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

2

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15

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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  $\chi^2$  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  $\chi^2$ .

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  $\chi^2$  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  $\chi^2$  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  $\chi^2$   
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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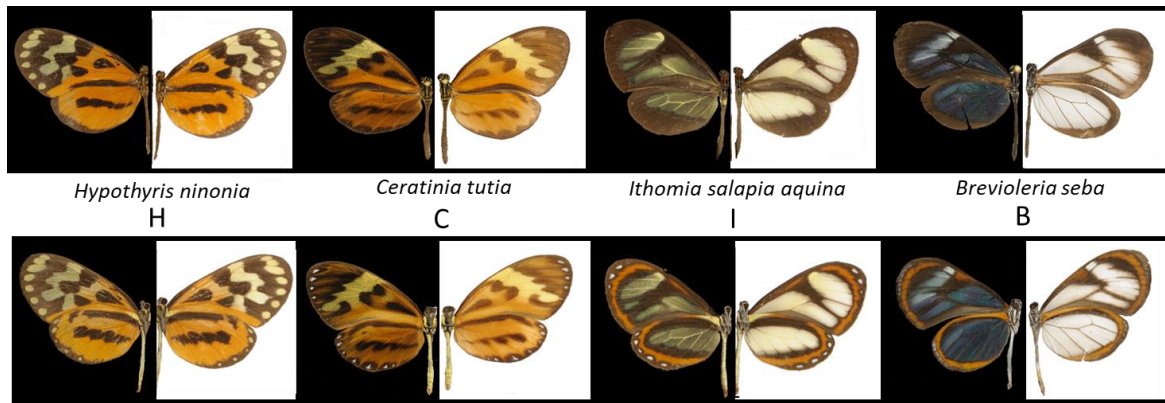
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666 **Figures**

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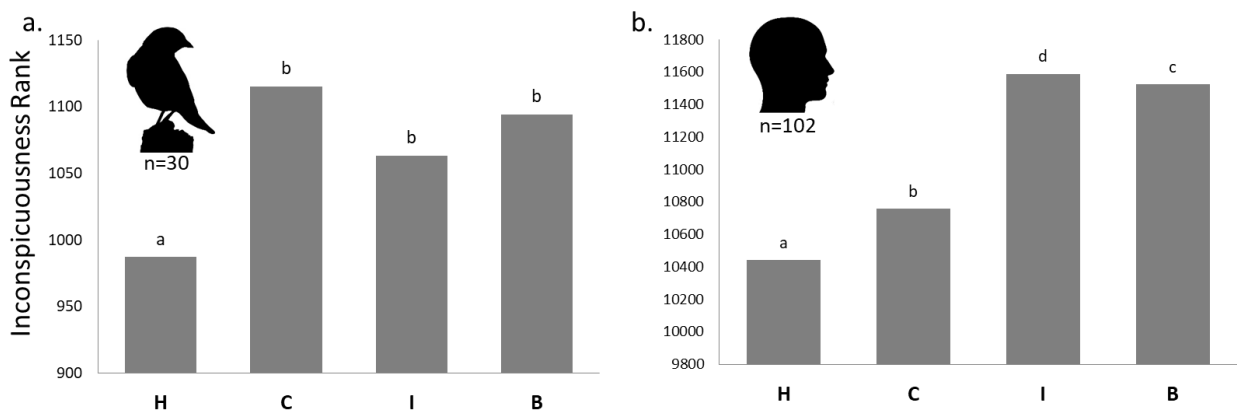
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**Figure 1.** Dorsal (top row) and ventral (bottom row) view of butterfly species used in the study (photographed against a black and a white background to show the location and degree of transparency in the wings). Wing transparency (transmission 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* (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). ©

