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

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ABSTRACT

The sensory drive theory predicts that signals, sensory systems, and signaling behavior should coevolve. Variation in the sensory systems of prey and predators may explain the diversity of color signals, such as color polymorphism. The spider *Gasteracantha cancriformis* (Araneidae) possesses several conspicuous color morphs. The aim of the present study was to assess whether the color polymorphism of *G. cancriformis* may be maintained by pressure from multiple signal receivers, such as prey and predators with distinct color vision systems. Although, the multiple receivers world is a more realistic scenario, it has received little attention. In orb-web spiders, the prey attraction hypothesis states that conspicuous colors are prey lures that increase spider foraging success via flower mimicry. However, in highly defended species, conspicuous colors could also be a warning signal to predators. We used color vision modelling to estimate chromatic and achromatic contrast of *G. cancriformis* morphs as perceived by potential prey and predator taxa. Our results revealed that individual prey and predator taxa perceive the conspicuousness of morphs differently. For instance, the red morph is perceived as quite conspicuous to lepidopteran prey and avian predators, but not by other insects. Therefore, the multiple prey and predator hypotheses may explain the evolution of color polymorphism in *G. cancriformis*. However, flower mimicry hypothesis was weakly corroborated. Other parameters that are not evaluated by color vision models, such as

distance, shape, angle, and pattern geometry could also affect the perception of color morphs by both prey and predators and thereby influence morph survival.

KEY WORDS: *Gasteracantha cancriformis*; Araneidae; Visual communication; Aposematism; Luring; Prey attraction

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

In the context of visual signaling, the distinct visual systems of prey and predators may play a role in the evolution and maintenance of color polymorphisms (Ruxton et al., 2004; White and Kemp, 2015). Animal communication involves the generation, emission transmission, and processing of the signal by a receiver, in which an appropriate response is elicited (Endler 1993). Any factors that affect these steps can influence signal efficiency and, as a result, affect the direction of communication evolution (Endler 1993). Thus, the diversity of signals are likely influenced by variation in the sensory systems of receivers.

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 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

62 bright color patterns of this species are thought to resemble symmetric flower patterns
63 that may attract bees, owing to the innate preference of bees for symmetry (Chiao et al.,
64 2009). Moreover, yellow patches on the spider's body may be perceived as food resources
65 by flowers visitors (Tso et al. 2004). Besides being attractive to pollinators, the yellow
66 patches on the species' body also seems to attract hymenopteran predators. Therefore, it
67 is possible that there is a trade-off between foraging success and predation risk in
68 polymorphic populations in which some morphs are more cryptic than others (Fan et al.,
69 2009).

70 The predators of orb-web spiders possess very distinct visual systems. Birds, for
71 example, are tetrachromats, whose photoreceptors are most sensitive to ultraviolet-violet,
72 blue, green, and red (Hart 2001), whereas spider hunting wasps, such as members of the
73 Sphecidae, are trichromats, whose photoreceptors are most sensitive to ultraviolet, blue,
74 and green (Peitsch, 1992; Briscoe and Chittka, 2001). Similarly, the insect prey of orb-web
75 spiders also vary in their types of color vision. For example, bees are trichromats with
76 spectral sensitivities that are similar to those of sphecid wasps (Briscoe and Chittka, 2001),
77 whereas some lepidopterans are tetrachromats, and some dipterans possess
78 photoreceptors with five different sensitivity peaks (Schnaitmann et al., 2013). Therefore,
79 the maintenance of spider color polymorphism may result not only from a trade-off
80 between prey attraction and capture success but also from selective pressure from multiple
81 receivers (Endler, 1992; Ruxton et al., 2004; White and Kemp, 2015)

82 The orb-web spider *G. cancriformis* constructs large webs and rests in the web
83 hub during the day (Levi, 1978). Females of the species possess a hard abdomen with
84 three pairs of spines and vary in color, with some morphs quite conspicuous to human
85 observers (Levi, 1978; Gawryszewski and Motta, 2012). The ventral side of females are
86 mostly black, sometimes with small bright spots. In one studied population, the dorsal
87 side of females possessed black or reddish spines and four different color patterns: yellow,
88 white (without UV reflectance), red, and a combination of black and white (white patches
89 reflects UV; Gawryszewski 2007; Gawryszewski and Motta, 2012). Adult females measure
90 from 5 to 7 mm in length and 10 to 13 mm in width (Muma, 1971), whereas the males
91 are brownish, small, and do not exhibit chromatic variation (Levi, 1978). The prey
92 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). Nonetheless, it remains the possibility that each color morphs attracts preferentially specific types of prey. Furthermore, 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.

Considering that the same "color" may be perceived as cryptic or conspicuous by different species (Endler and Mappes 2004), each color morph of a 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). Therefore, it is plausible that the variation of color among individuals within a population is affected by a diverse range of interactions that leads to different selection process. To date, the role of multiple predators on the evolution of prey coloration has been approached by theoretical models (Endler and Greenwood, 1988; Endler and Mappes, 2004). Endler and Greenwood (1988) models, for instance, indicated that a stable polymorphisms might evolve in the presence of anti-apostatic (positive frequency-dependent) from different predators, given that predators perceive prey conspicuousness differently and there is a covariance between the relative degree of crypsis and the degree of frequency-dependent selection by each predator.

In this study we aimed to explore old and new hypothesis that could potentially explain the maintenance of color polymorphism in a spider species. 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, which posits that the spiders attract prey *via* aggressive mimicry of flower colors and that each color morph mimics a different flower color. In addition, (3) the *multiple predator hypothesis* posits that the conspicuous colors found in spiders could serve as warning signals to predators and that color polymorphism could evolve and be maintained if each color morph is adapted to the vision of a specific predator.

MATERIALS AND METHODS

Color vision model

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).

To estimate the perception of *G. cancriformis* color morphs by distinct predators and prey groups, we used the color vision model proposed by Chittka (1992). Although this model has been only validated with behavioral experiments on bees, its general form allow us to apply it for other taxa (e.g. Thery and Casas 2002). There are other models of color vision (Vorobyev and Osorio, 1998; Endler and Mielke, 2005), but when applied correctly, their results tend to be highly correlated (Gawryzewski, 2017). The Chittka (1992) model requires four inputs: (1) the irradiance reaching the observed object, (2) the observer photoreceptor excitation curves, (3) the background reflectance to which photoreceptors are adapted to, and (4) the reflectance curve of the observed object. First, the sensitivity factor R was determined for each photoreceptor, as follows:

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

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 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):

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

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

150 For tetrachromat organisms (Théry and Casas, 2002):

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

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

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

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

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

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

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

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

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

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

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

157 The color spaces are chromaticity diagrams and, thus, do not estimate the
158 achromatic contrast between objects. Nonetheless, achromatic contrasts can be important
159 visual cues used by both prey and predators. In bees, achromatic contrast is more
160 important than chromatic cues for objects that subtend a visual angle smaller than ~15°,
161 which means that bees have to be very close to flowers in order to use their color vision
162 for discrimination tasks (Giurfa et al., 1997). Similarly, birds use achromatic contrast in
163 detection of small objects (Osorio et al., 1999). We estimated the achromatic contrast as
164 the excitation (Eq. 3) of the photoreceptor responsible for achromatic discrimination in
165 each organism (Chittka and Kevan 2005).

