1   Mate detection in a territorial butterfly – the effect of background and luminance contrast

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Abstract

Many animals search for potential mates or prey using a perch-and-sally strategy. The success of

such a strategy will depend on factors that affect the observer's ability to detect a passing

resource item. Intrinsic factors (e.g. eye structure and physiology) have received much recent

attention but less is known about effects on object detection in nature and extrinsic factors such

as size, coloration and speed of a passing object and the background against which the object is

viewed. Here, we examine how background affects the detection of butterfly models by perched

males of the butterfly *Asterocampa leilia* in the field. We test the hypothesis that male choice of

perch site in nature will influence the contrast between the object and background against which

it is viewed, and that this will influence success in detecting the object. We also test the effect of

contrast by manipulating the brightness of the object and presenting butterfly models of different

reflectance (ranging from black to white). We found an effect of model luminance, with dark

models being most likely to elicit a response regardless of background. Further, there was an

effect of background type with models viewed against blue sky eliciting the highest response.

Perceived luminance contrast correlates to behavior; highly contrasting objects are more

frequently detected. This study expands our understanding of visual system performance and has

implications for our understanding of the behavior and evolutionary ecology of perching species.

Key words: Lepidoptera; mate locating behavior; perching; sexual selection; perching;

territoriality; vision

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Introduction

Many animals visually search for resources such as potential mates or prey using a perch-and-sally strategy. This strategy involves individuals waiting at some vantage point on the lookout for mates or prey passing by and then sallying out to intercept, investigate and pursue any objects of potential interest. Perch-and-sally tactics are a widespread and conspicuous component of animal behavior in both invertebrates and vertebrates (Atkins 1980; Thornhill & Alcock 1983; Remsen Jr & Robinson 1990). This strategy contrasts with patrolling (actively searching) as a way to locate mates or prey and the perch-patrol dichotomy in mate locating behavior and hunting strategies is well established in the literature on insect behavioral ecology (Scott 1974; Ehrlich 1984; Rutowski 1991; Corbet 1999; Wiklund 2003; Corbet & May 2008).

Even though perching as a mate locating tactic or hunting strategy is widespread, little research has been done to understand the factors that contribute to success in detecting, intercepting and pursuing moving objects of interest. This is peculiar because the behavior is strikingly similar among all perching species and success in this activity should have a huge effect on the performer's fitness. A more comprehensive understanding of the proximate factors that influence male's success in mate searching or hunting, especially factors influencing visual mate detection, will give us more insight in the potential adaptive features of mate-locating behavior and mating system structure in insects.

Both *intrinsic* and *extrinsic* factors will influence a percher's visual detection of passing objects. Over the last few decades our understanding of intrinsic factors, such as visual system performance, has increased due to studies of eye structure, optics and electrophysiology (Land & Nilsson 2012). However, less attention has been given to the role of extrinsic factors such as

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61   object size, coloration, pattern and shape, the speed of the object, the properties of the

62   background against which the object is viewed, perch location, and the positioning of the

63   observer in relation to the object and its path. These factors will cause variation in ambient light

64   levels, contrast between the object and the background and blurring effects, which are all known

65   to be key elements in visual acuity and target detection (Land 1997; Rutowski 2003). Intrinsic

66   factors studied in controlled laboratory environments have been the focus of attention in the last

67   few decades. However, extrinsic factors are ideally examined under field conditions, where

68   experiments are logistically more difficult and more difficult to control but yield results more

69   directly relatable to what the animals are doing in nature. In species that use a perching strategy

70   to find mates or prey, we expect strong selection on behavior to adapt to environmental, extrinsic

71   factors, in order to optimize success in mate searching or hunting.

72

73   One of the relatively few species that has been studied in the field to understand visual aspects of

74   perching behavior is the Empress Leilia butterfly, *Asterocampa leilia*. Males of this nymphalid

75   species perch in the morning from about 8 to 11 AM on or adjacent to the larval food plant,

76   desert hackberry (*Celtis pallida*), and wait for females to pass by (Austin 1977; Rutowski &

77   Gilchrist 1988). From his perch, the male flies out to investigate passing objects. Conspecific

78   females are pursued and courted, whereas conspecific males are chased away from the perching

79   site (Rutowski & Gilchrist 1988).

80

81   During the course of a morning's activity, males of *A. leilia* can adopt two different perching

82   positions: (1) on the ground, next to a hackberry tree, or (2) on the hackberry tree at a height of

83   about 0.85 meters, the same height at which *A. leilia* females fly through the habitat (Rutowski

84   2000). Males start the morning perching on ground but shift to perching off ground, on hackberry

85 trees as temperatures rise (Rutowski *et al.* 1991). The change of male's perch preference is driven

86 by thermoregulatory issues; ground temperatures can exceed the tolerable thermal maximum for

87 *A. leilia*, while temperatures are lower at 0.85 m, the typical perch height of *A. leilia* (Rutowski *et*

88 *al.* 1994). Perch location influences the male's ability to detect passing objects. In a field study,

89 Rutowski *et al.* (2001) presented butterfly models to perched males and found that males on

90 ground were more likely to initiate pursuit of a passing model than those off ground. The body

91 posture of males perched on the ground (body pitch upward about 30 degrees and facing away

92 from the sun) is different from that of males perched on the ground (body pitch slightly down,

93 facing out of bush; Rutowski 2000). This difference in body posture with perch location means

94 the equatorial acute zone of their eyes (Rutowski and Warrant 2002) is directed slightly

95 differently relative to the likely flight path of conspecifics. However, this difference suggests that

96 males on the ground should be less likely to detect conspecifics passing through the territory,

97 contrary to what was observed.

98

99 Alternatively Rutowski *et al.* (2001) argued that differences in the rate of response was most

100 likely because males perching above ground are likely to view passing conspecifics against a

101 relative dark background of nearby vegetation, while males perching on ground are likely to have

102 the relatively bright and uniform blue sky as the background. Rutowski *et al.* (2001) crudely

103 tested this hypothesis by increasing the flight height of the models, to change the potential

104 viewing background for perched males. And indeed, this change increased the proportion of

105 males that responded to the models (Rutowski *et al.* 2001). However, the authors did not explore

106 this issue further.

107

108 Other studies have also shown that background affects the detection of passing conspecifics or

109 prey (Labhart & Nilsson 1995; Switzer & Eason 2000) but few studies have further investigated

110 what properties of object and background are important. Animal sense motion of a passing target

111 as temporally correlated changes in stimulation in sets of photoreceptors (Cronin *et al.* 2014).

