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

Nathalia X. Gonçalves[1,2], Felipe M. Gawryszewski[1]

[1]Evolution and Sensory Ecology Lab, Departamento de Genética, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Campus Samambaia, Goiânia, GO, 74690-900, Brazil,

[2]Pós-Graduação em Ecologia e Evolução, Universidade Federal de Goiás, Campus Samambaia, Goiânia, GO, 74690-900, Brazil.

*Author for correspondence (xg.nathalia@gmail.com)

Running title: Orb-web spider color polymorphism

18   **Summary statement:** We assessed the role of both prey and predator vision on the

19   evolution and maintenance of color polymorphism and color conspicuousness of the

20   orb-web spider *Gasteracantha cancriformis* (Araneidae).

21

22   **ABSTRACT**

23   The sensory drive theory predicts that signals, sensory systems, and signaling behavior

24   should coevolve. Variation in the sensory systems of prey and predators may explain

25   the diversity of color signals, such as color polymorphism. The spider *Gasteracantha*

26   *cancriformis* (Araneidae) possesses several conspicuous color morphs. The aim of the

27   present study was to assess whether the color polymorphism of *G. cancriformis* may be

28   maintained by pressure from multiple signal receivers, such as prey and predators with

29   distinct color vision systems. In orb-web spiders, the prey attraction hypothesis states

30   that conspicuous colors are prey lures that increase spider foraging success via flower

31   mimicry. However, in highly defended species, conspicuous colors could also be a

32   warning signal to predators. We used color vision modelling to estimate chromatic and

33   achromatic contrast of *G. cancriformis* morphs as perceived by potential prey and

34   predators taxa. The results revealed that individual prey and predator taxa perceive the

35   conspicuousness of morphs differently. Therefore, the multiple prey and multiple

36   predator hypotheses may explain the evolution of color polymorphism in *G.*

37   *cancriformis*. However, the spider's coloration did not resemble flower coloration,

38   which suggests that the species' conspicuousness is not the result of flower mimicry.

39   Other parameters that are not evaluated by color vision models, such as distance, shape,

40   angle, pattern geometry, and contour, could also affect the perception of color morphs

41   by both prey and predators and thereby influence morph survival.

42

43   **INTRODUCTION**

44   The evolution and maintenance of color polymorphism have traditionally been

45   attributed to apostatic selection (Clarke, 1979). Assuming that predators form a search

46   image (Tinbergen, 1960), the advantage of rarity promotes the coexistence of multiple

47   prey types and stabilizes polymorphisms (Bond, 2007). Nonetheless, other adaptive and

48   non-adaptive explanations for the evolution and maintenance of color polymorphisms

49 have been proposed (Gray and McKinnon, 2007). For instance, gene flow between
50 populations with distinct phenotypes that are favored by natural selection could
51 maintain polymorphism within the individual populations (Farkas et al., 2013; Gray and
52 McKinnon, 2007).

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

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

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

80   violet, blue, green, and red (Hart, 2001), whereas spider hunting wasps, such as

81   members of the Sphecidae, are trichromats, whose photoreceptors are most sensitive to

82   ultraviolet, blue, and green (Peitsch, 1992; Briscoe and Chittka, 2001). Similarly, the

83   insect prey of orb-web spiders also vary in their types of color vision. For example, bees

84   are trichromats with spectral sensitivities that are similar to those of sphecid wasps

85   (Briscoe and Chittka, 2001), whereas some lepidopterans are tetrachromats, and some

86   dipterans possess photoreceptors with five different sensitivity peaks (Schnaitmann et al.,

87   2013). Therefore, the maintenance of spider color polymorphism may result not only

88   from a trade-off between prey attraction and capture success but also from selective

89   pressure from multiple receivers (Endler, 1992; Ruxton et al., 2004; White and Kemp,

90   2015). Color perception depends on both the signal reflectance and observer visual

91   system, as well as on the background reflectance spectrum and ambient light intensity

92   (Endler, 1990). Physiological models of color vision include all these factors and have

93   been effective for objectively studying animal coloration (i.e., independent of human

94   subjective assessment; Renoult, 2015).

95   The orb-web spider *G. cancriformis* constructs large webs and rests in the hub of

96   the web during the day (Levi, 1978). Females of the species possess a hard abdomen

97   with three pairs of spines and vary in color, with some morphs quite conspicuous to

98   human observers (Levi, 1978; Gawryszewski and Motta, 2012). The ventral side of

99   females are mostly black, sometimes with small bright spots. In one studied population,

100   the dorsal side of females possessed black or reddish spines and four different color

101   patterns: yellow, white, red, and a combination of black and white (Gawryszewski 2007;

102   Gawryszewski and Motta, 2012). Adult females measure from 5 to 7 mm in length and

103   10 to 13 mm in width (Muma, 1971), whereas the males are brownish, small, and do not

104   exhibit chromatic variation (Levi, 1978). The prey attraction hypothesis does not seem

105   to explain the coloration of the orb-web spider *Gasteracantha cancriformis* (Araneidae),

106   since both naturally bright morphs and yellow-painted individuals failed to capture

107   more prey than either naturally cryptic morphs or black-painted individuals

108   (Gawryszewski and Motta, 2012). Although evidence is still needed, Edmunds and

109   Edmunds (1983) suggested that the conspicuous body coloration of *Gasteracantha*

110   spiders might serve as a warning signal to predators. The aim of the present study was

111   to investigate three hypotheses for the evolution and maintenance of color

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

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

114   morphs are perceived differently by different prey taxa and that each color morph is

115   adapted to lure a specific type of prey, and (2) the *multiple mimic model* hypothesis,

116   which posits that the spiders attract prey *via* aggressive mimicry of flower colors and that

117   each color morph mimics a different flower color. In addition, (3) the *multiple predator*

118   *hypothesis* posits that the conspicuous colors found in spiders could serve as warning

119   signals to predators and that color polymorphism could evolve and be maintained if

120   each color morph is adapted to the vision of a specific predator.

