

1 **Transparency reduces predator detection in chemically protected clearwing butterflies**

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18

19 **Abstract**

20 1. Predation is an important selective pressure and some prey have evolved warning
21 colour signals advertising unpalatability (i.e. aposematism) as an antipredator
22 strategy. Unexpectedly, some butterfly species from the unpalatable tribe Ithomiini
23 possess transparent wings, an adaptation rare on land but common in water where it
24 helps avoiding predator detection.

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

35 **Introduction**

36 Predation is an important selective pressure and a strong evolutionary force shaping prey
37 coloration. As a way to avoid predator detection, some prey have evolved colours and
38 textures that mimic those of the background, hence rendering them cryptic (Endler, 1988). In
39 midwater environments where there is nowhere to hide, crypsis can be achieved by different
40 means, including transparency (Johnsen, 2014). Transparency is common in aquatic
41 organisms where it has been shown to decrease detectability by visual predators, enabling
42 prey to blend in with their environment (Kerfoot, 1982; Langsdale, 1993; Tsuda, Hiroaki, &
43 Hirose, 1998; Zaret, 1972). By contrast, transparency is generally rare in terrestrial
44 organisms, except for insect wings, which are made of chitin, a transparent material. The lack
45 of pigments in these wings is sometimes accompanied by anti-reflective nanostructures that
46 render them highly transparent, such as in cicadas and damselflies (Watson, Myhra, Cribb, &
47 Watson, 2008; Yoshida, Motoyama, Kosaku, & Miyamoto, 1997). However, Lepidoptera
48 (named after ancient Greek words for scale – *lepis* – and wing *-pteron*) are an exception as
49 their wings are generally covered with colourful scales that are involved in intraspecific

50 communication (Jiggins C. D., Estrada C., & Rodrigues A., 2004), thermoregulation
51 (Miaoulis & Heilman, 1998), water repellence (Wanasekara & Chalivendra, 2011), flight
52 enhancement (Davis, Chi, Bradley, & Altizer, 2012), and antipredator strategies such as
53 crypsis (Stevens & Cuthill, 2006), masquerade (Suzuki, Tomita, & Sezutsu, 2014) and
54 aposematism (i.e. advertisement of unpalatability, 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 They are thought to be unpalatable due to the accumulation of pyrrolizine alkaloids collected
59 from Asteraceae, Boraginaceae and Apocynaceae plants (Brown, 1984, 1985). Many of these
60 clearwing butterflies represent classic examples of aposematic prey, whereby bright colour
61 patterns – often with orange, yellow and black - advertise their unprofitability to predators
62 (Mappes, Marples, & Endler, 2005; Poulton, 1890). Bright contrasting and aposematic
63 coloration is likely to be the ancestral state in the group, since sister lineages (Tellerveni and
64 Danaini) are opaque and aposematic (Freitas & Brown, 2004). However, transparency has
65 evolved to some degree in approximately 80% of clearwing butterfly species, even though
66 many retain minor opaque and colourful wing elements (Beccaloni, 1997; Elias, Gompert,
67 Jiggins, & Willmott, 2008; Jiggins, Mallarino, Willmott, & Bermingham, 2006). Since
68 transparency is often associated with crypsis, for example in aquatic organisms (Johnsen,
69 2014), transparency may have evolved in these butterflies to reduce their detectability by
70 predators.

71

72 To determine if transparency in clearwing butterflies decreases detectability by visual
73 predators, we compared predator detection of four Ithomiini species that differed in the
74 amount of transparency of their wings (Fig S1): *Hypothesis ninonia* (largely opaque),

75 *Ceratinia tutia* (transparent but brightly coloured), *Ithomia salapia* (transparent with a pale
76 yellow tint) and *Brevioleria seba* (transparent without colouration other than a white band in
77 the forewing). Given the proportion of light that is transmitted through the butterfly wing of
78 the different species (Fig S2), we predicted that the opaque species *Hypothyris ninonia*
79 should be easiest to detect, followed by the transparent but coloured *Ceratinia tutia*. Finally,
80 the more transparent butterfly species *Ithomia salapia* and *Brevioleria seba* should be the
81 least detectable. We tested our predictions using two complementary behavioural
82 experiments, further supported by a vision modelling approach.

