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

31 distance, shape, angle, and pattern geometry could also affect the perception of color

32 morphs by both prey and predators and thereby influence morph survival.

33

34 KEY WORDS: *Gasteracantha cancriformis*; Araneidae; Visual communication;

35 Aposematism; Luring; Prey attraction

36

37 **INTRODUCTION**

38 The evolution and maintenance of color polymorphism have traditionally been attributed

39 to apostatic selection (Clarke, 1979). Assuming that predators form a search image

40 (Tinbergen, 1960), the advantage of rarity promotes the coexistence of multiple prey types

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

42 explanations for the evolution and maintenance of color polymorphisms have been

43 proposed (Gray and McKinnon, 2007). For instance, gene flow between populations with

44 distinct phenotypes that are favored by natural selection could maintain polymorphism

45 within populations (Farkas et al., 2013; Gray and McKinnon, 2007).

46   In the context of visual signaling, the distinct visual systems of prey and predators

47 may play a role in the evolution and maintenance of color polymorphisms (Ruxton et al.,

48 2004; White and Kemp, 2015). Animal communication involves the generation, emission

49 transmission, and processing of the signal by a receiver, in which an appropriate response

50 is elicited (Endler 1993). Any factors that affect these steps can influence signal efficiency

51 and, as a result, affect the direction of communication evolution (Endler 1993). Thus, the

52 diversity of signals are likely influenced by variation in the sensory systems of receivers.

53   Many orb-web spiders exhibit conspicuous coloration. Although sexual selection

54 is a common explanation for bright coloration in other taxa such as birds (Ryan, 1990),

55 this scenario is less likely to happen in orb web spiders, because they have limited visual

56 acuity (Foelix, 2011). *Argiope argentata* (Araneidae), for instance, seems to possess only

57 one photoreceptor (Tiedemann, 1986). The prey attraction hypothesis states that the

58 bright coloration of some spiders lures insects, possibly by mimicking flower coloration

59 (e.g. Craig and Ebert, 1994; Hauber, 2002). The hypothesis has been empirically tested

60 several times, and most studies have found support for it. The polymorphic *Nephila*

61 *pilipes* (Nephilidae) present a melanic and a bright colored morph (Tso et al., 2004). The

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

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

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 (without UV reflectance), red, and a combination of black and white (white patches reflects UV; 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

93   *Gasteracantha cancriformis* (Araneidae), since both naturally bright morphs and yellow-

94   painted individuals failed to capture more prey than either naturally cryptic morphs or

95   black-painted individuals (Gawryszewski and Motta, 2012). Nonetheless, it remains the

96   possibility that each color morphs attracts preferentially specific types of prey.

97   Furthermore, although evidence is still needed, Edmunds and Edmunds (1983) suggested

98   that the conspicuous body coloration of *Gasteracantha* spiders might serve as a warning

99   signal to predators.

100   Considering that the same "color" may be perceived as cryptic or conspicuous by

101   different species (Endler and Mappes 2004), each color morph of a polymorphic

102   populations may represent an adaptation to particular visual systems of prey or predator

103   species (Endler, 1992; Ruxton et al., 2004; White and Kemp, 2015). Therefore, it is

104   plausible that the variation of color among individuals within a population is affected by

105   a diverse range of interactions that leads to different selection process. To date, the role

106   of multiple predators on the evolution of prey coloration has been approached by

107   theoretical models (Endler and Greenwood, 1988; Endler and Mappes, 2004). Endler and

108   Greenwood (1988) models, for instance, indicated that a stable polymorphisms might

109   evolve in the presence of anti-apostatic (positive frequency-dependent) from different

110   predators, given that predators perceive prey conspicuousness differently and there is a

111   covariance between the relative degree of crypsis and the degree of frequency-dependent

112   selection by each predator.

