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1 **Transparency reduces predator detection in mimetic clearwing butterflies**

2

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15

16 Keywords: aposematic, bird, citizen science, crypsis, detectability, experiment, Ithomiini,
17 vision modelling

18

19 **Abstract**

20 1. Predation is an important selective pressure and some prey have evolved conspicuous
21 warning signals that advertise unpalatability (i.e. aposematism) as an antipredator
22 defence. Conspicuous colour patterns have been shown effective as warning signals,
23 by promoting predator learning and memory. Unexpectedly, some butterfly species
24 from the unpalatable tribe Ithomiini possess transparent wings, a feature rare on land
25 but common in water, known to reduce predator detection.

- 26 2. We tested if transparency of butterfly wings was associated with decreased
27 detectability by predators, by comparing four butterfly species exhibiting different
28 degrees of transparency, ranging from fully opaque to largely transparent. We tested
29 our prediction using both wild birds and humans in behavioural experiments. Vision
30 modelling predicted butterfly detectability to be similar for these two predator types.
- 31 3. In concordance with predictions, the most transparent species were almost never
32 found first and were detected less often than the opaque species by both birds and
33 humans, suggesting that transparency enhances crypsis. However, humans were able
34 to learn to better detect the more transparent species over time.
- 35 4. Our study demonstrates for the first time that transparency on land likely decreases
36 detectability by visual predators.

37 **Introduction**

38 Predation is an important selective pressure and a strong evolutionary force shaping prey
39 colouration. Some prey have evolved colours and textures that mimic those of the
40 background, hence rendering them cryptic (Endler, 1988) and reducing predator detection. In
41 midwater environments, where there is nowhere to hide, crypsis can be achieved by different
42 means, including transparency (Johnsen, 2014). Transparency is common in aquatic
43 organisms where it has been shown to decrease detectability by visual predators, enabling
44 prey to blend in with their environment (Kerfoot, 1982; Langsdale, 1993; Tsuda, Hiroaki, &
45 Hirose, 1998; Zaret, 1972). By contrast, transparency is generally rare in terrestrial
46 organisms, except for insect wings, which are made of chitin, a transparent material.
47 However, Lepidoptera (named after ancient Greek words for scale – *lepis* – and wing -
48 *pteron*) are an exception as their wings are generally covered with colourful scales that are
49 involved in intraspecific communication (Jiggins, Estrada, & Rodrigues, 2004),
50 thermoregulation (Miaoulis & Heilman, 1998), water repellence (Wanasekara & Chalivendra,

51 2011), flight enhancement (Davis, Chi, Bradley, & Altizer, 2012), and antipredator
52 adaptations such as crypsis (Stevens & Cuthill, 2006), masquerade (Suzuki, Tomita, &
53 Sezutsu, 2014) and aposematism (i.e. advertisement of unpalatability by the means of
54 conspicuous colouration, Mallet & Singer, 1987).

55

56 Ithomiini (Nymphalidae: Danainae), also known as clearwing butterflies, are some of the
57 most abundant butterflies in Neotropical forests (Willmott, Willmott, Elias, & Jiggins, 2017).

58 Ithomiini species are considered to be unpalatable to some extent due to the accumulation of
59 pyrrolizidine alkaloids collected from Asteraceae, Boraginaceae and Apocynaceae plants
60 (Brown, 1984, 1985). Pyrrolizidine alkaloids, naturally present in Ithomiini butterflies,
61 *Oreina* beetles, or artificially added to mealworms, have been reported to effectively deter
62 predation by birds (Brown & Neto, 1976). Many Ithomiini represent classic examples of
63 aposematic prey, whereby bright wing colour patterns – including orange, yellow and black -
64 advertise their unprofitability to predators (Mappes, Marples, & Endler, 2005; Nokelainen,
65 Hegna, Reudler, Lindstedt, & Mappes, 2011; Poulton, 1890). Ithomiini butterflies are also
66 involved in mimicry with other aposematic species such as several *Heliconius* butterflies
67 (Beccaloni, 1997). Bright contrasting and aposematic colouration is likely to be the ancestral
68 state in the group, since most species in sister lineages (Tellerveni and Danaini) are opaque
69 and aposematic (Freitas & Brown, 2004). However, transparency has evolved to some degree
70 in approximately 80% of clearwing butterfly species, even though many retain minor opaque
71 and colourful wing elements (Beccaloni, 1997; Elias, Gompert, Jiggins, & Willmott, 2008;
72 Jiggins, Mallarino, Willmott, & Bermingham, 2006). Similarly to cicadas and damselflies,
73 transparency in these butterfly wings is sometimes enhanced by anti-reflective nanostructures
74 (Siddique, Gomard, & Hölscher, 2015; Watson, Myhra, Cribb, & Watson, 2008; Yoshida,
75 Motoyama, Kosaku, & Miyamoto, 1997). Since transparency is often associated with crypsis,

76 for example in aquatic organisms (Johnsen, 2014), transparency in these butterfly may
77 decrease detectability by predators.

78

79 To determine if transparency in clearwing butterflies decreases detectability by visual
80 predators, we compared predator detection of four Ithomiini species that differ in the amount
81 of transparency of their wings (Fig 1): *Hypothyris ninonia* (largely opaque and brightly
82 coloured), *Ceratinia tutia* (brightly coloured and translucent), *Ithomia salapia* (transparent
83 with a pale yellow tint and an opaque contour) and *Brevioleria seba* (transparent without
84 colouration other than a white band in the forewing and an opaque contour). Given the
85 proportion of light that is transmitted through the butterfly wing of the different species (Fig
86 S1), we predicted that the opaque species *Hypothyris ninonia* should be the easiest to detect,
87 followed by the translucent species *Ceratinia tutia*. Finally, the more transparent butterfly
88 species *Ithomia salapia* and *Brevioleria seba* should be the least detectable. However, it is
89 also possible that the coloured opaque elements of the transparent species, such as the white
90 band in *B. seba* and the opaque contour found in most of these species, enhance detection.
91 We tested our predictions using two complementary behavioural experiments involving birds
92 and humans, and further supported by a vision modelling approach.

93

94 Detectability of butterflies was first tested using wild great tits (*Parus major*) as model bird
95 predators. Great tits are sensitive to UV wavelengths (UVS vision in Ödeen, Håstad, &
96 Alström, 2011). Their vision is similar to that of naturally occurring Ithomiini predators such
97 as the houtouc motmot (*Momotus momota*, Pinheiro et al., 2008), the fawn-breasted tanager
98 (*Pipraeidea melanonota*, Brown & Neto, 1976) or the rufous-tailed tanager (*Ramphocelus*
99 *carbo*, Brower et al., 1963). However, unlike Neotropical insectivorous birds, great tits are
100 naïve to ithomiine butterflies and have not learned to associate their colour patterns to

101 toxicity. As a bird's propensity to attack prey is the result of both prey detection and
102 motivation to attack the prey, we also performed behavioural experiments using human
103 participants, which can be useful in disentangling these two factors. Differences in colour
104 perception between great tits and humans include the presence of a fourth single cone type
105 receptor (instead of three cones in humans) that extend the great tits' sensitivity into the UV
106 light spectrum (Hart, 2001), and oil droplets that refine colour discrimination in birds
107 (Vorobyev, 2003). However, neither humans or birds are able to detect linear polarization,
108 which excludes the use of polarization cues to detect and discriminate between butterfly
109 species (Foster et al., 2018; Greenwood, Smith, Church, & Partridge, 2003; Melgar, Lind, &
110 Muheim, 2015; Montgomery & Heinemann, 1952). Moreover, humans have been found to be
111 good predictors of insect prey survival in the wild (Penney, Hassall, Skevington, Abbott, &
112 Sherratt, 2012). Finally, models of predator vision (both for birds and humans) were used to
113 complement behavioural experiments and infer the relative detectability of each butterfly
114 species based on their contrast against the background.

115 **Materials and Methods**

116 **Butterflies used for the behavioural experiments**

117 Specimens of the four Ithomiini species used in both experiments – which, in order of
118 increasing transparency are *Hypothyris ninonia*, *Ceratinia tutia*, *Ithomia salapia aquina*,
119 *Brevioleria seba* (see Figs 1 and S1) – were collected in Peru in 2016 and 2017, along the
120 Yurimaguas - Moyobamba road (-6.45°, -76.30°). Butterflies were kept dry in glassine
121 envelopes until use. In behavioural experiments, a single real hindwing and a single real
122 forewing were assembled into artificial butterflies using glue and a thin copper wire to attach
123 the artificial butterfly to a substrate (see Fig S2 for an example). These artificial butterflies

124 mimicked real Ithomiini butterflies at rest, with wings closed and sitting on plant leaves (a
125 typical posture for resting butterflies).

126 **Behavioural experiments using wild birds**

127 Behavioural experiments took place in August and September 2017 at the Konnevesi
128 Research Station (Finland). Thirty wild-caught great tits (*Parus major*) were used. Birds were
129 caught using spring-up-traps and mist-nets, individually marked with a leg band and used
130 only once. Each bird was housed individually in an indoor cage (65x65x80 cm) and were fed
131 with seeds and water *ad libitum*, except during training and experiments. During training,
132 birds were given mealworms attached to butterfly wings (see Training section). Birds were
133 deprived of food for up to 2 hours before the experiment to increase their motivation to hunt.

134

135 *Training.* In indoor cages, birds were taught that all four species of butterflies were similarly
136 palatable by offering them laminated wings of four butterflies (one of each species) with a
137 mealworm attached to the copper wire. Wings were laminated during training only, using
138 transparent thin plastic so as to minimize damage and enabling us to re-use the wings
139 between trials. Butterflies were presented to the birds in the absence of vegetation during
140 training so as to enhance the association between butterfly colour patterns and fully edible
141 prey. When birds had eaten all four prey items (one of each species), a new set was presented.
142 Training ended when birds had eaten 3 sets of butterflies. No time constraint was imposed for
143 training and most birds completed it in less than 4 hours.

144

145 In order to familiarise birds with the experimental set-up, which was novel to them, they were
146 released in the experimental cage by groups of two to four birds for approximately one hour
147 the day before the experiment. Oat flakes, seeds and mealworms were dispersed over leaves

148 and vegetation so as to encourage searching for edible items in locations similar to where
149 butterflies would be placed during the experiment.

150

151 *Experiments.* The experimental set-up consisted of a 10m x 10m cage that had tarpaulin walls
152 and a ceiling of whitish dense net that let in natural sunlight. Butterflies were disposed in a 5
153 x 5 grid, delimited by poles all around the borders and a rope defining rows and columns (see
154 Fig S3). Five specimens of each species (20 specimens in total) were placed in the grid, one
155 per cell. Before each trial, butterflies were photographed over graph paper, used as a scale to
156 measure butterfly size on Image J (Rueden et al., 2017). Butterflies were pinned on top of
157 meadowsweet leaves (*Filipendula ulmaria*) that had naturally grown in the outdoor cages.
158 Butterflies were always put in similar places within the cell and could be easily seen from a
159 nearby pole. Butterfly position was randomized but care was taken in 1) leaving the 5 cells
160 closest to the observer empty as birds tended to avoid this area, 2) avoiding having more than
161 two specimens of the same species in the same row or column, and 3) having two specimens
162 of the same species in neighbouring cells. This ensured that all species were evenly
163 represented along the grid. This random configuration was reshuffled between trials.