83

84 Detectability of butterflies was first tested using wild great tits (*Parus major*) as model bird
85 predators. Great tits are highly sensitive to UV wavelengths (UVS vision in Ödeen, Håstad,
86 & Alström, 2011). Their vision is similar to that of naturally occurring Ithomiini predators
87 like the houtouc motmot (*Momotus momota*, Pinheiro, Medri, & Salcedo, 2008), the fawn-
88 breasted tanager (*Pipraeidea melanonota*, (Brown & Neto, 1976) or the rufous-tailed tanager
89 (*Ramphocelus carbo* (Brower, Brower, & Collins, 1963). However, great tits are naïve to
90 ithomiine butterflies and they do not associate their colour patterns to toxicity. As bird
91 responses are the result of both prey detection and motivation to attack the prey, we
92 performed behavioural experiments using human participants, which can prove to be useful
93 in disentangling these two factors. Despite differences in colour perception between humans
94 and birds (both of which are visual predators), humans have been found to be good predictors
95 of prey survival in the wild (Penney, Hassall, Skevington, Abbott, & Sherratt, 2012). Finally,
96 models of predator vision (both for birds and humans) were used to complement behavioural
97 experiments and infer the relative detectability of each butterfly species based on their
98 contrast against the background.

99 **Materials and Methods**

100 **Butterflies used for the behavioural experiments**

101 Specimens of the four Ithomiini species used in both experiments – which, in order of
102 increasing transparency are *Hypothyris ninonia*, *Ceratinia tutia*, *Ithomia salapia aquina*,
103 *Brevioleria seba* (see Figs S1, S2) – were collected in Peru in 2016 and 2017, along the
104 Yurimaguas - Moyobamba road (-6.45°, -76.30°). Butterflies were kept dry in glassine
105 envelopes until use. In behavioural experiments, a single real hindwing and a single real
106 forewing were assembled into artificial butterflies using glue and a thin copper wire to attach
107 the artificial butterfly to a substrate (see Fig S3 for an example). These artificial butterflies
108 mimicked real Ithomiini butterflies at rest, with wings closed and sitting on plant leaves (a
109 typical posture for resting butterflies).

110 **Behavioural experiments using wild birds**

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

123

124 *Training.* In their indoor cage, birds were taught that all four species of butterflies were
125 similarly palatable by offering them wings of four butterflies (one of each species) with a
126 mealworm attached to the copper wire. Butterfly wings used for training were laminated with
127 transparent thin plastic to minimize damage so that these wings could be re-used when
128 possible. As birds typically do not consume butterfly wings but only butterfly body (here
129 dead mealworm), wing toxicity or unpalatability did not influence their training. Butterflies
130 were presented to the birds in the absence of any vegetation during training. When birds had
131 eaten all four prey items (one of each species), a new set was presented. Training ended when
132 birds had eaten 3 sets of butterflies. No time constraint was imposed for training and most
133 birds completed it in less than 4 hours.

134

135 In order to familiarise birds with the experimental set-up, which was novel to them, they were
136 released in the experimental cage by groups of two to four birds for approximately one hour
137 the day before the experiment. Oat flakes, seeds and mealworms were dispersed over leaves
138 and vegetation to encourage searching for edible items in locations similar to where
139 butterflies would be placed during the experiment.

140

141 *Experiments.* The experimental set-up consisted of a 10m x 10m cage that had tarpaulin walls
142 and a ceiling of whitish dense net that let in natural sunlight. Butterflies were disposed in a 5
143 x 5 grid, delimited by poles all around the borders and a rope defining rows and columns (see
144 Fig S4). Two extra poles were placed in the grid centre to increase the appeal of this area for
145 birds. Five specimens of each species (20 specimens in total) were placed in the grid, one per
146 cell, following a block randomization for each row and column and ensuring that all species
147 were evenly represented along the grid. This random configuration was reshuffled between
148 trials (i.e. randomized block design). The 5 cells closest to the observer were left empty as

149 birds tended to avoid this area. For each trial, an observer, hidden to the birds, watched from
150 outside the cage through a small window and took notes of which butterfly species were
151 attacked and in which order. A GoPro camera also recorded the experiments. A butterfly was
152 considered detected only if a bird directly approached it to grab, including when the attack
153 failed. No bird was seen hesitating the attack once it started it. Experiments took place
154 between 9 am and 5 pm. Before each trial, the radiance of ambient light (coming from the sun
155 and sky) was measured using an Ocean Optics spectrophotometer in the same location each
156 time. We computed the total radiance (TR) over the range of 300-700 nm of bird spectral
157 sensitivity to account in statistical analyses for the level of ambient light intensity associated
158 to each experimental trial. Further information on weather conditions (cloudy, sunny, etc)
159 were also noted. Experiments ended when a bird had eaten half of the available butterflies (ie.
160 10 butterflies) or after 2 hours, whichever happened first. Wings were occasionally re-used if
161 they had not been damaged.

