1 **Ambient light and mimicry as drivers of wing transparency in Lepidoptera**

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13 14 **Abstract**

15 Transparency reduces prey detectability by predators. While transparent aquatic
16 species hold higher transparency levels as the light availability of their habitat
17 increases, less is known about such variation in terrestrial species. Lepidoptera
18 species exhibiting transparent wings display various levels of transparency. Using
19 two complementary approaches, we explore how the evolution of different
20 transparency degrees relates to habitat openness, activity rhythm and mimicry
21 syndrome (bee/wasp versus dead-leaf mimic). First, by exposing artificial moth-like
22 prey to wild avian predators in a range of habitat openness, we show that survival is
23 lower in more open habitats. We also found that less transparent morphs are more
24 attacked than more transparent ones, regardless of habitat openness degree. Second,
25 by analysing the evolution of wing features and ecological traits in 107 clearwing

26 species, we found that diurnal species transmit more light than nocturnal species
 27 under certain conditions (when considering only forewings, at smaller clearwing
 28 surfaces and at larger wing lengths) and that species flying in open habitats and
 29 exhibiting large percentages of clearwing surface transmit slightly more light than
 30 those flying in closed habitats, although this is reversed at smaller percentages of
 31 clearwing surfaces. Additionally, bee/wasp mimics are more often diurnal and have
 32 higher and less variable light transmittances than dead-leaf mimics, which are more
 33 often nocturnal. Flying during the day, in open habitats and mimicking insects with
 34 transparent wings seem to promote high light transmittance under certain
 35 circumstances. Activity rhythm, habitat openness and species interactions play a
 36 crucial role in determining transparency design on land.

37

38 **Keywords:** transparency, irradiance, mimicry, nocturnality, Lepidoptera, Batesian
 39 mimicry, habitat openness

40

41 **Introduction**

42 Transparency is common in aquatic environments where it reduces detectability by
 43 predators, especially in the pelagic environment where there is nowhere to hide
 44 (Johnsen 2014). The amount of light transmitted by transparent tissues varies
 45 between organisms. Experiments and modelling have shown that in brighter light
 46 conditions, the visual performance of aquatic predators is higher and only high
 47 levels of transparency confer protection to prey. Conversely, at dim light conditions
 48 that limit visual performance, a large range of transparency levels give equal
 49 protection (Figure S1, Johnsen and Widder 1998).

50 On land, transparency is present in only a handful of lineages, among which
 51 insects. In Lepidoptera, transparency is based on diverse microstructures (scales
 52 varying in morphology, insertion on the membrane, and colouration) and
 53 nanostructures (on scales and on the wing membrane), producing transmittance
 54 levels ranging from 10 to 90% (Gomez et al. 2021; Pinna et al. 2021). The triggers of
 55 the evolution of such diversity are still unexplored. Predation is likely important, as
 56 transparency efficiently reduces prey detectability (Arias et al. 2019, 2020; McClure
 57 et al. 2019). As in water, the visual performance of terrestrial predators is higher in
 58 bright than in dim light conditions, as shown in birds (Hodos et al. 1976; Kassarov
 59 2003; Lind et al. 2013). We can thus hypothesize that diurnal terrestrial transparent
 60 species living in more open habitats - with brighter light conditions - would transmit
 61 more light compared to species flying at night and/or living in more closed habitats -
 62 with dimmer light conditions-. In addition, at low light availability, different levels of
 63 transmittance could confer similar protection against predators. Therefore, we
 64 expect that nocturnal and/or closed-habitat species would show larger variation in

65 light transmittance, as neither high nor low transmittance levels are strongly
66 selected.