121

MATERIALS AND METHODS

Color vision model

124   To estimate the perception of *G. cancriformis* color morphs by distinct predators and

125   prey groups, we used the color vision model proposed by Chittka (1992). The model

126   requires four inputs: (1) the irradiance reaching the observed object, (2) the observer

127   photoreceptor excitation curves, (3) the background reflectance to which photoreceptors

128   are adapted to, and (4) the reflectance curve of the observed object. First, the sensitivity

129   factor R was determined for each photoreceptor, as follows:

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

130   where $I_B(\lambda)$ is the spectral reflectance function of the background, $S(\lambda)$ is the spectral

131   sensitivity function of each photoreceptor, and $D(\lambda)$ is the illuminant irradiance

132   spectrum. Secondly, the quantum flux P (relative amount of photon catch) is calculated,

133   as follows:

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

134   where $I_S(\lambda)$ is the spectral reflectance function of the stimulus. Assuming that the

135   maximum excitation of a photoreceptor is 1, the phototransduction process is

136   determined by:

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

137   Stimuli spectra are projected in specific color spaces. The coordinates of each

138   spectrum are calculated using photoreceptor excitations, as follows (Chittka et al., 1994):

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

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

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

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

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

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

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

141   as follows:

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

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

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

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

142   Chromatic contrast between a color stimulus and background, or between two

143   color stimuli, is calculated as the Euclidean distance (ΔS) between two points in color

144   space, as follows:

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

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

146   The color spaces are chromaticity diagrams and, thus, do not estimate the

147   achromatic contrast between objects. Nonetheless, achromatic contrasts can be

148   important visual cues used by both prey and predators. In bees, achromatic contrast is

149   more important than chromatic cues for objects that subtend a visual angle smaller than

150   ~15°, which means that bees have to be very close to flowers in order to use their color

151   vision for discrimination tasks (Giurfa et al., 1997). Similarly, birds use achromatic

152   contrast in detection of small objects (Osorio et al., 1999). We estimated the achromatic

153   contrast as the excitation (Eq. 3) of the photoreceptor responsible for achromatic

154   discrimination in each organism (Chittka and Kevan, 2005).

155   For our modeling, we used the reflectance data of *G. cancriformis* color morphs

156   that was collected during a previous study (Gawryszewski, 2007; Gawryszewski and

157   Motta 2012). These data have already been used to estimate the visual contrast of the

158   yellow and white morphs and the black and white morphs from the perspective of *Apis*

159   *mellifera* (Gawryszewski and Motta 2012). The spiders were collected from a Brazilian

160   savanna physiognomy, namely Cerrado *sensu stricto,* which is characterized by shrubs

161   and trees of 3 to 8 m tall that are contorted and possess thick, fire-resistant bark, a crown

162   cover of >30%, and additional herbaceous vegetation (Oliveira-Filho and Ratter, 2002).

163   The background reflectance was estimated from the average reflectance of leafs, leaf

164   litter, bark, and grasses that were collected from the same area as the spiders

165   (Gawryszewski and Motta, 2012). As illuminant spectrum, we used the International

166   Commission on Illumination (CIE) standard illuminant of D65, which is comparable to

167   open areas, such as the Brazilian savanna (Chittka, 1996).

168

Multiple prey hypothesis

169

170   Using the model described above, we estimated the chromatic and achromatic

171   conspicuousness of the *G. cancriformis* morphs (yellow, white, red, and white parts of

172   the black and white morph) to a suit of potential prey: *A. mellifera* (Hymenoptera,

173   Apidae), *Drosophila melanogaster* (Diptera, Drosophilidae), and *Fabriciana adippe*

174   (Lepidoptera, Nymphalidae). We selected these insect orders because they are

175   commonly intercepted by orb-webs in field experiments (Craig and Ebert, 1994; Tso et

176   al., 2002) and represent the diversity of visual systems among insects (Briscoe and

177   Chittka, 2001). For *A. mellifera* and *D. melanogaster*, we used photoreceptor sensitivity

178   curves from the literature (Peitsch et al., 1992; Schnaitmann et al., 2013). It was recently

179   shown that, together with R7-R8 photoreceptors, R1-R6 photoreceptors contribute to

180   color vision in *D. melanogaster* (Kelber and Henze, 2013; Schnaitmann et al., 2013).

181   Therefore, we included the R1-R6 photoreceptor curve, treating *D. melanogaster* as a

182   pentachromat. The graphical curves were extracted directly from the figures of relevant

183   publications using DataThief III version 1.7 (Tummers, 2006). For *Fabriciana adippe,*

184   however, no photoreceptor sensitivity curves are available, so electrophysiological

185   measurements of photoreceptor sensitivity peaks (λ_{max}; Eguchi et al., 1982) were used to

186   estimate the photoreceptor curves (for details see Govardovskii et al., 2000).

187   For achromatic contrast, bees only use the green photoreceptor (Giurfa et al.,

188   1996), whereas flies only use the outer photoreceptors (R1-R6; Kelber & Henze, 2013).

189   Because the exact mechanism used by lepidopterans for achromatic discrimination is

190   incompletely understood, we assumed that they employ the same mechanism as in

191   bees. The color hexagon model assumes that photoreceptors respond to half their

192   maximum for the background they are adapted to, so that the photoreceptor excitation

193   for background is equivalent to 0.5 units (Chittka, 1992).

194   The multiple prey hypothesis predicts that different prey taxa perceive color

195   morphs differently. To assess whether each spider morph was perceived differently by

196   prey species, we constructed two linear mixed models, one for chromatic contrast and

197   one for achromatic contrast. Either chromatic (ΔS) or achromatic contrast were used as

198   the dependent variable, and spider morph and prey taxon were used as the

199   independent variables (contrast = spider morph $\times$ observer). The spider morph was

200   defined as yellow, white, red, or black and white, and the observers were defined as

201   hymenopteran, dipteran, or lepidopteran. Individual spiders were used as random

202   effects. Normality and homogeneity were verified by visual inspection of quantile-

203   quantile and residuals vs. fitted values plots. We computed all nested models and used

204   the Akaike Information Criterion to select the best model.

205   As a reference point, we used a color discrimination threshold of ΔS = 0.11,

206   which is the threshold value below which trained bees are unable to distinguish different

207   flower colors (Chittka, 1996). However, discrimination thresholds are variable and can

208   change depending on the study species, learning conditions, previous experience,

209   background coloration, whether the task involves discrimination between colors or

210   detection against the background, and whether objects are compared sequentially or

211   simultaneously (Avarguès-Weber & Giurfa, 2014).

212

Multiple mimic models hypothesis

214   To test the multiple mimic models hypothesis, we compared how flowers and spider

215   morphs are perceived by prey. We gathered all flower reflectance data available in the

216   Floral Reflectance Database (FReD; Arnold et al., 2010), excluding reflectance data

217   from lower flower parts, leaves, bracts, stamens, the inner parts of bell-shaped flowers,

218   and unknown items, as well as spectrum files that did not cover 300 to 700 nm. Most

219   species in the database have only one reflectance spectrum, and for species with

220   multiple reflectance spectra, we randomly selected a single spectra. We did not average

221   the reflectance of these species because there was no information available on whether

222   these measurements referred to different individuals or different parts of single flowers.

223   In total, we gathered reflectance data from 859 plant species.

224   We calculated a matrix of chromaticity distances between average color loci of

225   each spider color morph and each individual flowers species and plotted these values

226   using a Kernel density estimation. If spiders are mimicking some group of flower colors,

227   we would expect to find at least one peak near or below the theoretical detection

228   threshold of 0.11. We also followed the same procedure for the achromatic difference

229   between individual flowers and average color loci of each color morph. If spiders and

230   flowers are similar in the achromatic dimension, we would expect to find a peak near

231   0.5.