162

163 After the experiment, the probability of a bird being present in a given grid cell was
164 calculated as a proxy for the probability of an attack occurring in that grid cell. To do so, 10-
165 minute intervals from each recording, selected based either on the maximum attack rate or
166 when the bird was seen actively exploring the cage, were revised so as to calculate the
167 proportion of time birds spent on poles situated next to each cell. A total of 87% of all attacks
168 started from the pole closest to the grid cell, while all other attacks were initiated from a pole
169 situated only one grid cell further away. Thus, the probability of visiting a given cell was the
170 sum of the time spent on each neighbouring pole, divided by the number of either “close”
171 (immediately next to) or “distant” (one grid cell removed) neighbouring cells and multiplied
172 by either 0.87 or 0.13 (depending on the distance to the pole). As such, those cells closest to
173 the poles and those at the edge of the cage were most likely to be visited by birds.

174

175 *Statistical analyses.* Differences in the total number of butterflies of each species that were
176 attacked were compared by fitting generalised linear mixed effect models (GLMM), with bird
177 identity as a random factor. A binomial distribution was used for the response variable
178 (attacked or not), and the butterfly species, butterfly size, the probability of being attacked for
179 a given cell, trial duration, age and sex of the bird, time to first attack, first butterfly species
180 found, weather (as a qualitative variable), and total radiance (TR) were all selected as
181 explanatory variables. The best fitting model was selected based on minimization of Akaike's
182 Information Criteria (AIC), assuming that models differing by two units or less were
183 statistically indistinguishable (Anderson, Burnham, & White, 1998). Coefficients and
184 standard errors were computed using a restricted maximum likelihood approach and a Wald z
185 test was used to test for factor significance.

186

187 Most birds fed willingly on all butterflies located on the borders of the grid. Given that
188 butterfly species distribution was random and reshuffled between trials, the four species were
189 similarly represented in those cells (Fig S5), so no bias was expected. The order of attack for
190 each species was tested by ranking the "inconspicuousness" of each butterfly species based
191 on the order in which butterflies were found and how many of them were detected (Ihalainen,
192 Rowland, Speed, Ruxton, & Mappes, 2012). To do so, the position of each butterfly that was
193 attacked for each species and the total number of non-attacked butterflies of each species
194 multiplied by 11 (i.e. the maximum number of butterflies that could be found + 1) were
195 added. This inconspicuousness rank distinguishes between those species found first and in
196 higher numbers (lower values of inconspicuousness) from those found last and in lower
197 numbers (higher values of inconspicuousness). For example, if a bird captured two *H.*
198 *ninonia* second and fifth in the sequence of captured prey, this species gets a rank value of

199 $2+5+3 \times 11=40$ for that trial. We fitted a linear mixed effect model to test for differences in
200 rank for each species, assuming a normal distribution, with rank as the response variable, bird
201 individual as a random factor and butterfly species, age and sex of the bird, date, time until
202 first attack, first butterfly species found, weather as a qualitative variable, and total radiance
203 (TR) as explanatory variables. Again, the best fitting model was selected using AIC
204 minimization.

205

206 We also tested whether differences in the rank of species inconspicuousness could be due to a
207 differential attack probability for each species, i.e. whether species more likely to be attacked
208 were more often placed on cells more likely to be visited. To do so, the probability of a bird
209 being in proximity to a grid cell containing one of the five artificial butterflies of each species
210 was averaged for each trial. An ANOVA was then used to compare the probability of attack
211 for each butterfly species.

212

213 Finally, to test whether birds created a “search image” (i.e. improved in finding butterflies of
214 a given species), the number of butterflies of each species found consecutively was counted.
215 Results were compared among butterfly species using a χ^2 test. Additionally, whether finding
216 some species improved the bird’s ability to find others was tested. For each combination of
217 two species, we calculated how many times a butterfly of species 1 was found after a
218 butterfly of species 2. Differences between them were tested using a χ^2 test. All analyses were
219 performed in R (R Foundation for Statistical Computing, 2014).

220

221 **Behavioural experiments using human participants**

222 Between mid-November and early December 2017, visitors of the Montpellier botanical
223 garden (France) were invited to take part in an experiment where they searched for artificial

224 butterflies. Before each trial, participants were shown pictures of various ithomiine
225 butterflies, both transparent and opaque, but of species different than those used in the
226 experiments, so as to familiarize them with what they would be searching for. Anonymous
227 personal data was collected from each participant, including gender, age group (A1: <10
228 years, A2: 11-20 y, A3: 21-30 y, A4: 31-40 y, A5: 41-50 y and A6: >51 years), and vision
229 problems. A participant did the experiment only once.