67 Other visual anti-predatory defences might also be segregated according to
68 light availability (activity rhythm and habitat openness). Predation risk can be
69 reduced by mimicking unprofitable prey (batesian mimicry, Bates 1862) such as
70 bees and wasps, or inedible elements such as leaves (masquerade, Skelhorn et al.
71 2010), species and items that are more common in habitats with different light
72 conditions. Several small clearwing moths and butterflies exhibit yellow and dark
73 stripes on their bodies and highly transparent wings (example in Fig. 1a), resembling
74 harmful species for predators, such as bees and wasps, that often live in open
75 habitats (Grundel et al. 2010; Yamaura et al. 2012), and bee/wasp mimics should do
76 as well. Other clearwing Lepidoptera exhibit brownish colorations that combined
77 with transparent surfaces that are presumably perceived as “holes” can resemble
78 dead-leaves (example of a potential dead-leaf mimic in Fig. 1b) that are probably
79 highly common in closed habitats where lots of dead leaves fall down. High light
80 transmittance should be selected in bees, wasps and their mimics because the
81 reduction of detectability already conferred by their small body sizes, can be
82 enhanced by higher levels of transparency (Gomez et al. 2021), and because light
83 conditions are brighter in open habitats. By contrast, dead-leaf mimics might show a
84 larger variation in body size, and we expect that they would exhibit a broader
85 diversity of light transmittance, as a range of transmittance levels in poor light
86 conditions can contribute to mimic leaves in decomposition and confer similar
87 protection against predators. The expected differences in light transmittance should
88 also be associated to variation in morphology and coloration of wing scales, with
89 absent or reduced scales for bee/wasp mimics, while all scale types should be

probably found in dead-leaf mimics. Therefore, mimicry syndromes that are probably segregated in habitats with different light availability could also drive different transmittance optima in bee/wasp and in dead-leaf mimics.

Here, we investigate whether differences in transmittance found in Lepidoptera clearwing species are related to light availability and/or mimicry syndrome using two complementary approaches. First, we tested whether the difference in efficiency of different transmittance levels at reducing detectability is lower in closed habitat compared to open habitat, by carrying out a fieldwork experiment with artificial moth-like prey and natural predator communities. Second, using actual specimens of butterflies and moths, we tested whether differences in light transmission could be explained by species daytime rhythm, habitat and/or mimicry, by conducting comparative analyses at broad interspecific level. We took advantage of the large dataset of Lepidoptera species analysed by Gomez et al (2021), added species ecological traits (habitat, daytime rhythm and mimicry, known for 107 species) and explore the correlation and coevolution between them and light transmittance, and clearwing morphological (wing size, proportion of clearwing surface, and fore-/hindwing) and structural traits (scale characteristics). Together, these analyses help understanding the evolution of light transmission on terrestrial species exhibiting transparency.

Material and Methods

1. Field experiment

1.a. Artificial butterfly Elaboration

To test for the efficiency of light transmittance at reducing detectability in habitats with different light availability, we elaborated plain grey artificial butterflies with

115 paper wings and a malleable edible body (see Figure 2 for pictures of the artificial
116 moths). Following the general methodology described in Arias *et al* (2020),
117 butterflies did not replicate any real local butterfly, but mimicked a general grey
118 moth with closed wings (i. e., a triangular shape of 25mm high by 36mm wide for a
119 surface of 450mm²). Artificial moth colour was chosen based on the bark colour of
120 evergreen oak (See ESM for further details). Artificial grey was chromatically similar,
121 but lighter, to the evergreen oak trunk. This achromatic mismatch allowed us to test
122 transparency as crypsis enhancer in imperfectly cryptic artificial prey. Moths were
123 built combining mat laminated paper wings (as described in ESM). Four types of
124 artificial butterflies were used, here listed in order of increasing proportion of light
125 transmittance: completely opaque butterfly “C”; poorly transparent butterfly with 6
126 layers of transparent film “T6”; highly transparent butterfly with a single layer of
127 transparent film “T1”; and fully transparent butterfly with no film in the transparent
128 zones “T0”. For the butterflies that included transparent elements, two triangular
129 windows of 252mm² each (56% of total artificial moth surface) were cut down from
130 the grey triangle and the remaining part was either left empty (T0) or glued on top
131 of one (T1) or six (T6) layers of 3M of transparency film for inkjet printing. This
132 transparent film was chosen as it is highly transparent, even in the UV range of the
133 spectrum, and going from 1 to 6 transparent layers permitted a reduction of 50% of
134 the transmittance between treatments (Fig. S2). The upper part of transparent areas
135 was coated with a transparent matte varnish to reduce its shininess, making them
136 more similar to real Lepidoptera transparent wings, which often exhibit nano
137 antireflective structures (Pinna et al. 2021; Pomerantz et al. 2021). We added an
138 edible malleable grey body on top of the wings that permitted registering and
139 distinguishing bird, insect and mammal marks. Further details in the measurement

of colour and transparency spectra, as well as in the fabrication of prey bodies can be found in ESM.

