, 2008) or, for
spiders with hard integument and spines, conspicuous colors may be a warning signal to
predators (Gawryszewski & Motta, 2012; Vanderhoff et al., 2008). We found support to
850 the aposematism hypothesis because black spider models had a higher probability of
presenting marks than yellow and red spider models. Other pieces of evidence also point
to the warning signal function of conspicuous colors in *Gasteracantha* spp. For instance,
birds foraging near webs usually caught spiders except for *Gasteracantha curvispina*, even
if their webs were close (Edmunds & Edmunds 1983). Similarly, predation by lizards has

855 also been reported to affect negatively some orb-web spider species, but not populations
of *Gasteracantha cancriformis* (Schoener & Toft, 1983), and individuals of the genus
Gasteracantha seems to be avoided by hunting wasps (Elgar & Jebb, 1999).

The multiple predators hypothesis for the evolution of color polymorphism,
nevertheless, was not confirmed by our results. We found a great variability of markings,
860 and our inference about their origins were limited. Only a few of them were caused by
birds. This scarcity may be due to their higher visual acuity, which enables them to
discriminate spider models from real spiders (Pinto, 1997), or even because this spider
species is indeed avoided by birds regardless of color. Furthermore, spider predation by
birds seems to be rare compared to wasp predation. It was estimated that only one wasp
865 species consumes 30 times more spiders than 15 bird species (Blackledge, Coddington, &
Gillespie, 2003).

When holding prey, Hymenopterans predators use mandibles and forelegs
(Coville & Coville, 1980) to facilitate transport, and they also sting and paralyze prey
(Dean, Nyffeler, & Sterling, 1988), leaving paired mandibles markings or small holes of
870 the ovipositor (Schwartz, 2001). In order to carry prey to the nest, they may also leave
models deformed due to multiple bites. However, markings from arthropods in general
may result in holes, paired mandibles markings or considerable damage (Low et al., 2014),
which was an obstacle to our analysis, because non-predatory insects could have left
impressions on models and be misinterpreted with hunting wasps markings. But still, if
875 markings were from insects that do not hunt spiders, we would expect that colorful models
presented more markings than black models, considering that some insect taxa have
preference for yellow, usually associated to valuable flower resources, such as pollen
(Campbell, Bischoff, & Lord, 2010; Gollan, Ashcroft, & Batley, 2011; Lunau, 2000, 2014;
Papiorek, Junker, Melo, & Sazima, 2016; Reisenman & Giurfa, 2008).

880 The evolution and maintenance of coloration may be the result of multiple
selection pressures; aposematism being only one of them (Stevens & Ruxton, 2012). In
the wood tiger moth (*Parasemia plantaginis*), for instance, predators avoid hunting yellow
males of *P. plantaginis* more than white males, but the latter have more benefits on mating
(Stevens & Ruxton, 2012). *Oophaga pumilio* is also an aposematic species and other

885 morphs are maintained in population due to female preferences (Stevens & Ruxton, 2012).

Morphs may also benefit from the use of different microhabitats, maximizing thermoregulatory effects or camouflage against predators (Forsman, Ahnesjo, Caesar, & Karlsson, 2008). Thermoregulatory behavior had been suggested for few orb-web spiders: 890 *Argiope argentata* (Robinson & Robinson, 1978), *Nephila clavipes* and *Nephila pilipes* (Robinson & Robinson, 1976), *Verrucosa arenata* (Rao & Mendoza-Cuenca, 2016), and *Gasteracantha spp.* (Robinson & Robinson, 1978). 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 895 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 & 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 & Hearing, 900 2008) and commercial sunscreen lotions (Antoniou et al., 2008). Camouflage seems less likely to occur in *Gasteracantha cancriformis*, since some of the morphs are quite conspicuous to bird and wasps (chapter 1 of this dissertation). Even so, evaluating if morphs occupy specific microhabitats and if properties of this location provide concealment is still required. For instance, considering that the contrasting pale and dark 905 patches in the black and white morph give the impression to break up spider silhouette, protection to predator may be achieved through disruptive coloration in this morph. Also, the white morph of *Gasteracantha curvispina* were more frequent in shady locations, whereas dark morphs were more common in open areas (Edmunds & Edmunds, 1983). Another possible scenario is that selective pressures from predators favors a 910 monomorphic warning signal, but on the other hand, prey learning favors rare morphs, because they would learn more easily to avoid conspicuous common morphs.