230

231 *Experimental set-up.* Artificial butterflies (N=10 of each of the four species, for a total of 40
232 butterflies) again consisted of one forewing and one hindwing assembled with copper wire
233 and placed on leaves, but without the mealworm used in the bird experiments. These
234 butterflies were set-up along two corridors in a forest-like understory habitat of similar
235 vegetation and light conditions. Butterfly order followed a block randomisation, with five
236 blocks each consisting of eight butterflies (i.e. two per species). This ensured that observers
237 were similarly exposed to the four species all throughout the experimental transect. Whether
238 a butterfly was placed on the left or right side of the corridor was also randomised. Both order
239 and corridor side were changed daily. Participants could start the path from either end of the
240 set-up and were given unlimited time to complete the trial. However, they could only move
241 forward on the path. Only one participant was allowed in the path at any given time, and they
242 were accompanied by an observer who recorded which butterflies were found. Trials ended
243 when the participant had completed both corridors.

244

245 *Statistical analyses.* Differences in the total number of butterflies found for each species was
246 tested by fitting GLMMs. A binomial distribution for the response variable (either found or
247 not) was assumed, and participant identity was set as a random factor, butterfly species, first
248 species found, butterfly position, corridor, left or right side of the path, time of day, gender

249 and age of the participant, duration of the experiment, and their interactions, were all used as
250 explanatory variables. A minimization of Akaike's Information Criteria (AIC) was used to
251 select the best model, assuming that models differing by two units or less were statistically
252 indistinguishable (Anderson et al., 1998). Coefficients and standard errors were computed
253 using a restricted maximum likelihood approach and a Wald z test was used to test for factor
254 significance. Whether specific species were more frequently detected was also tested using a
255 χ^2 test. Similarly as for the experiments using birds, a GLMM was also fitted under the same
256 conditions, but using the butterfly species inconspicuousness rank (similarly calculated as for
257 the bird experiments) as a response variable.

258

259 Finally, whether humans found several butterflies of the same species consecutively (perhaps
260 because they formed a "species search image") was also tested. A χ^2 test was used to compare
261 the number of butterflies of each species that were found consecutively. Whether finding
262 individuals of a species increased the likelihood of finding other species was also tested. For
263 each combination of two species, we calculated how many times a butterfly of the first
264 species was found immediately after a butterfly of a second species. Differences between the
265 frequencies of these combinations were tested using a χ^2 test, comparing observed results and
266 the frequency at which each possible pair of species was placed consecutively in the original
267 experimental setup. All analyses were performed in R (R Foundation for Statistical
268 Computing, 2014).

269

270 Colour measurements and vision modelling can be found in electronic supplementary
271 material (additional materials and methods).

272 **Results**

273 **Behavioural experiments using wild birds**

274 Birds took anywhere between 1 and 37 minutes (average: 7.54 ± 8.96 min) after release into
275 the experimental cage before initiating an attack. For three of the birds, the experiment ended
276 without having eaten 10 butterflies in the allocated 2 hours. The other 27 birds took between
277 11 and 112 minutes to attack 10 butterflies (mean time to attack 10 butterflies: 40.76 ± 26.23
278 min). Considering all trials, similar percentages of butterflies for each species was found by
279 birds (54% of *H. ninonia* butterflies (the most colourful species), 48.7% for *C. tutia*
280 (colourful but transparent species), 46.7% for *I. salapia* (yellow-tinted butterfly) and 49.3%
281 of *B. seba* butterflies (most transparent species).

282

283 The model that best explained whether butterflies were attacked or not only included the
284 probability of occupancy of a given grid cell by a bird, time of the first attacked and the grid
285 cell occupied by the butterfly (AIC = 610.42, Delta AIC = 4.8142 with a model that
286 additionally included butterfly species, Table S1). Butterflies were more likely to be attacked
287 where birds visited most often ($z = 2.93$, $p = 0.003$). By contrast, the inconspicuousness rank of
288 a butterfly species was best explained by a model including average probability of occupancy
289 by a bird and butterfly species as explanatory variables (AIC = 765.73, Delta AIC = 2.53 with
290 a model including the species that was attacked first, Table S2). *H. ninonia*, which was the
291 most colourful species, was usually detected in the first prey attacked ($t = -3.15$, Fig 1a, Table
292 S2). Moreover, which species were found first closely matched their transmission properties:
293 *H. ninonia*, followed by *C. tutia*, *I. salapia* and *B. seba* ($X^2 = 11.07$, $df = 3$, $p = 0.011$, Table
294 S3). When comparing species distribution along the grid, artificial butterflies that were
295 attacked were in grid cells with moderate to high bird occupancy rate ($F = 0.82$, $df = 3$, $p =$
296 0.485 , Fig S5).