Céline Houssin



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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](http://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:  $7.54 \pm 8.96$  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 ( $12.75 \pm 4.68$  butterflies found per participant) and took between 7.5 and 37 minutes to  
191 complete both corridors ( $18.04 \pm 6.5$  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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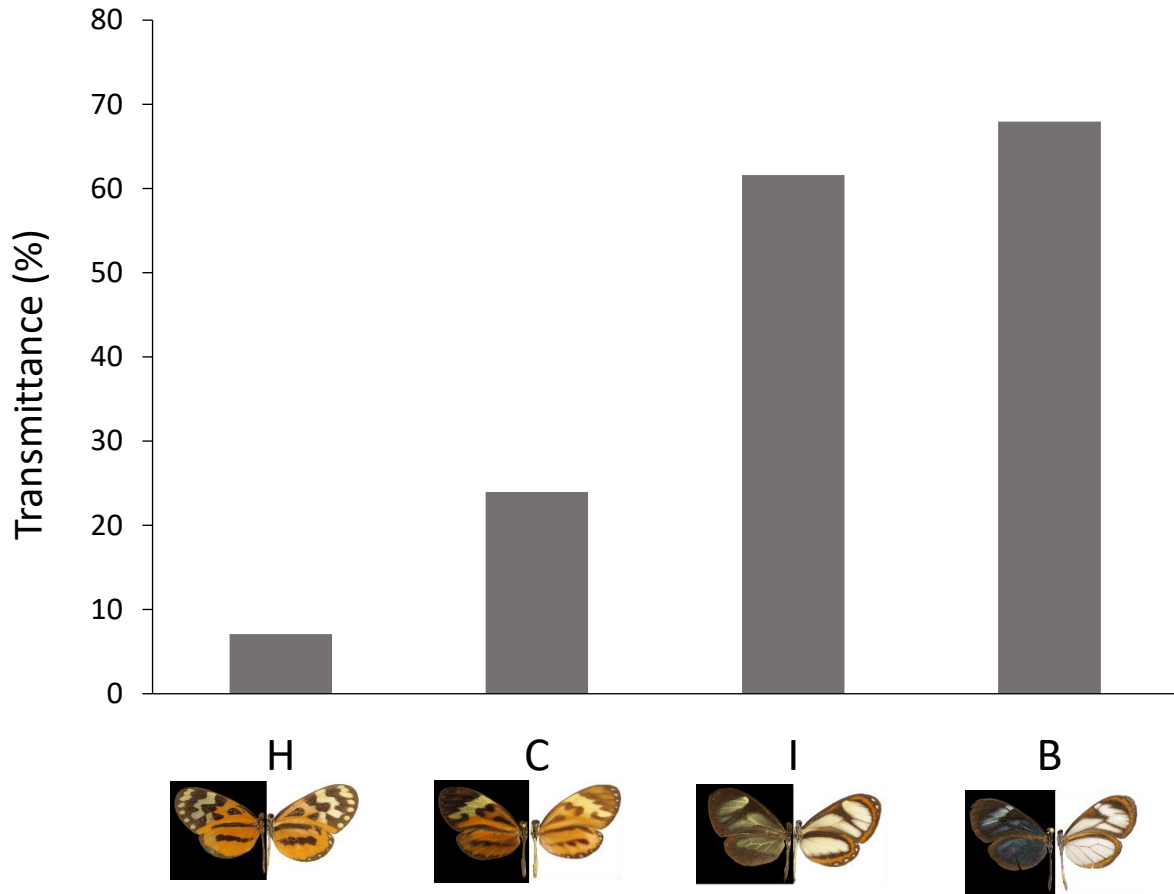
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277

### Electronic supplementary Material

278 **Figures**

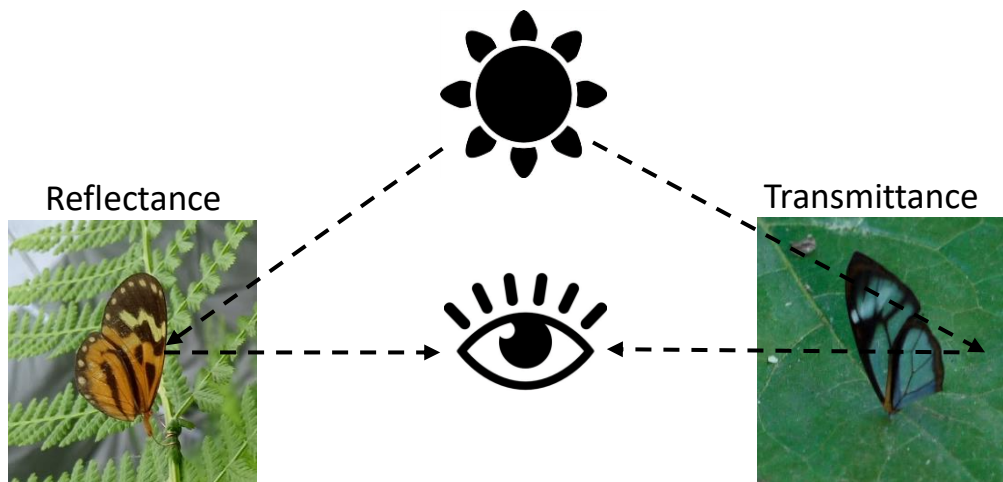
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280