164

165 For each trial, an observer, hidden to the birds, watched from outside the cage through a
166 small window and took notes of which butterfly species were attacked and in which order. A
167 GoPro camera also recorded the experiments. A butterfly was considered detected only if a
168 bird directly approached to attack it, including when the attack failed. No bird was seen
169 hesitating during an attack once it had initiated it. Experiments took place between 9 am and
170 5 pm. Before each trial, the radiance of ambient light (coming from the sun and sky) was
171 taken by spectrophotometry in the same location. We computed the total radiance (TR) over
172 the bird's spectral sensitivity, which range from 300-700 nm, to account for the intensity of

173 ambient light associated to each experimental trial in the statistical analyses. Further
174 information on weather conditions (cloudy, sunny, etc) was also recorded. Experiments ended
175 when a bird had eaten half of the available butterflies (ie. 10 butterflies) or after 2 hours,
176 whichever happened first. Wings were occasionally re-used if they had not been damaged.

177

178 To control for any positional effect on overall species detection, we computed the probability
179 of a bird being present in a given grid area. To do so, a 10-minute interval of each recorded
180 trial was selected and revised to calculate the proportion of time birds spent on the different
181 poles. The time intervals were possible for all trials as they all lasted at least 10 minutes and
182 were selected either as a result of the birds actively attacking prey or actively exploring the
183 cage during that time, based on notes taken by the observer. These probabilities were later
184 used to divide the grid into four main areas according to bird occupancy: furthest and closest
185 corner to the observer, grid border and grid centre (Fig S4a). Most birds fed willingly on all
186 butterflies located on the borders of the grid. Given that butterfly species distribution was
187 random and reshuffled between trials, the four species were similarly represented throughout
188 the grid (Fig S4b), so no bias was expected. For more details about permits, husbandry
189 conditions, training and experiments, see Supplementary Material.

190

191 **Behavioural experiments using human participants**

192 Between mid-November and early December 2017, visitors of the Montpellier botanical
193 garden (France) were invited to take part in an experiment where they searched for artificial
194 butterflies. Before each trial, participants were shown pictures of various ithomiine butterfly
195 species, both transparent and opaque, different from those used in the experiments to
196 familiarize them with what they would be searching for. Anonymous personal data was
197 collected from each participant, including gender, age group (A1: <10 years, A2: 11-20 y,

198 A3: 21-30 y, A4: 31-40 y, A5: 41-50 y and A6: >51 years), and vision problems. Each
199 participant attempted the experiment only once.

200

201 *Experimental set-up.* As with the behavioural bird experiments, artificial butterflies (N=10 of
202 each of the four species, for a total of 40 butterflies) consisted of one real forewing and one
203 real hindwing assembled with copper wire and placed on leaves, but without the mealworm
204 used in the bird experiments. These butterflies were set-up along two corridors in a forest-like
205 understory habitat of similar vegetation and light conditions. Butterfly order followed a block
206 randomisation, with five blocks each consisting of eight butterflies (i.e. two of each species;
207 see Fig S5). This ensured that observers were similarly exposed to the four species all
208 throughout the experimental transect. Whether a butterfly was placed on the left or right side
209 of the corridor was also randomised and both order and corridor side were changed daily.
210 Participants could start the path from either end of the set-up and were given unlimited time
211 to complete the trial. However, they could only move forward on the path. Only one
212 participant was allowed in the path at any given time, and they were accompanied by an
213 observer who recorded which butterflies were found. Trials ended when the participant had
214 completed both corridors.

215

216 **Statistical analyses.**

217 Experiments using birds and humans were analysed independently. Differences in the total
218 number of butterflies of each species that were attacked by predators (for the sake of
219 simplicity we use ‘attacked’ hereafter for both birds and humans) were compared by fitting
220 generalised linear mixed effect models (GLMM), with bird/human identity as a random
221 factor. A binomial distribution was used for the response variable (attacked or not). For the
222 experiments using birds, butterfly species, butterfly size, trial duration, age and sex of the

223 bird, time to first attack, first butterfly species attacked, butterfly position on the grid (corner
224 –furthest or closest to the observer-, grid border, grid centre), weather (as a qualitative
225 variable), and total radiance (TR), as well as their interactions, were all included as
226 explanatory variables. For human trials, butterfly species, first species attacked, butterfly
227 position, corridor, left or right side of the path, time of day, gender and age of the participant,
228 duration of the experiment, and their interactions, were all used as explanatory variables. In
229 each case, the best fitting model was selected based on minimization of Akaike’s Information
230 Criteria (AIC), assuming that models differing by two units or less were statistically
231 indistinguishable (Anderson, Burnham, & White, 1998). Coefficients and standard errors
232 were computed using a restricted maximum likelihood approach and a Wald z test was used
233 to test for factor significance.

234

235 In addition to the total number of butterflies attacked per species, an “inconspicuousness”
236 rank was calculated for each butterfly species, as done in a previous study (Ihalainen,
237 Rowland, Speed, Ruxton, & Mappes, 2012). This ranking takes into consideration both the
238 specimens that were attacked and those that were not for each species. Lower values are
239 assigned to those specimens that were attacked (from 1 to 10, according to the sequence of
240 overall prey discovery), and higher values are given to those specimens that were not
241 attacked (all unnoticed specimens are given a value of 11: the maximum number of
242 butterflies that could be attacked before the experiment ended + 1). For example, if a bird
243 captures two *H. ninonia* second and fifth in the sequence of captured prey, leaving three
244 specimens unnoticed (out of a total of 5 placed in the cage), this species gets a rank value of
245 $2+5+(3 \times 11)=40$ for that trial. This inconspicuousness rank distinguishes species attacked first
246 and in higher numbers (lower values of inconspicuousness) from those attacked last and in
247 lower numbers (higher values of inconspicuousness). We fitted a linear mixed effect model to

248 test for differences in rank for each species, assuming a normal distribution, with rank as the
249 response variable. We fitted independent models for birds and human experiments. For bird
250 experiments, bird individual was considered a random factor, and butterfly species, age and
251 sex of the bird, date, time until first attack, first butterfly species attacked, weather as a
252 qualitative variable, and total radiance (TR) were explanatory variables. For humans,
253 participant identity was a random factor, and butterfly species, first species attacked, time of
254 day, gender and age of the participant, duration of the experiment, and their interactions, were
255 all explanatory variables. Again, the best fitting model was selected using AIC minimization.
256 GLMMs were fitted using *nlme* (Pinheiro, Bates, DebRoy, Sarkar, & R Core team, 2009) and
257 *lme4* (Bates, Maechler, Bolker, & Walker, 2015, p. 4) packages for R. Moreover, whether
258 specific species were more frequently detected first by either birds or humans was tested
259 using a χ^2 test.

260

261 Additionally for birds, we tested whether butterfly location in the grid could explain
262 differences in the overall species' detection, i.e. whether species more likely to be attacked
263 were more often placed on areas more likely to be visited. To do so, the frequency per species
264 on the four different grid zones was compared using a χ^2 .

265

266 Finally, we tested whether birds and humans created a “search image” (i.e. improved ability
267 in finding butterflies of a given species after encountering a similar one) by counting the
268 number of butterflies of each species attacked consecutively. Results were compared among
269 butterfly species using a χ^2 test. Additionally, whether finding some species improved a
270 bird's or a human's ability to find others was also tested. For each combination of two
271 species, we calculated how many times a butterfly of species 1 was found after a butterfly of
272 species 2. Differences between combinations of butterfly species found by birds were tested

273 using a χ^2 test. For humans, observed results and the frequency at which each possible pair of
274 species was placed consecutively in the original experimental setup were compared using a χ^2
275 test. All analyses were performed in R (R Foundation for Statistical Computing, 2014).

276

277 **Colour measures and vision modelling**

278 Finally, models of predator vision (both for birds and humans) were used to complement
279 behavioural experiments and infer the relative detectability of each butterfly species based on
280 their contrast against the background. First, we measured colour (i.e. reflectance) and
281 transmission properties (i.e. transmittance of transparent wing areas) using
282 spectrophotometry. Vorobyev & Osorio's discriminability model (1998) was then used to
283 calculate the contrast between butterfly and background for birds and humans. Detailed
284 methods for measurements and vision modelling can be found in the electronic
285 supplementary material (additional materials and methods).

286

287 **Results**

288 **Behavioural experiments using wild birds**

289 The model that best explained whether butterflies were attacked or not included only the time
290 required before the first attack and the cage area in which the butterfly was located (Table
291 S1). Butterflies were most likely to be attacked when located in the furthest corners and in the
292 borders than in the rest of the cage ($z = 9.13$, $p < 0.001$). By contrast, the inconspicuousness
293 rank of a butterfly species was best explained by a model including butterfly species as an
294 explanatory variable (Table S2). Which species was attacked first closely matched wing
295 transmission properties: *H. ninonia*, the fully opaque species, followed by the translucent *C.*
296 *tutia*, the transparent and yellow-tinted *I. salapia* and the most transparent species in our
297 study, *B.seba* ($X^2 = 11.07$, $df = 3$, $p = 0.011$; Table S3). *Hypothyris ninonia*, which was the

298 most colourful species, was usually the first species attacked ($t = -3.15$, $p = 0.002$, Fig 2a;
299 Tables S2 and S3). Species distribution along the four different grid zones was similar ($X^2 =$
300 6.19, $df = 9$, $p = 0.72$; Fig. S4b).

301

302 Generally, birds did not attack several butterflies of the same species consecutively (Fig S6a).
303 In the rare instances when they did, no differences between species was found ($X^2 = 0.6$, $df =$
304 3, $p = 0.90$) suggesting that birds did not form a “search image” for any of the butterfly
305 species. No combination of species attacked consecutively at high frequencies were found
306 either ($X^2 = 10.88$, $df = 11$, $p = 0.45$).

307

308 **Behavioural experiments using human participants**

309 Younger participants found more butterflies than older ones (number of butterflies: $z = -2.34$,
310 $p = 0.019$; Fig S7a). Additionally, participants found more butterflies earlier than later in the
311 afternoon (number of butterflies: $z = -2.80$, $p = 0.005$; Fig S7a). Generally, the more time
312 participants spent on the experiment, the more butterflies they found (number of butterflies: z
313 $= 5.21$, $p < 0.001$), although this was most significant for women (number of butterflies: $z = -$
314 2.96 , $p = 0.003$), Fig S7b). Participants found more butterflies on the corridor that had
315 slightly larger vegetation cover (number of butterflies: $z = 3.14$, $p = 0.002$). Participants also
316 found more butterflies at the end rather than at the start of the experiment (number of
317 butterflies: $z = 3.70$, $p < 0.001$, Tables S4), most likely because they became accustomed to
318 the set-up and what they were searching for.