297

298 Generally, birds did not find several butterflies of the same species consecutively (Fig S6a).
299 In the rare instances that they did, no differences between species was found ($X^2 = 0.6$, $df = 3$,
300 $p = 0.90$) suggesting that birds did not form a “search image” for any of the butterfly species.
301 No combination of species was attacked consecutively at high frequencies either ($X^2 = 10.88$,
302 $df = 11$, $p = 0.45$).

303

304 **Behavioural experiments using human participants**

305 A total of 102 volunteers participated in the experiment (63 men and 39 women, with
306 10:11:21:18:31:11 in the A1:A2:A3:A4:A5:A6 age classes). 19 volunteers ran the experiment
307 before 13h30, 35 between 13h30 and 16h, and 48 after 16h. Participants found between 5 and
308 28 of the 40 butterflies (12.75 ± 4.68 butterflies found per participant) and took between 7.5
309 and 37 minutes to walk both corridors (18.04 ± 6.5 minutes spent in average per participant).
310 For all trials combined, participants found 42.5% of *H. ninonia* butterflies (the most colourful
311 species), 38% of *C. tutia* (colourful but transparent species), 23.54% of *I. salapia* (yellow-
312 tinted butterfly) and 28.63% of *B. seba* butterflies (most transparent species).

313

314 Younger participants found more butterflies than older ones (number of butterflies: $z = -2.34$;
315 butterfly species rank: $t = -1.36$). Additionally, participants found more butterflies earlier
316 than later in the afternoon (number of butterflies: $z = -2.80$; inconspicuousness rank: $t = -$
317 1.77). However, this was most significant for younger participants (inconspicuousness rank: t
318 $= 1.32$, Fig S7a). Generally the more time participants spent in the experiment, the higher the
319 number of butterflies they found (number of butterflies: $z = 5.21$; inconspicuousness rank: $t =$
320 4.03), although this was most significant for women (number of butterflies: $z = -2.96$,
321 inconspicuousness rank: -2.83 , Fig S7b). There was a corridor effect, likely due to differences
322 in the overall cover of vegetation (number of butterflies: $z = 3.14$; inconspicuousness rank: t

323 = -3.52). Participants also found more butterflies at the end rather than at the start of the
324 experiment (number of butterflies: $z = 5.21$; inconspicuousness rank: $t = 4.03$, Tables S3 and
325 S4), most likely because they became accustomed to the set-up and what they were searching
326 for.

327

328 The inconspicuousness rank of butterfly species was affected by time of day and the day of
329 the experiment (Fig 1b, Table S5). Species rank was highest earlier in the day ($t = -1.77$).
330 Moreover, older participants omitted fewer butterflies at later hours ($t = 1.32$).

331

332 Participants were more likely to find opaque butterflies than transparent ones, following the
333 order *H. ninonia* (H), *C. tutia* (C), *B. seba* (B) and *I. salapia* (I) (H>C, I, B: number of
334 butterflies: $z = 5.73$; inconspicuousness rank: $t = -7.11$; C>B: number of butterflies: $z = 0.03$;
335 inconspicuousness rank: $t = -1.65$; B>I: number of butterflies: $z = 2.37$, Table S4;
336 inconspicuousness rank: $t = 2.68$, Fig 1b). However, the gain in detection with increasing
337 time spent searching was highest for the most transparent species ($z = -2.75$, Fig 2, Fig S7).
338 *H. ninonia* was also the species most frequently found first, followed by *C. tutia*, *B. seba* and
339 *I. salapia* ($X^2 = 19.5$, $df = 3$, $p < 0.001$, Table S3).

340

341 Differences were also found in the consecutive order in which butterflies were found.
342 Participants were more likely to find two consecutive butterflies of the same species when
343 they were colourful (*H. ninonia* -50 times- and *C. tutia* -58 times) than when they were
344 transparent (*B. seba* -32 times- or *I. salapia* -18 times; $X^2 = 29.14$, $df = 3$, $p < 0.001$). *B. seba*
345 and *H. ninonia* were found up to four consecutive times in a single trial. Some species were
346 also more likely to be found consecutively after another species. The most opaque butterflies
347 *H. ninonia* and *C. tutia* (found 278 times consecutively), and the transparent species *B. seba*

348 and *I. salapia* (found 186 times consecutively), were found consecutively more frequently
349 than any of the other possible combinations after correcting for the number of butterflies
350 found for each species ($X^2 = 170.95$, $df = 5$, $p < 0.001$). These observed frequencies
351 significantly differed from the position that butterflies occupied on the original set-up along
352 the path ($X^2 = 79.12$, $df = 11$, $p < 0.001$, Fig S6b).