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

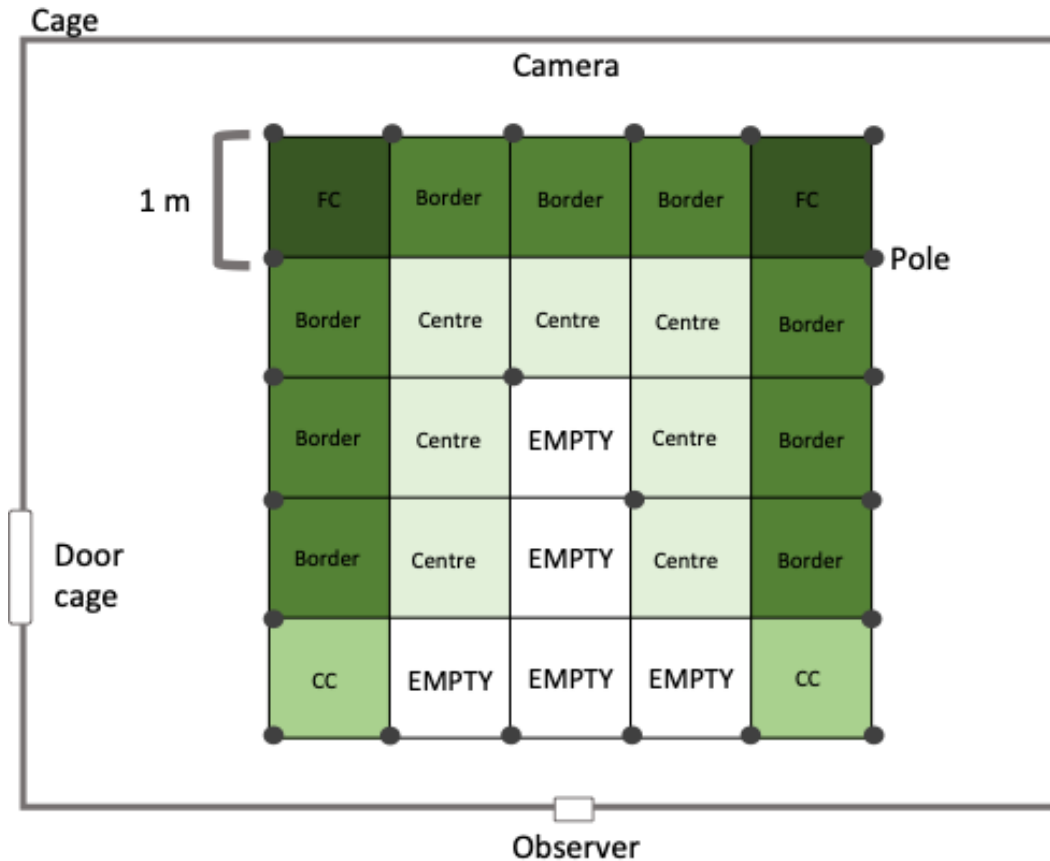
291 and transmitted again through the wing before reaching the observer's eye. Butterflies shown

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).