Finally, other processes that may contribute to color polymorphism maintenance such as gene flow and heterozygote advantage (Forsman et al., 2008; Gray & McKinnon, 2007) are frequently ignored in behavioral studies of coloration. For instance, a balance 915 between gene flow and divergent selection acts on the camouflage of the walking stick

insect *Timema cristinae*, causing morphological and spatial variation (Farkas, Mononen, Comeault, Hanski, & Nosil, 2013; Gray & McKinnon, 2007). Color polymorphism could also be maintained in cases of overdominance, where heterozygous phenotypes have greater fitness than homozygous phenotypes (e.g. horn size in wild Soay sheep – Johnston
920 et al., 2013 – and color polymorphism in buzzards – Krüger, Lindström, & Amos, 2001).

In conclusion, the prey attraction hypothesis was not corroborated by our results, but we found evidence for warning coloration in *Gasteracantha cancriformis*. The multiple prey or multiple predators hypothesis for color polymorphism maintenance were not corroborated. Other adaptive and non-adaptive processes may be involved in the
925 evolution and maintenance of polymorphism. Knowing microhabitat distribution and the inheritance pattern of polymorphic species are essential to fully understand color polymorphism beyond the behavioral approach.

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Tables

Table 1. Summary of prey capture GLMs (log transformed data) and proportion of web damage LMs (square-root transformed data), from the prey experiment. The lowest AIC models are bolded. Models are sorted by Δ AIC values.

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Models	df	Δ AIC
Prey capture GLMs		
Prey capture ~ prey taxa	4	0.0
Prey capture ~ morph + prey taxa	7	14.4
Prey capture ~ morph * prey taxa	10	26.3
Prey capture ~ 1	3	214.6
Prey capture ~ morph	6	226.5
Web damage LMs		
Final web damage ~ Initial web damage + Morph	6	0
Final web damage ~ Initial web damage * morph	9	3.8

Final web damage ~ Initial web damage	3	9.4
Final web damage ~ 1	2	60.4
Final web damage ~ morph	5	60.6

Table 2. General Linear Mixed Model results (log transformed) comparing the number of prey capture depending on the prey taxa (diptera or other insects).

	Estimate	SE	df	t-value	p-value
Intercept	1.956097	0.037134	119	52.67678	<0.0001
Prey taxa: other	-1.10122	0.055801	89	-19.7349	<0.0001

935 Table 3. Linear Model results (square root transformed) of web damage area explained by the different spider models (yellow, red, black, no model), taking into account the initial web damage (10 a.m.). Coefficients in comparison to the webs without model.

	Estimate	SE	t-value	p-value
Intercept	0.48823	0.035424	13.78242	<0.0001
Web damage 10 a.m.	0.544794	0.058815	9.262831	<0.0001
Yellow morph	-0.05259	0.020703	-2.54004	0.012925
Black morph	-0.08076	0.020591	-3.92196	0.000179
Red morph	-0.05306	0.020276	-2.61667	0.010528

940 Table 4. Delta Akaike Information Criterion (Δ AIC) of Generalized Linear Models computed for the number of attack markings, according to spider models (yellow, red, black) and location (FAL, PNB). Models are sorted by Δ AIC values. The lowest AIC model is bolded.

Model	df	Δ AIC
Markings ~ Morph + Location	4	0.0
Markings ~ Location	2	1.8
Markings ~ Morph * Location	6	3.9
Markings ~ Morph	3	6.1
Markings ~ 1	1	7.8

945 Table 5. Results of Generalized Linear Model (binomial) of proportion of markings with covariation of spider models (yellow, red, black) and location (PNB, FAL). Coefficients in comparison to the yellow model and FAL.

	Estimate	SE	z-value	p-value
Intercept	-1.15551	0.19991	-5.78017	7.46E-09
Black model	0.511908	0.242136	2.11414	0.034503
Red model	0.030682	0.25612	0.119797	0.904644
Location: PNB	-0.56846	0.20226	-2.81051	0.004946