113   In this study we aimed to explore old and new hypothesis that could potentially

114   explain the maintenance of color polymorphism in a spider species. The aim of the

115   present study was to investigate three hypotheses for the evolution and maintenance of

116   color polymorphism, using *G. cancriformis* as a model. Two derivations from the prey

117   attraction hypothesis include (1) the *multiple prey hypothesis*, which posits that each color

118   morph is adapted to lure a specific type of prey, which posits that the spiders attract prey

119   *via* aggressive mimicry of flower colors and that each color morph mimics a different

120   flower color. In addition, (3) the *multiple predator hypothesis* posits that the conspicuous

121   colors found in spiders could serve as warning signals to predators and that color

122   polymorphism could evolve and be maintained if each color morph is adapted to the

123   vision of a specific predator.

124

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:

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

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:

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

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:

$$E = P/(P + 1) \tag{3}$$

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

$$X1 = \sin 60° (E_3 - E_1) \tag{4}$$

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

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 Xi (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

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 (λ_{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

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):

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

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

271   reflectances;

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

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;

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

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

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 $\Delta S =$

324 0.11 and $\Delta S = 0.06$ 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

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

442   study of various orb-web spider species that also used color vision models found that, as

443   perceived by dipterans and hymenopterans, the colors of spiders are very similar to those

444   of flowers (White et al., 2016). Both pieces of evidence are circumstantial. They may only

445   reflect the diversity of flower colors and spider colors. Additionally, in the Brazilian

446   savanna, *G. cancriformis* is abundant during the transition from the wet to the dry season,

447   which overlaps partially the flowering period of woody plants (Oliveira, 1998; Gouveia

448   and Fefili, 1998). Flowering peaks in these plants is related to pollinators occurrence, that

449   is around April to October (Oliveira, 1998; Gouveia and Fefili, 1998). Therefore, flower

450   coloration mimicry would be an advantageous foraging strategy to spiders that are

451   abundant during this period of the year. However, a field experiment conducted with *G.*

452   *cancriformis* showed that color had no effect on prey capture success (Gawryzewsky and

453   Motta, 2012). Furthermore, it could be possible that insects do not represent a strong

454   selection force, considering that most of taxa only perceive chromatic contrast when they

455   are very close to the object (Giurfa et al., 1997).

456

Multiple predators hypothesis

458   The results of the present study do not strongly support the multiple predator

459   hypothesis for the maintenance of color polymorphism in *G. cancriformis*, as the spider

460   morphs present the same order of conspicuousness in both the chromatic and achromatic

461   dimensions. Even so, red morphs are particularly more conspicuous to a bird than to a

462   wasp. Therefore, this signal could be targeting bird predators but would appear relatively

463   inconspicuous to a hymenopteran predator and prey. In contrast, the white and yellow

464   morphs are highly detectable by both predator taxa. The colors of two of the four *G.*

465   *cancriformis* morphs (yellow and red) are typical of aposematic species (Endler and

466   Mappes, 2004). Conspicuous coloration is especially advantageous when it increases the

467   mismatch with the background and facilitates predator learning (Endler and Greenwood,

468   1988). Spiders of the genus *Gasteracantha* possess spines and a hard abdomen. Moreover,

469   the hunting wasp *Sceliphron laetum* avoids provisioning initial instars with *Gasteracantha*

470   spiders (Elgar and Jebb, 1999). Morphological and behavioral defenses that make

471   ingestion difficult along with the species' bright colors constitute aposematism (Endler and

472   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

504   can have black, orange, yellow or iridescent spots, that influence on female attraction, but

505   they vary in frequency and size accordingly with predation risk (Endler, 1983). Lastly,

506   flowers polymorphism is also influenced by multiple receivers as in the wild radish

507   (*Raphanus sativus*). Plants that produce anthocyanin – a defense component - and plants

508   that do not produce vary in coloration. Therefore, herbivores may use coloration as cue

509   to find the anthocyanin-recessive morphs (Irwin et al., 2003).