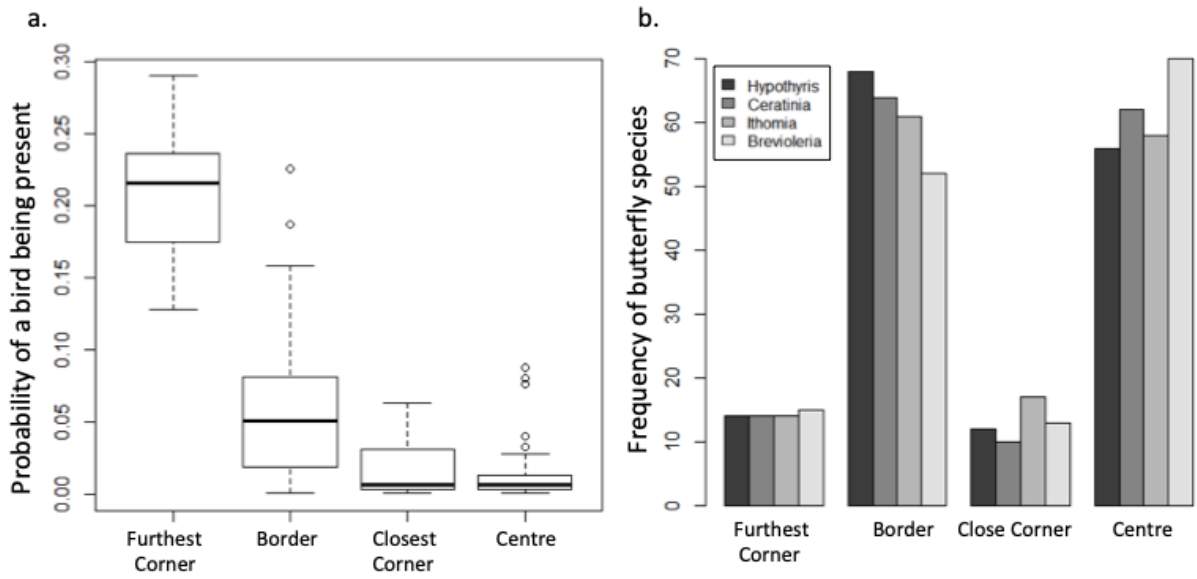
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297 Figure S3. Top view of the experimental arena in the outdoor cage used for the bird  
 298 experiments. This arena was located within a cage made of tarpaulin walls and a ceiling  
 299 consisting of a whitish dense net. The cage had a door to 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



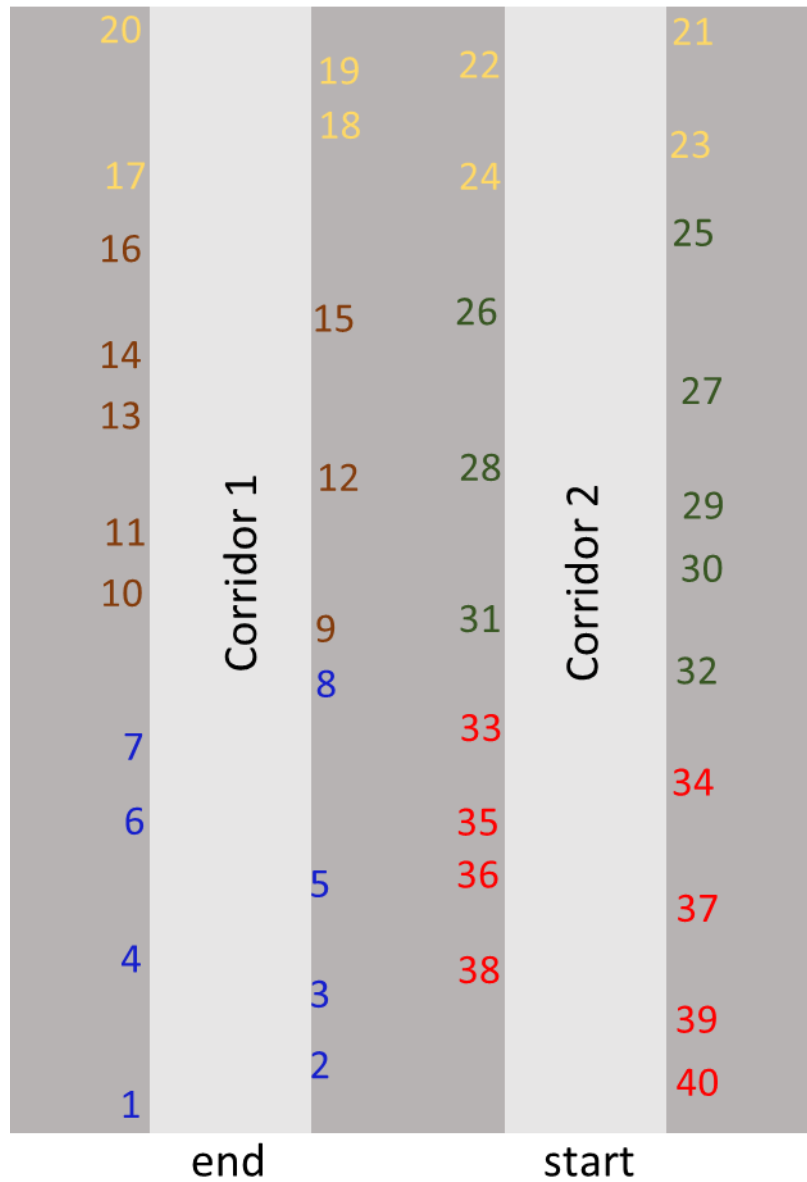
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312 Figure S4. Probability of a bird occupying different grid zones (a) and distribution of butterfly