112 Hence, the luminance of a target and its contrast with the luminance from a background will

113 affect the magnitude of such change in stimulation and thereby significantly affect visual

114 detectability, where a high contrast increases the change in signal and thereby detectability

115 (Buser & Imbert 1992). For an insect, this means that when luminance contrast between an object

116 and its background is high, the viewer will be able to see that object from further away, and at a

117 given distance, detect smaller objects (Mazokhin-Porshnyakov 1969; e.g. Lehrer & Bischof

118 1995). Here we experimentally explore the nature and implications of object-background contrast

119 in nature, with *Asterocampa leilia* as the subject. We investigate how background contributes to

120 variation in luminance contrast between a passing object and the background against which it is

121 viewed and how this will influence visual mate detection in nature. We further test the hypothesis

122 that visual mate detection is influenced by contrast by manipulating the brightness and color of

123 the object, and consequently also manipulating contrast. We presented butterfly models of

124 different reflectances (ranging from black to white) to perched males. We further, and more

125 quantitatively than previously, tested the prediction that the type of background a male chooses

126 by selecting a perch site in nature will influence the contrast of the object and that the choice of

127 perch site thereby also will influence his success in detecting the object. We predict that a perch

128 site that create a high contrast between the object and the background will increase detectability

129 and perch sites with a low contrast between the object and the background will decrease

130 detectability.

131

Material and methods

Study site

The experiments were conducted in May-June and September-October 2013 in the upper Sonoran

Desert in the Mazatzal Mountains of central Arizona, USA. The site, known as Round Valley (N

33.79784, W 111.4877), is in the floodplain of Sycamore Creek and has been used in previous

studies of *Asterocampa leilia* (Rutowski & Gilchrist 1988; Rutowski et al. 1991; Rutowski 2000;

Rutowski et al. 2001). The larval foodplant of *A. leilia*, the desert hackberry tree (*Celtis pallida*),

is abundant in this area.

Butterfly model presentation device and general experimental procedure

Butterfly models were presented to perched males with an apparatus that has been used in

previous studies of mate detection in territorial butterflies (Rutowski *et al.* 2001; Bergman &

Wiklund 2009) and consisted of two metal poles, positioned eight meters apart, and each with an

attached pulley. The pulley on one of the poles was turned by a small electrical motor controlled

by a portable remote control. A loop of thin (0.65mm in diameter) nylon line was strung between

the pulleys and a model attached with an 8 cm line to the loop. The attached butterfly model was

presented to a male at a height of 0.85 m above ground. When the motor was activated, the model

quickly accelerated to a speed of about 4.5 m/s. For each presentation we measured the time it

took for the model to get from one pole to the other, i.e. to travel eight meters. We calculated the

speed of the model and only recorded the male's response if the speed of the model was between

4 and 5 m/s, the typical flight speed for this species. All models were made of 2 mm thick

cardboard. The models were constructed to match the size (45 mm wingspread) and shape of an

A. leilia with wings spread.

156   We used four different types of butterfly models hereafter referred to as *black*, *gray*, *white* and

157   *tan*, respectively. The black and gray models were painted with an acrylic paint (Model Master

158   Acryl, ©*The Testor Corp.*), the white model consisted of white filter paper glued to the cardboard

159   model and the tan model consisted only of unpainted cardboard. The four butterfly models varied

160   in their reflectance spectra (figure 1) (for description of the measuring methods see *Reflectance*

161   section below): the black, gray and white butterfly models had a flat reflectance curve where the

162   black reflected least and white reflected most light between 300 and 700 nm. The average

163   reflectance spectrum of the tan butterfly model (26.6 +/- 2.7 %) was similar to the gray butterfly

164   model (27.6 +/- 1.3%) but had a shift towards longer wavelengths and thereby the coloration also

165   matched the overall ventral wing surface reflectance of *A. leilia* females (Figure S1). Further, tan

166   model average reflectance (26.6 +/- 2.7%) was not significantly different from female ventral

167   hindwing average reflectance (21.0 +/- 2.33%, t-test: $t_4 = 1.88$, $P = 0.13$).

168

169   Each model presentation proceeded as follows. We located a male in the field, perched either on

170   the ground or on a hackberry tree, and set up the presentation apparatus near him. The flight path

171   of the model was set up so that it was perpendicular to a line between the perching male and the

172   midpoint of the model flight path. The distance between the midpoint of the flight path and the

173   perching male was between 1 and 2 meters for all presentations, which is a range of distances

174   where males of *A. leilia* respond most frequently to passing objects of the size and shape

175   presented (Rutowski *et al.* 2001). Once the apparatus was activated and the model moved from

176   one pole to the other we recorded the following information: (1) whether the male responded to

177   the passing model by flying up and pursuing the model, (2) the direction that the male faced

178   relative to north just before the model was presented, (3) the compass bearing of the flight path,

179   (4) the compass bearing of a line from the perching male to the nearest point of the flight path

180   and (5) the perch height of the male.

181

182   To characterize the background against which the butterfly model was viewed by the male in a

183   presentation, we took panoramic photos with a Casio EX-ZR100 camera of the model flight path

184   from the vantage point of the male's perch (Figure 2). This was done for all presentations during

185   the experiment.

186

187   Although the apparatus for presenting butterfly models was relatively mobile, the number of

188   potential males available on any given day was limited in time and space (cf. Rutowski et al.

189   1996; 2001). To maximize data yield per unit effort, after setting up the apparatus at a perching

190   site we presented, in randomized order, as many of the four types of butterfly models as we

191   could. However, we never presented the same model type to a specific male more than once. We

192   were able to present all four model types to 78% of the males and conducted on average 3.51

193   presentations per individual male. In total, we presented models for 51 different males and we did

194   179 presentations.

195

196   Occasionally, due to high flight activity, resident males engaged in territorial contests with

197   intruder males between different model presentations; these occurrences led to uncertainties of

198   whether the same male was present for all model presentations at a given site. However, in

199   territorial interactions the original resident most often wins the fight and returns to the perch site

200   (Rutowski & Gilchrist 1988; Kemp & Wiklund 2001) making a change in resident unlikely.

201   Moreover, even though males of *A. leilia* rarely occupy a perch site for more than one day

202   (Rutowski & Gilchrist 1988), we never presented models for males on the same perch site on

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203 subsequent days and we alternated experimental areas within Round Valley to avoid running the

204 same male twice. However, sometimes the male left or changed perch site after taking off. Hence,

205 some of the males did not experience all four models and the sample sizes might vary between

206 groups. This also means that across the four model types the observed responses were not

207 completely independent. However, within any model type all responses are to our best knowledge

208 independent.

209

210 *Analyses and statistical methods*

211 To estimate the main background for each presentation we used the images taken after each

212 presentation. In the images we overlaid the "flight" path of the model. Since earlier studies on *A.*

213 *leilia* have shown that the male's ability to detect passing objects is strongly affected by distance

214 and that perched males cannot detect a natural sized gray butterfly model at a distance larger than

215 3 meters (Rutowski *et al.* 2001), we excluded parts of the flight path that were further away than

216 3 meters from the perched male. In the images we identified three different background

217 categories against which the "flight" path was viewed: sky, vegetation and sand. With ImageJ

218 (http://imagej.nih.gov/ij/) we measured (in number of pixels) the proportion of the "flight" path

219 seen against sky, vegetation and sand respectively and the category with highest proportion was

220 considered the main background for that presentation (figure 2).