Figures

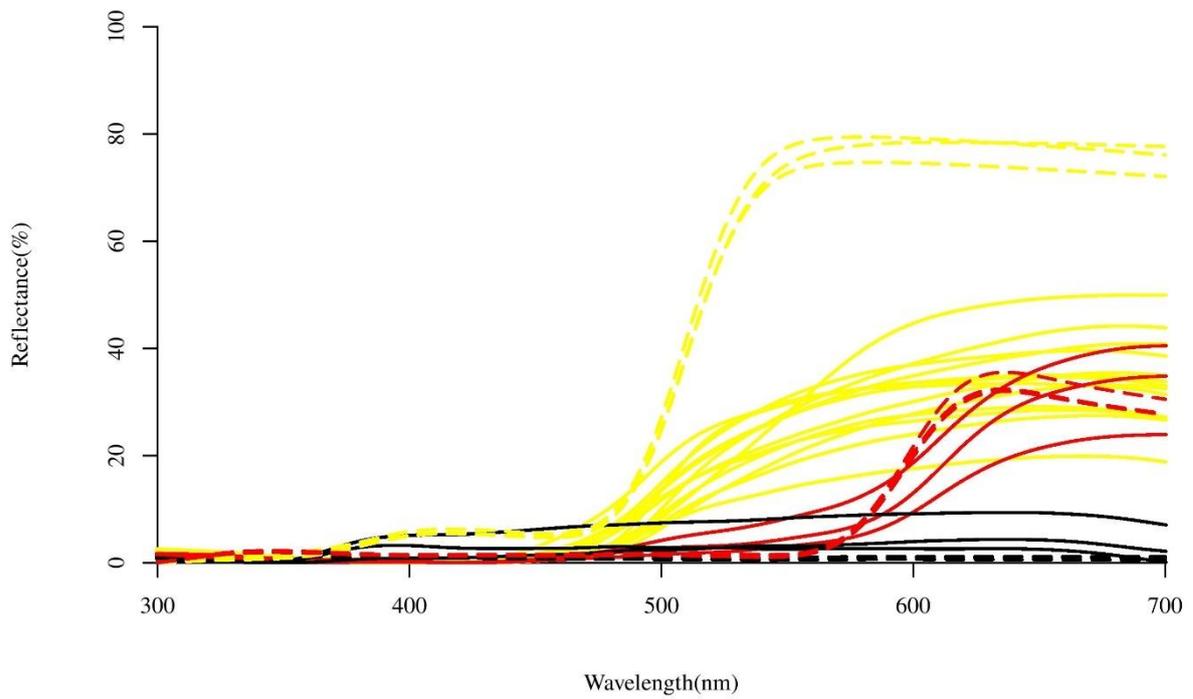