510   Here, we present a small step of the multiple receivers hypothesis on the evolution

511   of color polymorphism, multiple functions may also maintain this variation, although it

512   remains to be tested. Variation of signal receivers alone may not be sufficient to explain

513   color polymorphism, and gene flow may act together on the maintenance of color

514   variation (Gray and McKinnon, 2007). We only considered chromatic and achromatic

515   discrimination, but color pattern geometry, shape, contour, size, angle, texture, and

516   distance of visual detection (Troscianko et al., 2009) may also influence the behavior of

517   both prey and predators toward spiders since different species use distinct visual cues for

518   stimuli detection and recognition (Théry and Gomez, 2010). Furthermore, color vision

519   models do not include other perceptual mechanisms, such as cognition, color

520   categorization, past experiences, or memory imprecision (Renoult et al., 2015), even

521   though these factors may affect detectability and, consequently, influence the survival rate

522   of morphs in different ways (Théry and Gomez, 2010). Additionally, non-adaptive

523   explanations, such as overdominance and allele equilibrium in absence of selection, are

524   often ignored when studying polymorphisms in an ecological perspective. Finally,

525   predation experiments, field experiments that evaluate prey taxa caught by the different

526   spider morphs, and ecological data on abudance and composition of prey and predators

527   populations that occur sympatrically with *G. cancriformis* are paramount to validate and

528   complement the findings of the present study.

529

Acknowledgments

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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530

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
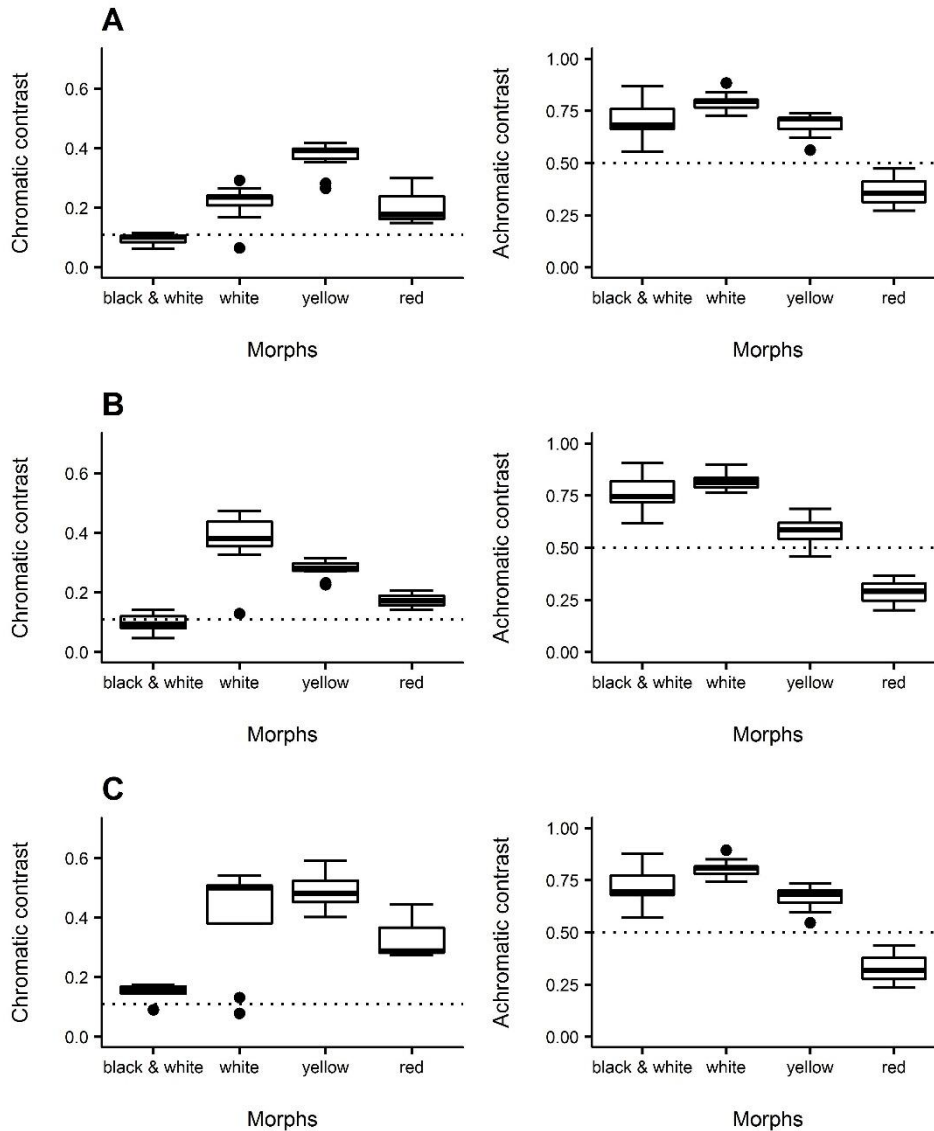
Fig. 1. Chromatic (left) and achromatic (right) contrasts of four *Gasteracantha cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) when viewed against a Brazilian savanna background by prey with distinct visual systems. (A) *Apis mellifera* (Hymenoptera). (B) *Drosophila 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).