221

222 To analyze the effect of butterfly model type and background we used Generalized Estimating

223 Equations (GEE) with a binary logistic model. We used the response by the male (take-off or no

224 take-off) as a binary dependent variable and butterfly model type with background as predictors.

225 Since we have several presentations for the same male, male identity was used as a subject

226 variable and model type was consequently treated as a within-subject variable. As the interaction

227   between butterfly model type and background was non-significant it was excluded from the

228   model when testing for the main effects of model type and background. The GEE model was run

229   in SPSS 22. For testing the differences in response between the four model types we performed

230   pairwise McNemar's test between each combination of model type. Four model types resulted in

231   six pairwise tests and the α-value was adjusted with a sequential Bonferroni-correction.

232

233   *Reflectance*

234   We collected leaves from hackberry and mesquite trees (*Prosopis velutina*) along with sand and

235   rocks from the field sites in Round Valley and transported them back to the lab. Vegetation

236   reflectance measurements were taken immediately to ensure that the vegetation retained its

237   natural reflectance. We measured reflectance of 20 hackberry leafs from 4 trees, 10 mesquite

238   leafs from two plants, 10 dishes of sand and each model used in the behavioral trials. For each

239   plant, we measured reflectance three times on the upper and lower surfaces of leaves. To

240   characterize the color of each model we measured reflectance at five points on the model and

241   averaged the spectra. We measured the ventral hind wing reflectance of three *A. leilia* females to

242   confirm that the tan model was similar in coloration to female *A. leilia* individuals. The ventral

243   surface of *A. leilia* is not homogenous, however butterflies that employ a perch and sally mate

244   location strategy are highly unlikely to have sufficient visual acuity to resolve specific internal

245   pattern elements of potential mates, given they are a small, moving target relative to their field of

246   view. With this in mind, we measured across the hind wing with a sampling area of 1 cm. To

247   collect all reflectance spectra we used a bifurcated reflectance probe (Ocean Optics) with the

248   collecting fiber connected to a spectroradiometer (USB2000, Ocean Optics, Dunedin, FL, USA)

249   and the illuminating fiber connected to a Pulsed Xenon light source (Ocean Optics). All

250   reflectance measurements were collected in a dark room with the probe positioned perpendicular

251   to the target surface and were measured relative to a spectralon diffuse reflectance white standard

252   (Labsphere, Inc., North Sutton, NH, USA). The output of the spectroradiometer was collected

253   with SpectraSuite software (Ocean Optics) and then raw spectral data from 300 to 700nm were

254   binned into one nanometer bins with CLR (Montgomerie 2008). Reflectance spectra were first

255   averaged for each plant and then all plants were averaged resulting in one average vegetation

256   reflectance. Similarly, all sand reflectance spectra were averaged for an overall sand reflectance,

257   and each butterfly model's reflectance spectra were averaged.

258

259   *Irradiance*

260   Light environment (ambient irradiance) measurements were collected on 7 November 2013 from

261   five perching locations where butterfly models were presented in Round Valley. Irradiance

262   measurements were used with reflectance to estimate the radiance from the models, and the

263   radiance from background as viewed by male *A. leilia* in behavioral trials. The measurements

264   were made when the sky was clear from 10:00am to 12:30pm. Ambient irradiance was measured

265   separately for the butterfly models and background (i.e. sand, vegetation and blue sky). We used

266   a cosine-corrected irradiance probe (Ocean Optics, Dunedin, FL, USA) connected to a

267   spectroradiometer (USB 2000, Ocean Optics) connected to a laptop running SpectraSuite

268   software (Ocean Optics). The radiance of the butterfly models is determined by the ambient

269   irradiance from the hemisphere in the direction of the perched male, multiplied by the diffuse

270   reflectance of the model. We aimed the cosine-corrected irradiance probe at where the viewer

271   would be perched during a butterfly model presentation (figure S2). Hence, when taking the

272   relevant irradiance measurements that contributed to butterfly model radiance (via reflection from

273   the model) the probe was held at the position in the horizontal plane where the butterfly model

274   would pass, at a distance of 1 m from the perch, and aimed at the hemisphere which includes the

12

275   potential perching point of the male (figure S2). The radiance reflected from the background is

276   determined by the light environment from the hemisphere facing the direction of the butterfly

277   model, and the perched male. When taking irradiance measurements that contribute to

278   background radiance (via reflection) the probe was held at the position in the horizontal plane

279   closest to the background type (sand or vegetation), and aimed at the hemisphere which includes

280   the butterfly model (figure S2). Because males can perch either on the hackberry tree or on the

281   ground in front of the tree, both positions were used for taking the irradiance measurements.

282   Because males perched on hackberry exhibit a wide range of orientations, with no significant

283   mean vector in relation to sun azimuth or point of compass in the field (Rutowski *at al.* 1991), the

284   five perch site locations selected were focused on hackberry trees oriented 90° relative to sun

285   azimuth (99 +/- 44°). To characterize backgrounds of blue sky we took irradiance measurements

286   of blue sky background that was relevant to the orientation of males perched on ground

287   (Rutowski et al. 1991), the probe was held at the position of perched males, and aimed at blue

288   sky, 180 degrees from sun azimuth. As with reflectance spectra, we binned the irradiance data

289   into 1 nm bins from 300 to 700nm using CLR (Montgomerie 2008). Irradiance was then

290   converted to photon flux units relevant for vision (photons/cm^2/sec).

291

292   *Visual System Model*

293   We modeled *A. leilia* luminance contrast by calculating achromatic contrast using a von Kries

294   term that assumes receptor adaptation *sensu* Morehouse & Rutowski (2010). Insects have been

295   found to detect motion using luminance contrast from a single spectral photoreceptor type with

296   peak absorption (λ_{Rmax}) in the green region (Briscoe and Chittka 2001) Therefore, we

297   incorporated a visual pigment with λ_{Rmax} of 530nm found in a related nymphalid species,

298 *Vanessa cardui* (Briscoe *et al.* 2003) - photoreceptor absorbances are unknown for *A. leilia* - and

299 a rhodopsin template (Govardovskii 2000) into our model. We then calculated luminance

300 contrasts between butterfly models and backgrounds according to a log linear model of

301 photoreceptor quantum catch with a von Kries transformation for receptor light adaptation:

$$q_G = \ln\left(\frac{Q_G}{Q_{GB}}\right)$$

302 Where q_G is the quantum catch of a green photoreceptor with peak sensitivity at 530nm.

$$Q_G = \int R(\lambda)I(\lambda)A_G(\lambda)d\lambda$$

$$Q_{GB} = \int R_B(\lambda)I(\lambda)A_G(\lambda)d\lambda$$

303 $R(\lambda)$ is the averaged reflectance from a target, R_B is the averaged reflectance from a background

304 type, $I(\lambda)$ is the relevant ambient irradiance (photons/cm^2/sec), and $A_G(\lambda)$ is the modeled

305 photoreceptor absorbance. We integrated these equations from 300-700nm. All background types

306 are either diffusely reflecting surfaces (sand and vegetation), or diffuse light sources (blue sky),

307 allowing direct comparisons. The calculated luminance contrasts between various butterfly

308 models and backgrounds were compared to the response patterns of males in the field in order to

309 test the prediction that high contrast should result in a high probability of males responding.