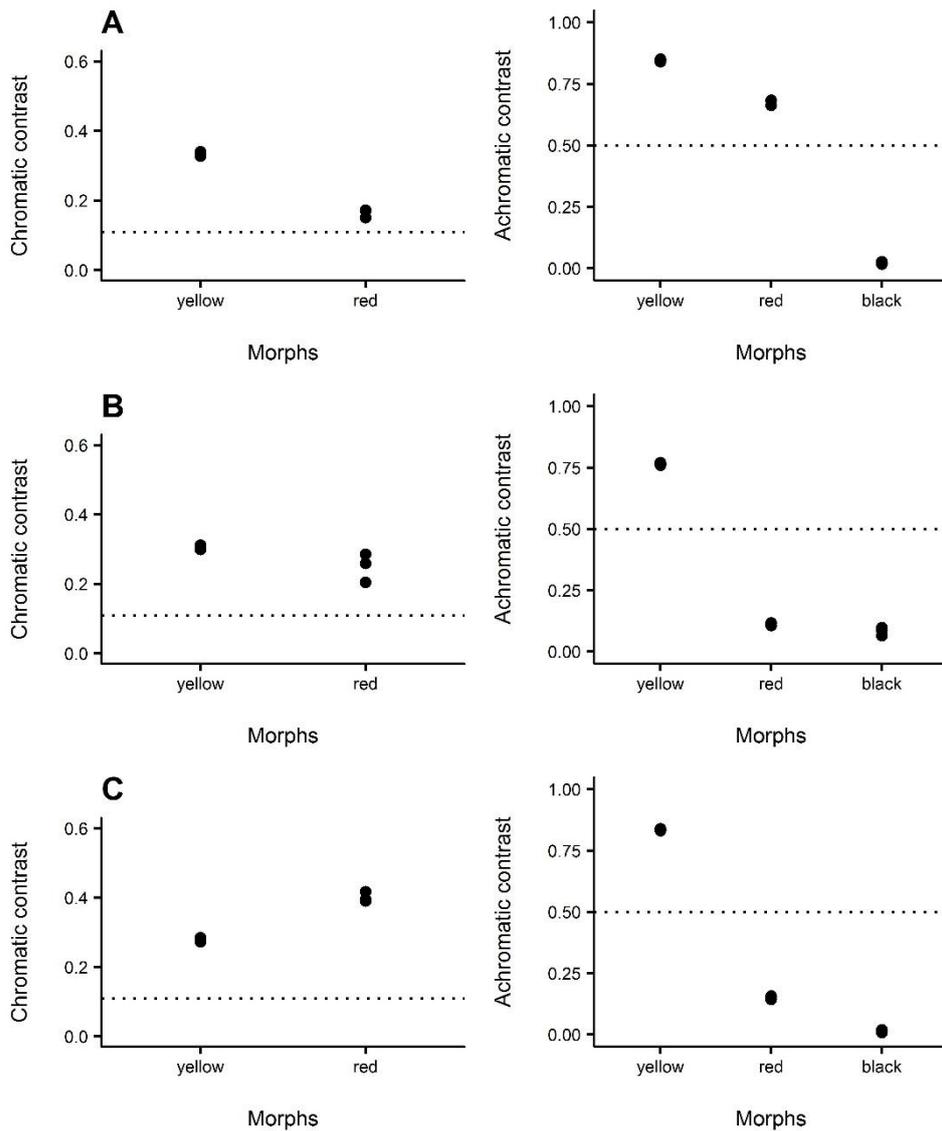
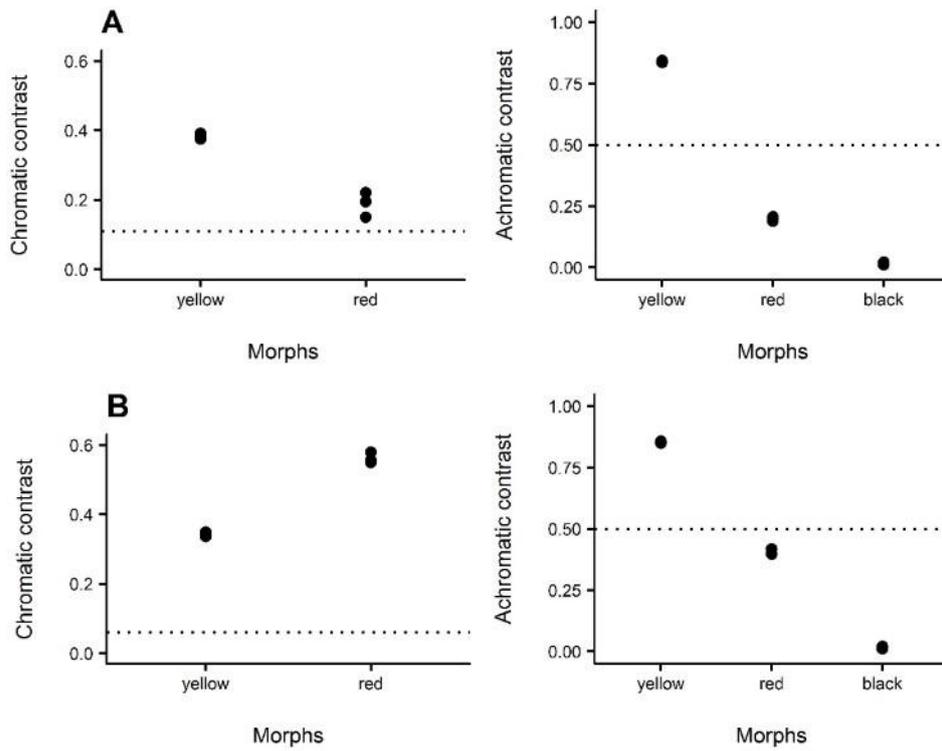