232

Multiple predator hypothesis

234   The methodology used to investigate the multiple predator hypothesis methodology was

235   very similar to that used for the multiple prey hypothesis, except that we used predator

236   species in our models. As predators, we considered the bird *Parus caeruleus* (Paridae)

237   and the wasp *Philanthus triangulum* (Sphecidae), since birds and wasps are the main

238   predators of orb-web spiders (Rayor, 1996; Foelix, 2010), are visually guided hunters,

239   and have distinct color vision systems. For *P. caeruleus*, we used photoreceptor

240   sensitivity curves available in the literature (Hart, 2001), and for *P. triangulum*, we used

241   photoreceptor sensitivity peaks to estimate photoreceptor sensitivity curves (data

242   available in Briscoe and Chittka, 2001; see Govardovskii et al. 2000 for estimation of

243   sensitivity curves from sensitivity peaks).

244   The multiple predator hypothesis predicts that different predator taxa perceive

245   color morphs differently. To assess this prediction, we established two linear mixed

246   models, one for chromatic contrast and one for achromatic contrast. Either chromatic (Δ

247   S) or achromatic contrast were used as the dependent variable, and spider morph and

248   predator taxon were used as the independent variables (contrast = spider morph $\times$

249   observer). The spider morph was defined as yellow, white, red, or black and white, and

250   individual spiders were used as random effects. Normality and homogeneity were

251   verified by visual inspection of quantile-quantile and residuals vs. fitted values plots. We

252   computed all nested models and used the Akaike Information Criterion to select the

253   best model.

254   As in the multiple prey hypothesis, we used discrimination thresholds as

255   reference points. For the chromatic contrast, we considered color discrimination

256   thresholds of $\Delta S = 0.11$ and $\Delta S = 0.06$ for the wasp (Dyer and Chittka, 2004) and bird

257   (Théry et al., 2005), respectively. For the achromatic contrast, we considered double

258   cones in birds (Hart, 2001), and assumed green photoreceptors for wasps, as in bees.

259   Visual modeling calculations were conducted using the 'colourvision' R package

260   (Gawryszewski, 2017). Linear mixed models were performed using the 'nlme' package

261   (Pinheiro et al., 2016), and graphs were plotted using the 'ggplot2', 'ggExtra', and

262   'gridExtra' packages (Wickham, 2009; Attali, 2016; Auguie, 2016; R Core Team, 2015).

263

264   **RESULTS**

265   **Multiple prey hypothesis**

266   For chromatic contrast, the model that included the interaction between spider morph

267   and prey taxon presented the lowest AIC value (Table 1). The yellow morph presented

268   the highest ΔS value for *A. mellifera* and *F. adippe* vision, whereas the white spider

269   presented the highest ΔS value for *D. melanogaster*, followed by the yellow morph (Fig.

270   1). The white patch of the black and white spiders presented a ΔS value that was very

271   close to the theoretical discrimination threshold for all prey species (Fig. 1). The red

272   spiders presented ΔS values near the theoretical discrimination threshold for *A. mellifera*

273   and *D. melanogaster*, but not for *F. adippe* (Fig. 1). For prey achromatic contrast, the

274   model that included the interaction between variables presented the lowest AIC value

275   (Table 1). For all prey groups, the white morph had the highest excitation value,

276   followed by the black and white, yellow, and red morphs, respectively (Fig. 1). The

277   model coefficients are provided in the supplementary material (Table S1 and S2).

278

Multiple mimic models hypothesis

280   For *A. mellifera*, *D. melanogaster*, and *F. adippe*, only the white patch of the black and

281   white morph had a density peak near the theoretical discrimination threshold of 0.11

282   when compared to all flower reflectance spectra (Fig. 2). For all three prey taxa, the

283   white morph showed a scattered density, with higher densities varying from 0.2 until 0.8

284   in Euclidean distance units. Other morphs showed more homogeneous density curves,

285   for *A. mellifera*, the red morph peak was closer to the discrimination threshold

286   (Euclidean distance around 0.2) than the yellow morph (Euclidean distance around 0.4).

287   Red and yellow morphs had similar density peaks of Euclidean distance, around 0.3 in

288   *D. melanogaster* vision. For *F. adippe*, both peaks were distant from the discrimination

289   threshold, more than 0.4. In the achromatic dimension, the red morph was the only

290   one with a density peak near 0.5 (Fig. 3). All three prey taxa had similar density curves

291   for the white, yellow and red morphs, hanging from 0 to 0.2. Except for *D.*

292   *melanogaster*, which presented a scattered density distribution for the yellow morph.

293

Multiple predator hypothesis

295   For the chromatic contrast, the model with interaction between variables presented the

296   lowest AIC value (Table 1). The black and white morph presented the lowest ΔS value

297   for both predators (Fig. 4A,B; Table S3). The yellow morph was the one with highest ΔS

298   value for *P. caeruleus*, though white and red morphs presented similar values (Fig. 4A).

299   For *P. triangulum*, the white spider morph presented the highest ΔS value, followed by

300   the yellow and red morphs, and the red morph was near the theoretical discrimination

301 threshold of 0.11 (Fig. 4B). For the achromatic contrast, the model that included the

302 interaction between variables presented the lowest AIC value (Table 1), even though

303 the values of the two predator species were very similar. For *P. caeruleus*, the white

304 morph had the highest excitation value, followed by the yellow, black and white, and

305 red morphs, respectively (Fig. 4C). The white morph also had the highest excitation

306 value for *P. triangulum*, followed by the black and white, yellow, and red morphs,

307 respectively (Fig. 4D). The model coefficients are provided in the supplementary

308 material (Table S3 and S4)

309

310 **DISCUSSION**

311 Our statistical analyses suggested that the multiple prey and multiple predator

312 hypotheses, but not the flower mimicry hypothesis, are plausible explanations for the

313 evolution of color polymorphism in *G. cancriformis*. If conspicuous colors in orb-web

314 spider attracts prey, then polymorphism may occur in luring systems by multiple mimic

315 models that act as sensory traps for particular prey taxa. Conversely, polymorphism

316 may occur as a result of multiple receiver biases, where each morph lures specific prey

317 taxa that possess specific sensory, neural, or perceptual biases (White and Kemp, 2015).

318 In *G. cancriformis*, spider morphs conspicuousness is perceived differently by prey

319 species. The yellow and white morphs were the most conspicuous to all prey taxa. The

320 former being more contrasting from the background for honeybee color vision, and the

321 latter, for flies. Whereas, the red morph, although inconspicuous for honeybee and flies,

322 showed high detectability for butterflies. Furthermore, yellow spider morphs could

323 experience higher foraging success as a result of insects preference for yellow signals.