310

311 **Results**

312 *Effect of butterfly model type*

313 Across presentations, regardless of background and perch locations, model type strongly affected

314 the probability that a male responded by leaving his perch to chase the model (Figure 3a; Table

315 1). 87% of the males responded when the black model was presented but only 26% responded to

316 presentations of the white model. 52% of the males responded to the gray model and 60%

317   responded to the tan colored model. The response to the black model was significantly higher

318   than the response to the three other model types (Table 2). Likewise, the response to the white

319   model was significantly lower than all other models (Table 2). However, there was no difference

320   in male response to gray and tan colored models (Table 2).

321

322   *Effect of perch location*

323   Male perch location also affected the male's probability of responding to the presented models.

324   As reported in Rutowski *et al.* (2001) males that were perched on the ground were more likely to

325   respond to the presented models than males perched on a hackberry tree (Figure 3b; Chi-square

326   test: $X^2 = 7.87$, $P = 0.005$). This was true for all four model types, but seemingly stronger in the

327   tan colored model (Figure 3b). When the tan colored model was presented for males perching on

328   ground, 86% of the males responded. When the tan colored model was presented for males

329   perching off ground, significantly fewer males, only 44% responded by sallying after the model

330   (Fisher's exact test: $P = 0.017$).

331

332   *Effect of background*

333   In this study, we have described the background type as one of three discrete categories: sky,

334   sand or vegetation. In 54% of the presentations, the male viewed the passing model against a

335   background that consisted of 90-100% of a single background type. The background never

336   contained less than 50% of a single background type. We find an overall effect of the main

337   background type on the probability that male's would respond to the presented models (Figure

338   3c; Table 1). Males that viewed models against a background of mostly sky were more likely to

339   respond (87%) than males which viewed the model with vegetation (45%), or sand (50%) as the

340   background (Figure 3c). There was no effect of interaction between butterfly model and

15

341   background type (Table 1). The strength of the background effect differed slightly between

342   model types but was strongest for the tan colored model (Figure 3c).

343

344   *Luminance contrast*

345   The luminance contrast between the presented butterfly model and the background varied with

346   model type. The contrast was highest against all backgrounds for the black model. The contrast

347   was lower for the other three models with small differences between the models (Figure 4a).

348   However, the white model differed from the other three models in the directionality of the

349   contrast. The white model often had a higher luminance than the background while the tan, grey

350   and black models most often were darker than the background. Consequently, the absolute

351   contrast between the white model and the background were similar to the contrast between the

352   grey model and the background, and the tan model and the background, but with an essential

353   difference in the directionality of the contrast. Nevertheless, the contrast values were largely

354   correlated with the behavioral responses for three of the four models (figure 4a). The most

355   contrasting butterfly model (black) generated the highest response from males, while the models

356   with a lower contrast generated a lower behavioral response from males.

357

358   The luminance contrast also varied with background. Here, the contrasts between models and

359   background are greatest when the background is blue sky than when the background is sand or

360   vegetation (figure 4b). Again the pattern of change in contrast with background parallels the

361   behavioral data where across model types males responded most frequently when blue sky was

362   the background than when sand or vegetation were the background (cf. figure 3c). But again,

363   there is little difference in absolute contrast between the white model and the three background

364 types (figure 4b), but reversal in contrast directionality. When the white model is viewed against

365 the vegetation or the sand it had a higher luminance than the background.

366

367 **Discussion**

368 Our results show that both the brightness of an object as well as the background against which it

369 is viewed strongly affects male probability of detecting the object and initiating a pursuit flight.

370 Perched males are most likely to detect and pursue dark objects such as the black models when

371 they pass by. Moreover, objects were most likely to be detected when viewed against a bright

372 uniform background such as blue sky compared to a background of vegetation or sand. Our

373 modeling of perceived brightness contrast between the presented butterfly models and the

374 background correlates with the behavioral data, at least for three of the four butterfly models.

375 Hence, we argue that the effect of model brightness and background type can be largely

376 explained by differences in achromatic luminance contrast and that a high contrast between

377 object and background facilitates visual detection of small fast moving objects. However, the

378 response to white objects is far lower than would be expected by our measured luminance

379 contrast.

380

381 *Characterization of background*

382 We acknowledge a few potential sources of error in our measurement techniques. First, we did

383 not determine the background against which a male was viewing the model at the moment he

384 responded. However, not knowing this is mitigated by two features of the backgrounds data.

385 First, in 42% of all presentations from the male's perspective there was only one background

386 type, sky, sand or vegetation, along the model flight path. Second, in cases where there were

387 mixtures of backgrounds there was often one background type that dominated the scene. Also,

17

388   when there was a mixture of backgrounds, the response from the males was not significantly

389   different than when there was only a single background (table S1). Hence, we contend that using

390   the main background is a good estimation of what the background was at the point the male

391   responded.

392

393   Second, our background categories gloss over complexity within categories. Especially

394   vegetation is likely to be a matrix of bright and dark parts. The result would be that the luminance

395   contrast between a passing object and the background would shift repeatedly and quickly when

396   viewed by the male. It is unknown how this would affect visual detection. However, this effect of

397   this variation is mitigated by the fact that the acuity of male eyes is such that this variation will

398   not be resolved, especially when the background is several meters or more away. The perceived

399   luminance variation will effectively be averaged, resulting in a fairly constant luminance contrast

400   of a passing object. Switzer and Eason (2000) found that in a territorial dragonfly, intruders

401   viewed against distant vegetation were more readily detected than intruders viewed against near

402   vegetation.

403

404   Lastly, our field irradiance measurements used to calculate this contrast may not have captured

405   accurately all of the background light which was present. Specifically our measurements omitted

406   the skylight, a major extrinsic factor in desert ecosystems, which would contribute to background

407   luminance. Further, the light contributing to background luminance comes from a larger field of

408   view than is represented by measuring vector irradiance, which by necessity is restricted to the

409   hemisphere in which a cosine-corrected collector is directed. Nevertheless, the effect of scattered

410   background light is predicted to increase perceived luminance contrast of objects which have less

411   luminance than the background (black, tan and gray models), but also to decrease the perceived

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412   contrast of objects which have a higher luminance than the background (white model). This

413   would explain why the measured luminance contrast of the white target is higher than predicted

414   by behavioral responses, and the contrast for the three other models is slightly lower than

415   predicted by the behavioral responses, as seen in Figure 4a. Further, we find that the deviation is

416   larger when the background was sand, or vegetation (Figure 4b), which could be explained due to

417   a stronger effect of scattered skylight. As overall levels of direct illumination decrease, the

418   proportion of scattered skylight contributing to ambient illumination is higher.