319

320 Participants were more likely to find opaque butterflies than transparent ones, following the
321 order *H. ninonia* (H), *C. tutia* (C), *B. seba* (B) and *I. salapia* (I) (H>C, I, B: number of
322 butterflies: $z = 5.73$, $p < 0.001$; inconspicuousness rank: $t = -3.96$, $p < 0.001$; C>B:

323 inconspicuousness rank: $t = -4.81$, $p < 0.001$; B>I: inconspicuousness rank: $t = -1.325$, $p <$
324 0.001 ; Tables S4 and S5; Fig 2b). However, the gain in detection with increasing time spent
325 searching was highest for the most transparent species ($z = -2.75$, $p = 0.006$, Fig S7c).
326 *Hypothyris ninonia* was also the species most frequently found first, followed by *C. tutia*, *B.*
327 *seba* and *I. salapia* ($X^2 = 19.5$, $df = 3$, $p < 0.001$, Table S3). More butterflies of each species
328 were found when *C. tutia* was found first ($t = -3.96$, $p < 0.001$).

329

330 There were also differences in the consecutive order in which butterflies were found.
331 Participants were more likely to find two consecutive butterflies of the same species when
332 they were colourful (*H. ninonia* -50 times- and *C. tutia* -58 times) than when they were
333 transparent (*B. seba* -32 times- or *I. salapia* -18 times; $X^2 = 29.14$, $df = 3$, $p < 0.001$).
334 *Brevioleria seba* and *H. ninonia* were found consecutively up to four times in a single trial.
335 Some species were also more likely to be found consecutively after another species. The two
336 most opaque butterflies *H. ninonia* and *C. tutia* (found 278 times consecutively), and the two
337 transparent species *B. seba* and *I. salapia* (found 186 times consecutively), were found
338 consecutively more frequently than any of the other possible combinations after correcting
339 for the number of butterflies found for each species ($X^2 = 170.95$, $df = 5$, $p < 0.001$). These
340 observed frequencies differed significantly from expected as a result of their physical position
341 along the path ($X^2 = 79.12$, $df = 11$, $p < 0.001$, Fig S6b).

342

343 **Models of bird and human vision**

344 The achromatic weighted contrast between butterfly colour patches and green-leaf
345 background were similar for both birds and humans (mean achromatic contrast for birds:
346 H=3.81, C= 3.15, I=2.31, B=2.11; for humans: H=5.25, C=4.35, I=3.58, B=3.86; Fig S8). For
347 both observers, *H. ninonia* (the most colourful species) followed by *C. tutia* (colourful but

348 translucent species) contrasted the most against the leaves, while the transparent butterflies (*I.*
349 *salapia* for humans and *B. seba* for birds) were the least contrasting. Butterflies seem to be
350 more chromatically detectable by birds than for humans (mean chromatic contrast for
351 humans: H = 0.44, C = 0.37, I = 0.25, B = 0.22). For the chromatic contrast seen by birds, *C.*
352 *tutia*, followed by *H. ninona* were the most contrasting, whereas *B. seba* and *I. salapia* were
353 the least contrasting (mean chromatic contrast for birds: H = 2.02, C = 2.05, I = 1.30, B =
354 1.38). For further details of the experiment results, see the Electronic Supplementary
355 Material.

356 **Discussion**

357 **Transparency reduces detectability**

358 As initially predicted based on wing transmittance, and as demonstrated by our behavioural
359 experiments and visual modelling results, transparency decreases butterfly detectability.
360 Interestingly, detection by human participants was similar to that of naïve birds, as shown in
361 other studies (Beatty, Bain, & Sherratt, 2005; Sherratt, Whissell, Webster, & Kikuchi, 2015),
362 providing further support for using human participants to measure predator detection.
363 Surprisingly, experimental results from the bird experiments differed slightly from
364 predictions based on the measures of transmittance of transparent patches and results
365 obtained from the vision models. For instance, according to the transmittance and the
366 chromatic contrast measured between butterflies and their background, birds should have
367 detected *C. tutia* more easily than the two more transparent species. Indeed, semi-transparent
368 objects should be more easily detected than fully transparent objects at short distances and
369 when more light is available (Johnsen & Widder, 1998), such as conditions present during
370 our experiments. Yet this transparent but brightly coloured species was detected at rates
371 similar to those of the most transparent species, perhaps because transparent butterflies were
372 more easily detected and attacked by birds than we predicted (e. g., if an opaque contour

373 enhances detectability of otherwise transparent prey). Alternatively, the semi-transparent *C.*
374 *tutia* could have been less detectable by birds, because it shows less strongly delimited
375 contours than those of the most opaque species *H. ninonia*. Perhaps this hampered its
376 detection as occurs in disruptively coloured prey (Honma, Mappes, & Valkonen, 2015;
377 Stevens & Cuthill, 2006). These contradicting results highlight the importance of combining
378 both modelling and behavioural experiments to better understand the evolution of
379 transparency and other prey defences.

380

381 **Transparency in potentially unpalatable butterflies?**

382 Our results demonstrate that transparency can effectively reduce prey detectability in
383 ithomiine butterflies, where several species have been experimentally demonstrated to be
384 chemically-protected (Brown, 1985; Trigo et al., 1996). This is surprising as aposematic
385 colour patterns, rather than inconspicuousness, are more common in toxic and unpalatable
386 prey (Mappes et al., 2005; Poulton, 1890; Ruxton, Sherratt, & Speed, 2004). In fact,
387 conspicuousness is positively correlated with toxicity or unpalatability in some species and
388 can thus be an honest indicator of prey defences (Arenas, Walter, & Stevens, 2015; Blount,
389 Speed, Ruxton, & Stephens, 2009; Maan & Cummings, 2012; Prudic, Skemp, & Papaj, 2007;
390 Sherratt & Beatty, 2003). Moreover, predators learn more quickly to avoid unpalatable prey
391 when colours are more conspicuous (Gittleman & Harvey, 1980; Lindstrom, Alatalo,
392 Mappes, Riipi, & Vertainen, 1999). This might suggest that the evolution of transparency in
393 these butterflies is the result of a loss or a reduction in unpalatability. If this is the case, the
394 existence of mimicry rings of transparent clearwing butterflies remains unexplained, as this is
395 usually the result of convergence of warning signals promoted by the positive frequency-
396 dependent selection exerted by predators (Willmott et al., 2017). Alternatively, if defences
397 are costly, prey may invest in either visual or chemical defences (Darst, Cummings, &

398 Cannatella, 2006; Speed & Ruxton, 2007; Wang, 2011), as such options have been shown to
399 afford equivalent avoidance by predators (Darst et al., 2006). In which case, transparency
400 should instead be associated with an increase in unpalatability. This relationship between
401 transparency and chemical defences in clearwing butterflies remains to be explored.

402

403 Alternatively, transparency may lower detection and function as a primary defence, with
404 aposematism taking over as a secondary defence if the prey is detected. Indeed, transparent
405 butterflies were not completely cryptic for either birds or humans. In fact, although birds
406 detected the most colourful species first, in total they found a similar number of both
407 colourful and transparent butterflies. Moreover, humans appear to learn to detect and perhaps
408 remember common elements between the more transparent species, which might be the result
409 of a search image. As such, Ithomiini butterflies may be cryptic from afar, but perceived as
410 conspicuous from up close. The combination of crypsis and conspicuousness has also been
411 shown for other defended prey (Järvi, Sillén-Tullberg, & Wiklund, 1981; Sillén-Tullberg,
412 1985). For example, toxic salamanders of the genus *Taricha* are generally cryptic, only
413 revealing their warning coloured underbelly when threatened (Johnson & Brodie, 1975). In
414 Ithomiini, conspicuous elements such as opaque areas that delineate the edges and contrast
415 with the background likely increase detection, as has been shown for artificial moths (Stevens
416 & Cuthill, 2006). Furthermore, pigmentary or structurally produced opaque colours, such as
417 the white band in *B. seba*, may also enhance butterfly detection. This suggests, as do our
418 results and the occurrence of co-mimics in natural habitats, that these butterflies may reduce
419 the cost of conspicuousness using transparency in addition to maintaining the benefits of
420 detectable warning signals. Further behavioural experiments testing the distance at which
421 Ithomiini butterflies are detected are needed to shed further light on the function of
422 aposematism in less conspicuous prey.

423

424 Finally, transparency may have evolved as an additional protection against birds such as adult
425 kingbirds (*Tyrannus melancholicus*, Pinheiro, 1996) which are able to tolerate their chemical
426 defences. Indeed, both theoretical (Endler & Mappes, 2004) and experimental (Mappes,
427 Kokko, Ojala, & Lindström, 2014; Valkonen et al., 2012) studies have shown that weak
428 warning signals (not overtly conspicuous) can evolve and be maintained in communities
429 where predators vary in their probability of attacking defended prey. Larvae of *Dryas iulia*
430 butterflies, pine sawfly larvae (*Neodiprion sertifer* for example), and shield bugs
431 (Acanthosomatidae, Heteroptera) are only a few of the examples that exist of unpalatable
432 species that display weak visual warning signals (see Endler & Mappes, 2004). As in the
433 polymorphic poison frog *Oophaga granulifera*, clearwing species may reflect a continuum
434 between aposematism and crypsis, possibly shaped by differences in the strength of predator
435 selection as a result of the frequency of naïve predators and/or the variation in predator
436 sensitivities to chemical compounds (Willink, Brenes-Mora, Bolaños, & Pröhl, 2013). A
437 thorough characterization of unpalatability, microhabitat and predator communities would be
438 useful in better understanding conditions that promote the evolution of transparency.

439

440 **Conclusions**

441 Our study, which combines behavioural experiments with different predators and vision
442 modelling, provides important insights into the complex role transparency may play in
443 predator defences of terrestrial aposematic organisms. We show for the first time that
444 transparency results in the reduction of detectability of terrestrial prey. We also demonstrate
445 that Ithomiini butterflies may in fact be decreasing the costs of conspicuousness, while still
446 retaining visual elements that are recognised as warning signals. Future studies exploring the

447 efficiency of combining transparency and warning signals in decreasing predation risk will
448 further contribute to our understanding of the evolution of cryptic elements in aposematic
449 prey.

450

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464

465 **Author contribution**

466 DG, ME, JM and MA designed the study; ME, MM and DG collected the butterfly samples;
467 MA, SG, ON, ME and JM performed the experiments; DG and CD did the optical
468 measurements; MA, DG and ME analysed the data; MA, DG, MM, ME, ON, SG and JM
469 wrote the manuscript. Authors have none conflict of interest to declare.