324 Yellow pollen and yellow flower centers, both of which are likely adapted to insect

325 visual systems, are among the most common traits of floral color patterns (Papiorek et

326 al., 2016; Lunau, 2000). On the other hand, red morphs are conspicuous to

327 lepidopterans and may be effective for attracting them. Therefore, the distinct

328 perceptions of insect taxa support the multiple prey hypothesis as a mechanism for the

329 evolution of color polymorphism in stationary predators.

330   For the achromatic dimension, the statistical analyses also suggested an

331 interaction between spider morph and prey taxon. However, there was very little

332 difference between the morphs when viewed by different prey. Therefore, the
333 differences in achromatic contrast between prey perception may be statistically
334 significant but not biologically relevant. A recently proposed scenario for the evolution
335 of color polymorphism is that different morphs exploit different visual channels in prey.
336 The yellow morphs of *G. fornicata* would benefit from stimulating the dipteran
337 chromatic channel, whereas white morphs would benefit from stimulating the
338 achromatic channel (White and Kemp, 2016). However, this is inconsistent with *G.*
339 *cancriformis* because the spider morphs presented similar levels of achromatic
340 detectability by all the prey taxa examined.

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

359 The results of the present study also support the multiple predator hypothesis for
360 the maintenance of color polymorphism in *G. cancriformis*, as the spider morphs
361 conspicuousness was perceived differently by predator species in both the chromatic
362 and achromatic dimensions. The red morph could be targeting bird predators but

363   would appear relatively inconspicuous to a hymenopteran predator. In contrast, the

364   white and yellow morphs are highly detectable by both predator taxa. The colors of two

365   of the four *G. cancriformis* morphs (yellow and red) are typical of aposematic species

366   (Endler and Mappes, 2004). Conspicuous coloration is especially advantageous when it

367   increases the mismatch with the background and facilitates predator learning (Endler

368   and Greenwood, 1988). Spiders of the genus *Gasteracantha* possess spines and a hard

369   abdomen. Moreover, the hunting wasp *Sceliphron laetum* avoids provisioning initial

370   instars with *Gasteracantha* spiders (Elgar and Jebb, 1999). Morphological and

371   behavioral defenses that make ingestion difficult may also constitute an aposematic

372   signal, along with the species' bright colors, which enhance predator memory and

373   learning (Endler and Greenwood, 1988; Ruxton et al., 2004). Though aposematism is

374   not commonly reported in spiders (Oxford and Gillespie, 1998), Brandley et al. (2016)

375   conducted an experiment with black widow models and found that models with red

376   markings were more likely to be avoided by birds than all black models. If spider

377   morphs are conspicuous to prey but do not resemble flowers, it is possible that the

378   conspicuousness of *G. cancriformis* reflects a trade-off between an antipredatory strategy

379   and foraging success, in which common spiders morphs would be avoided by prey.

380   Thermoregulation is another possible explanation for color polymorphism in

381   orb-web spiders. In *Verrucosa arenata* (Araneidae), yellow and white morphs exhibited

382   different thermal responses when exposed to artificial heat. The white morphs absorbed

383   less visible radiation and shorter wavelengths than the yellow morphs, which could

384   protect the spiders' internal tissue from the harmful effects of UV radiation (Rao and

385   Mendoza-Cuenca, 2016). However, absorbing pigments are likely to offer protection

386   against UV damage (Gawryszewski et al., 2015), which is analogous to the melanin

387   found in the human epidermis (Brenner and Hearing, 2008) and commercial sunscreen

388   lotions (Antoniou et al., 2008).

389   We only considered chromatic and achromatic discrimination, but color pattern

390   geometry, shape, contour, size, angle, texture, and distance of visual detection

391   (Troscianko et al., 2009) may also influence the behavior of both prey and predators

392   toward spiders since different species use distinct visual cues for stimuli detection and

393   recognition (Théry and Gomez, 2010). Furthermore, color vision models do not include

394   other perceptual mechanisms, such as cognition, color categorization, past experiences,

395   or memory imprecision (Renoult et al., 2015), even though these factors may affect

396   detectability and, consequently, influence the survival rate of morphs in different ways

397   (Théry and Gomez, 2010). Additionally, non-adaptive explanations, such as

398   overdominance and alele equilibrium is absence of selection, are often ignored by when

399   studying polymorphisms in an ecological perspective. Finally, predation experiments

400   and field experiments that evaluate the prey taxa caught by the different spider morphs

401   are paramount to validate and complement the findings of the present study.

402

406

407   **Competing interests**

408   No competing interests declared.

409

413

414   **References**

415

416   **Antoniou, C., Kosmadaki, M. G., Stratigos, A. J. and Katsambas, A. D.** (2008).

417   Sunscreens–what's important to know. *J. Eur. Acad. Dermatol. Venereol.*, **22(9)**,

418   1110-1119.

419   **Arnold, S. E. J., Faruq, S., Savolainen, V., McOwan, P. W. and Chittka, L.** (2010).

420   FReD: The Floral Reflectance Database — A Web Portal for Analyses of Flower

421   Colour. *PLoS ONE* **5**(12): e14287. doi:10.1371/journal.pone.0014287.

422   **Avarguès-Weber, A. and Giurfa, M.** (2014). Cognitive components of color vision in

423   honey bees: how conditioning variables modulate color learning and

424   discrimination. *J. Comp. Physiol. A*, **200(6)**, 449-461.

425 Attali, D. (2016). *R package 'ggExtra': Add marginal histograms to 'ggplot2', and more
426   'ggplot2' enhancements* (version 0.6). https://CRAN.R-project.org/package=ggExtra

427 Auguie, B. (2016). *R package 'gridExtra': Miscellaneous functions for "grid" graphics*
428   (version 2.2.1). https://CRAN.R-project.org/package=gridExtra

429 Bond, A. B. (2007). The Evolution of Color Polymorphism: Crypticity, Searching
430   Images, and Apostatic Selection. *Annu. Rev. Ecol. Evol. Syst.*, **38**, 489–514.

431 Brandley, N., Johnson, M. and Johnsen, S. (2016). Aposematic signals in North
432   American black widows are more conspicuous to predators than to prey. *Behav.*
433   *Ecol.*, p.arw014.

434 Brenner, M. and Hearing, V. J. (2008). The protective role of melanin against UV
435   damage in human skin. *Photochem. and photobiol.,* **84(3),** 539-549.

436 Briscoe, A. D. and Chittka, L. (2001). The evolution of color vision in insects. *Annu.*
437   *Rev. Entomol.*, **46**, 471-510.

438 Chiao, C.-C., Wu, W.-Y., Chen, S.-H. and Yang, E.-C. (2009). Visualization of the
439   spatial and spectral signals of orb-weaving spiders, Nephila pilipes, through the
440   eyes of a honeybee. *J. Exp. Biol.*, **212**, 2269–78.

441 Chittka, L. (1992). The colour hexagon: a chromaticity diagram based on photoreceptor
442   excitations as a generalized representation of colour opponency. *J. Comp. Physiol.*
443   *A*, **170**(5), 533-543.