353

354 **Models of bird and human vision**

355 The achromatic weighted contrast between butterfly colour patches and green-leaf
356 background were similar for both birds and humans (mean achromatic contrast for birds:
357 $H=3.81$, $C= 3.15$, $I=2.31$, $B=2.11$; for humans: $H=5.25$, $C=4.35$, $I=3.58$, $B=3.86$. Fig S8). For
358 both observers, *H. ninonia* followed by *C. tutia* (colourful and transparent butterfly)
359 contrasted the most against the leaves, while transparent butterflies (*I. salapia* for humans
360 and *B. seba* for birds), were the least contrasting. Butterflies seem to be more chromatically
361 detectable by birds than for humans (mean chromatic contrast for humans: $H = 0.44$, $C =$
362 0.37 , $I = 0.25$, $B = 0.22$). For the chromatic contrast seen by birds, *C. tutia*, and then *H.*
363 *ninona* was the most contrasting, whereas *B. seba* and finally *I. salapia* were the least (mean
364 chromatic contrast for birds: $H = 2.02$, $C = 2.05$, $I = 1.30$, $B = 1.38$).

365 **Discussion**

366 **Transparency reduces detectability**

367 As initially predicted based on wing transmittance, and as demonstrated by our behavioural
368 experiments and visual modelling results, transparency decreases butterfly detectability.
369 Interestingly, detection by human participants was similar to naïve birds, as has been shown
370 in other studies (Beatty, Bain, & Sherratt, 2005; Sherratt, Whissell, Webster, & Kikuchi,
371 2015), providing further support to using human participants to measure predator detection.
372 Surprisingly, experimental results from the bird experiments differed slightly from

373 predictions made based on the measures of transmittance of transparent patches and results
374 obtained from the vision models. For instance, according to the transmittance and the
375 chromatic contrast measured between butterflies and their background, birds should have
376 probably detect *C. tutia* more easily than the two more transparent species. Indeed, semi-
377 transparent objects should be more easily detected than fully transparent objects at short
378 distances and when more light is available (Johnsen & Widder, 1998), such as experimental
379 conditions present during bird experiments. Yet this transparent but brightly coloured species
380 was detected at similar rates as the most transparent species. One possible explanation is that
381 this species possesses disruptive colouration; indeed, wing contours of this species are less
382 strongly delimited than that of the other species and a disrupted outline may hamper detection
383 (Honma, Mappes, & Valkonen, 2015; Stevens & Cuthill, 2006). These contradicting results
384 highlight the importance of combining both modelling and behavioural experiments to better
385 understand the evolution of transparency and other prey defences.

386

387 **Transparency in a toxic butterfly?**

388 Our results demonstrate that transparency can effectively reduce prey detectability in
389 chemically-protected ithomiine butterflies. This is surprising as aposematic colour patterns,
390 rather than inconspicuousness, are more common in toxic and unpalatable prey (Mappes et
391 al., 2005; Poulton, 1890; Ruxton, Sherratt, & Speed, 2004). In fact, conspicuousness is often
392 positively correlated with toxicity or unpalatability and can thus be an honest indicator of
393 prey defences (Arenas, Walter, & Stevens, 2015; Blount, Speed, Ruxton, & Stephens, 2009;
394 Maan & Cummings, 2012; Prudic, Skemp, & Papaj, 2007; Sherratt & Beatty, 2003), and
395 predators learn faster to avoid unpalatable prey when colours are more conspicuous
396 (Gittleman & Harvey, 1980; Lindstrom, Alatalo, Mappes, Riipi, & Vertainen, 1999). This
397 might suggest that the evolution of transparency in these butterflies is the result of a loss in

398 unpalatability. If this is the case, the existence of mimicry rings of transparent clearwing
399 butterflies remains unexplained, as this is usually the result of convergence of warning
400 signals promoted by the positive frequency-dependent selection exerted by predators
401 (Willmott et al., 2017). Alternatively, if defences are costly, prey may invest in either visual
402 or chemical defences (Darst, Cummings, & Cannatella, 2006; Speed & Ruxton, 2007; Wang,
403 2011). Such strategies have been shown to afford equivalent avoidance by predators (Darst et
404 al., 2006). Transparency may therefore be associated with an increase in unpalatability.
405 Unfortunately, the relationship between transparency and the degree of chemical defences in
406 clearwing butterflies is yet unknown.