470

471 Data available from the Dryad Digital Repository (Arias *et al.* 2019).

472

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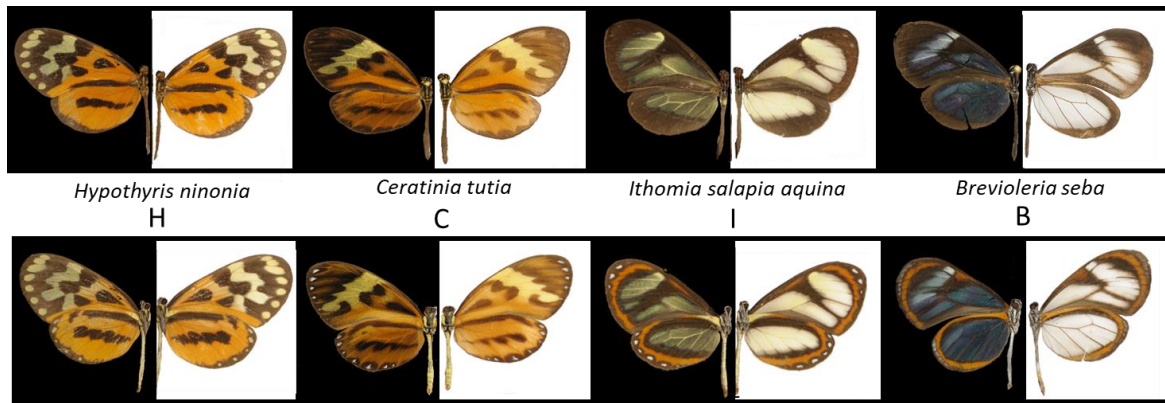
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666 **Figures**

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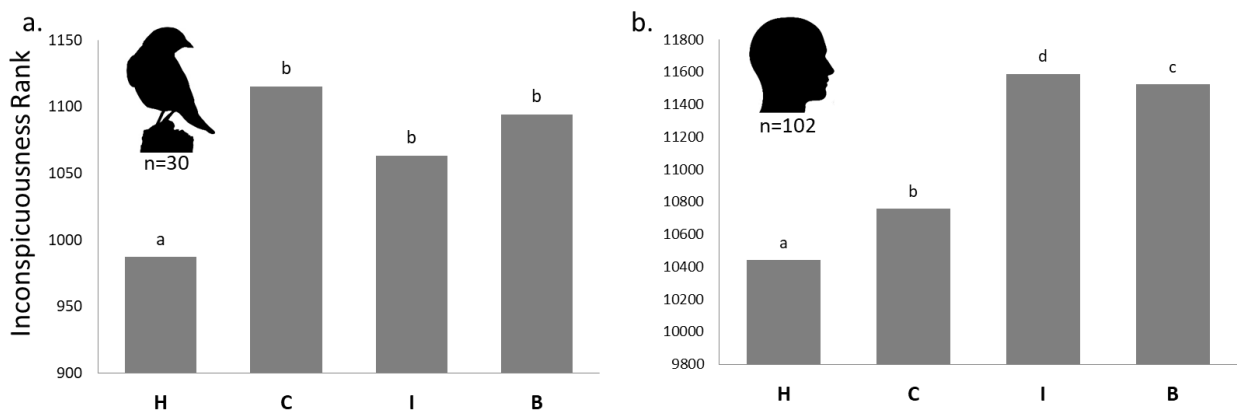
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Figure 1. Dorsal (top row) and ventral (bottom row) view of butterfly species used in the study (photographed against a black and a white background to show the location and degree of transparency in the wings). Wing transparency (transmission and area occupied by transparent patches) increases from left (most opaque) to right (most transparent): *Hypothyris ninonia* (largely opaque), *Ceratinia tutia* (translucent but brightly coloured), *Ithomia salapia aquina* (transparent with a pale yellow tint and black wing contour), *Brevioleria seba* (transparent without colouration other than a white band in the forewing and a black wing contour). ©

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Figure 2. Sum of the inconspicuousness rank for each butterfly species calculated from the behavioural experiments using a) great tits and b) humans. Species for which butterflies were detected first and most often by birds or humans have lower values of “inconspicuousness”

683 rank". Butterfly transparency increases from left to right: *H. ninonia* (H), *C. tutia* (C), *I.*
684 *salapia* (I), and *B. seba* (B). Letters above the bars mean significant differences below 0.05.
685

Electronic supplementary Material

Additional materials and methods and results.

Detailed behavioural experiments

Behavioural experiments using wild birds

Behavioural experiments took place in August and September 2017 at the Konnevesi Research Station (Finland) under permit from the National Animal Experiment Board (ESAVI/9114/04.10.07/2014) and the Central Finland Regional Environment Centre (VARELY/294/2015). Thirty wild-caught great tits (*Parus major*) were used, including 3 juveniles and 10 adult females, and 8 juveniles and 9 adult males. Birds were caught using spring-up-traps and mist-nets, individually marked with a leg band and used only once. Each bird was housed individually in an indoor cage (65x65x80 cm), with a 12:12 photoperiod. Birds were fed with peanuts, sunflower seeds, oat flakes and water *ad libitum*, except during training and experiments. During training, birds were given mealworms attached to butterfly wings (see Training section). Birds were deprived of food for up to 2 hours before the experiment to increase their motivation to hunt. Most birds were kept in captivity for less than a week, after which they were released at their capture site.

Training. In indoor cages, birds were taught that all four species of butterflies were similarly palatable by offering them laminated wings of four butterflies (one of each species) with a mealworm attached to the copper wire. Wings were laminated during training only, using transparent thin plastic so as to minimize damage and enabling us to re-use the wings between trials. Butterflies were presented to the birds in the absence of vegetation during training so as to enhance the association between butterfly colour patterns and fully edible prey. When birds had eaten all four of the prey items (one of each species), a new set was presented. Training

25 ended when birds had eaten 3 sets of butterflies. No time constraint was imposed for training
26 and most birds completed it in less than 4 hours.

27

28 In order to familiarise birds with the experimental set-up, which was novel to them, they were
29 released in the experimental cage by groups of two to four birds for approximately one hour
30 the day before the experiment. Oat flakes, seeds and mealworms were dispersed over leaves
31 and vegetation to encourage searching for edible items in locations similar to where butterflies
32 would be placed during the experiment. After an hour, no visible oat flakes, seed or
33 mealworms could be found in the cage.

34

35 *Experiments.* The experimental set-up consisted of a 10m x 10m cage that had tarpaulin walls
36 and a ceiling of whitish dense net that let in natural sunlight. Butterflies were dispersed in a 5
37 x 5 grid, delimited by poles all around the borders and a rope defining rows and columns (see
38 Fig S3). Two extra poles were placed in the grid centre to increase the appeal of this area for
39 birds. Five specimens of each species (20 specimens in total) were placed in the grid, one per
40 cell. Butterflies were pinned to the top of meadowsweet leaves (*Filipendula ulmaria*) that had
41 naturally grown in the outdoor cages. Butterflies were always put in similar places within the
42 cell and could be easily seen from a nearby pole. Before the experiment, butterflies were
43 photographed over graph paper, used as a scale to measure butterfly size on Image J (Rueden
44 et al., 2017). Butterfly position was randomized but care was taken in 1) leaving the 5 cells
45 closest to the observer empty as birds tended to avoid this area, 2) avoiding having more than
46 two specimens of the same species in the same row or column, and 3) having two specimens
47 of the same species in neighbouring cells. This ensured that all species were evenly
48 represented along the grid. This random configuration was reshuffled between trials.

49

50 For each trial, an observer, hidden to the birds, watched from outside the cage through a small
51 window and took notes of which butterfly species were attacked and in which order. A GoPro
52 camera also recorded the experiments. A butterfly was considered detected only if a bird
53 directly approached to attack it, including when the attack failed. No bird was seen hesitating
54 during an attack once it had initiated it. Experiments took place between 9 am and 5 pm.
55 Before each trial, the radiance of ambient light (coming from the sun and sky) was measured
56 using an Ocean Optics spectrophotometer in the same location. We computed the total
57 radiance (TR) over the bird's spectral sensitivity, which range from 300-700 nm, to account
58 for the intensity of ambient light associated with each experimental trial in the statistical
59 analyses. Further information on weather conditions (cloudy, sunny, etc) was also recorded.
60 Experiments ended when a bird had eaten half of the available butterflies (ie. 10 butterflies) or
61 after 2 hours, whichever happened first. Wings were occasionally re-used if they had not been
62 damaged.

63

64 To control for any positional effect on overall species detection, we computed the probability
65 of a bird being present in a given grid area. To do so, a 10-minutes interval of each recorded
66 trial was selected and revised to calculate the proportion of time birds spent on the different
67 poles. The time intervals were possible for all trials as they all lasted at least 10 minutes and
68 were selected either as a result of the birds actively attacking prey or actively exploring the
69 cage during that time, based on notes taken by the observer. A total of 87% of all attacks
70 started from the pole closest to the grid cell, while all other attacks were initiated from a pole
71 situated only one grid cell further away. Thus, the probability of visiting a given cell was
72 calculated based on the amount of time spent by the bird on each pole, the number of "close"
73 (immediately next to) or "distant" (one grid cell removed) poles and the probability of visiting

74 them (0.87 for close grid cells and 0.13 for distant grid cells). These probabilities were later
75 used to divide the grid into four main areas according to bird occupancy: furthest and closest
76 corner to the observer, grid border and grid centre (Fig S4a). Most birds fed willingly on all
77 butterflies located on the borders of the grid. Given that butterfly species distribution was
78 random and reshuffled between trials, the four species were similarly represented in those
79 cells (Fig S4b), so no bias was expected.

80

81 **Colour and optical measurements**

82 Both the transmittance and reflectance of the transparent and opaque wing elements
83 respectively, were measured using spectrophotometry. All measurements were taken using a
84 spectrophotometer (Starline Avaspec-2048 L, Avantes) and a deuterium halogen lamp
85 (Avalight DHS, Avantes) emitting in the 300-700 nm range, including UV, to which birds but
86 not humans are sensitive (Chen & Goldsmith, 1986). To measure transmittance, illumination
87 and collection fibres were separated (FC-UV200-2-1.5 x 100, Avantes), aligned, and the wing
88 held perpendicularly at an equal distance of ~2mm from each fibre. Measurements were done
89 relative to a white reference (lights turned on with no sample) and a dark reference (light
90 turned off with no sample).

91 To measure reflectance, an optic probe (FC-UV200-2-1.5 x 100, Avantes) merging
92 illumination and collection angles was used. The fibre was kept in place with a small black
93 chamber that allowed measurements of reflection at 0°, perpendicularly to wing surface.
94 Samples were again placed at ~2mm from the fibre in front of a light trap to avoid parasitic
95 illumination and reflection. Measurements were relative to a white reference (WS2, Avantes)
96 and a dark reference (light on with the light trap in front). Measurements of both the forewing
97 and the hindwing were taken for one individual of each species. For each wing, 5 measures of

98 transmittance in different transparent areas and 1 measure of reflectance for each colourful
99 patch were taken. Values of transmittance were averaged, and both values of reflectance and
100 transmittance were used to predict butterfly detectability, as a result of the wings' optical
101 properties, by the "predators" used in the two different behavioural experiments.

102

103 **Models of bird and human vision**

104 We used vision modelling to predict detectability of butterfly species for both birds and
105 humans. Birds and humans are unable to detect linear polarization, and to form spatial images
106 of this property as they do with brightness or colour (Foster et al., 2018; Greenwood, Smith,
107 Church, & Partridge, 2003; Melgar, Lind, & Muheim, 2015; Montgomery & Heinemann,
108 1952); hence, birds and humans can use only brightness and colour to detect specimens and
109 discriminate between butterfly species.