537
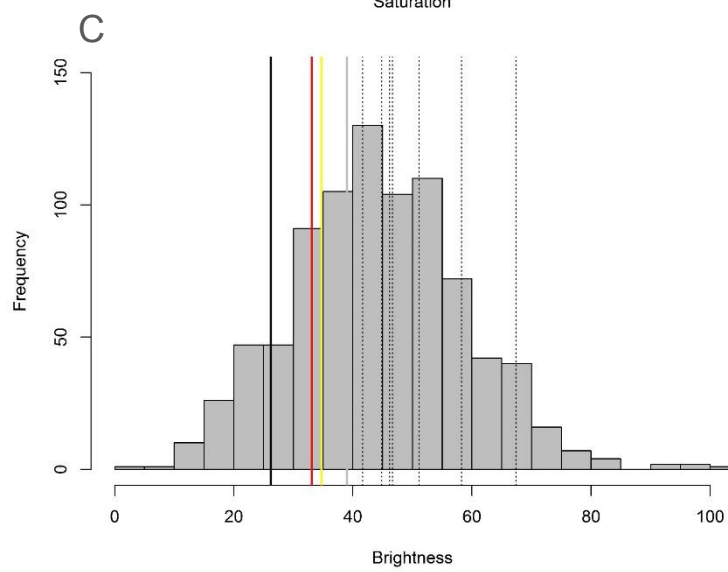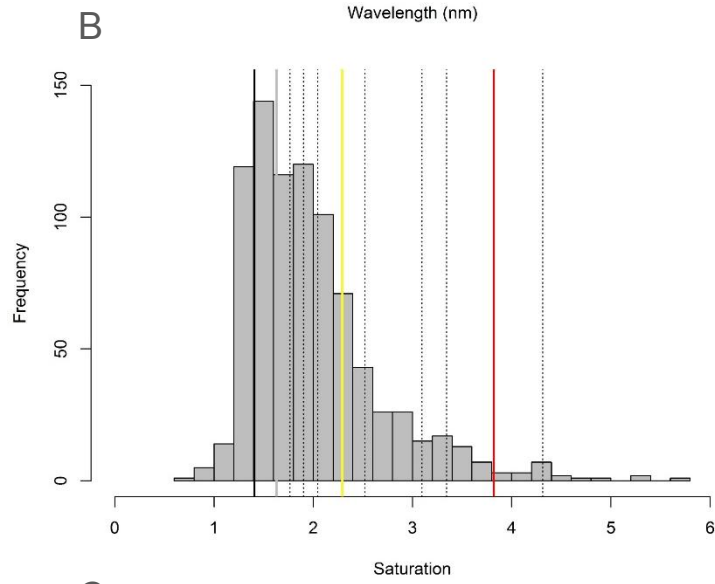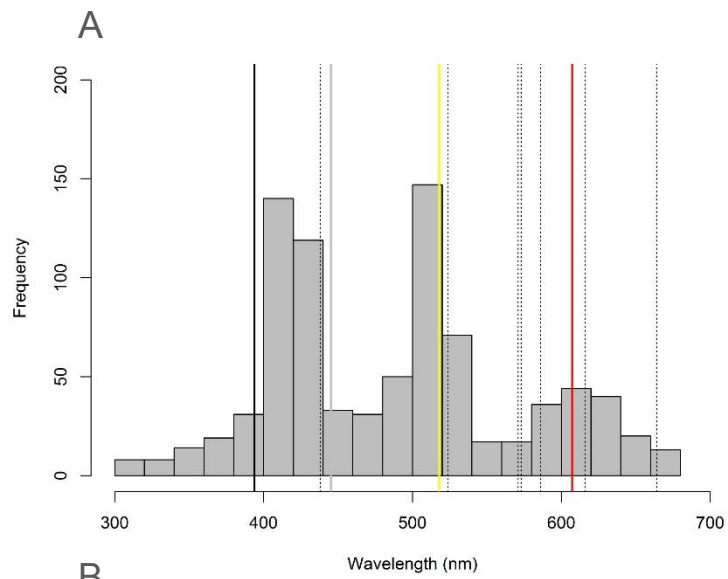
538