313 specimens in the different zones (b).

314

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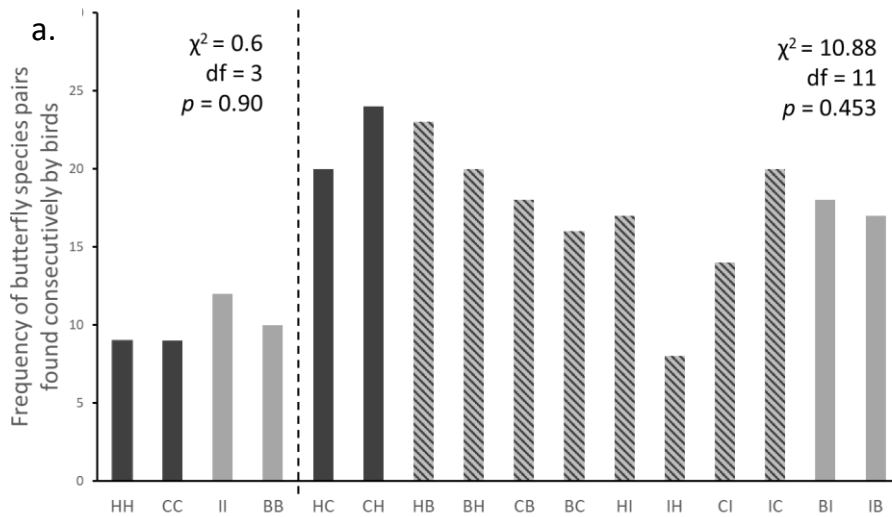
317 Figure S5. Example of an experimental trial with human participants. Numbers represent the  
 318 order in which butterflies were distributed. The colours of the numbers represent the blocks  
 319 that were randomised, and consisted of two butterflies of each species. Participants could start  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