110 The contrast perceived by birds and humans for each element of the butterfly colour pattern
111 was calculated using Vorobyev & Osorio's discriminability model (1998). As butterflies were
112 placed on leaves of living plants for all behavioural experiments, they all had green leaves as
113 background. The reflectance of an average green leaf (calculated using the average of 86
114 different leaves from tropical species (Gomez & Théry, 2007)) transmitted through the
115 transparent wing patches was therefore used. For behavioural experiments using birds as
116 predators, butterflies were seen against leaves in open habitat conditions (under direct
117 sunlight). Hence, for the bird vision model, we used open habitat ambient light conditions
118 (large gaps where sun is visible, similar to conditions present in the outdoor aviaries where we
119 performed bird experiments, Gomez & Théry, 2007), and blue tit photoreceptors, including
120 oil droplets that enhance colour discrimination (Misha Vorobyev, 2003), with relative cone
121 densities of 1: 1.92: 2.68: 2.7 for UVS:SWS:MWS:LWS (Hart, Partridge, Cuthill, & Bennett,

122 2000). We used a Weber fraction of 0.1 for the chromatic response (as reported for Pekin
123 robin *Leiothrix lutea* in (Maier & Bowmaker, 1993)) and 0.2 for the brightness response (as
124 the average reported values for known bird species (Lind, Karlsson, & Kelber, 2013)). In
125 behavioural experiments with humans, we used forest shade ambient light conditions (the
126 forest path we used for the experiment was typical of forest understorey, Gomez & Théry,
127 2007), and human photoreceptors (www.cvrl.org ; interpolated every nm) with relative cone
128 densities of 1:16:32 for SWS:MWS:LWS (Walraven, 1974). We also used a Weber fraction
129 of 0.018 for LWS in chromatic vision (Wyszecki & Stiles, 1982), and 0.11 for brightness
130 (Scholtyssek, Kelber, & Dehnhardt, 2008).

131 Colour and brightness contrast of butterflies resting on leaves were modelled for both bird and
132 human vision. For transparent wing areas (transparent patches for *I. salapia* and *B. seba*), the
133 ambient light was assumed to be transmitted by the wing, reflected on the leaf, and again
134 transmitted by the wing to reach the eye of the observer (see Fig S2). For opaque wing areas
135 (all coloured patches of *H. ninonia* and *C. tutia*, and the colourful opaque elements found in
136 the two transparent species), the ambient light had to be reflected by the wing to reach the eye
137 of the observer (see Fig S2). All contrasts were computed using the *pavo* package (Maia,
138 Eliason, Bitton, Doucet, & Shawkey, 2013) in R (R Foundation for Statistical Computing,
139 2014). Standardized weighed averages across all areas, weighed for the patch size and
140 standardized for the size of the individuals, were then calculated for chromatic and achromatic
141 contrast between species and green-leaf background.

142

143 **Detailed statistical analyses and results**

144 **Behavioural experiments using wild birds**

145 Birds took anywhere between 1 and 37 minutes (average: 7.54 ± 8.96 min) after release into
146 the experimental cage before initiating an attack. For three of the birds, the experiment ended
147 without having eaten 10 butterflies in the allocated 2 hours. The other 27 birds took between
148 11 and 112 minutes to attack all 10 butterflies (mean time to attack 10 butterflies: $40.76 \pm$
149 26.23 min). For all the trials combined, birds attacked 54% of the *H. ninonia* butterflies (the
150 most colourful species), 48.7% of the *C. tutia* (colourful but transparent species), 46.7% of the
151 *I. salapia* (transparent yellow-tinted butterfly) and 49.3% of the *B. seba* butterflies (most
152 transparent species).

153 To test whether birds detected different numbers of butterflies per species, a linear mixed
154 model, including bird ID as a random factor, was fitted. A binomial distribution was used for
155 the response variable (attacked or not), and the butterfly species, butterfly size, trial duration,
156 age and sex of the bird, time to first attack, first butterfly species found, butterfly position on
157 the grid (corner –furthest or closest to the observer-, grid side, grid centre), weather (as a
158 qualitative variable), and total radiance (TR), as well as their interactions, were all selected as
159 explanatory variables. The best fitting model was selected based on minimization of Akaike's
160 Information Criteria (AIC), assuming that models differing by two units or less were
161 statistically indistinguishable (Anderson, Burnham, & White, 1998). The best fitted model,
162 shown in Table S1, included time to first attack, and the position of the butterfly on the grid
163 (furthest or closest corner, border, centre. Fig. S3). According to the results, butterflies were
164 more likely to be attacked when they were in grid zones with a higher probability of a
165 predator being present, when a predator initiated attacks earlier in the experimental trial, and
166 when butterflies were located in the furthest corners from the observer. Thus, similar numbers
167 of butterflies were attacked between species (as species was not part of the best fitting model).

168

169 We also calculated an “inconspicuousness rank” that included the order in which butterflies
170 were found and the number of butterflies that were not attacked for each species (i.e.
171 inconspicuousness rank: Ihalainen, Rowland, Speed, Ruxton, & Mappes, 2012). For example,
172 if a bird captured two *H. ninonia* second and fifth in the sequence of captured prey, this
173 species gets a rank value of $2+5+3 \times 11=40$ for that trial. Therefore, highly conspicuous species
174 are characterized by lower inconspicuous rank values. We fitted a linear mixed effect model
175 to test for differences in rank for each species, assuming a normal distribution, with rank as
176 the response variable, bird individual as a random factor and butterfly species, age and sex of
177 the bird, date, time until first attack, first butterfly species found, weather as a qualitative
178 variable, and total radiance (TR) as explanatory variables. Again, the best fitting model was
179 selected using AIC minimization. According to the best fitted generalised linear mixed model,
180 butterfly species explained the variation in inconspicuous rank (Table S2). Butterflies were
181 more conspicuous when they were opaque, such as those belonging to the *H. ninonia* species.
182 In addition to the strong spatial distribution effect on butterfly attacks (detected on the number
183 of butterflies found), transparency was found to decrease butterfly detection.

184

185 **Behavioural experiments using human participants**

186 A total of 102 volunteers participated in the experiment (63 men and 39 women, with
187 10:11:21:18:31:11 in the A1 (<10): A2 (11-20): A3 (21-30): A4 (31-40): A5 (41-50): A6
188 (>51) age classes). Of these, 19 volunteers did the experiment before 13h30, 35 between
189 13h30 and 16h, and 48 after 16h. Participants found between 5 and 28 of the 40 butterflies
190 (12.75 ± 4.68 butterflies found per participant) and took between 7.5 and 37 minutes to
191 complete both corridors (18.04 ± 6.5 minutes spent in average per participant). For all the
192 trials combined, participants found 42.5% of the *H. ninonia* butterflies (the most colourful
193 species), 38% of the *C. tutia* (colourful but translucent species), 23.54% of the *I. salapia*

194 (transparent yellow-tinted butterfly) and 28.63% of the *B. seba* butterflies (most transparent
195 species).

196

197 Similar statistical analyses were performed for human experiments. First, a linear mixed
198 model was fitted to test for differences in the total number of butterflies per species that were
199 found, assuming a binomial distribution for the response variable (either found or not) and
200 including participant's ID as random factor. Butterfly species, first species found, butterfly
201 position, corridor, left or right side of the path, time of day, gender and age of the participant,
202 duration of the experiment, and their interactions, were all used as explanatory variables. A
203 minimization of Akaike's Information Criteria (AIC) was used to select the best model,
204 assuming that models differing by two units or less were statistically indistinguishable
205 (Anderson et al., 1998). According to the best fitted model (Table S3), participants found
206 more opaque butterflies (*H. ninonia*) than any other species ($z = 5.73, p < 0.001$). More
207 butterflies were found earlier than later in the day ($z = -2.80, p = 0.005$), by men ($z = 3.40, p$
208 < 0.001) and by younger participants ($z = -0.237, p = 0.019$). Smaller but significant effects
209 were found for: trial duration, the order in which butterflies were found, and the interactions
210 between species and trial duration, trial duration and gender, and time of day, age and gender.

211 As in the bird experiments, we also tested whether the order in which butterflies were found,
212 and the number of butterflies that were missed for each species, were related to differences in
213 transparency (i.e. inconspicuousness rank), assuming a Gaussian distribution for the
214 inconspicuousness rank, participant ID as a random factor, and butterfly species, first species
215 found, time of day, gender and age of the participant, duration of the experiment, and their
216 interactions, were all used as explanatory variables. The best fitted linear mixed model (Table
217 S5) shows that the most opaque butterfly species, *H. ninonia*, was the most conspicuous
218 followed by *C. tutia* and *B. seba*. More butterflies were detected when transparent butterflies,

219 especially *B. seba* and *I. salapia*, were detected first ($t = -12.085$, $p = 0.004$). Fewer
220 butterflies were missed in trials that were done on the second day ($t = -1.98$, $p = 0.05$). As for
221 birds, transparency decreases butterfly detection by humans.

222

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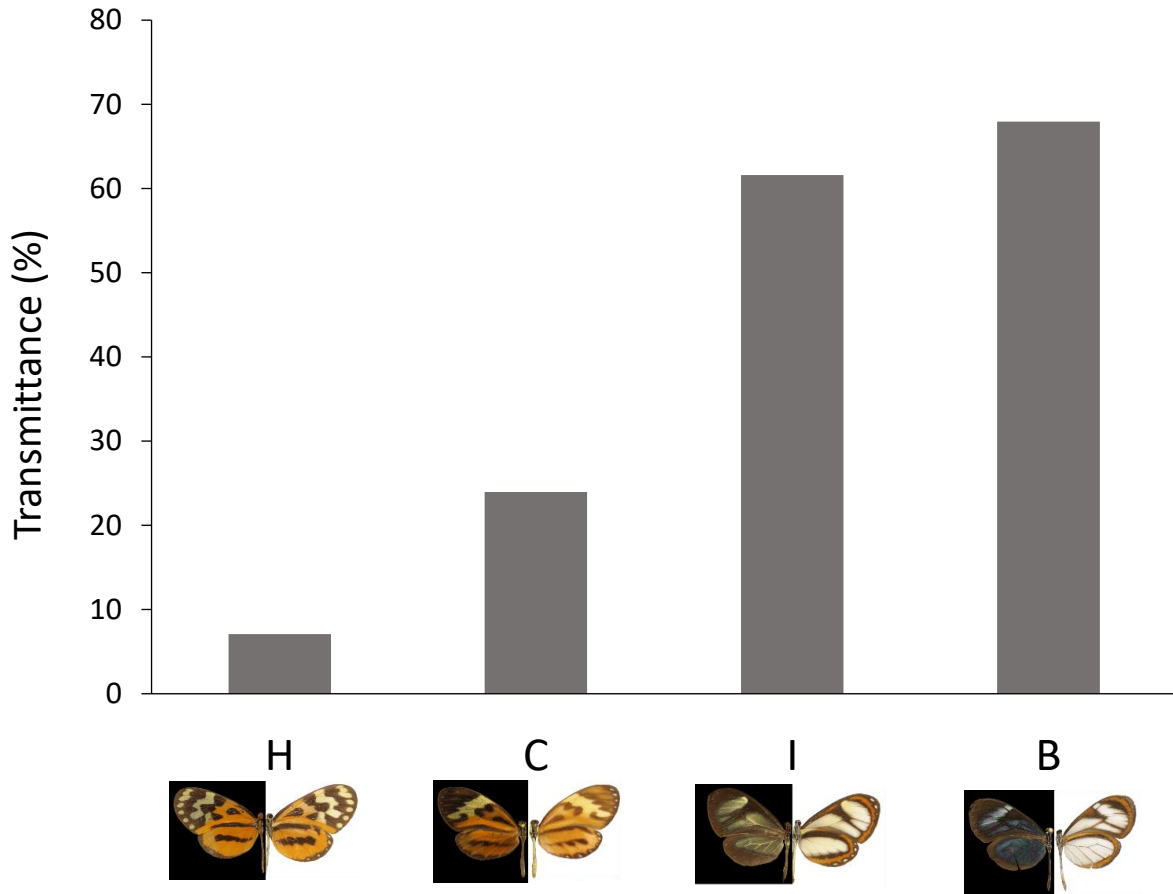
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277