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
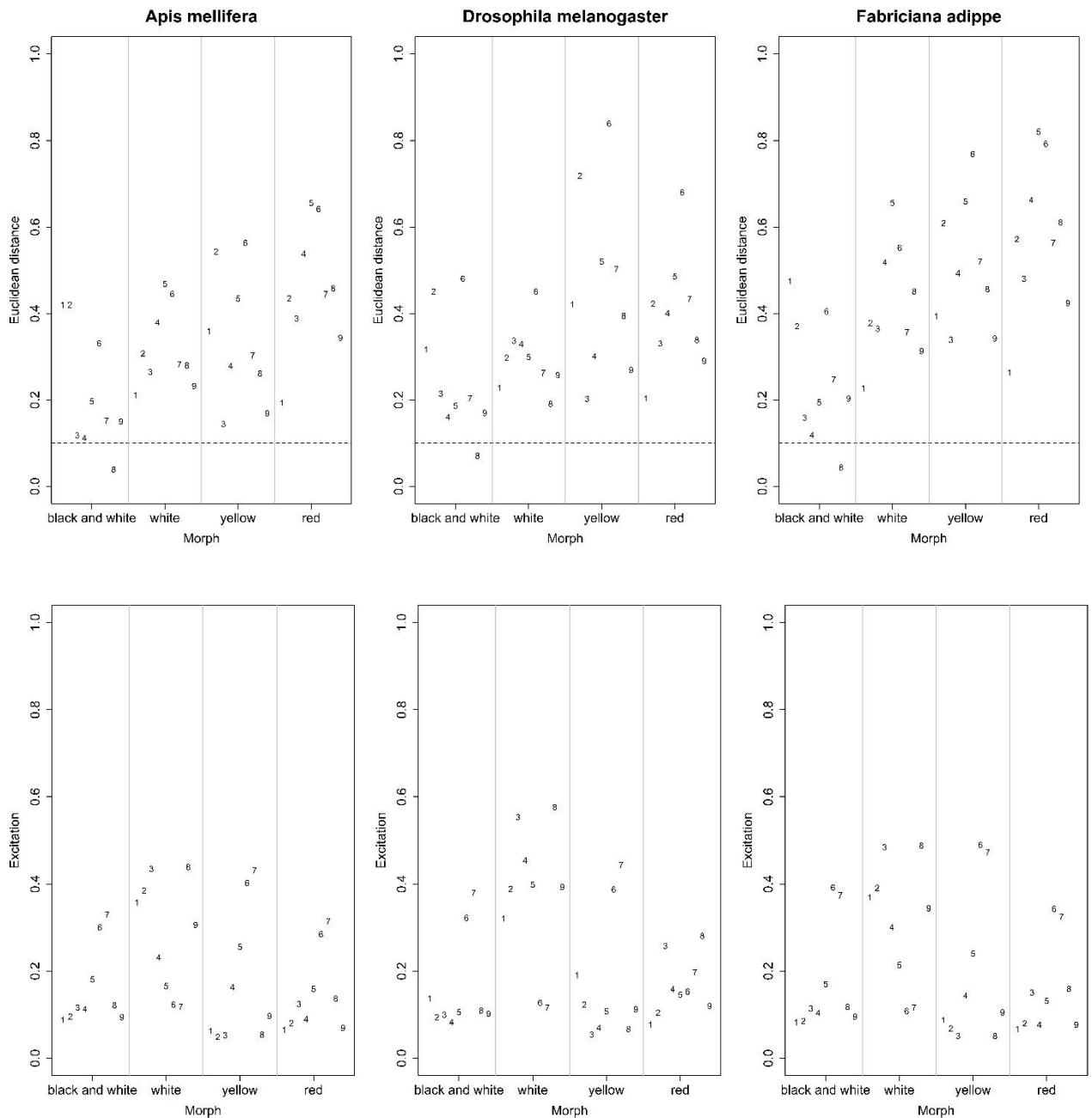
545