444 Chittka, L. (1996). Optimal sets of color receptors and color opponent systems for
445   coding of natural objects in insect vision. *J. Theor. Biol.,* **181**(2), 179-196.

446 Chittka, L. and Kevan, P. G. (2005). Flower colour as advertisement. *Pract. Pollinat.*
447   *Biol.,* 157–230.

448 Chittka, L., Shmida, A., Troje, N. and Menzel, R. (1994). Ultraviolet as a component of
449   flower reflections, and the colour perception of hymenoptera. *Vision Res.*, **34**,
450   1489–1508.

451 Clarke, B. C. (1979). The evolution of genetic diversity. *Proc. R. Soc. Lond.*, **205**, 453-
452   474.

453 Craig, C. L. and Ebert, K. (1994). Colour and Pattern in Predator–Prey Interactions:
454   The Bright Body Colours and Patterns of a Tropical Orb-Spinning Spider Attract
455   Flower-Seeking Prey. *Funct. Ecol.*, **8**, 616–620.

456 **Dyer, A. G. and Chittka, L.** (2004). Fine colour discrimination requires differential
457   conditioning in bumblebees. *Naturwissenschaften*, **91**(5),224-227.

458 **Edmunds, J. and Edmunds, M.** (1983). The defensive mechanisms of orb weavers
459   (Araneae: Araneidae) in Ghana, West Africa. In: *Proc. 9th Int. Cong. Arachnol.*,
460   Panama, 73-89.

461 **Eguchi, E., Watanabe, K., Hariyama, T. and Yamamoto, K.** (1982). A comparison of
462   electrophysiologically determined spectral responses in 35 species of Lepidoptera.
463   *J. Insect Physiol.*, **28**, 675–682.

464 **Elgar, M. A. and Jebb, M.** (1999). Nest provisioning in the mud-dauber wasp Sceliphron
465   laetum (F. Smith): Body mass and taxa specific prey selection. *Behaviour*, **136**, 147-
466   159.

467 **Endler, J. A.** (1990). On the measurement and classification of colour in studies of
468   animal colour patterns. *Biol. J. Linn. Soc.*, **41**, 315–352.

469 **Endler, J. A.** (1992). Signals, signal conditions, and the direction of evolution. *Am. Nat.*
470   S125-S153.

471 **Endler, J. A.** (1993). Some general comments on the evolution and design of animal
472   communication systems. *Philos. Trans. R. Soc. B Biol. Sci.*, **340**, 215–225.

473 **Endler, J. A. and Greenwood, J.** (1988). Frequency-dependent predation, crypsis and
474   aposematic coloration [and Discussion]. *Philos. Trans. R. Soc. Lond. B*, **319**, 505-
475   523.

476 **Endler, J. A. and Mappes, J.** (2004). Predator mixes and the conspicuousness of
477   aposematic signals. *Am. Nat.*, **163**, 532–547.

478 **Fan, C. M., Yang, E. C. and Tso, I. M.** (2009). Hunting efficiency and predation risk
479   shapes the color-associated foraging traits of a predator. *Behav. Ecol.* **20**, 808–816.

480 **Farkas, T. E., Mononen, T., Comeault, A. A., Hanski, I., and Nosil, P.** (2013). Evolution
481   of camouflage drives rapid ecological change in an insect community. *Curr*
482   *Biol*, **23**(**19**), 1835-1843.

483 **Foelix, R. F.** (2010). *Biology of Spiders*. Oxford: Oxford University Press.

484 **Gawryszewski, F. M.** (2007). Policromatismo e *stabilimentum* em *Gasteracantha*
485   *cancriformis* (Araneae, Araneidae): caracterização e as hipóteses da atração de
486   presas e da proteção da teia. *Masters Thesis*, *Universidade de Brasília*.

487   Gawryszewski, F. M. (2017). Colour vision models: a practical guide, some simulations,

488   and colourvision R package. BioRxiv, **doi:** https://doi.org/10.1101/103754

489   Gawryszewski, F. M. and Motta, P. C. (2012). Colouration of the orb-web spider

490   Gasteracantha cancriformis does not increase its foraging success. *Ethol. Ecol. Evol.*

491   **24**, 23–38.

492   Gawryszewski, F. M., Birch, D., Kemp, D. J. and Herberstein, M. E. (2015). Dissecting

493   the variation of a visual trait: the proximate basis of UV-Visible reflectance in crab

494   spiders (Thomisidae). *Funct. Ecol.*, **29(1)**, 44-54.

495   Giurfa, M., Vorobyev, M., Kevan, P., and Menzel, R. (1996). Detection of coloured

496   stimuli by honeybees: minimum visual angles and receptor specific contrasts. *J*

497   *Comp Physiol A*, **178(5)**, 699-709.

498   Giurfa, M., Vorobyev, M., Brandt, R., Posner, B. and Menzel, R. (1997). Discrimination

499   of coloured stimuli by honeybees: alternative use of achromatic and chromatic

500   signals. *J. Comp. Physiol. A*, **180**, 235–243.

501   Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G. and Donner, K. (2000). In

502   search of the visual pigment template. *Vis. Neurosci.*, **17**, 509–528.

503   Gray, S. M. and McKinnon, J. S. (2007). Linking color polymorphism maintenance and

504   speciation. *Trends Ecol. Evol.* **22**, 71–79.

505   Hart, N. S. (2001). The visual ecology of avian photoreceptors. *Prog. Retin. Eye*

506   *Res.*, **20**(5), 675-703.

507   Hauber, M. E. (2002). Conspicuous colouration attracts prey to a stationary

508   predator. *Ecol. Entomol.* **27**, 686 - 691.

509   Kelber, A. and Henze, M. J. (2013). Colour vision: Parallel pathways intersect in

510   *Drosophila. Curr. Biol.* **23(23)**, R1043-R1045.

511   Levi, H. W. (1978). The American orb-weaver genera Colphepeira, Micrathena and

512   Gasteracantha North of Mexico (Araneae, Araneidae). *Bull. Mus. Comp. Zool.*

513   **148**: 417-442.

514   Lunau, K. (2000). The ecology and evolution of visual pollen signals. *Plant Syst.*

515   *Evol.*, **222**(1), 89-111.

516   Muma, M. H. (1971). Biological and behavioral notes on *Gasteracantha cancriformis*

517   (Arachnida: Araneidae). *Fla. Entomol.* **54**, 345-351.

518   O'Hanlon, J.C. (2014). The roles of colour and shape in pollinator deception in the
519   orchid mantis Hymenopus coronatus. *Ethology*, **120(7)**, 652-661.

520   O'Hanlon, J. C., Holwell, G. I. and Herberstein, M. E. (2013). Pollinator deception in
521   the orchid mantis. *Am. Nat.*, **183(1)**, 126-132.