Electronic supplementary Material

278 **Figures**

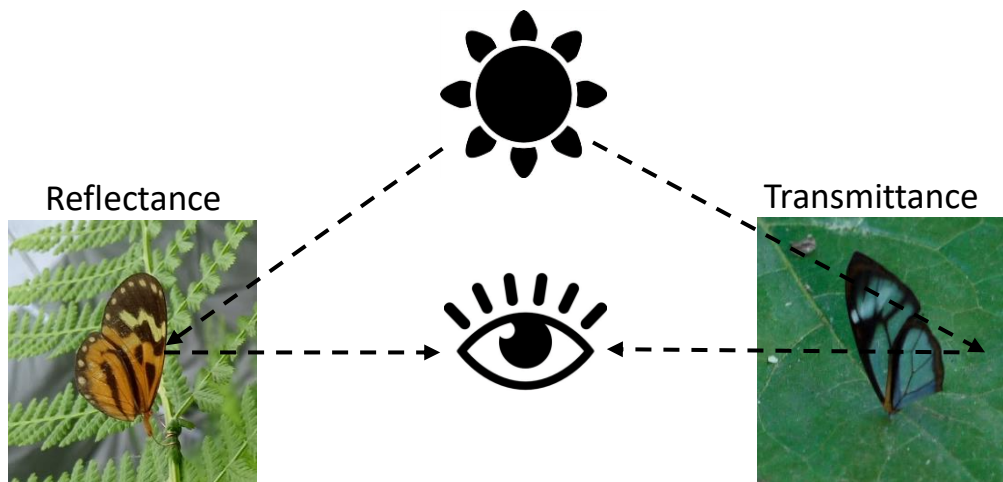
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281 Figure S1. Average transmittance values per butterfly species: the lower the value, the more
282 opaque the wing. The least detectable species are therefore expected to be the most
283 transparent *I. salapia* (I) and *B. seba* (B), as they have the highest transmittance values.

284



286

287 Figure S2. Diagram of how reflectance and transmittance were calculated for vision models.

288 Light reflection of opaque wing elements, as seen on the left of the figure, assumes only

289 reflection of the wing surface. Light transmission of transparent wing elements, as seen on the

290 right of the figure, assumes that light is transmitted through the wing, reflected by the leaves

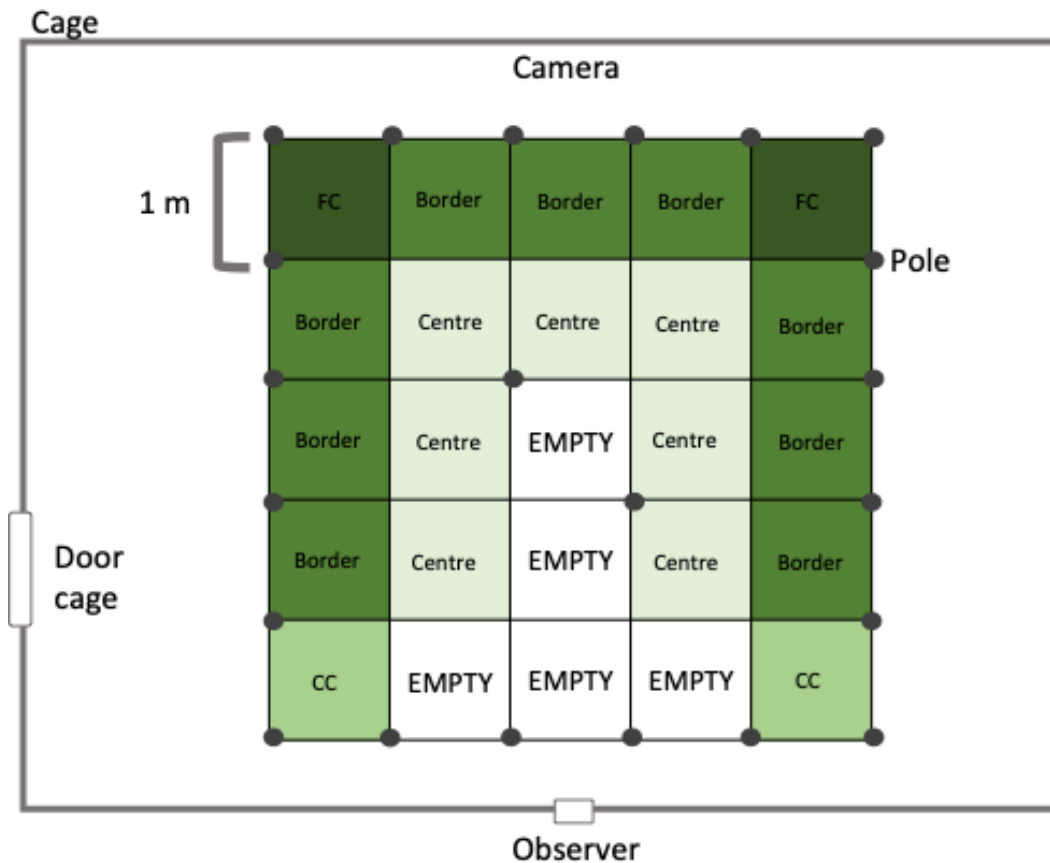
291 and transmitted again through the wing before reaching the observer's eye. Butterflies shown

292 were those used in behavioural experiments and consisted of real natural wings attached

293 together in the appropriate position with a thin wire. A mealworm was attached to those

294 artificial butterflies that were used for the experiments with birds (shown on the left).

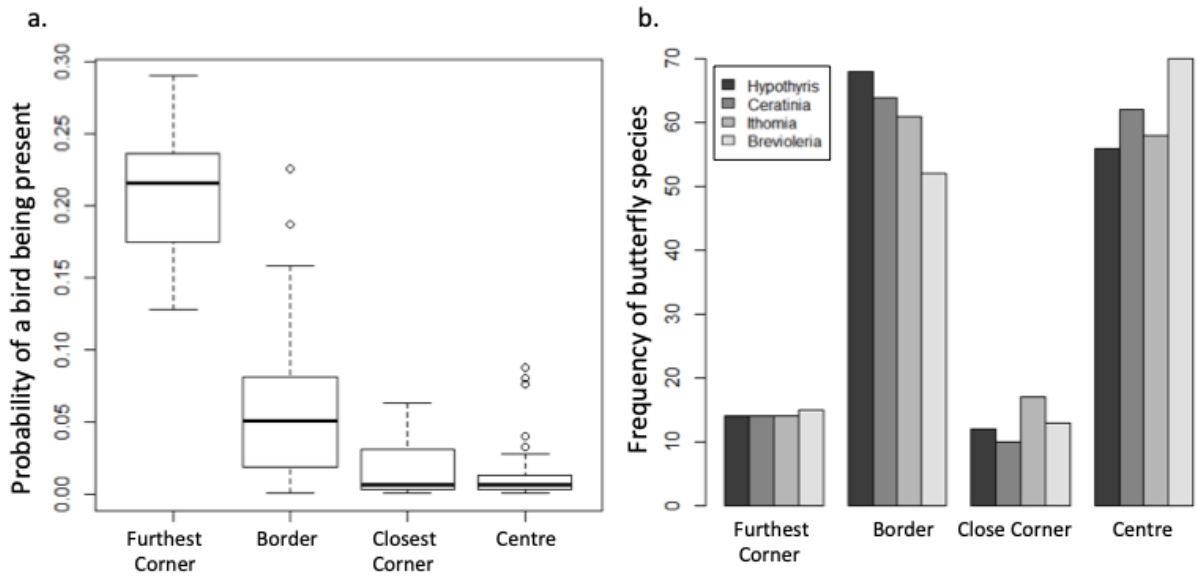
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297 Figure S3. Top view of the experimental arena in the outdoor cage used for the bird
 298 experiments. This arena was located within a cage made of tarpaulin walls and a ceiling
 299 consisting of a whitish dense net. The cage had a door to access the arena, which was closed
 300 during the experiment, and a small opening from which birds were released and where the
 301 observer could monitor the experiment (its location is indicated as “observer” in the diagram).
 302 Dots correspond to poles, which delimited the experimental arena, and rope was used to
 303 create the grid layout. Two additional poles were placed in the centre of the arena. A total of
 304 20 artificial butterflies (5 per species) were placed on the green squares (one per square), and
 305 never on the “empty” cells, which were avoided by birds, likely due to the proximity of the
 306 observer. Cells were divided into four main categories, according to a decreasing probability
 307 of being visited by a bird: FC (corner furthest to the observer), grid border, CC (corner closest
 308 to the observer) and grid centre. We used a camera, located opposite the observer, to record
 309 the experiment.

310



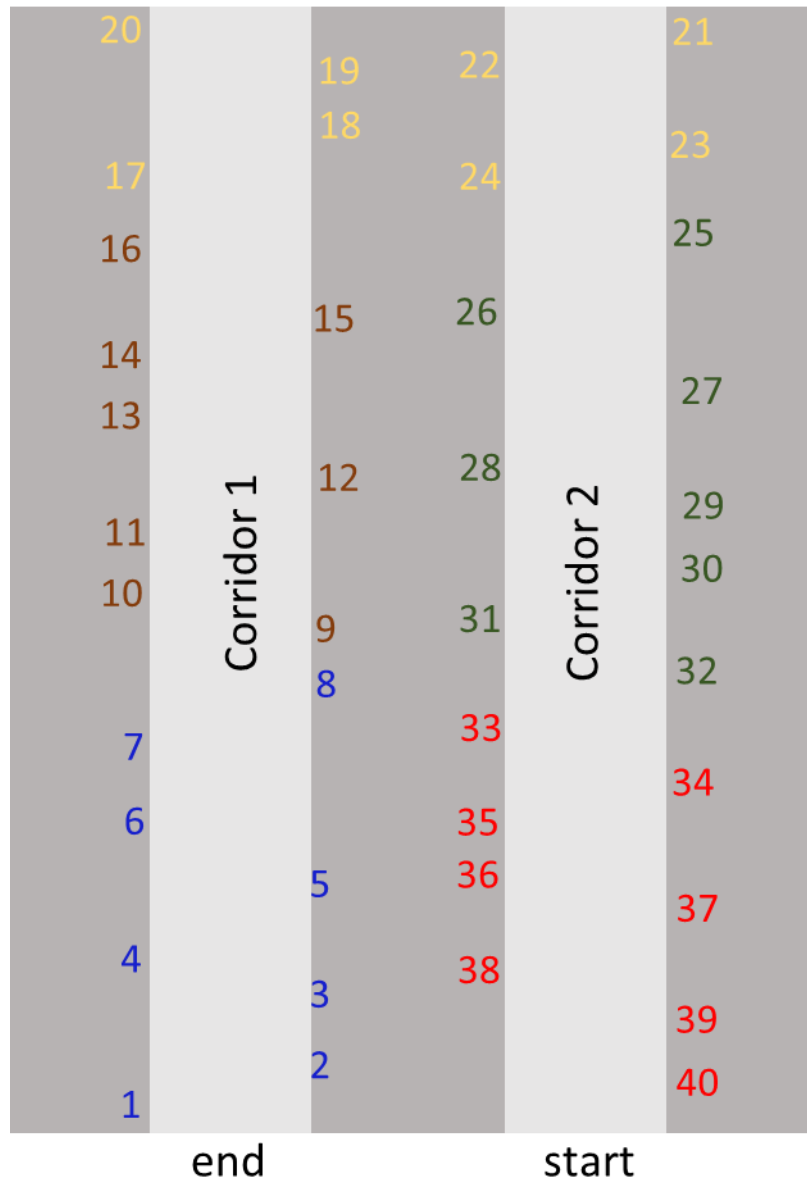
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312 Figure S4. Probability of a bird occupying different grid zones (a) and distribution of butterfly

313 specimens in the different zones (b).