Fig. 1. Reflectance spectra of spider morphs (solid lines; yellow, N = 13; red, N = 3; black, N = 7) and paints (dashed lines; yellow, N = 3; red, N = 3; black, N = 3).



950 Fig. 2. Chromatic (left) and achromatic (right) contrasts of model paints (yellow, N = 3; red, N = 3; black, N = 3) predicted by visual modeling according to the vision of *Apis mellifera* (A), *Drosophila melanogaster* (B), and *Fabriciana adippe* (C), considering Brazilian savanna as background. Dotted lines represent the discrimination threshold for chromatic contrast (0.11) and the maximum excitation for achromatic contrast (0.5).



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Fig. 3. Chromatic (left) and achromatic (right) contrasts of model paints (yellow, N = 3; red, N = 3; black, N = 3) predicted by visual modeling according to the vision of *Philanthus triangulum* (A), *Parus caeruleus* (B), considering Brazilian savanna as background. Dotted lines represent the discrimination threshold for chromatic contrast (0.11) and the maximum excitation for achromatic contrast (0.5).

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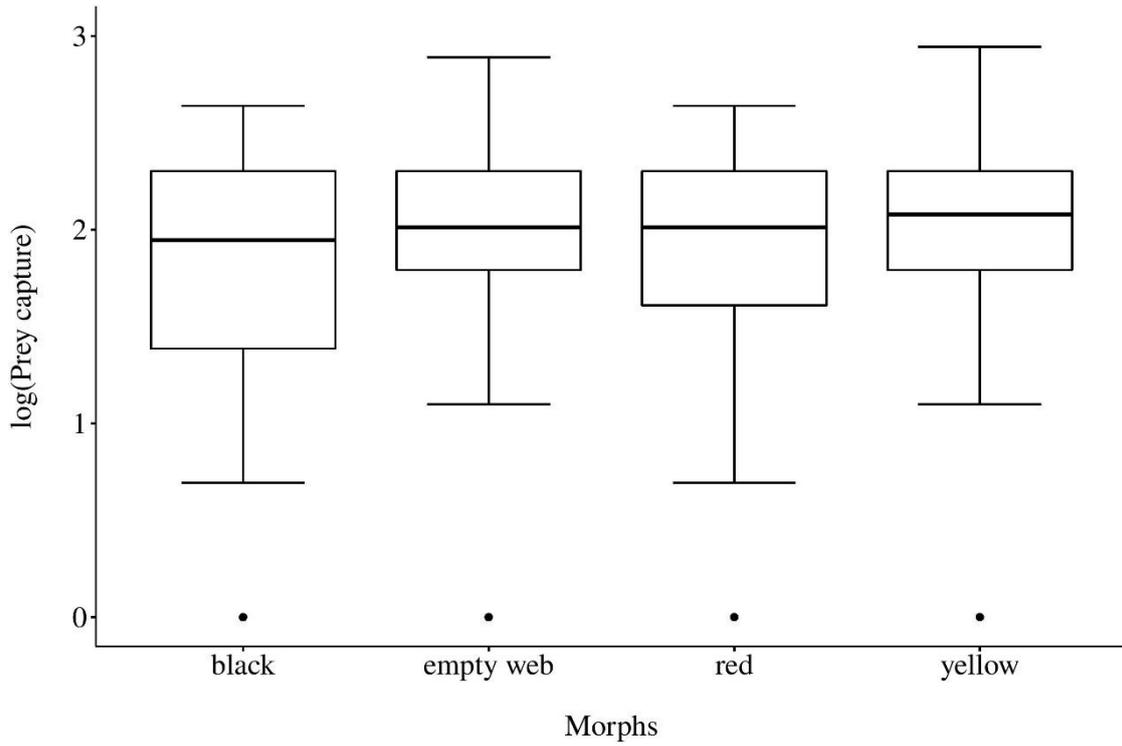


Fig. 4. Number of prey captured (log transformed) in the four treatments of the prey attraction experiment (black, N = 30; empty web, N = 31; red, N = 30; yellow, N = 31).