407

408 Alternatively, transparency may lower detection and function as a primary defence, with
409 aposematism taking over as a secondary defence if the prey is detected. Indeed, transparent
410 butterflies were not completely cryptic for either birds or humans. In fact, birds found a
411 similar number of both colourful and transparent butterflies, and humans appear to learn to
412 detect and perhaps remember common elements between the more transparent species, what
413 might be the result of a search image. As such, Ithomiini butterflies may be cryptic from afar,
414 but perceived as conspicuous from up close (Gamberale-Stille, Bragee, & Tullberg, 2009;
415 Tullberg, Merilaita, & Wiklund, 2005). A dual strategy of crypsis and conspicuousness has
416 been described for other prey, including defended prey (Järvi, Sillén-Tullberg, & Wiklund,
417 1981; Kang, Zahiri, & Sherratt, 2017; Sillén-Tullberg, 1985) For example, toxic salamanders
418 of the genus *Taricha* are generally cryptic, only revealing their warning coloured underbelly
419 when threatened (Johnson & Brodie Jr, 1975). In Ithomiini, conspicuous (or potentially
420 conspicuous) elements can be found on even transparent species, and most species possess
421 opaque areas delineating the edges and contrasting with the background, most likely
422 increasing detection (Stevens & Cuthill, 2006). This, combined with our results and the

423 occurrence of co-mimics in natural populations, suggests that these butterflies may reduce the
424 cost of conspicuousness using transparency in addition to maintaining the benefits of
425 detectable warning signals. Behavioural experiments testing the distance at which Ithomiini
426 butterflies are detected are needed to shed further light on the function of aposematism in less
427 conspicuous prey.

428

429 Finally, transparency may have evolved as an additional primary protection against birds
430 such as adult kingbirds (*Tyrannus melancholicus*, C. E. G. Pinheiro, 1996) which are able to
431 tolerate their chemical defences. Indeed, both models (Endler & Mappes, 2004) and
432 experiments (Mappes, Kokko, Ojala, & Lindström, 2014; Valkonen et al., 2012) have shown
433 that weak warning signals (not overtly conspicuous) can evolve and be maintained in
434 communities where predators vary in their probability of attacking defended prey. Larvae of
435 *Dryas julia* butterflies, pine sawfly larvae (*Neodiprion sertifer* for example), and shield bugs
436 (Acanthosomatidae, Heteroptera) are some of the several examples of unpalatable species
437 that display weak visual warning signals (see Endler & Mappes, 2004). Similar to the
438 polymorphic poison frog *Oophaga granulifera*, clearwing species may reflect a continuum
439 between aposematic and cryptic strategies, possibly shaped by differences in the strength of
440 predator selection as a result of the frequency of naïve predators and/or the variation in
441 predator sensitivities to chemical compounds (Willink, Brenes-Mora, Bolaños, & Pröhl,
442 2013). A thorough characterization of unpalatability, microhabitat and predator communities
443 would be useful in better understanding conditions that promote the evolution of transparency
444 in Ithomiini.

445

446 **Conclusions**

447 Our study, combining behavioural experiments with different predators and vision modelling,
448 provides comparative insights into the complex role transparency may play in anti-predator
449 defences of aposematic organisms. We show for the first time that transparency is an
450 effective strategy for the reduction of detectability of terrestrial prey. We also demonstrate
451 that Ithomiini butterflies may in fact be decreasing the costs of conspicuousness, while still
452 retaining visual elements that are recognised as warning signals. Future studies exploring the
453 efficiency of combining transparency and warning signals in decreasing predation risk will
454 further contribute to understanding the evolution of cryptic elements in aposematic prey.

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467

468 **Author contribution**

469 DG, ME, JM and MA designed the study; ME, MM and DG collected the butterfly samples;
470 MA, SG, ON, ME and JM did the experiments; DG and CD took the optical measurements;