166 For our modeling, we used the reflectance data of *G. cancriformis* color morphs
167 that was collected during a previous study (for reflectance curves see figure 1.8 in

168 Gawryszewski, 2007, and figure 5 in Gawryszewski and Motta 2012). These data have
169 already been used to estimate the visual contrast of the yellow, white and the black and
170 white morphs from the perspective of *Apis mellifera* (Gawryszewski and Motta 2012). The
171 spiders were collected from a Brazilian savanna physiognomy, namely Cerrado *sensu*
172 *stricto,* which is characterized by shrubs and trees of 3 to 8 m tall that are contorted and
173 possess thick, fire-resistant bark, a crown cover of >30%, and additional herbaceous
174 vegetation (Oliveira-Filho and Ratter 2002). The background reflectance was estimated
175 from the average reflectance of leafs, leaf litter, bark, and grasses that were collected from
176 the same area as the spiders (see figure 5 in Gawryszewski and Motta, 2012). To avoid
177 issues with negative values and unrealistic positive values we adjusted the reflectance data
178 by subtracting the reflectance values by the minimum value of each measurement. As
179 illuminant spectrum, we used the International Commission on Illumination (CIE)
180 standard illuminant of D65, which is comparable to open areas, such as the Brazilian
181 savanna (Chittka, 1996).
182 Visual modeling calculations were conducted using the 'colourvision' R package
183 (Gawryszewski, 2017). Linear mixed models were performed using the 'nlme' (Pinheiro
184 et al., 2016,) and 'lme4' packages (Bates et al. 2015), graphs were plotted using the
185 'ggplot2', 'ggExtra', 'gridExtra', and 'pavo' packages (Wickham, 2009; Maia et al., 2013;
186 Attali, 2016; Auguie, 2016; R Core Team, 2015), and R^2 values were computed using the
187 package 'piecewiseSEM' (Nakagawa and Schielzeth, 2013).
188
189 **Multiple prey hypothesis**
190 Using the model described above, we estimated the chromatic and achromatic
191 conspicuousness of the *G. cancriformis* morphs (yellow, white, red, and white patches of
192 the black and white morph) to a suit of potential prey: *A. mellifera* (Hymenoptera,
193 Apidae), *Drosophila melanogaster* (Diptera, Drosophilidae), and *Fabriciana adippe*
194 (Lepidoptera, Nymphalidae). Those species are not necessarily sympatric with *G.*
195 *cancriformis*. However, these insect orders are commonly intercepted by orb-webs in field
196 experiments (Craig and Ebert 1994; Tso et al. 2002) and represent the diversity of visual
197 systems among insects (Briscoe and Chittka, 2001). Variation within of wavelength of
198 maximum sensitivity is small in Hymenoptera is very little, except for ants and in

199 Lepidoptera most species present four photoreceptor spectral curves (Briscoe and Chittka,
200 2001). For Diptera, the number of photoreceptors is not so conservative among species
201 and there are not many studies on the color vision of this taxon (Briscoe and Chittka,
202 2001; Lunau, 2014). Visual modeling work have usually considered Diptera as a
203 tetrachromatic organism (White and Kemp, 2016; White et al., 2016; O'Hanlon et al.
204 2014). However, recent work showed that *Drosophila melanogaster* use a fifth
205 photoreceptor for color vision (Schnaitmann et al., 2013). Although it remains to be tested
206 whether this species behave as pentachromat we decided to explore this possibility and
207 modelled this is species using five spectral curves for color vision.

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

218 For achromatic contrast, bees only use the green photoreceptor (Giurfa et al.,
219 1996), whereas flies only use the outer photoreceptors (R1-R6; Kelber & Henze, 2013).
220 Because the exact mechanism used by lepidopterans for achromatic discrimination is
221 incompletely understood, we assumed that they employ the same mechanism as in bees.
222 The color hexagon model assumes that photoreceptors respond to half their maximum
223 for the background they are adapted to, so that the photoreceptor excitation for
224 background is equivalent to 0.5 units (Chittka, 1992).

225 The multiple prey hypothesis predicts that different prey taxa perceive color
226 morphs differently. To assess whether each spider morph was perceived differently by
227 prey species, we constructed two linear mixed models, one for chromatic contrast and
228 one for achromatic contrast. Either chromatic (ΔS) or achromatic contrast were used as
229 the dependent variable, and spider morph and prey taxon were used as the independent

230 variables (contrast = spider morph × observer). The spider morph was defined as yellow,
231 white, red, or black and white, and the observers were defined as hymenopteran,
232 dipteran, or lepidopteran. Individual spiders were used as random effects. Normality and
233 homogeneity were verified by visual inspection of quantile-quantile and residuals vs. fitted
234 values plots. We computed all nested models and used the Akaike Information Criterion
235 (AIC) to select the best model. Marginal and conditional R^2 were estimated according to
236 the recommendations of Nakagawa and Schielzeth (2013).

237 As a reference point, we used a color discrimination threshold of $\Delta S = 0.11$, which
238 is the threshold value below which trained bees are unable to distinguish different flower
239 colors (Chittka, 1996). However, discrimination thresholds are variable and can change
240 depending on the study species, learning conditions, previous experience, background
241 coloration, whether the task involves discrimination between colors or detection against
242 the background, and whether objects are compared sequentially or simultaneously
243 (Avarguès-Weber & Giurfa, 2014). It should also be noted that threshold values were not
244 behaviorally validated for other taxa.

245

246 **Flower mimicry hypothesis**

247 To test the multiple mimic models hypothesis, we compared how flowers and spider
248 morphs are perceived by prey. We gathered all flower reflectance data available in the
249 Floral Reflectance Database (FReD; Arnold et al., 2010), excluding reflectance data from
250 lower flower parts, leaves, bracts, stamens, the inner parts of bell-shaped flowers, and
251 unknown items, as well as spectrum files that did not cover 300 to 700 nm. Most species
252 in the database have only one reflectance spectrum, and for species with multiple
253 reflectance spectra, we randomly selected a single spectrum. We did not average the
254 reflectance of these species because there was no information available on whether these
255 measurements referred to different individuals or different parts of single flowers. In total,
256 we gathered reflectance data from 859 plant species. We grouped flowers visually
257 according to the 10 categories proposed by Chittka et al. (1994), considering whether they
258 reflect or absorb in four spectral ranges, UV (300-400 nm), blue (400-500 nm), green (500-
259 600 nm) and red (600-700 nm). We deleted three spectral curves that did not seem to fit
260 in any of these categories. A caveat of this analysis is that these flowers are not necessarily

261 sympatric to *Gasteracantha cancriformis*. However, flowers spectral curves variation are
262 subtle, because there is a constraint on flower pigments blending (Chittka and Menzel,
263 1992; Chittka et al. 1994). In addition, we computed reflectance curves from different
264 countries available in FReD database. A qualitative analysis strongly suggests that they all
265 have similar shapes (Fig. S1).