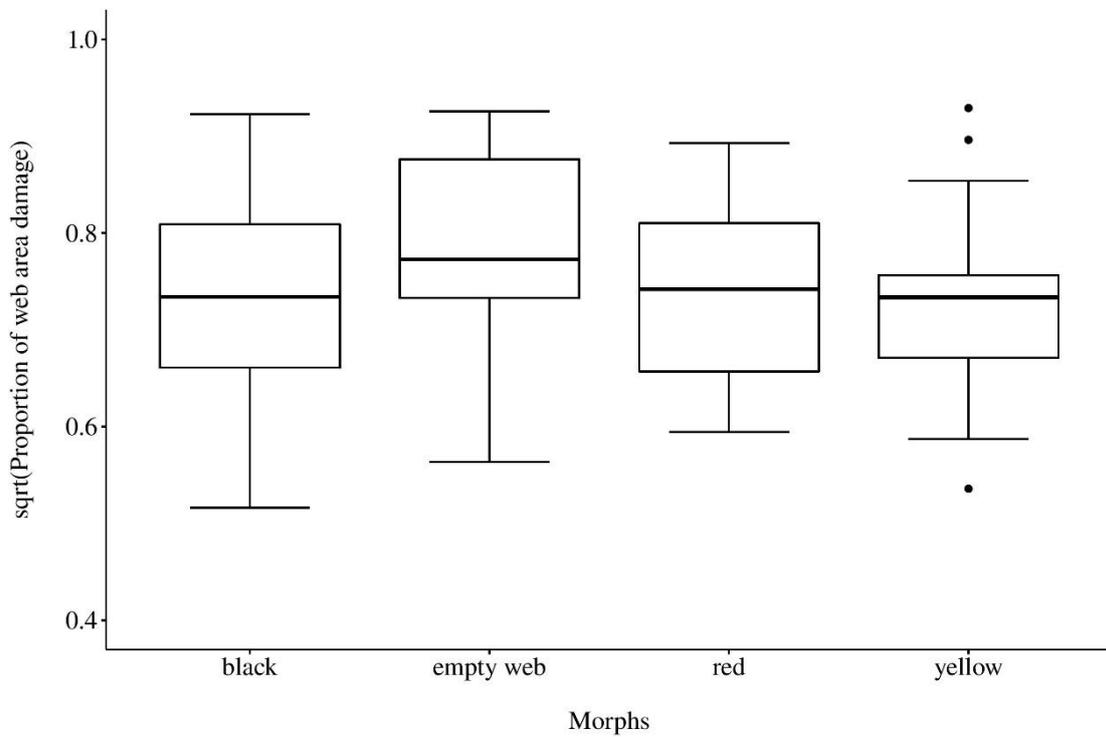


Fig. 5. Web damaged area proportion (square-root transformed), observed at 4 p.m., in the four treatments (black, N = 23; empty web, N = 20; red, N = 24; yellow, N = 22) of prey attraction experiment.

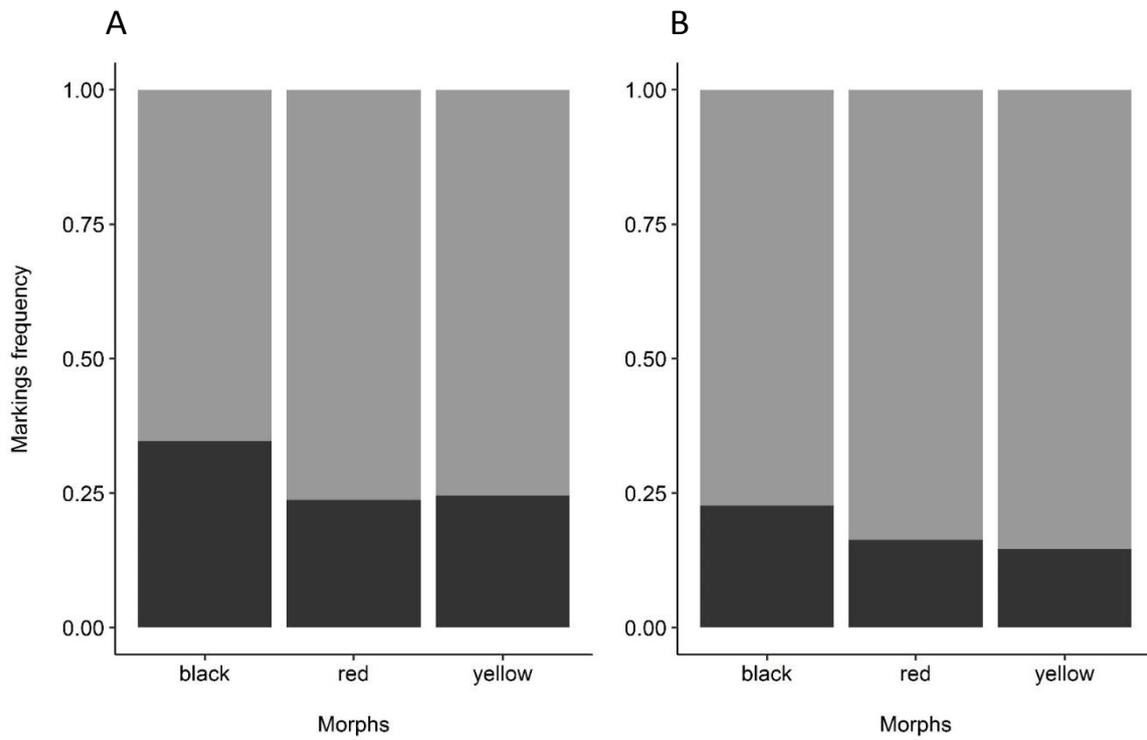


Fig. 6. Proportion of morphs that presented attack marks in the predation experiment, conducted in (A) Fazenda Água Limpa (FAL; yellow models, N = 98; black models, N = 98; red models, N = 97) and (B) Brasília National Park (PNB; yellow models, N = 96; black models, N = 97; red models, N = 98). Dark grey bars represent attacked models and light grey bars represent non-attacked models.