419

420   *Visual modeling and luminance contrast*

421   By using physiological models of color processing we explored the role that perceived luminance

422   contrast between object and background might play in explaining these results. The models we

423   used are built on several assumptions. First, the model assumes that the spectral sensitivity of *A.*

424   *leilia* is similar to that of *Vanessa cardui*. Second, the model assume that *A. leilia* use mainly

425   their green receptor for target detection. It is well known that bees use their green receptor for

426   target detection (Giurfa & Vorobyev 1997, 1998) but it is still largely unknown how this works in

427   butterflies. However, which visual pathway (chromatic or achromatic) is used for target detection

428   in bees depends on the angle subtended by the target. If a target subtends an angle above 30°,

429   color vision is used for target detection and recognition. But if a target is small and close to

430   detection limits (subtends less than 5° in the visual field) detection is guided by the long

431   wavelength photoreceptor class alone (Giurfa & Vorobyev 1997, 1998). The long wavelength

432   photoreceptor is used to see the intensity differences between the object and the background, a

433   mechanism also referred to as "green contrast" (Giurfa *et al.* 1996). Behavioral studies also

434   suggest that butterflies use achromatic vision and target-background intensity contrast for some

435   behavioral tasks, such as landing on flowers (Koshitaka *et al.* 2011). In our study we did not find

436   any difference in the males' response to the gray model and the tan colored model (Table 2;

437   Figure 3a). The gray and the tan colored models are similar in brightness but differ in spectral

438   properties (Figure 1). The similarity in response to the gray and tan model may indicate that *A.*

439   *leilia* also uses an achromatic rather than a chromatic visual pathway to detect small passing

440   objects. Based on this and considering that the butterfly models at the distance presented in our

441   study subtended less than 5° in the perched male's visual field, we conclude that males use only

442   their long wave receptor for detection of the presented models. Hence, given that the assumptions

443   made by our visual model are correct, we can infer that perched males of *A. leilia* use mainly

444   luminance and not color contrast to detect flying females and other males.

445

446   *Contrast directionality and response to white objects*

447   The comparison of the behavioral data and the measured luminance contrast between the

448   butterfly models and the background show that the white model deviates from the correlation

449   between behavior and measured luminance contrast. As mentioned above, it is likely that our

450   natural irradiance measurements were unable to accurately capture all of the background light

451   which was present and that we thereby underestimate the background luminance perceived by the

452   perched males. However, there is also a small possibility that there is an additional, biological

453   explanation for this mismatch. Two other studies, where artificial butterfly-shaped models have

454   been presented to perched butterfly males in the field, reported a very low response to white

455   objects (Stride 1957; Tinbergen *et al.* 1972). The species studied were both nymphalids

456   (*Hipparchia semele* and *Hypolimnas misippus*) and Stride (1957) proposed an adaptive

457   explanation to the low response to white objects. Since perching behavior involves costly

458   approach flights and investigation of essentially anything that comes into the visual field, a

459   mechanism that allows discrimination would be an advantage for the male. Approach inhibition

460   based on brightness of the object would avoid pursuit of butterflies in the family Pieridae, a

461   family with many species of white butterflies (Stride 1957). Even though an underestimation of

462   background luminance seems likely to explain the low response to white models in *A. leilia*, if is

463   not impossible that there is a general approach inhibition towards white objects in perching

464   species and that this could be the result of undescribed visual system response that detect the

465   direction of contrast rather than only the magnitude and affects a male's decision to take off and

466   pursue a passing object.

467

468   *Conclusions*

469   Studies like the one presented here, that examine how extrinsic factors such as background affect

470   visual detection under natural settings complement previous studies on intrinsic factors, i.e. how

471   visual systems work and how morphological and physiological features affect visual

472   performance. By taking the experiment to the field we can test hypotheses about how natural

473   variation and real environmental factors affect visual system performance. Studies like this are

474   also important to understand the behavioral and evolutionary ecology of a perch-and-sally

475   strategy. Males of *A. leilia* can affect their success at detecting and pursuing passing females by

476   choosing a perch site that facilitate this task. By studying this in wild males found on their natural

477   perch sites we can understand the variation in real environmental factors and how this will

478   structure selection for choosing preferred perch sites. In an earlier study, Bergman and Wiklund

479   (2009) studied visual mate detection in the speckled wood butterfly (*Pararge aegeria*), a species

480   in which males defend mating territories in large sunspots on the forest floor (Davies 1978;

481   Wickman & Wiklund 1983). Bergman and Wiklund (2009) showed that males enhance their

482   chance of visually detecting passing females by preferring to perch in large sunspots over small

483   ones. In this system the background varies little between perch sites, but the level of ambient

484 illumination in the sunspot facilitates visual detection of passing females, presumably because a

485 female entering the sunspot will be illuminated by the sun, appear brighter and thereby contrast

486 more with the dark forest background. Further, a female passing a male sitting in a large sunspot

487 will be illuminated by the sun for a longer time than a female passing a male in a small sunspot,

488 and thereby more likely to be detected. Similar to *A. leilia*, males of *P. aegeria* can increase the

489 chance of viewing a passing female at a high contrast to the background by the choice of perch

490 site. We argue that this and other extrinsic factors have large effects on the success of perch-and-

491 sally tactics and ultimately on the evolution of perch site preferences.

492

498

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595

Figure legends

597 *Figure 1*

598 Reflectance spectra of the four butterfly models presented to perched males of *Asterocampa*

599 *leilia*.

600 *Figure 2*

601 Background classification images taken after presentations of butterfly models to males of

602 *Asterocampa leilia*. Each photo was taken from the exact spot the male was perched. The black

603 line shows the flight path of the model. *(a)* represents a background consisting of mainly sky, *(b)*

604 represents a background consisting of mainly sand and *(c)* represents a background consisting of

605 mainly vegetation.

606 *Figure 3*

607 The relationship between the proportion of perched males responding as a function of model

608 type: (A) for all presentations, (B) for all males as a function of perch location, and (C) for all

609 males as a function of main background type. For all graphs, the number adjacent to each data

610 point is the number of presentations with that model type.

611 *Figure 4*

612 (A) The relationship between the proportion of perched males responding as a function of model

613 type for all presentations (right y-axis), and luminance contrast as a function of model type (left

614 y-axis). (B) Luminance contrast as a function of model type and background.