266 The multiple mimic model hypothesis predicts that different colour morphs are
267 mimicking different flower colors. First, to evaluate color regardless of the observer, we
268 compared hue (Eq. 13), saturation (Eq. 14) and brightness (Eq. 15) of flowers and spiders
269 (Anderson and Prager, 2006):

$$H_3 = \lambda_{Rmid} \tag{13}$$

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

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

272 where R_{max} and R_{min} are the maximum and minimum reflectance points; and $B_2 =$
273 $\sum_{\lambda 300}^{\lambda 700} R_i/n_w$, where R_i is the reflectance corresponding to each wavelength point, and n_w
274 is the total wavelength intervals;

$$B_3 = R_{max} \tag{15}$$

275 where R_{max} is the maximum reflectance.

276 We estimated the chromatic difference between individual flowers and the mean
277 achromatic value for each color morph, and calculated the percentage of values below or
278 equal to the theoretical detection threshold of 0.11. Secondly, we computed chromatic
279 distances for spider morphs and flowers following the same steps as for the previous
280 section, considering the visual system of *A. mellifera*, *D. melanogaster* and *F. adippe*.
281 Then, we calculated a matrix of chromaticity distances between each individual spider
282 color morph and each individual flowers species.

283 To evaluate if each spider morph and flower category had similar perceptions to
284 each prey species, we constructed two linear mixed models, one for chromatic contrast
285 and one for achromatic contrast. Chromatic or achromatic contrast were used as the
286 dependent variable, and spider morph, flower category and prey taxon were used as the
287 independent variables (contrast = spider morph × flower category × observer). The spider
288 morph was defined as yellow, white, red, or black and white, flower category as '1' to

289 '10', and the observers were defined as hymenopteran, dipteran, or lepidopteran.
290 Individual spiders and individual flowers were considered as random effects. Normality
291 and homogeneity were verified as for the first hypothesis. We selected the best model
292 using AIC, and computed marginal and conditional R^2 for each model (Nakagawa and
293 Schielzeth, 2013).

294 As reference points, we used discrimination thresholds of $\Delta S = 0.11$, for the
295 chromatic contrast, and for the achromatic contrast, we assumed the excitation value of 0
296 for all the three insect taxa.

297

298 **Multiple predator hypothesis**

299 The methodology used to investigate the multiple predator hypothesis methodology was
300 very similar to that used for the multiple prey hypothesis, except that we used predator
301 species in our models. As predators, we considered the bird *Parus caeruleus* (Paridae)
302 and the wasp *Philanthus triangulum* (Sphecidae), since birds and wasps are the main
303 predators of orb-web spiders (Rayor, 1996; Foelix, 2010), are visually guided hunters, and
304 have distinct color vision systems. For *P. caeruleus*, we used photoreceptor sensitivity
305 curves available in the literature (Hart, 2001), and for *P. triangulum*, we used
306 photoreceptor sensitivity peaks to estimate photoreceptor sensitivity curves (data available
307 in Briscoe and Chittka, 2001; see Govardovskii et al. 2000 for estimation of sensitivity
308 curves from sensitivity peaks). Again, those species are not sympatric with *G. cancriformis*,
309 but we do not expect a great variation of photoreceptors types within hymenopterans
310 (Peitsch et al., 1992) nor Passeriformes (Hart, 2001).

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

320 We estimated marginal and conditional R^2 for the models as recommendations of
321 Nakagawa and Schielzeth (2013).
322 As in the multiple prey hypothesis, we used discrimination thresholds as reference
323 points. For the chromatic contrast, we considered color discrimination thresholds of ΔS =
324 0.11 and ΔS = 0.06 for the wasp (Dyer and Chittka, 2004) and bird (Théry et al., 2005),
325 respectively. For the achromatic contrast, we considered double cones in birds (Hart,
326 2001), and assumed green photoreceptors for wasps, as in bees, and compared values
327 obtained to the excitation of 0.5.
328
329 **RESULTS**
330 **Multiple prey hypothesis**
331 For chromatic contrast, the model that included the interaction between spider morph
332 and prey taxon presented the lowest AIC value (Table 1). The yellow morph presented
333 the highest ΔS value for *A. mellifera* and *F. adippe* vision, whereas the white spider
334 presented the highest ΔS value for *D. melanogaster*, followed by the yellow morph (Fig.
335 1). The white patch of the black and white spiders presented a ΔS value that was very
336 close to the theoretical discrimination threshold for all prey species (Fig. 1). The red
337 spiders presented ΔS values near the theoretical discrimination threshold for *A. mellifera*
338 and *D. melanogaster*, but not for *F. adippe* (Fig. 1). For prey achromatic contrast, the
339 model that included the interaction between variables presented the lowest AIC value
340 (Table 1). For all prey groups, the white morph had the highest excitation value, followed
341 by the black and white, yellow, and red morphs, respectively (Fig. 1). The model
342 coefficients are provided in the supplementary material (Table S1 and S2).
343
344 **Flower mimicry hypothesis**
345 We found three peaks of hue for the flowers, around 400, 500 and 600 nm, which are
346 similar to the average hue of spider morphs (Fig. 2A). The saturation metric had only one
347 peak for flowers, to which black and white, white and yellow spider morphs were close
348 (Fig. 2B). The brightness of flowers also only presented a single peak, and white, red and
349 yellow spider morphs had average brightness near to this peak (Fig. 2C).