522   Oliveira-Filho, A. T. and Ratter, J. A. (2002). Vegetation Physiognomies and Woody
523   Flora of the Cerrado Biome. *Cerrados Brazil Ecol. Nat. Hist. a Neotrop. Savanna*
524   91–120.

525   Osorio, D., Miklosi, A. and Gonda, Z. (1999). Visual ecology and perception of
526   coloration patterns by domestic chicks. *Evol. Ecol.* **13**(7-8), 673-689.

527   Oxford, G. S. and Gillespie, R. G. (1998). Evolution and ecology of spider coloration.
528   *Annu. Rev. Entomol.*, **43**, 619-643.

529   Papiorek, S., Junker, R. R., Alves-dos-Santos, I., Melo, G. A., Amaral-Neto, L. P., Sazima,
530   M., Wolowski, M., Freitas, L. and Lunau, K. (2016). Bees, birds and yellow
531   flowers: pollinator-dependent convergent evolution of UV patterns. *Plant*
532   *Biol.*, **18**(1), 46-55.

533   Peitsch, D., Fietz, A., Hertel, H., de Souza, J., Ventura, D. F. and Menzel, R. (1992).
534   The spectral input systems of hymenopteran insects and their receptor-based
535   colour vision. *J. Comp. Physiol. A,* **170**, 23–40.

536   Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Core Team (2016). *R package*
537   *'nlme':   Linear and Nonlinear Mixed Effects Models* (version 3.1-128).
538   http://CRAN.R-project.org/package=nlme>.

539   R Core Team (2015). R: A language and environment for statistical computing. R
540   Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

541   Rao, D. and Mendoza-Cuenca, L. (2016). The effect of colour polymorphism on
542   thermoregulation in an orb web spider. *Sci. Nat.,* **103**(7–8), 1–5.

543   Rayor, L. (1996). Attack strategies of predatory wasps (Hymenoptera: Pompilidae;
544   Sphecidae) on colonial orb web-building spiders (Araneidae: *Metepeira*
545   *Incrassata*). *J. Kans. Entomol. Soc.*, **69**, 67–75.

546   Renoult, J. P., Kelber, A. and Schaefer, H. M. (2015). Colour spaces in ecology and
547   evolutionary biology. *Biol Ver.*

548   Ruxton, G. D., Sherratt, T. N. and Speed, M. P. (2004). Avoiding Attack. First edition.

549   Oxford: Oxford University Press, USA.

550   **Schnaitmann, C., Garbers, C., Wachtler, T. and Tanimoto, H.** (2013). Color

551   discrimination with broadband photoreceptors. *Curr. Biol.*, **23**(23), 2375-2382.

552   **Tinbergen, L.** (1960). The natural control of insects in pine woods I. Factors influencing

553   the intensity of predation by songbirds. *Arch. Néerl. Zool.* **13**, 265h. N.

554   **Théry, M. and Gomez, D.** (2010). Insect colours and visual appearance in the eyes of

555   their predators. *Adv. Insect Physiol.* **38**, 267 - 353.

556   **Théry, M., Debut, M., Gomez, D. and Casas, J.** (2005). Specific color sensitivities of

557   prey and predator explain camouflage in different visual systems. *Behav. Ecol.*, **16**,

558   25-29.

559   **Troscianko, T., Benton, C. P., Lovell, P. G., Tolhurst, D. J. and Pizlo, Z.** (2009).

560   Camouflage and visual perception. *Philos. Trans. R. Soc. B Biol. Sci.*, **364**, 449 -

561   461.

562   **Tso, I. M.** (2013). Insect view of orb spider body colorations. In *Spider*

563   *Ecophysiology* (pp. 319-332). Springer Berlin Heidelberg.

564   **Tso, I., Tai, P., Ku, T., Kuo, C. and Yang, E.** (2002). Colour-associated foraging success

565   and population genetic structure in a sit-and-wait predator *Nephila maculata*

566   (Araneae: Tetragnathidae). *Anim. Behav.*, **63**, 175–182.

567   **Tso, I., Lin, C. and Yang, E.** (2004). Colourful orb-weaving spiders, *Nephila pilipes*,

568   through a bee's eyes. *J. Exp. Biol.*, **207**, 2631–2637.

569   **Tummers, B.** (2006). DataThief III. http://datathief.org/

570   **White, T. E., Dalrymple, R. L., Herberstein, M. E. and Kemp, D. J.** (2016). The

571   perceptual similarity of orb-spider prey lures and flower colours. *Evol. Ecol.*, 1-20.

572   **White, T. E. and Kemp, D. J.** (2015). Technicolour deceit: a sensory basis for the study

573   of colour-based lures. *Anim. Behav.* **105**, 231–243.

574   **White, T. E. and Kemp, D. J.** (2016). Colour polymorphic lures target different visual

575   channels in prey.

576   **Wickham, H.** (2009). *R package 'ggplot2': Elegant graphics for data analysis.* Springer-

577   Verlag New York.

578

579   **Table**

580

581   Table 1. Delta Akaike Information Criterion (AIC) of Linear Mixed Models of the

582   chromatic and achromatic contrasts of prey and predators.

| Model	df	ΔIC
Multiple prey hypothesis		
Chromatic dimension		
ΔS ~ morph*observer	17	0.0
ΔS ~ morph+observer	11	23.9
ΔS ~ observer	8	52.4
ΔS ~ morph	9	61.5
ΔS ~ 1	6	90.6
Achromatic dimension		
excitation ~ morph*observer	17	0.0
excitation ~ morph+observer	11	57.6
excitation ~ morph	9	72.2
excitation ~ observer	8	84.7
excitation ~ 1	6	100.2
Multiple predator hypothesis		
Chromatic dimension		
ΔS ~ morph*observer	13	0.0
ΔS ~ morph+observer	10	6.9
ΔS ~ observer	7	30.6
ΔS ~ morph	9	54.5
ΔS ~ 1	6	74.9
Achromatic dimension		
excitation ~ morph*observer	14	0.0
excitation ~ morph+observer	10	14.4
excitation ~ observer	7	21.1
excitation ~ morph	9	36.9
excitation ~ 1	6	43.9