314

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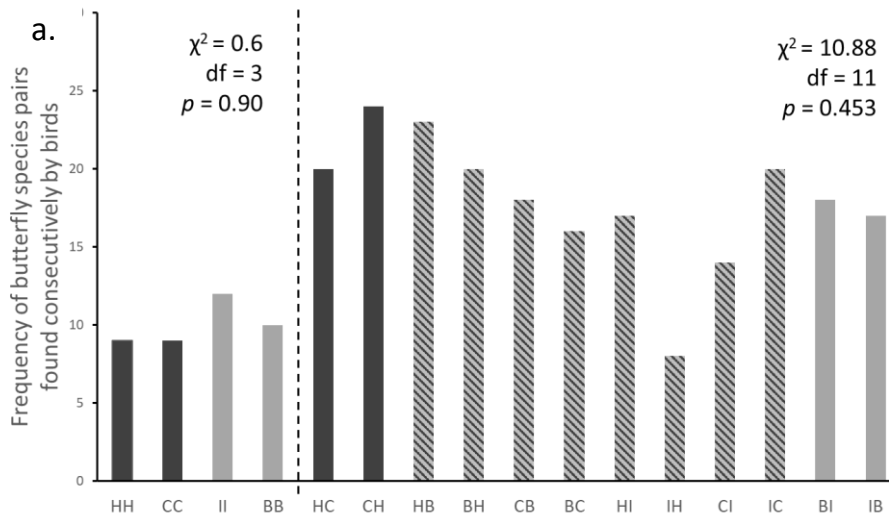
317 Figure S5. Example of an experimental trial with human participants. Numbers represent the
 318 order in which butterflies were distributed. The colours of the numbers represent the blocks
 319 that were randomised, and consisted of two butterflies of each species. Participants could start
 320 from either the first or the second corridor (the latter is shown on the diagram).

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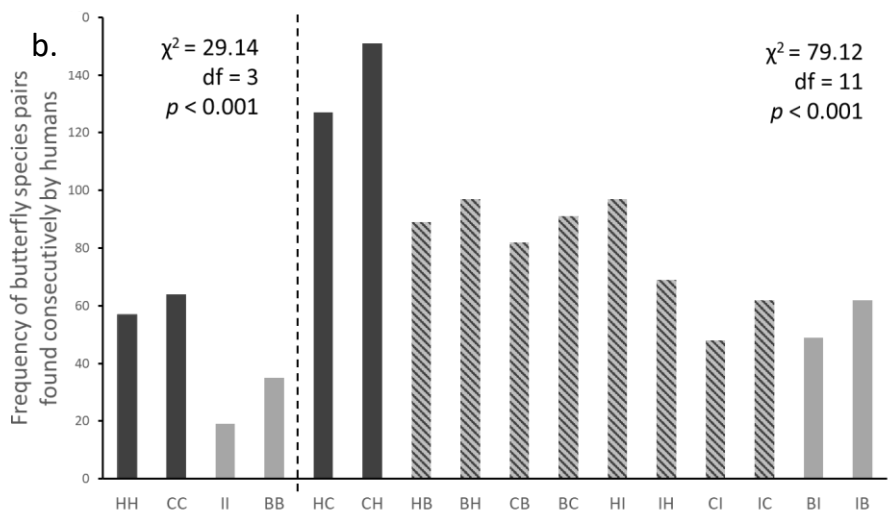
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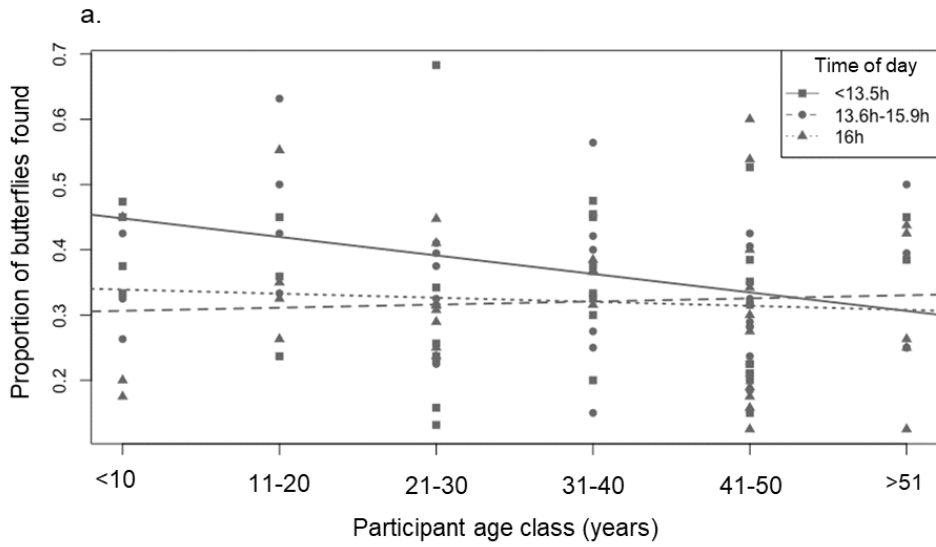


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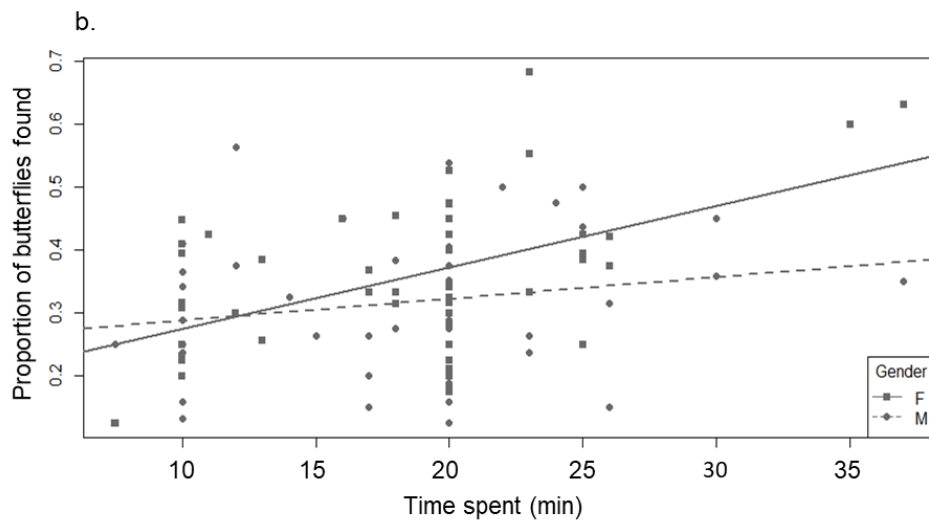


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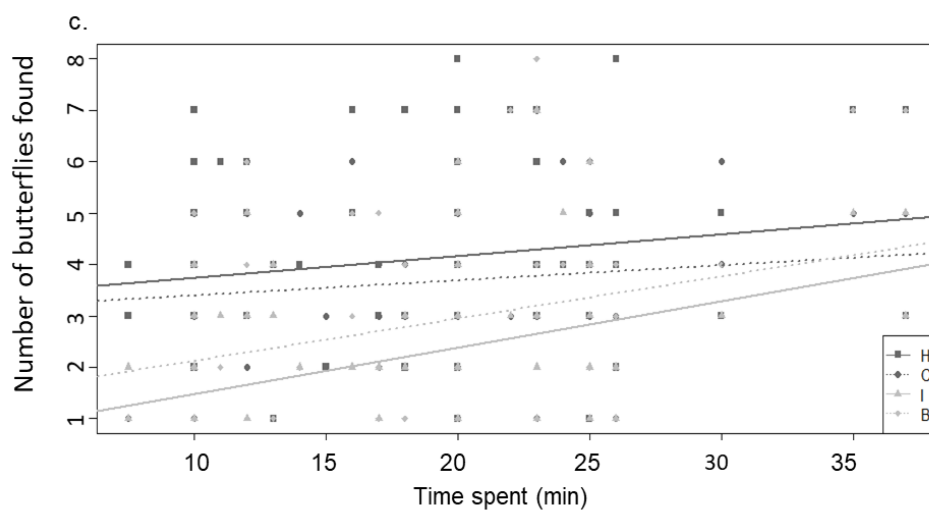
327 Figure S6. Frequency of pairs of butterflies of the same (left side of the dash line) or different
 328 species (right side of the dash line) found consecutively by a) birds and b) human participants.
 329 Dark bars represent pairs of the most colourful species (H and C), lighter bars represent pairs
 330 of the most transparent species (I and B) and light coloured bars with dashes represent pairs
 331 made up of one highly colourful and one highly transparent butterfly. The frequency with
 332 which butterfly pairs of the same species were found by both observers, and for pairs of
 333 different species found by birds, were compared using a chi-square test. The frequency with
 334 which pairs of butterflies of different species were found consecutively by human participants
 335 (bars on the right side of the dash line), were compared against the frequency of placing those
 336 different species consecutively in the experimental set-up. Butterfly species, from most
 337 opaque to most transparent, are (*H. ninonia* (H) > *C. tutia* (C) > *I. salapia* (I) ~ *B. seba* (B)).