350 For all three prey species, only the white patch of the black and white morph had
351 high percentage of values near the chromatic theoretical discrimination threshold of 0.11
352 when compared to all flowers reflectance spectra: 44.5% for *A. mellifera*, 16.8% for *D.*
353 *melanogaster,* and 35% for *F. addipe*. For the other spider morphs only a small proportion
354 of the Euclidean distances between flowers and morphs presented values < 0.11. For *A.*
355 *mellifera* only 1.6% of yellow morphs presented values lower than 0.11, 3.4% of white
356 morphs, and 4.8% of red morphs. For *D. melanogaster* only 2.4% of yellow morphs had
357 values lower than 0.11, 4.0 % of white spiders, and 3.0% of red morphs. For *F. addipe* this
358 values were 0.4%, 0.2%, and 0.5% respectively.
359 In the color vision model chromatic dimension, the statistical model with
360 interaction among the three variables (flower categories, spider morphs, and prey taxon)
361 had the lowest AIC (Table 2 and S3). In a general view, there seems to be a tendency of
362 growing contrast starting with the black and white morph followed by white, yellow and
363 red morphs (Fig. 3). Only the comparison of black and white spiders and the category '8'
364 of flowers (white flowers that reflect UV) was below the discrimination threshold of 0.11
365 for all prey taxa (Fig. 3). The categories '3' and '4' compared to black and white spiders
366 were slightly above 0.11 for *A. mellifera*, and '4' for *F. adippe* (Fig. 3). Some categories
367 were around 0.15, which may indicate that for these, flowers and spiders coloration may
368 be perceived as similar to flowers: categories '7' and '9' compared to the black and white
369 morph, and '3' compared to yellow spiders for *A. mellifera*; '4' and '9' compared to the
370 black and white morph for *D. melanogaster*; and '3' compared to black and white, for *F.*
371 *adippe* (Fig. 3). For the achromatic dimension, the statistical model with interaction among
372 all variables also had the lowest AIC (Table 2 and S4). Most of the groups had excitation
373 values around 0 and 0.2, regardless the spider morph and observer (Fig. 3).
374
375 **Multiple predator hypothesis**
376 For the chromatic contrast, the model with interaction between variables presented the
377 lowest AIC value (Table 1). The black and white morph presented the lowest ΔS value
378 for both predators (Fig. 4A,B; Table S5). The white morph was the one with highest ΔS
379 value for *P. caeruleus*, though yellow and red morphs presented similar values (Fig. 4A).
380 For *P. triangulum*, the white spider morph presented the highest ΔS value, followed by

381 the yellow and red morphs. The latter was near the theoretical discrimination threshold
382 of 0.11 (Fig. 4B). For the achromatic contrast, the model that included the interaction
383 between variables presented the lowest AIC value (Table 1), even though the values of
384 the two predator species were very similar. For *P. caeruleus*, the white morph had the
385 highest excitation value, followed by the yellow, black and white, and red morphs,
386 respectively (Fig. 4C). The white morph also had the highest excitation value for *P.*
387 *triangulum*, followed by the black and white, yellow, and red morphs, respectively (Fig.
388 4D). The model coefficients are provided in the supplementary material (Table S5 and
389 S6).
390

391 **DISCUSSION**

392 Our statistical analyses show that the majority of *G. cancriformis* morphs have a high
393 probability of being detected by potential prey and the degree of detectability varies
394 according to the receiver. Some spider morphs are also conspicuous for predators, but
395 the multiple predator hypothesis was partially corroborated, because the degree of
396 detectability between predators was similar. In addition, we offer some support for the
397 flower mimicry hypothesis.
398

399 **Multiple prey hypothesis**

400 In *G. cancriformis*, spider morphs conspicuousness is perceived differently by
401 prey species. The yellow and white morphs were the most conspicuous to all prey taxa.
402 The former being more contrasting from the background for honeybee color vision, and
403 the latter, for flies. The red morph, although inconspicuous for honeybee and flies,
404 showed high detectability for butterflies. A recent study using the receptor noise-limited
405 color vision model showed that insects prey perceive coloration of *Verrucosa arenata*
406 morphs differently, however the maintenance of color polymorphism does not seem to
407 be influenced by multiple prey as we suggested here. Yellow morphs of *V. arenata* have
408 higher chromatic contrast than white morphs for Diptera and Hymenoptera. Whereas in
409 the achromatic dimension the white morph had a higher contrast for both prey taxa
410 (Ajuria-Ibarra et al. 2017). Color morphs may, instead be influenced by other factors such

411 as different visual channels of relevant observers or illumination (Ajuria-Ibarra et al. 2017;
412 White and Kemp, 2016).
413 The hypothesis that different morphs exploit different visual channels in prey was
414 also proposed in another study to explain the evolution of color polymorphism in
415 *Gasteracantha fornicata*. The yellow morphs of *G. fornicata* would benefit from
416 stimulating the dipteran chromatic channel, whereas white morphs would benefit from
417 stimulating the achromatic channel (White and Kemp, 2016). For the achromatic
418 dimension, although the statistical analyses also suggested an interaction between spider
419 morph and prey taxon, spider morphs presented similar levels of achromatic detectability
420 comparing among prey taxa, therefore, this idea seems inconsistent with the multiple prey
421 hypothesis for *G. cancriformis.* However, when comparing chromatic and achromatic
422 contrasts of each prey taxa individually, we observe different detectabilities between the
423 two visual channels for the morphs. Therefore, the hypothesis of exploitation of different
424 visual channels of prey could be possible to explain the color polymorphism, as in White
425 & Kemp (2016), but possibly not enough to explain such a diverse color variation as occur
426 in *G. cancriformis*.
427
428 **Flower mimicry hypothesis**
429 Several authors have proposed flower mimicry hypothesis as a mechanism of prey
430 attraction by orb-web spiders *via* conspicuous body coloration (e.g. Craig and Ebert, 1994;
431 Hauber, 2002). However, the hypothesis has seldom been tested. Flower mimicry using
432 color vision modelling has been tested for orchid mantis (*Hymenopus coronatus*) prey
433 (O'Hanlon et al., 2013). Color vision modelling suggested that pollinators are unable to
434 distinguish the colors of the mantis and flowers, and a field experiment showed that the
435 mantis actually attracts more pollinators than flowers (O'Hanlon et al., 2013). Our results
436 showed that, considering only color metrics, most of the *G. cancriformis* morphs indeed
437 have similar coloration to flowers. However, when we modeled color perception to
438 potential prey, only the black and white morphs is similar to a category of white flowers.
439 Similarly to our results, in *G. fornicata* the white morphs seems to be indistinguishable
440 from sympatric flowers according to results of bee color vision modeling, but yellow
441 morphs and flowers were not perceptually different (Maia & White, 2017). 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). Both pieces of evidence are circumstantial. They may only reflect the diversity of flower colors and spider colors. Additionally, in the Brazilian savanna, *G. cancriformis* is abundant during the transition from the wet to the dry season, which overlaps partially the flowering period of woody plants (Oliveira, 1998; Gouveia and Fefili, 1998). Flowering peaks in these plants is related to pollinators occurrence, that is around April to October (Oliveira, 1998; Gouveia and Fefili, 1998). Therefore, flower coloration mimicry would be an advantageous foraging strategy to spiders that are abundant during this period of the year. However, a field experiment conducted with *G. cancriformis* showed that color had no effect on prey capture success (Gawryzewsky and Motta, 2012). Furthermore, it could be possible that insects do not represent a strong selection force, considering that most of taxa only perceive chromatic contrast when they are very close to the object (Giurfa et al., 1997).