583

584   **Figures**

585

586
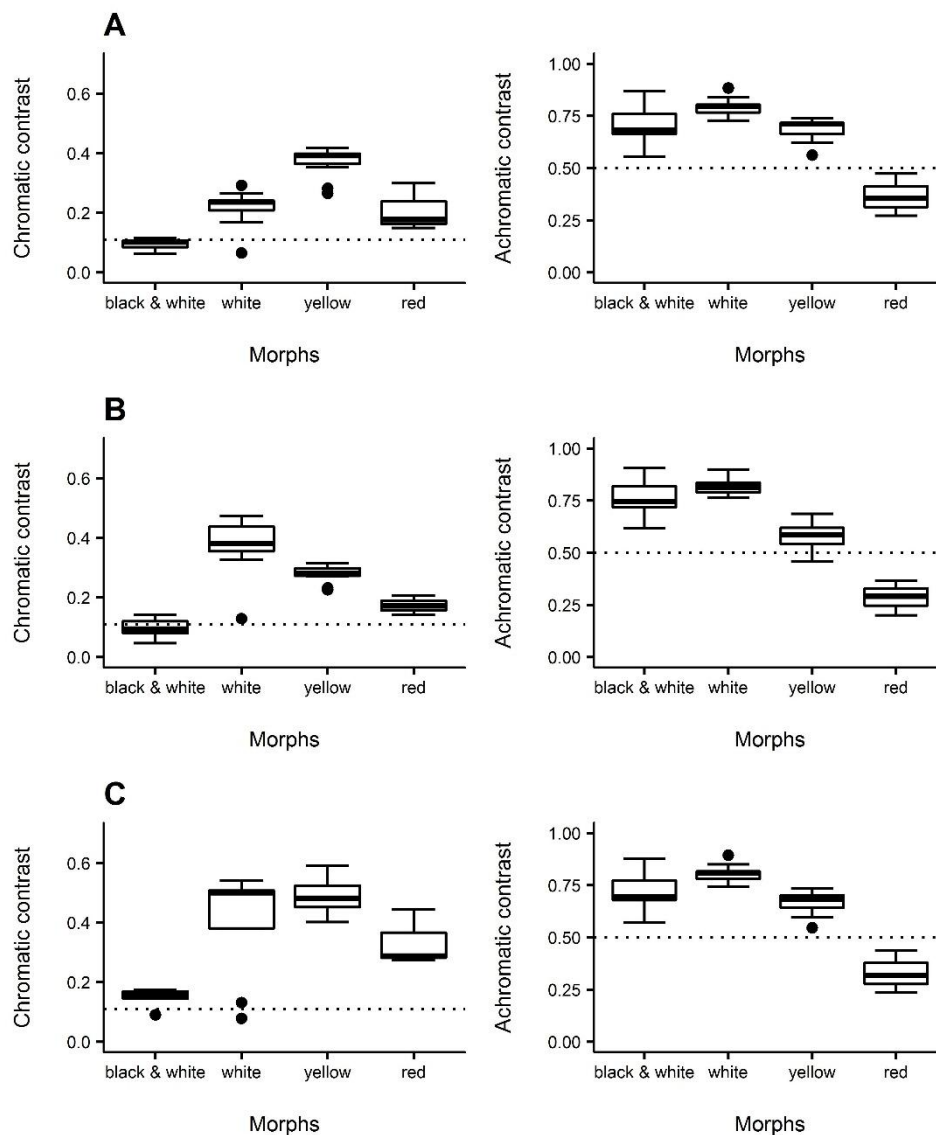
Fig. 1. Chromatic (left) and achromatic (right) contrasts of four *Gasteracantha cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3) when viewed against a Brazilian savanna background by prey with distinct visual systems. (A) *Apis mellifera* (Hymenoptera). (B) *Drosophila 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).
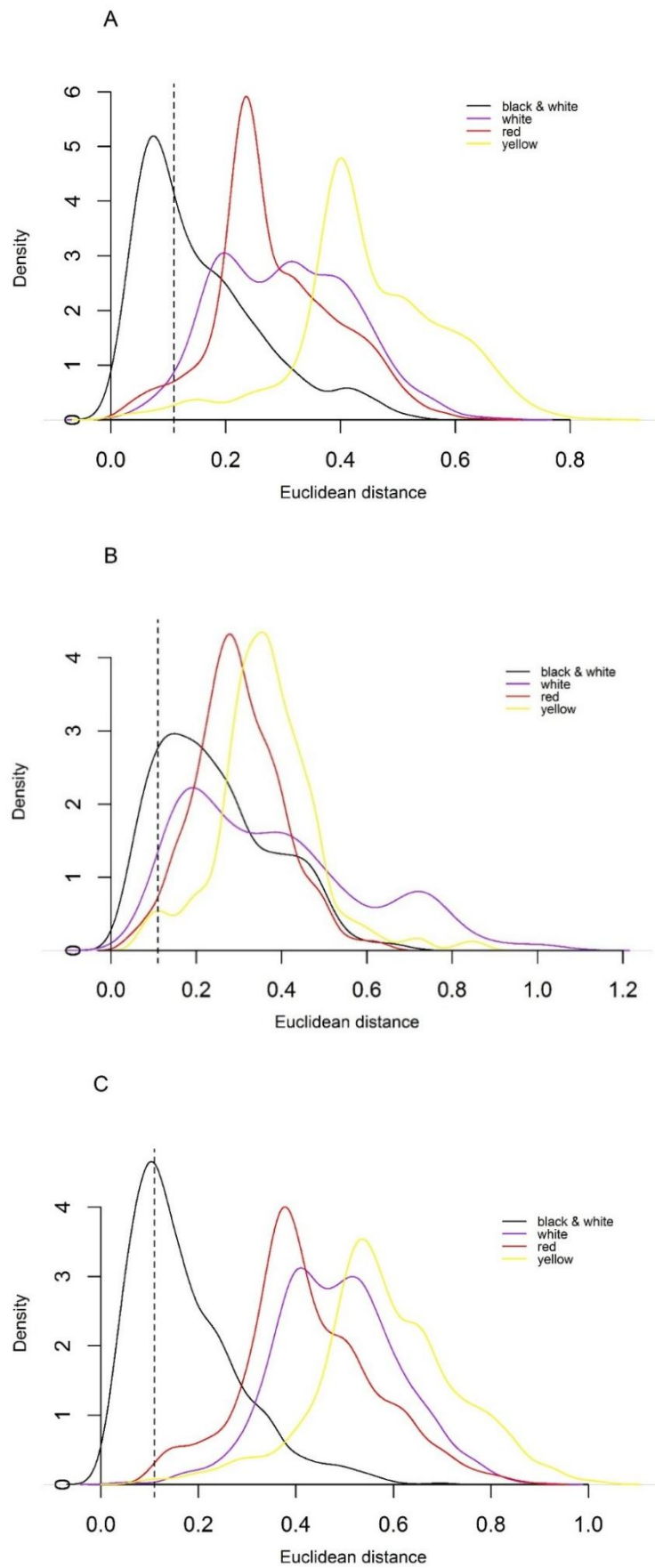
594

595

596

597

A

B

C

598

599   **Fig. 2**. Kernel density estimation of the Euclidean distance between four *Gasteracantha*

600   *cancriformis* morphs (black and white, N=6; white, N=10; yellow, N=13; and red, N=3)

601   and flower reflectance (N=859). Estimations were based on the photoreceptors of (A)

602   *Apis mellifera* (Hymenoptera), (B) *Drosophila melanogaster* (Diptera), and (C)

603   *Fabriciana adippe* (Lepidoptera). Dotted vertical line represents the crude