615

616 **Figure legends supplementary figures**

617 *Figure S1.*

618 Reflectance spectra of the tan butterfly model, presented to perched males of *Asterocampa leilia*,

619 and the ventral hindwing of *A.leilia* females. The gray areas show the 95% CI. The average

620   reflectance of the tan model is not significantly different from female ventral hindwing average

621   reflectance (t-test: $t_4 = 1.88$, $P > 0.05$).

622   *Figure S2.*

623   Diagram of irradiance and radiance of viewer, butterfly model and background. Black butterfly

624   represents a perched *A. leilia* male and grey butterfly represents presented butterfly model. Red

625   arrows represent radiance and blue arrows represent irradiance. Thick arrows represent the model

626   while dashed is for background. B represents background. *(a)* illustrates a male perched in the

627   hackberry tree viewing the model with a sand background. *(b)* illustrates a male perched on a

628   hackberry tree viewing the model against a vegetation background. *(c)* illustrates a male perched

629   on the ground viewing the model against the blue sky. *(d)* illustrates a male perched on the

630   ground viewing the model against a vegetation background.

631

632 **Tables**

633 *Table 1*

634 The results from a Generalized Equation Estimates (GEE) showing the effect of model type and

635 background on the probability of getting a response from a perched male to a presented butterfly

636 model.

Factor	d.f.	*Wald χ^2*	*P*
Model type	3	28.53	<0.001
Background type	2	7.51	0.023
Model type x Background type	6	5.83	0.44

637

638 *Table 2*

639 The result of six pairwise McNemara's tests between four butterfly model types. After correction

640 of the alpha-values with a sequential Bonferroni all pairwise tests are significant, except the

641 difference between the gray and the tan model, which is not significant.

		Model type			
		Black	Gray	Tan	White
Model type	Black		χ^2=12.07 P<0.001	χ^2=10.08 P=0.0015	χ^2=21.04 P<0.001
	Gray			χ^2=0.57 P=0.45	χ^2=6.72 P=0.0095
	Tan				χ^2=10.32 P=0.0013
	White				

642

643

644 **Supplementary table**

645 *Table S1*

646 The response to presented butterfly models by perched males of *A. leilia*. The proportion of males

647 that responded and that viewed the model against a single background type was not significantly

648 different from the proportion of males that responded which viewed the model against a mixture
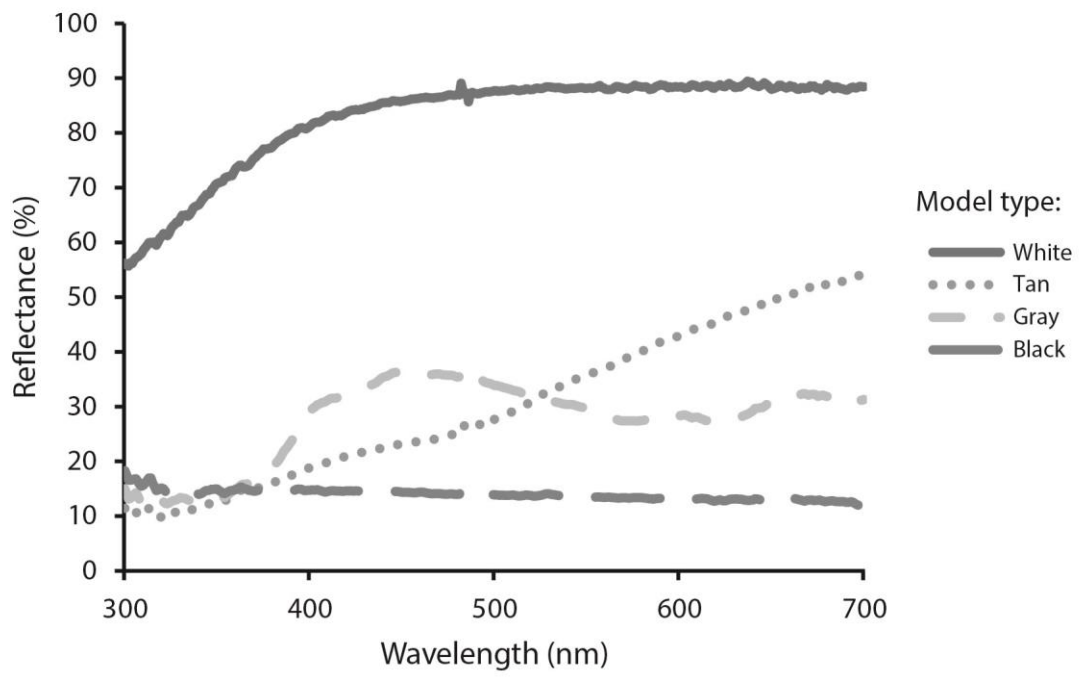
649 of background types.

Model type	Responded	Single background	Mixture of backgrounds	
Black	Yes	17	21	$\chi^2=1.00; p=0.32$
	No	4	2	
Gray	Yes	9	15	$\chi^2=0.06; p=0.81$
	No	9	13	
Tan	Yes	9	18	$\chi^2=3.38; p=0.07$
	No	11	7	
White	Yes	6	5	$\chi^2=1.90; p=0.17$
	No	10	22	