Multiple predators hypothesis

The results of the present study do not strongly support the multiple predator hypothesis for the maintenance of color polymorphism in *G. cancriformis*, as the spider morphs present the same order of conspicuousness in both the chromatic and achromatic dimensions. Even so, red morphs are particularly more conspicuous to a bird than to a wasp. Therefore, this signal could be targeting bird predators but would appear relatively inconspicuous to a hymenopteran predator and prey. In contrast, the white and yellow morphs are highly detectable by both predator taxa. The colors of two of the four *G. cancriformis* morphs (yellow and red) are typical of aposematic species (Endler and Mappes, 2004). Conspicuous coloration is especially advantageous when it increases the mismatch with the background and facilitates predator learning (Endler and Greenwood, 1988). Spiders of the genus *Gasteracantha* possess spines and a hard abdomen. Moreover, the hunting wasp *Sceliphron laetum* avoids provisioning initial instars with *Gasteracantha* spiders (Elgar and Jebb, 1999). Morphological and behavioral defenses that make ingestion difficult along with the species' bright colors constitute aposematism (Endler and Greenwood, 1988; Ruxton et al., 2004). Though aposematism is not commonly reported

473 in spiders (Oxford and Gillespie, 1998), Brandley et al. (2016) conducted an experiment
474 with black widow models and found that models with red markings were more likely to
475 be avoided by birds than all black models.

476 Color polymorphism may seem counterintuitive in aposematic species, but it may
477 occur when frequency-dependent selection is different among predators, for instance,
478 when a predator presents strong apostatic selection, while other predator has a strong anti-
479 apostatic selection (Endler and Greenwood, 1988). It is also possible when there is a
480 covariance between the relative crypsis of morphs for one predator and frequency-
481 dependent selection. However, contrarily to the first scenario, the equilibrium is unstable
482 (Endler and Greenwood, 1988). Besides, scenarios of overdominance, or equal fitness
483 from different selection pressures may also influence (Stevens and Ruxton, 2012). In *G.*
484 *cancriformis*, morphs have variable degrees of conspicuousness for a single predator or
485 for multiple predators, therefore they might be subject to distinct types of selection.

486 Not only selective pressures from prey and predators may influence color
487 polymorphism, but also thermoregulatory effects and the effect of illumination on the
488 signaler detectability (Rao and Mendoza-Cuenca, 2016; Rojas et al., 2014). Therefore,
489 polymorphism may result from multiple evolutionary forces, in which some morphs
490 signals their impalatability to predators, whereas other morphs are protected from certain
491 predators due to camouflage, meanwhile, they may benefit from thermoregulatory
492 behavior by occupying different microhabitats.

493 Most of studies focus on a single signal receiver, however, we could better
494 understand signal evolution if we considered that individuals interact with different kinds
495 of observers, whether they are mutualists or antagonists (Endler and Mappes, 2014;
496 Schaefer et al., 2004). The multiple receiver hypothesis has been evaluated in intersexual
497 and intrasexual relations (Guindre-Parker et al., 2012), signaler interaction with prey and
498 predators (Endler, 1983), and interaction with pollinators and herbivores (Irwin et al.,
499 2003). For instance, in the snow bunting (*Plectrophenax nivalis*), multiple achromatic
500 patches signal distinct information to females and males: wing coloration inform about
501 male immune response and reproductive performance, whereas plumage of the rectrices
502 and mantle convey information about territoriality and probable aggression (Guindre-
503 Parker et al., 2012). Multiple receivers also maintain guppy color polymorphism, males

can have black, orange, yellow or iridescent spots, that influence on female attraction, but they vary in frequency and size accordingly with predation risk (Endler, 1983). Lastly, flowers polymorphism is also influenced by multiple receivers as in the wild radish (*Raphanus sativus*). Plants that produce anthocyanin – a defense component - and plants that do not produce vary in coloration. Therefore, herbivores may use coloration as cue to find the anthocyanin-recessive morphs (Irwin et al., 2003).

Here, we present a small step of the multiple receivers hypothesis on the evolution of color polymorphism, multiple functions may also maintain this variation, although it remains to be tested. Variation of signal receivers alone may not be sufficient to explain color polymorphism, and gene flow may act together on the maintenance of color variation (Gray and McKinnon, 2007). We only considered chromatic and achromatic discrimination, but color pattern geometry, shape, contour, size, angle, texture, and distance of visual detection (Troscianko et al., 2009) may also influence the behavior of both prey and predators toward spiders since different species use distinct visual cues for stimuli detection and recognition (Théry and Gomez, 2010). Furthermore, color vision models do not include other perceptual mechanisms, such as cognition, color categorization, past experiences, or memory imprecision (Renoult et al., 2015), even though these factors may affect detectability and, consequently, influence the survival rate of morphs in different ways (Théry and Gomez, 2010). Additionally, non-adaptive explanations, such as overdominance and allele equilibrium in absence of selection, are often ignored when studying polymorphisms in an ecological perspective. Finally, predation experiments, field experiments that evaluate prey taxa caught by the different spider morphs, and ecological data on abudance and composition of prey and predators populations that occur sympatrically with *G. cancriformis* are paramount to validate and complement the findings of the present study.

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Competing interests

No competing interests declared.

Author contribution

NXG and FMG contributed to the design of the study. NXG wrote the manuscript and ran the statistical analyses. FMG supervised the analyses and commented on the manuscript.

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Tables

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

Model	df	ΔAIC	Marginal R^2	Conditional R^2
Multiple prey hypothesis				
Chromatic dimension				
ΔS ~ morph*observer	**17**	**0.0**	**0.87**	**0.96**
ΔS ~ morph+observer	11	23.9	0.89	0.95
ΔS ~ observer	8	52.4	0.33	0.96
ΔS ~ morph	9	61.5	0.50	0.51
ΔS ~ 1	6	90.6	0	0.53
Achromatic dimension				
excitation ~ morph*observer	**17**	**0.0**	**0.82**	**0.99**
excitation ~ morph+observer	11	57.6	0.77	0.86
excitation ~ morph	9	72.2	0.79	0.89
excitation ~ observer	8	84.7	0.004	0.60
excitation ~ 1	6	100.2	0	0.66
Multiple predator hypothesis				
Chromatic dimension				
ΔS ~ morph*observer	**13**	**0.0**	**0.86**	**0.99**
ΔS ~ morph+observer	10	6.9	0.88	0.99
ΔS ~ observer	7	30.6	0.29	0.99
ΔS ~ morph	9	54.5	0.58	0.58
ΔS ~ 1	6	74.9	0	0.63