Supplementary material

Table S1. Euclidean distances (mean \pm SE) between average color loci of each spider color morphs and paints used to manipulate spiders measured according to prey and predator chromatic and achromatic discrimination. There are no chromatic contrast values for black, because this spectrum does not have chromatic information.

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Observer	Morph	Chromatic (Mean \pm SE)	Achromatic
<i>Apis mellifera</i>	yellow	0.1144 \pm 0.0013	0.1292
	red	0.0610 \pm 0.0238	0.0431
	black	-	0.1988
<i>Drosophila melanogaster</i>	yellow	0.1806 \pm 0.0030	0.2062
	red	0.1722 \pm 0.0221	0.1834
	black	-	0.3158
<i>Fabriciana adippe</i>	yellow	0.1537 \pm 0.0038	0.1880
	red	0.1816 \pm 0.0115	0.1620
	black	-	0.3031
<i>Philathus triangulum</i>	yellow	0.1944 \pm 0.0041	0.1847
	red	0.0927 \pm 0.0295	0.1332
	black	-	0.2957
<i>Parus caeruleus</i>	yellow	0.1097 \pm 0.0035	0.1666
	red	0.2251 \pm 0.0102	0.0307
	black	-	0.2855

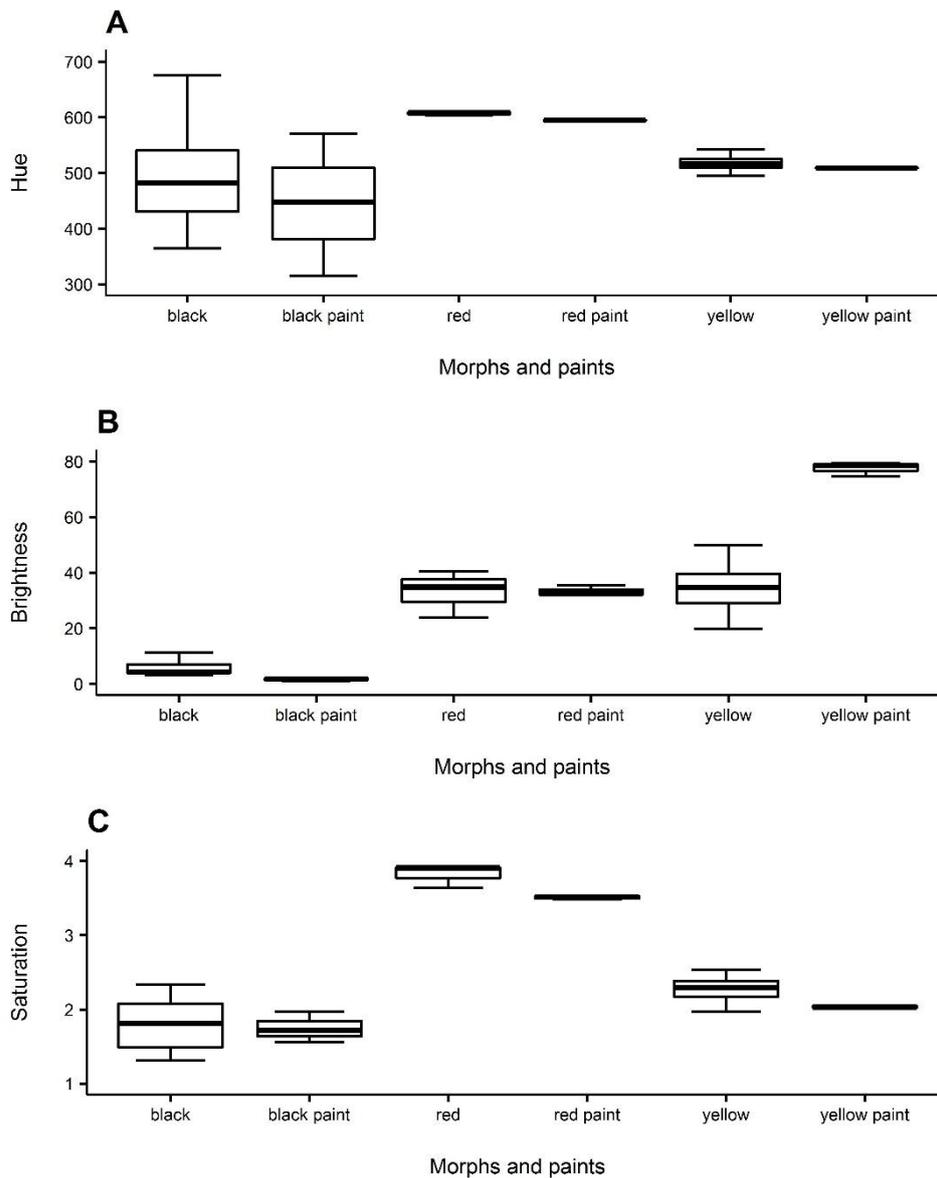


Figure S1. Comparison of color properties (hue, brightness and saturation) between paints used to color spider models (black, reference 320, N = 3; red, reference 350, N = 3; and yellow, reference 340, N = 3) and *Gasteracantha cancriformis* spiders (dark patches of black and white spiders, N = 7; red, N = 3; and yellow spiders, N = 13). (A) Hue of acrylic paints Acrylex and spider color morphs. (B) Brightness of acrylic paints Acrylex and spider color morphs. (C) Saturation of acrylic paints Acrylex and spider color morphs.

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Considerações finais

A aranha de teia orbicular *Gasteracantha cancriformis* apresenta padrões de coloração
conspícuos e polimorfismo de cor. A evolução e manutenção de tal variação cromática
990 pode ser influenciada por presas e predadores com sistemas visuais diferentes, já que um
mesmo morfo é percebido distintamente por potenciais presas e predadores.
Considerando apenas a matiz, a coloração conspícua se assemelha à coloração de flores.
Contudo, não influencia na captura de presas, visto que modelos dessa aranhas
apresentaram números similares de presas nas teias. Portanto, ao contrário de outras
995 aranhas de teia orbicular, para as quais a coloração chamativa é atribuída ao mimetismo
floral, para *G. cancriformis*, esta hipótese não é corroborada. Os morfos amarelo e
vermelho apresentam coloração típica de organismos aposemáticos e são conspícuos para