650

651

652 **Figures**

653 *Figure 1*

654
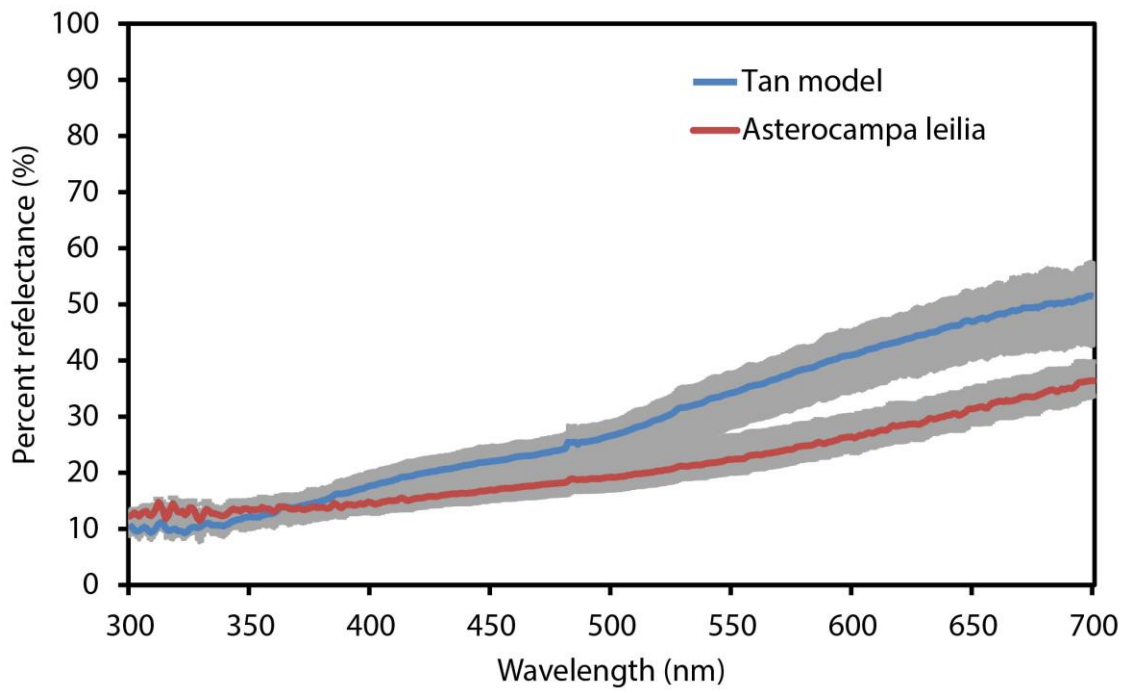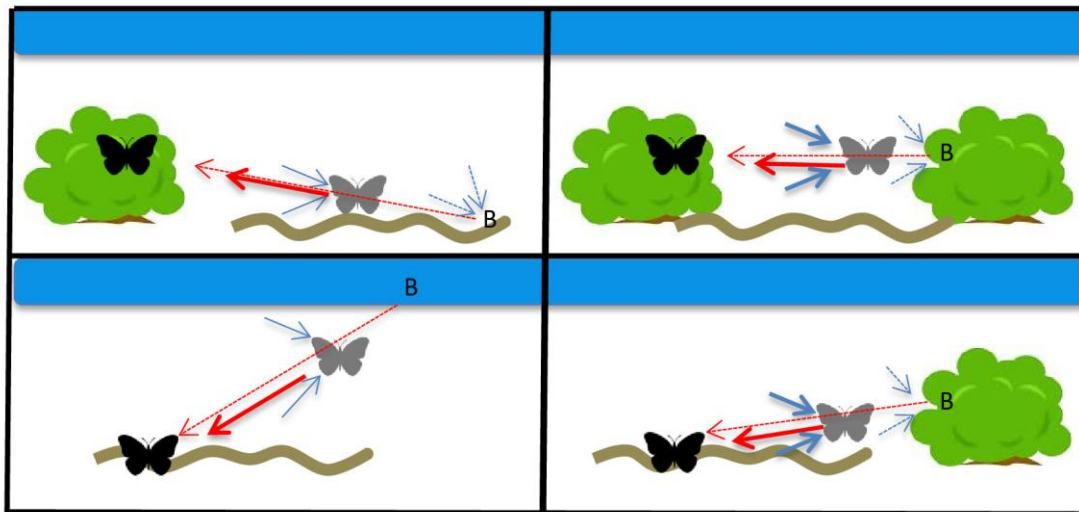
655