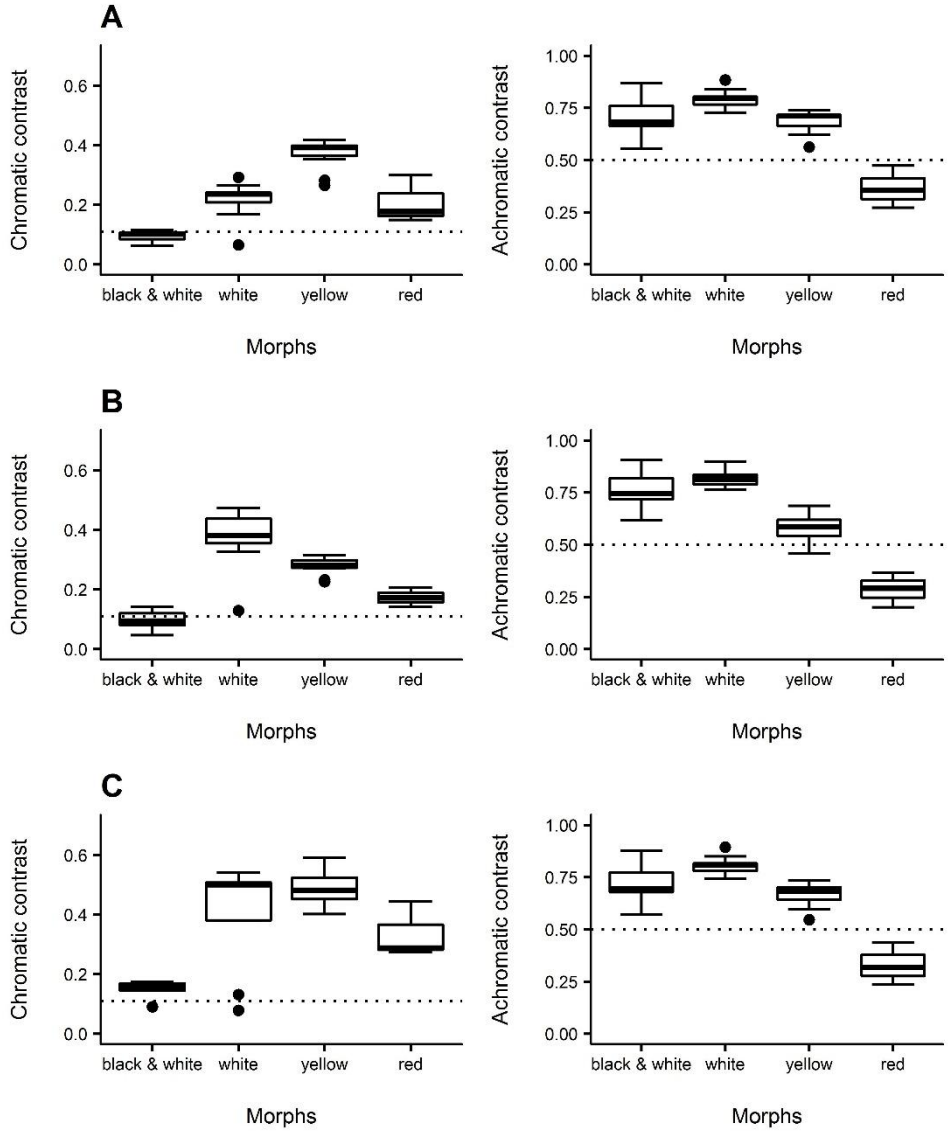
Achromatic dimension				
excitation ~ morph*observer	**14**	**0.0**	**0.78**	**1**
excitation ~ morph+observer	10	14.4	0.78	0.96
excitation ~ observer	7	21.1	0.007	0.86
excitation ~ morph	9	36.9	0.80	0.98
excitation ~ 1	6	43.9	0	0.92

Table 2. Delta Akaike Information Criterion (ΔAIC) and determination coefficients of Linear Mixed Models of the chromatic and achromatic contrasts between spider morphs and flower categories.

	df	ΔAIC	Marg. R^2	Cond. R^2
Chromatic dimension				
ΔS ~ morph * flower * observer + (1|IDflower) + (1|IDspider)	111	0	0.67	0.82
ΔS ~ morph * flower + observer + (1|IDflower) + (1|IDspider)	41	-22276.39	0.60	0.75
ΔS ~ morph * flower + (1|IDflower) + (1|IDspider)	39	-39316.11	0.55	0.70
ΔS ~ morph * observer + flower + (1|IDflower) + (1|IDspider)	23	-58911.74	0.46	0.61
ΔS ~ morph * observer + (1|IDflower) + (1|IDspider)	15	-59819.05	0.28	0.61
ΔS ~ morph + flower * observer + (1|IDflower) + (1|IDspider)	33	-62659.22	0.44	0.59
ΔS ~ Flower * observer + (1|IDflower) + (1|IDspider)	30	-62679.69	0.25	0.60
ΔS ~ morph + flower + observer + (1|IDflower) + (1|IDspider)	17	-64825.9	0.43	0.58
ΔS ~ flower + observer + (1|IDflower) + (1|IDspider)	14	-64846.37	0.24	0.58
ΔS ~ morph + observer + (1|IDflower) + (1|IDspider)	9	-65733.21	0.25	0.58
ΔS ~ observer + (1|IDflower) + (1|IDspider)	6	-65753.68	0.06	0.57
ΔS ~ morph + flower + (1|IDflower) + (1|IDspider)	15	-75210.75	0.37	0.52
ΔS ~ flower + (1|IDflower) + (1|IDspider)	12	-75231.22	0.18	0.52
ΔS ~ morph + (1|IDflower) + (1|IDspider)	7	-76118.06	0.20	0.52
ΔS ~ 1 + (1|IDflower) + (1|IDspider)	4	-76138.53	0	0.52
Achromatic dimension				
excitation ~ morph * flower * observer + (1|IDflower) + (1|IDspider)	111	0	0.49	0.64
excitation ~ morph * flower + observer + (1|IDflower) + (1|IDspider)	41	-13215.1	0.42	0.57
excitation ~ morph * flower + (1|IDflower) + (1|IDspider)	39	-14202.51	0.42	0.57
excitation ~ morph + flower * observer + (1|IDflower) + (1|IDspider)	33	-33305.63	0.30	0.45
excitation ~ morph * observer + flower + (1|IDflower) + (1|IDspider)	23	-33320.44	0.30	0.44
excitation ~ Flower * observer + (1|IDflower) + (1|IDspider)	30	-33331.64	0.12	0.45
excitation ~ morph * observer + (1|IDflower) + (1|IDspider)	15	-33835.46	0.20	0.45
excitation ~ morph + flower + observer + (1|IDflower) + (1|IDspider)	17	-35420.86	0.29	0.43
excitation ~ flower + observer + (1|IDflower) + (1|IDspider)	14	-35446.87	0.11	0.43
excitation ~ morph + observer + (1|IDflower) + (1|IDspider)	9	-35935.88	0.18	0.43
excitation ~ observer + (1|IDflower) + (1|IDspider)	6	-35961.89	0.05	0.43
excitation ~ morph + flower + (1|IDflower) + (1|IDspider)	15	-36160.68	0.28	0.43