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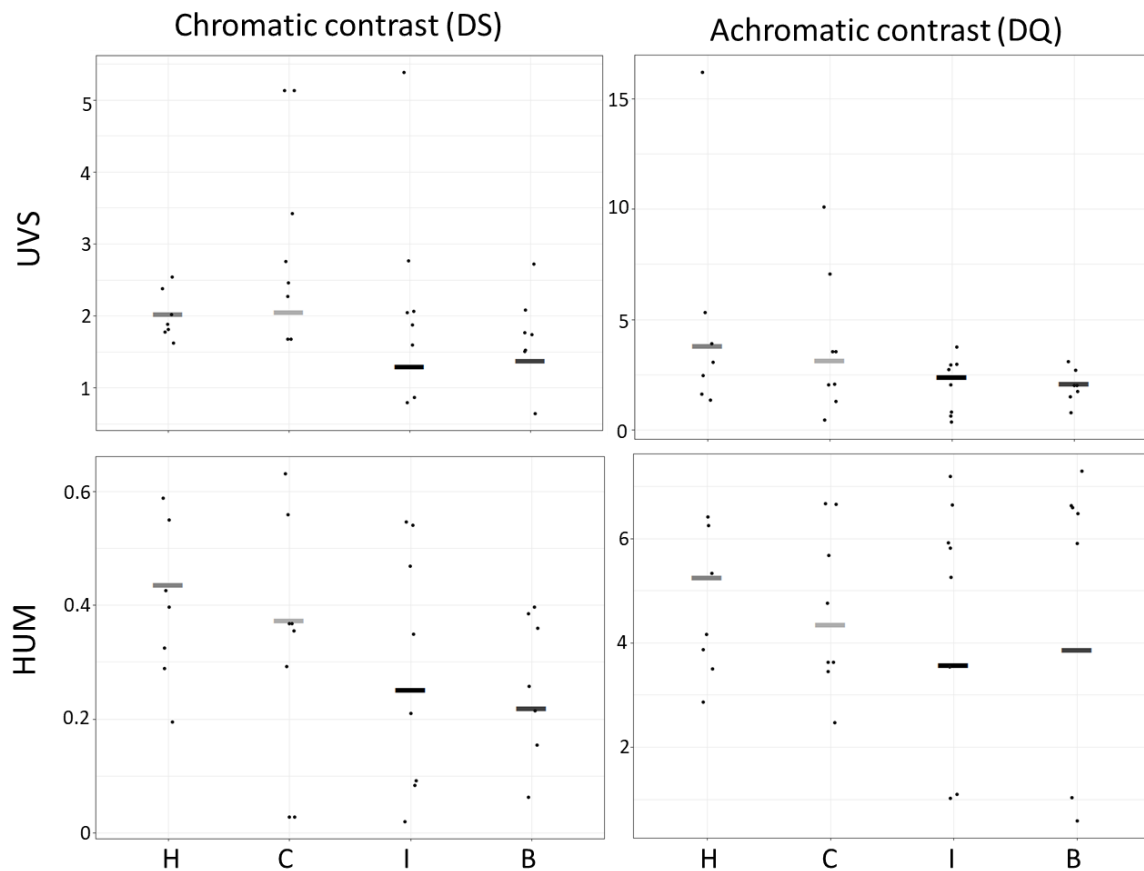


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343 Figure S7. Correlation between the proportion of butterflies found by human participants and
344 a) their age and the time of day at which trials were done (both shown as categorical data);
345 and b) the duration of the experiment and gender. c) The number of butterflies found for each
346 species according to the time spent completing the experiment by human participants. Factor
347 interactions that affected the total number of butterflies found (see Table S4) and butterfly
348 inconspicuousness rank (see Table S5) were also plotted. As such, regression lines shown in
349 panel **a** are for the proportion of butterflies found as a function of age for each interval of the
350 time of day (values for these intervals, and p-values testing for slopes different from zero, are:
351 <13.5h: $r^2 = 0.04$, $p = 0.206$; 13.6h-15.9h: $r^2 = 0.314$, $p = 0.75$; >16h: $r^2 = -0.013$, $p = 0.54$).
352 Regression lines shown in panel **b** are for the proportion of butterflies found as a function of
353 time spent by each gender (Women: $r^2 = 0.25$, $p < 0.001$; Men: $r^2 = 0.022$, $p = 0.12$).
354 Regression lines shown in panel **c** show that time spent on the experiment resulted in higher
355 numbers of butterflies found, especially for the transparent species (H: estimate slope= 0.043,
356 $r^2 = 0.014$, $p = 0.12$; C: estimate slope= 0.03, $r^2 = 0.005$, $p = 0.22$; I: estimate slope= 0.090, r^2
357 = 0.12, $p < 0.001$; B: estimate slope= 0.08, $r^2 = 0.07$, $p = 0.003$). Letters in the legend of panel
358 **c** stand for species names: *H.ninonia* (H), *C.tutia* (C), *I. salapia* (I), and *B. seba* (B). Butterfly
359 transparency increases from top to bottom of the legend (i.e. H<C<I<B).



361

362 Figure S8. Chromatic (DS, plots on the left) and achromatic (DQ, plots on the right) contrasts

363 (expressed in just noticeable difference units, JNDs) between butterfly wing colour patches

364 and a green-leaf background for blue tit vision under large gap light conditions (top) and for

365 humans under forest shade light conditions (bottom). Light conditions used in the models

366 were those present during each behavioural experiment. Each dot corresponds to the contrast

367 calculated between each given colour and the green-leaf background. Horizontal lines

368 represent a standardized weighed average across all areas, weighed by patch size and

369 standardized for butterfly size. Transparent parts were assumed to transmit leaf colour.

370 Opaque patches for all species were always considered in reflectance. *H. ninonia* (H) and *C.*371 *tutia* (C) were modelled under the “reflectance” scenario, while *I. salapia* (I) and *B. seba* (B),

372 the transparent species, were modelled under the “transmittance” scenario (see materials and

373 methods).

374 **Tables**

375 Table S1. Generalised linear mixed model (GLMM) results for the best-fitting model
 376 explaining the likelihood of butterflies being attacked in the bird experiments (binomial
 377 distribution).

Explanatory Variables	Estimate	Std. Error	z value	Pr(> z)
Intercept	0.443	0.16	2.76	0.006 *
Time of first attack	-0.03	0.01	-2.32	0.020 *
FurthestCorner&Border>all	1.24	0.14	9.13	<0.001 ***
FurthestCorner>Border	0.48	0.21	2.23	0.026 *
ClosestCorner >Centre	0.36	0.17	2.15	0.031 *

378

379 Explanatory variables are the time before the first attack and the general position on the grid
 380 (see Fig. S3). Comparisons correspond to: 1) more attacks on the grid borders and the corners
 381 located furthest from the observer than on the rest of the grid, 2) more attacks in corners than
 382 on the grid borders, 3) more attacks on corners located closest to the observer than in the
 383 centre of the grid. z corresponds to the values from the Wald z test used to test for factor
 384 significance. Symbols: ***p<0.001, *p<0.05.

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396 Table S2. Linear mixed model (LMM) results for the best-fitting model explaining the
 397 inconspicuousness rank for each species used in the bird experiments.

Explanatory Variables	Estimate	Std Error	t value	<i>p</i>
Intercept	41.775	0.539	77.53	<0.001 ***
Species.H>C,I,B	-0.981	0.311	-3.15	0.002 **
Species.C>B	0.411	0.880	0.47	0.641
Species.I>B	-0.455	0.880	-0.52	0.606

398 Butterfly species was the explanatory variable. Species from most opaque to most transparent
 399 are *H. ninonia* (*H*)> *C. tutia* (*C*) > *I. salapia* (*I*)~ *B. seba* (*B*). Symbols: ** p<0.01,
 400 ***p<0.001.

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402 Table S3. The number of times a species was found first in a given trial, either by birds or
 403 humans.

Species	Birds	Humans
<i>H. ninonia</i>	15	43
<i>C. tutia</i>	7	27
<i>I. salapia</i>	5	14
<i>B. seba</i>	3	18

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 408 Table S4. GLMM results for the best-fitting model explaining the number of butterflies found
 409 by human observers (binomial distribution).

Explanatory variables	Estimate	Std. Error	<i>z</i>	<i>p</i>
Intercept	-1.311	0.380	-3.45	<0.001 ***
Corridor	0.223	0.071	3.14	0.002 **
Species. H > C, I, B	0.616	0.108	5.73	<0.001 ***
Species. C > B	0.004	0.141	0.03	0.976
Species. B > I	0.221	0.162	1.37	0.171
Time of day	-0.416	0.149	-2.80	0.005 **
Gender M	1.031	0.304	3.40	<0.001 ***
Age Group	-0.237	0.101	-2.34	0.019 *
Time spent (min)	0.056	0.011	5.21	<0.001 ***
Butterfly block	0.092	0.025	3.70	<0.001 ***
Species. H > C, I, B: Time spent	-0.015	0.005	-2.75	0.006 **
Species. C > B: Time spent	0.005	0.007	0.69	0.488
Species. I > B: Time spent	0.004	0.008	0.56	0.573
Time spent : Gender M	-0.043	0.014	-2.96	0.003 **
Time of day: Gender F: Age Group	0.097	0.043	2.27	0.023 *
Time of day: Gender M: Age Group	0.047	0.039	1.22	0.223

410 Explanatory variables are: corridor, species, time of day, participant age and gender, order of
 411 butterfly position in the experimental sequence (butterfly block), time spent on the
 412 experiment, and the following interactions: species and time spent on the experiment, time of
 413 day, and participant age and gender, and time of day and gender. Butterfly species, from most
 414 opaque to most transparent are (H > C > I ~ B or *H. ninonia* > *C. tutia* > *I. salapia* ~ *B. seba*).
 415 *z* corresponds to the values from the Wald *z* test used to test for factor significance. Symbols:
 416 * *p* <0.05, ** *p*<0.01, ****p*<0.001.

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419 Table S5. LMM results for the best-fitting model explaining inconspicuousness rank for each
 420 species used in the experiments with human participants (Gaussian distribution).

Explanatory variables	Estimate	Std. Error	t value	<i>p</i>
Intercept	108.24	43.681	2.48	0.014 *
Species. H > C, I, B	-1.467	0.370	-3.96	<0.001***
Species. C > B	-5.042	1.048	-4.81	<0.001***
Species. I > B	5.114	1.048	4.88	<0.001***
First Found. C > H, I, B	-6.425	1.832	-3.51	0.0007 *
First Found. H > I, B	1.532	2.344	0.65	0.515
First Found. I > B	-2.761	5.687	-0.485	0.628
Date. Day1	10.782	12.894	0.836	0.405
Date. Day2	-20.890	10.527	-1.984	0.050 *
Date. Day3	-7.925	13.382	-0.592	0.555
Date. Day4	28.553	15.919	1.794	0.076
Date. Day5	-9.695	10.883	-0.891	0.375
Date. Day6 > Day 7	-5.428	33.586	-0.162	0.872
Time of day	4.554	11.226	0.406	0.686
Gender M	16.048	24.819	0.646	0.520
Age Group	6.552	7.436	0.88	0.381
Time of day: GenderM	-3.684	10.135	-0.363	0.717
Time of day: Age Group	-3.184	3.017	-1.05	0.294
GenderM: Age Group	-3.944	4.590	-0.86	0.392

421 Explanatory variables are: butterfly species, time of day, participant age and gender, first
 422 butterfly species found, date, and the interactions between: time of day and gender, time of
 423 day and age, gender and age. Butterfly species, from most opaque to most transparent are (H
 424 > C > I ~ B or *H. ninonia* > *C. tutia* > *I. salapia* ~ *B. seba*). t corresponds to the values from
 425 the t-test used to test for factor significance. Symbols: * $p < 0.05$, *** $p < 0.001$.

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