656   *Figure 2*

657

658
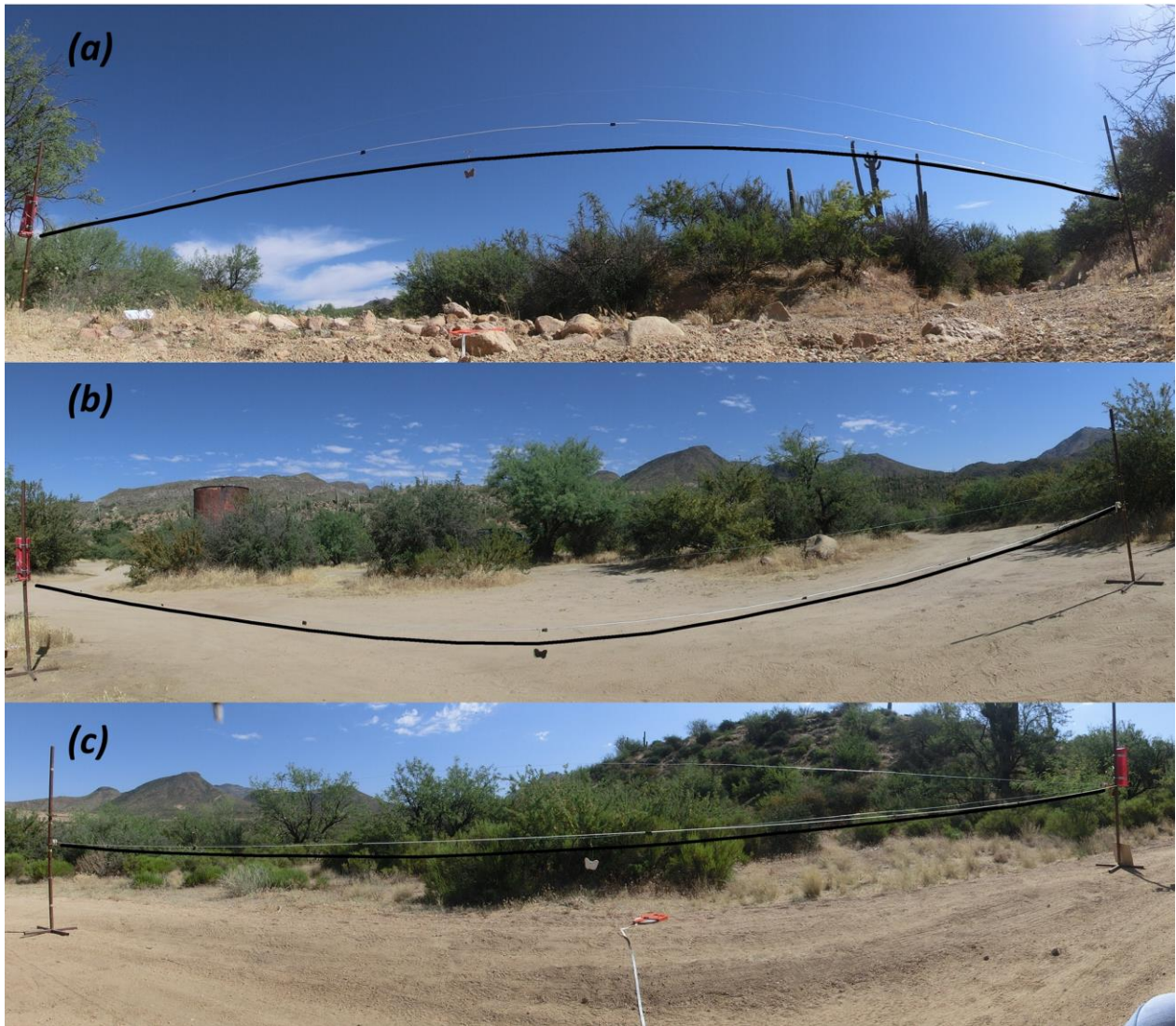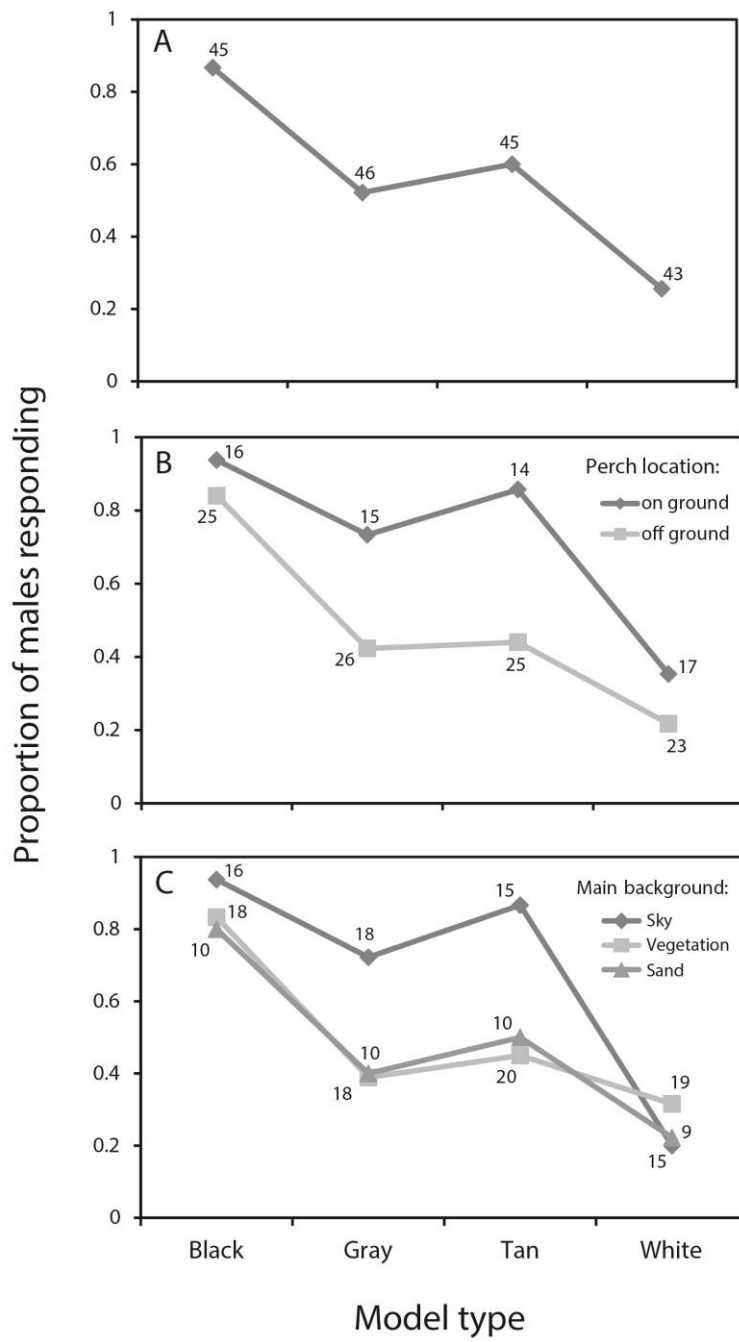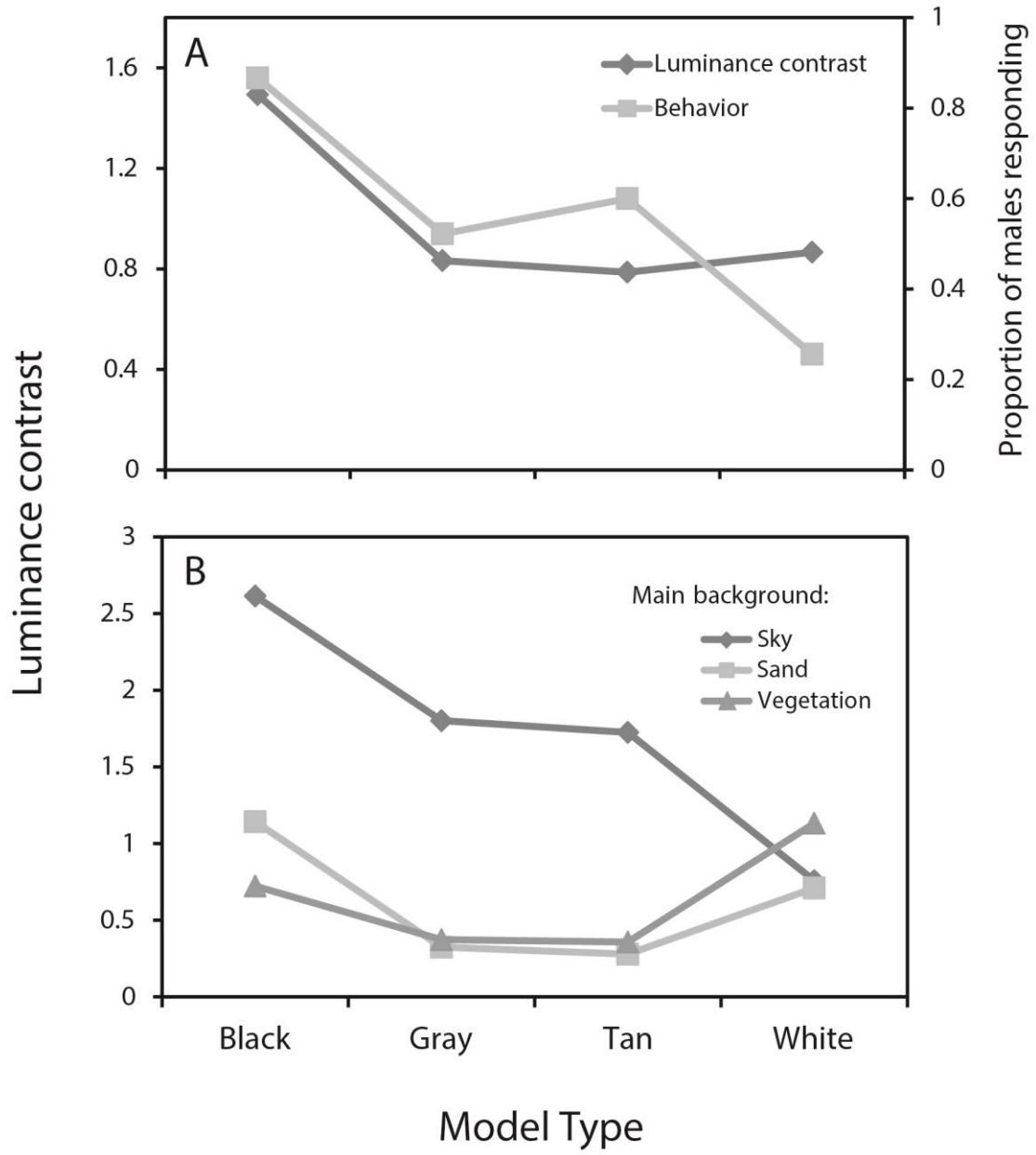
Figure 3

Figure 4

665   *Figure S1*

666

667   *Figure S2*

668