604   approximation of the color discrimination threshold.
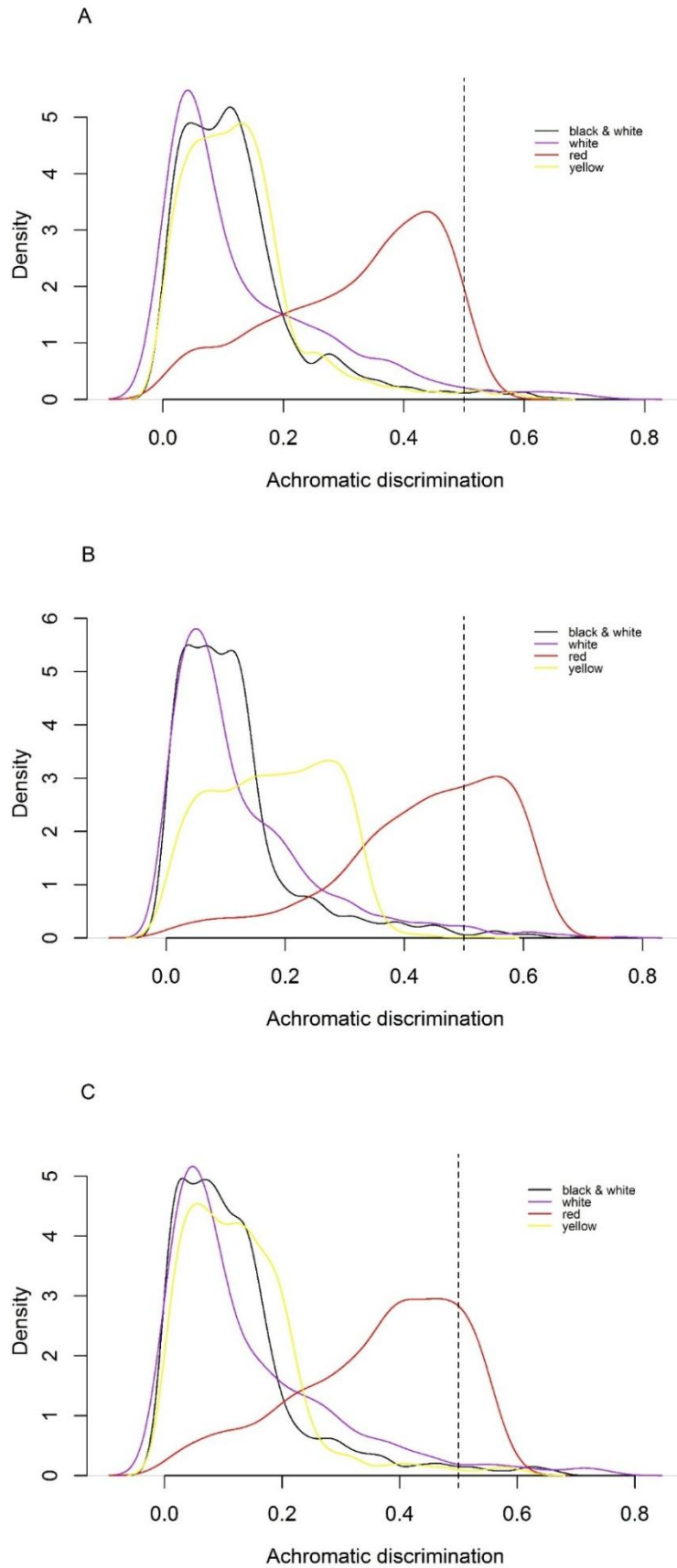
605

A

B

C

606

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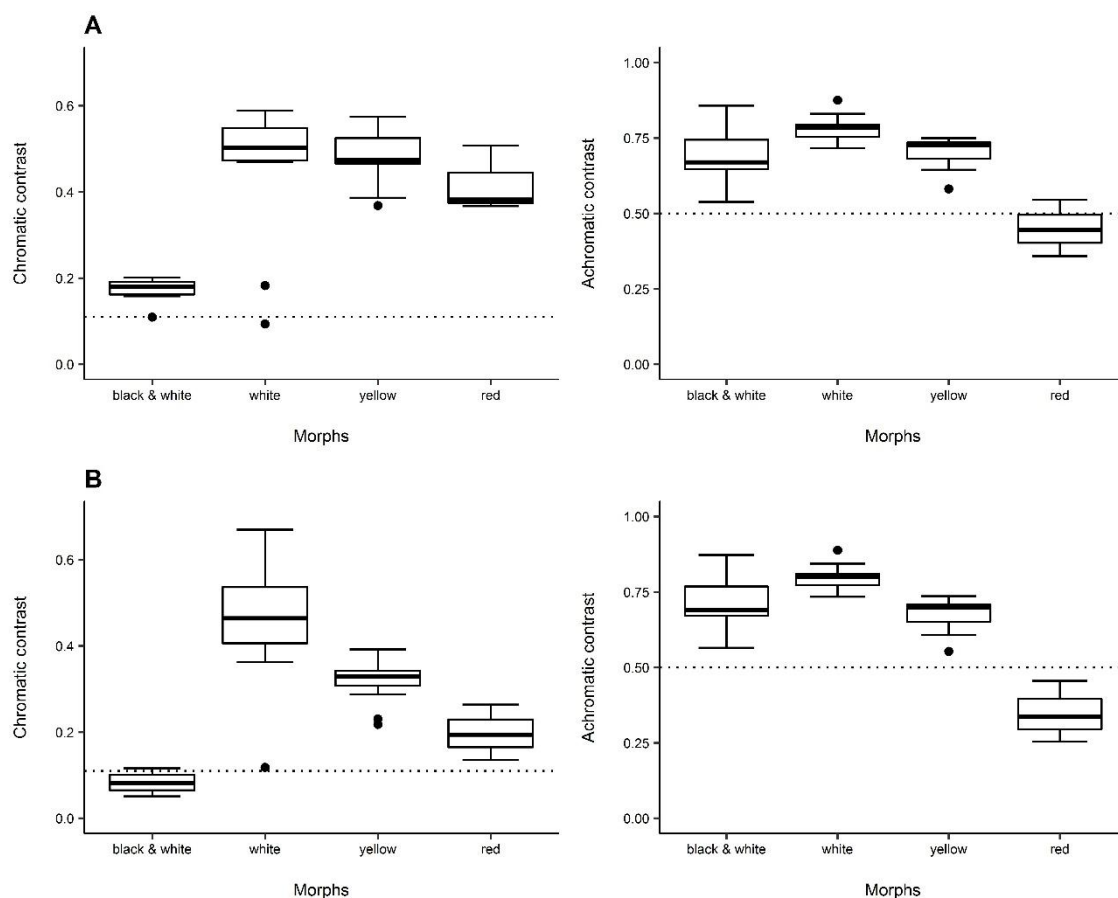612 excitation for the background.

613

614

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

622