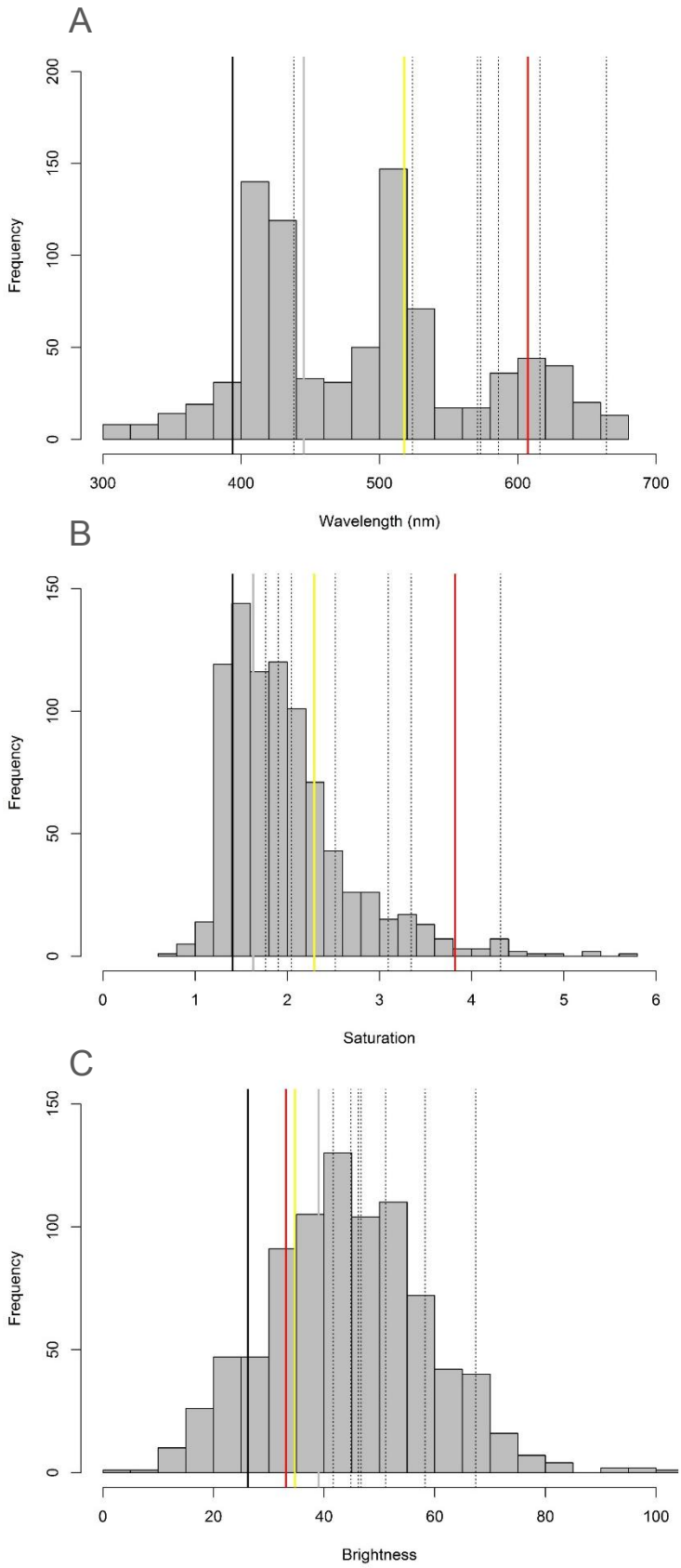
excitation ~ flower + (1 | IDflower) + (1 | IDspider)	12	-36186.69	0.10	0.43
excitation ~ morph + (1 | IDflower) + (1 | IDspider)	7	-36675.7	0.18	0.43
excitation ~ 1 + (1 | IDflower) + (1 | IDspider)	4	-36701.71	0	0.43

Figures



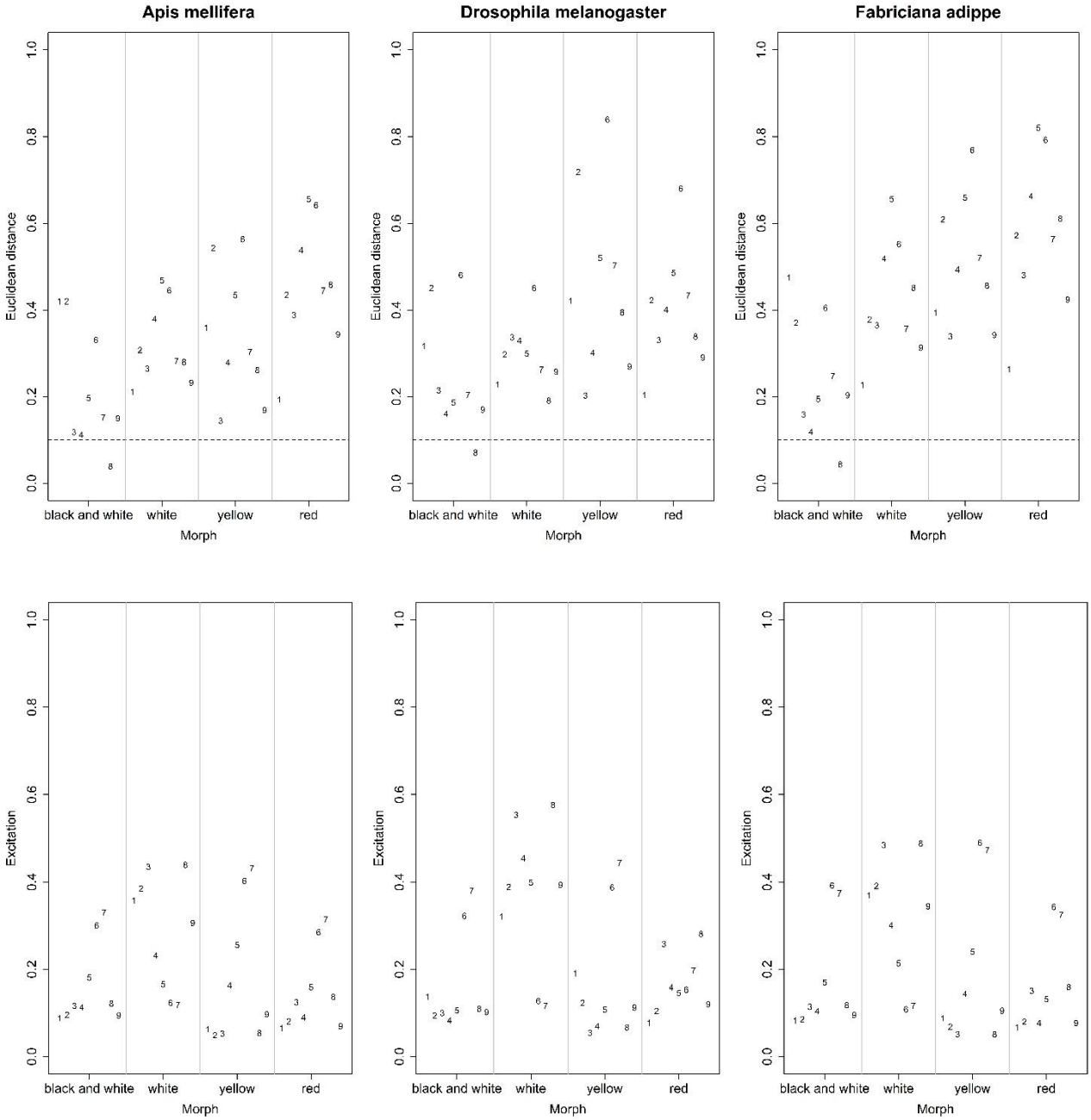
531 **Fig. 1.** Chromatic (left) and achromatic (right) contrasts of four *Gasteracantha cancriformis*
532 morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) when viewed
533 against a Brazilian savanna background by prey with distinct visual systems. (A) *Apis*
534 *mellifera* (Hymenoptera).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