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
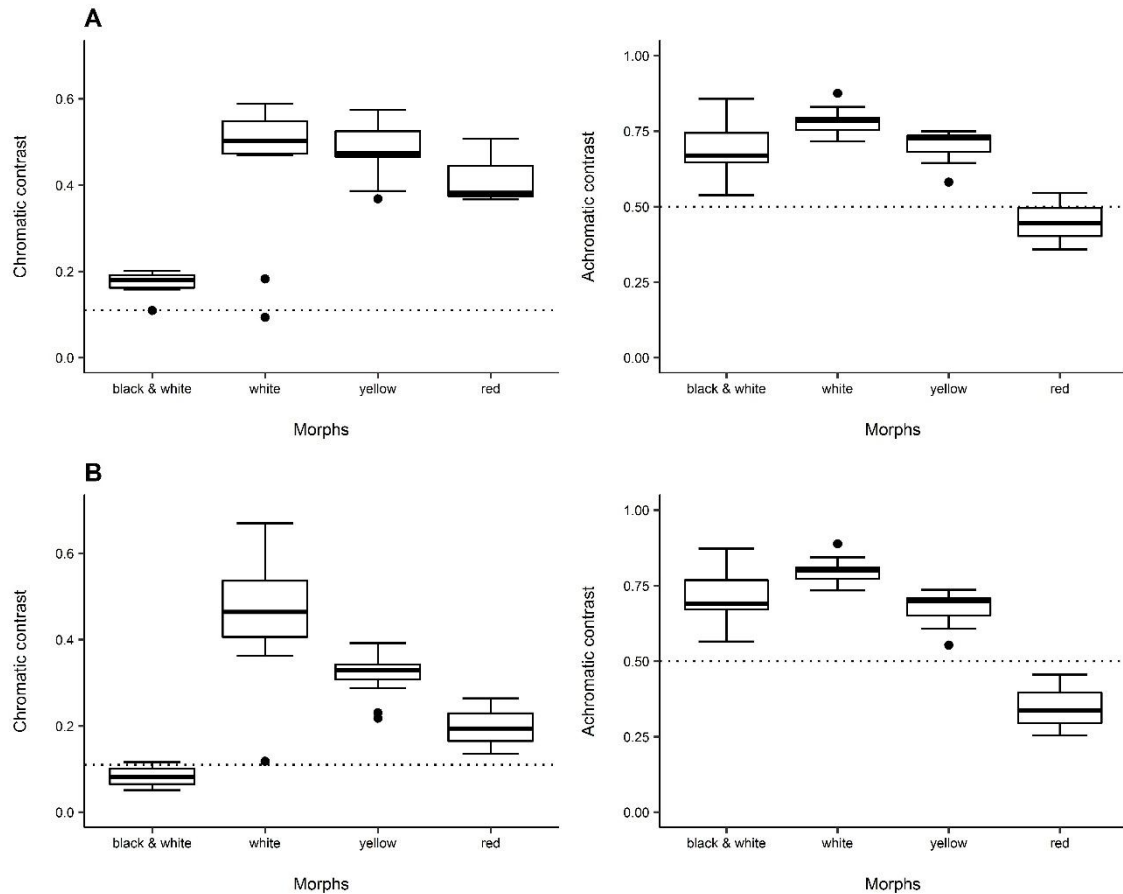
554

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).