321

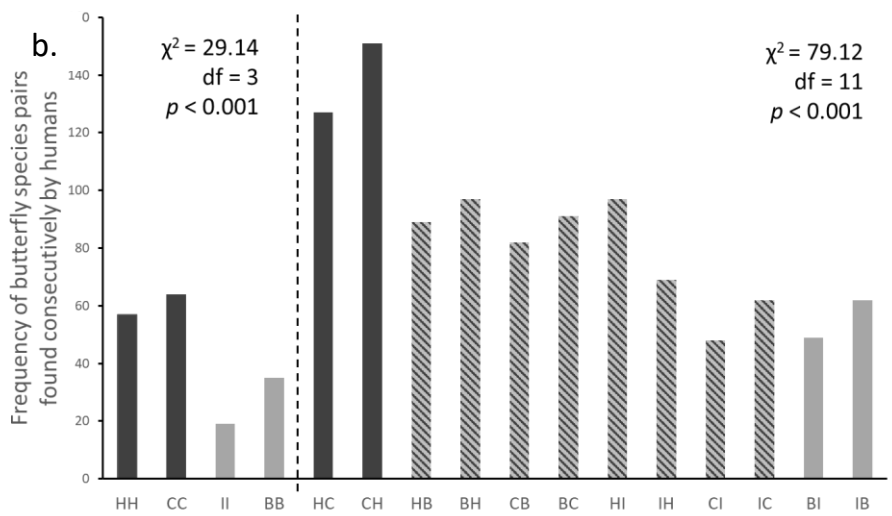
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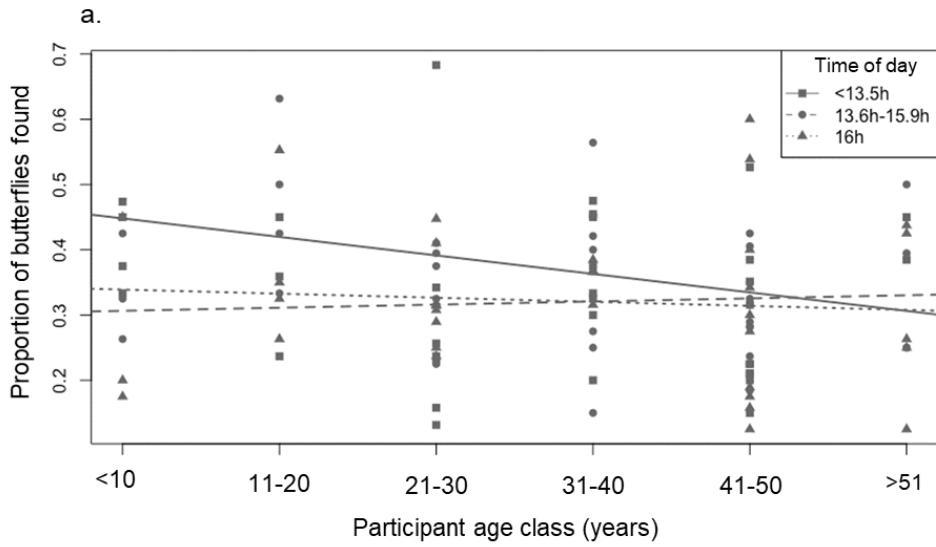


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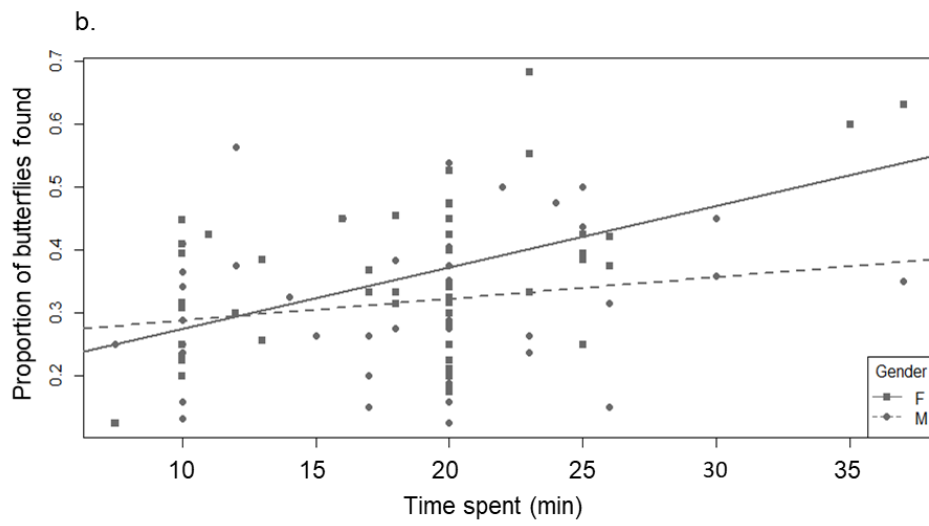