537


A
Frequency
0
50
100
150
200
300
400
500
600
700
Wavelength (nm)
B
Frequency
0
50
100
150
0
1
2
3
4
5
6
Saturation
C
Frequency
0
50
100
150
0
20
40
60
80
100
Brightness

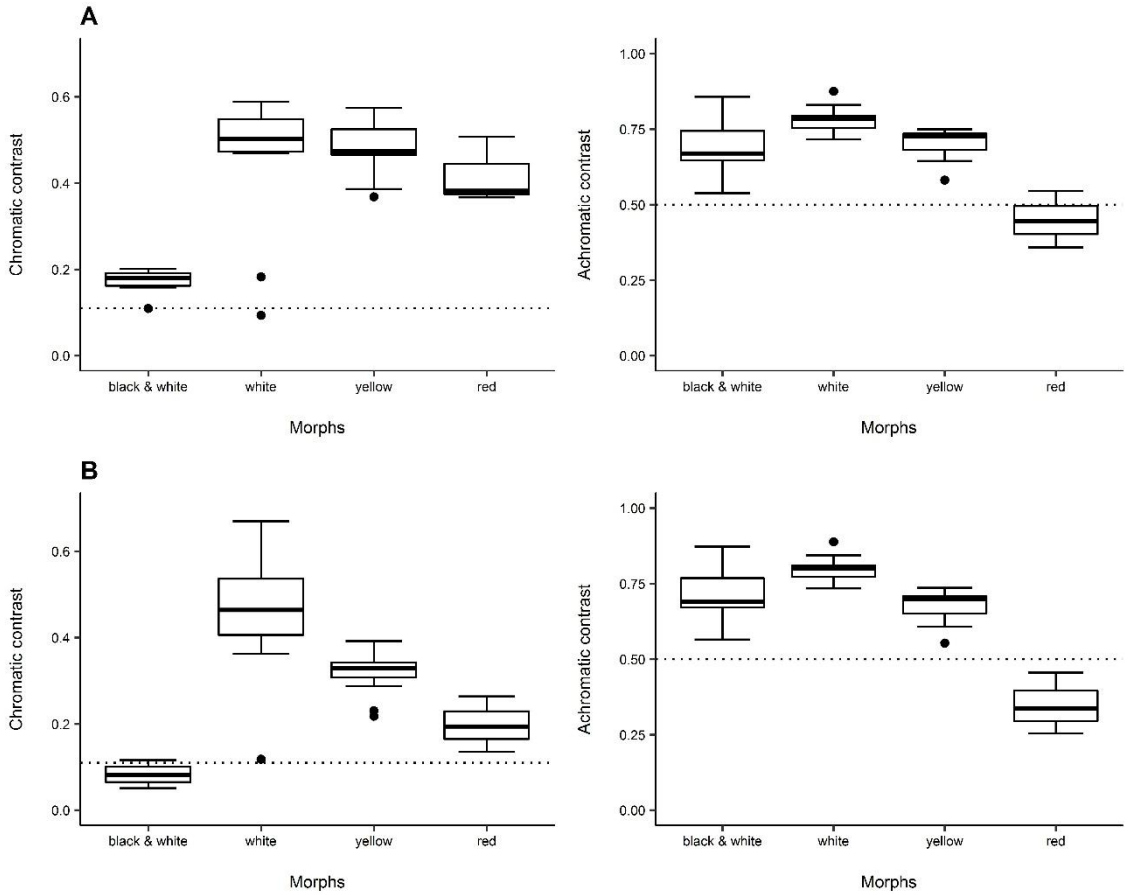
538

539 **Fig. 2.** Frequency of color properties of flowers (N = 859): (A) hue, (B) saturation, (C)
540 brightness. Average values of each *Gasteracantha cancriformis* morph are represented
541 with solid colored lines: black and white morph (black line; N = 6), white morph (gray
542 line, N = 10), yellow morph (yellow line, N = 13), red morph (red line, N = 3). Flowers
543 from the Brazilian Savanna (N = 7) are represented with dotted lines.
544



545
546 **Fig. 3.** Chromatic (upper) and achromatic (lower) contrasts of four *Gasteracantha*
547 *cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3)
548 when compared with ten flowers categories (Chittka et al., 1994) indicated by numbers.

549 These values were computed considering the Brazilian savanna as background and based
550 on three potencial prey: *Apis mellifera* (Hymenoptera; left), *Drosophila melanogaster*
551 (Diptera; middle), and *Fabriciana adippe* (Lepidoptera; right). Dotted vertical lines
552 represent the discrimination thresholds for chromatic contrast (0.11).
553



554
555 **Fig. 4.** Chromatic (left) and achromatic (right) contrasts of four *Gasteracantha cancriformis*
556 morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) when viewed
557 against a Brazilian savanna background by predators with distinct visual systems. (A)
558 *Parus caeruleus* (Passeriformes). (B) *Philanthus Triangulum* (Hymenoptera). Dotted
559 vertical lines represent the discrimination thresholds for chromatic contrast (0.06) and
560 photoreceptor excitation for background in achromatic contrast (0.5).