a visão de uma ave. O morfo vermelho apesar de não ser conspícuo na visão de vespas,
ainda assim poderia estar protegido de predação por camuflagem. Dessa forma, esses
1000 morfos poderiam ser mantidos na população devido à pressão de diferentes predadores,
morfos vermelhos sinalizariam impalatabilidade para aves, enquanto morfos amarelos,
para vespas. Porém, essas diferenças não foram observadas experimentalmente. Em
campo, modelos pretos de aranha apresentaram mais marcas de predação do que
modelos amarelos e vermelhos, também sugerindo que em *G. cancriformis* a coloração
1005 chamativa pode ser um sinal de advertência para predadores. Porém, a multiplicidade de
predadores por si só não explica a variação cromática em populações dessa espécie de
aranha. Conseqüentemente, o polimorfismo de cor em *G. cancriformis* pode ser uma
característica multi-funcional, onde morfos não aposemáticos seriam mantidos nas
populações devido a outras funções adaptativas, como camuflagem ou termorregulação.

1010 Apesar de parecer mais intuitivo que espécies aposemáticas sejam monomórficas,
algumas populações apresentam variação de conspicuidade (Stevens & Ruxton, 2012). O
polimorfismo em espécies aposemáticas pode ser explicado por sobredominância,
seleção negativa dependente de frequência e/ou fitness semelhante entre os morfos
devido à pressões seletivas distintas. O último caso pode ser exemplificado por *Oophaga*
1015 *pumilio*, no qual o polimorfismo geográfico é explicado pela combinação entre a pressão
exercida por predadores e a escolha de parceiros sexuais feita por fêmeas (Stevens &

Ruxton, 2012). Diferenças de intensidades luminosas e detectabilidade por predadores também podem influenciar a manutenção do polimorfismo em espécies aposemáticas como é o caso de *Dendrobates tinctorius* (Rojas et al., 2014). Portanto, o estudo do polimorfismo auxilia na compreensão de processos seletivos vantajosos para o sucesso ecológico de espécies e dinâmicas populacionais, como variação geográfica, fluxo gênico e ocupação de diferentes nichos (Forsman et al., 2008). Para compreender se outras forças seletivas e processos não-adaptativos atuam na evolução do polimorfismo de cor em *G. cancriformis* ainda é necessário conhecer a genética deste organismo, avaliar a distribuição em diferentes micro-habitats e a frequência de cada morfo ao longo do tempo e em diferentes localidades geográficas.

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