471 MA, DG and ME analysed the data; MA, DG, MM, ME, ON, SG and JM wrote the
472 manuscript.

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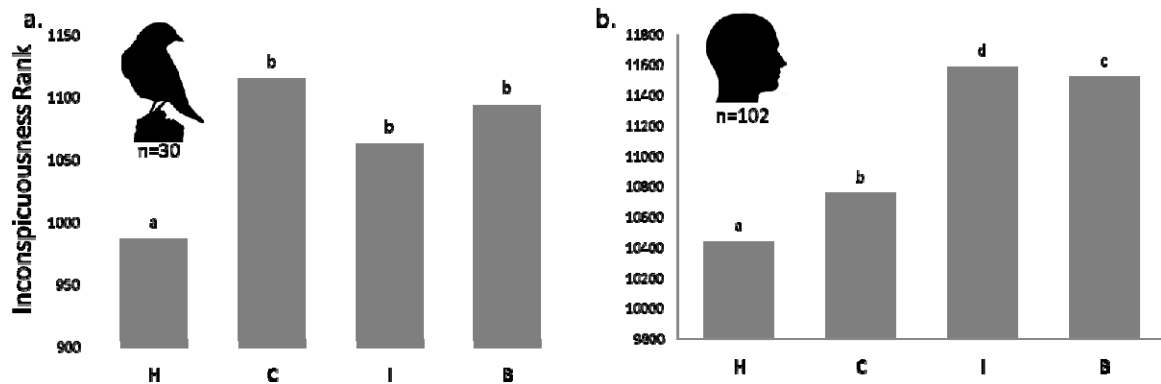
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639 **Figures**

640



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642 Figure 1. Sum of the inconspicuousness rank of each species for a) great tits and b) humans.

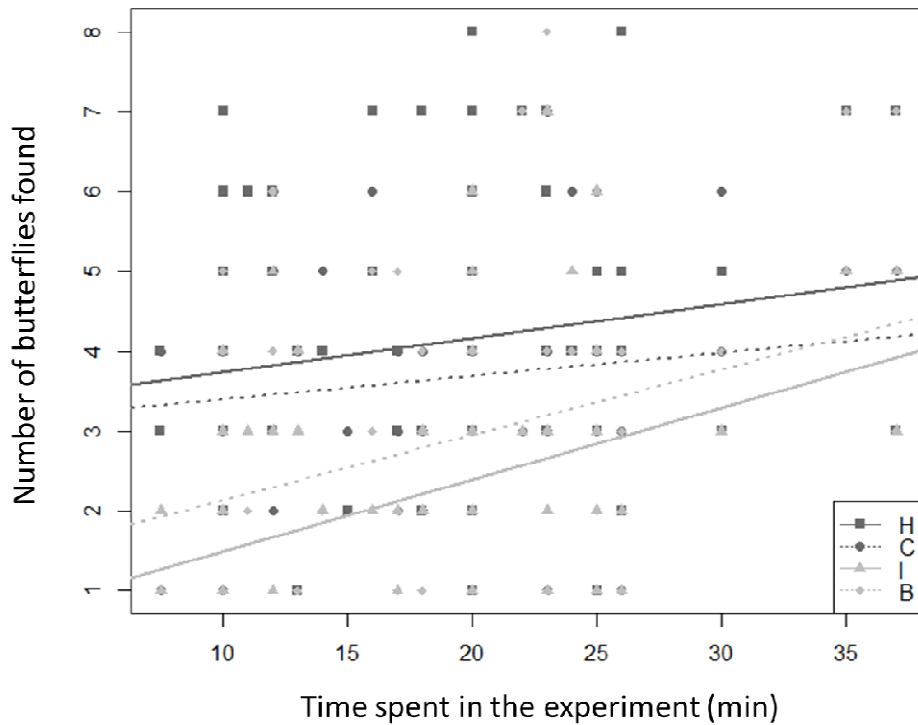
643 Species for which butterflies were detected first and most often by birds or humans have

644 lower values of “inconspicuousness rank”. Butterfly transparency increases from left to right:

645 *H. ninonia* (H), *C. tutia* (C), *I. salapia* (I), and *B. seba* (B). Letters above the bars mean

646 significant differences below 0.05.

647



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649

650 Figure 2. Number of butterflies found for each species according to the time spent completing
651 the experiment by human participants. As shown by the regression lines, spending more time
652 on the experiment resulted in higher numbers of butterflies found, especially for the
653 transparent species (H: estimate slope= 0.043, $r^2 = 0.014$, $p = 0.12$; C: estimate slope= 0.03, r^2
654 = 0.005, $p = 0.22$; I: estimate slope= 0.090, $r^2 = 0.12$, $p < 0.001$; B: estimate slope= 0.08, $r^2 =$
655 0.07, $p = 0.003$). Letters in the legend stand for species names: *H.ninonia* (H), *C.tutia* (C), *I.*
656 *salapia* (I), and *B. seba* (B). Butterfly transparency increases from top to bottom of the legend
657 (i.e. H<C<I<B).

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