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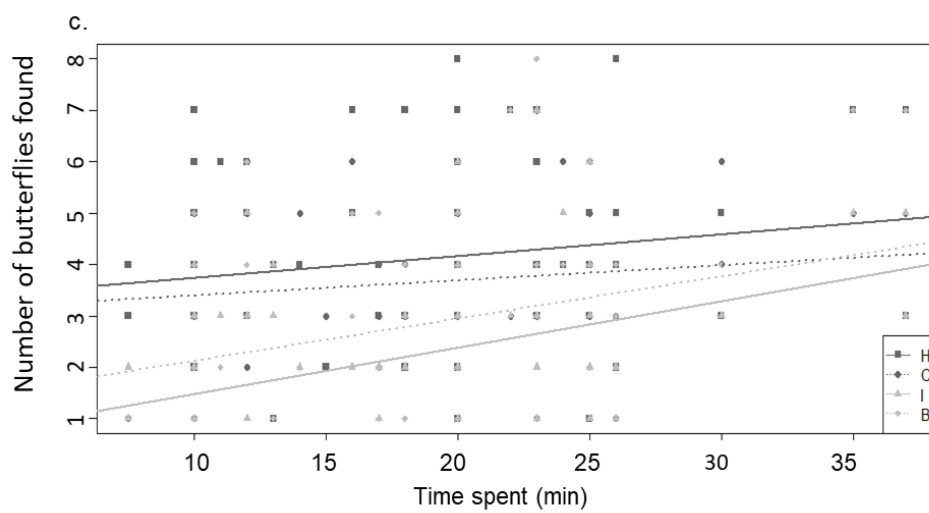
327 Figure S6. Frequency of pairs of butterflies of the same (left side of the dash line) or different  
 328 species (right side of the dash line) found consecutively by a) birds and b) human participants.  
 329 Dark bars represent pairs of the most colourful species (H and C), lighter bars represent 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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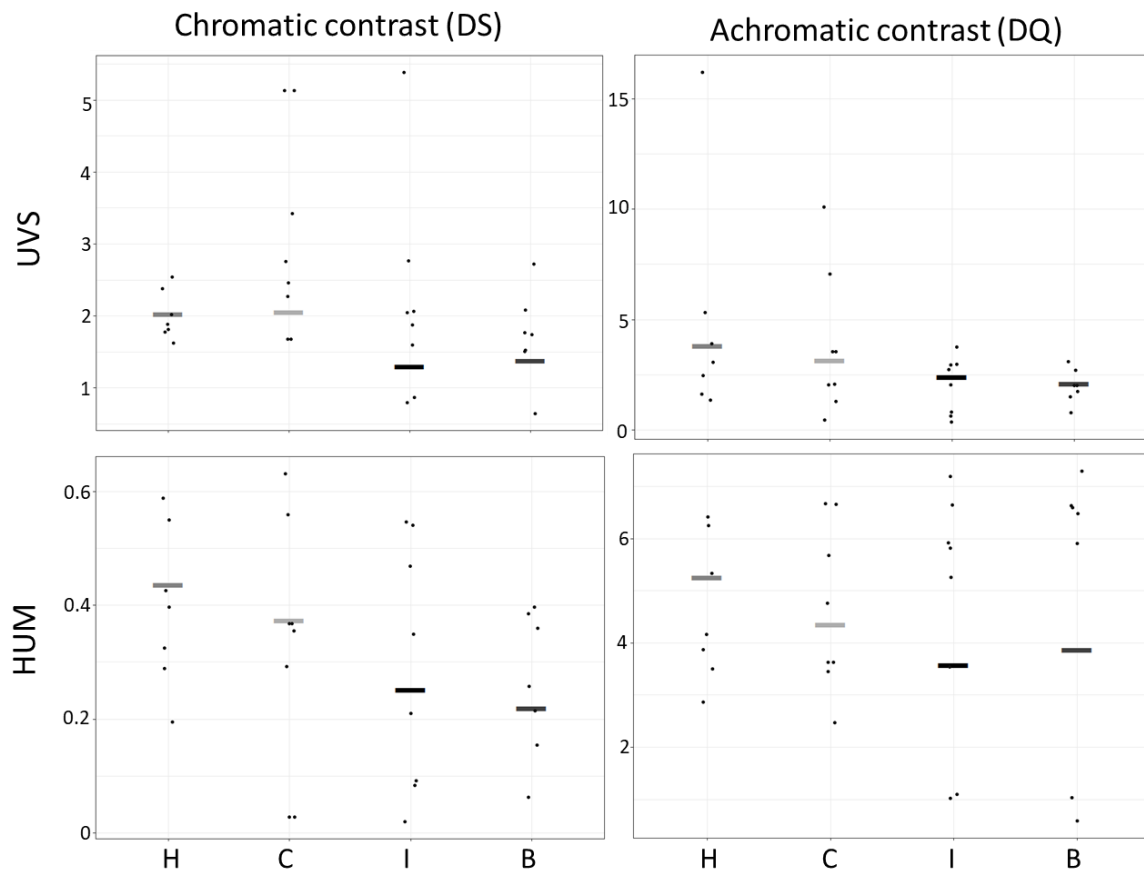


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342

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).



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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 *

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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.

401

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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