142

143 1.b. Experimental set up

Predation experiments were performed over four weeks between May and June 2018 at the zoological park of Montpellier (43.64°, 3.87°) and at La Rouvière forest (close to Montarnaud city 43.65°, 3.64°). Our experiment involved the local community of insectivorous birds and includes great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*), which are reported visual predators in other similar studies (Rowland et al. 2008; Stevens et al. 2008a). Artificial prey items were pinned every 10 metres on oak trunks of at least 10 cm of diameter and with few or no moss cover. Prey order was randomized by blocks of eight items, two per treatment, before starting the experiment. Prey were mostly disposed facing north and as perpendicular to the floor as possible, to reduce direct sunlight reflection on them that could damage prey bodies with the heat and that could increase transparency shininess. Prey were checked every 24h. After 96h all prey were removed. When V- or U- marks were detected, or when the body was missing without signals of invertebrate attacks (i.e. no body scraps left on the wings or around the butterfly on the trunk) prey were considered as attacked and wings or pins were removed. Instead, if invertebrate attacks were detected, or when prey was missing, it was replaced. Non-attacked prey items were considered as censored data.

Canopy cover was used to assess habitat openness. Once the experiment was over, we took pictures each 20 meters in the trails that were used for the experiment (at forest edges for the open habitat, inside the forest for the closed habitat, examples Fig. S3), using a Canon EOS 550D camera and a fisheye lens. Canopy cover

165 was further quantified using Fiji software (Schindelin et al. 2012), see Fig. S3 for the
166 distribution of canopy cover in the experimental sites).

167 To test how different treatments survive along the experiment according to
168 habitat openness, mixed effect and hierarchical (with block nested within location)
169 Cox Proportional Hazards models (Cox 1972) were applied using the *coxme* package
170 (Therneau 2020) in R (R Foundation for Statistical Computing 2014). Artificial type
171 and habitat openness were included as explanatory variables. Models including or
172 not their interaction were fitted and their fit was evaluated using the AIC criterion to
173 select the best model.

174

175 2. Comparative analyses of museum specimens

176 2. a. Species selection and phylogeny

177 To explore the association of light transmittance with different conditions across
178 multiple species we performed phylogenetic comparative analyses, since species are
179 not independent. We used the phylogeny of Lepidoptera species exhibiting
180 transparent areas in their wings by Gomez et al. (2021). In our analyses we included
181 107 species out of the 123 species included by Gomez et al, for which information
182 about the openness of their habitat was available. These species spanned 31 of the
183 124 existing Lepidoptera families.

184

185 2. b. Ecological data on species habitat, activity rhythm and mimicry type

186 Ecological information was collected from the literature, Lepidoptera experts and
187 our own knowledge for a total of 107 species for activity rhythm (57% of species are
188 diurnal (day-active) species), habitat (56% of species living in open habitats), and
189 mimicry status (14 species were bee/wasp mimics, 12 species were leaf mimics,

190 while the remaining 81 did not fall in either of those categories (hereafter referred to
191 as non-mimics although they include mimics involved in other butterfly mimetic
192 communities, Table S1 for references and Fig S4). Habitat was considered open
193 when it was described as “open habitat”, “meadow”, “small bushes”, “savannah”,
194 “riversides”, or “rocky grasslands”. When the term “forested areas”, “primary forest”,
195 “lowland forest”, “cloud forest” was mentioned the species was considered as closed
196 habitat species (for references see Table S1). The selected 107 species excluded
197 species that were reported to live in both open and closed habitats, or to fly at day
198 and night (for references see Table S1). Visual predators are mostly active during
199 the day and are supposed to be the main selective agents in the evolution of visual
200 anti-predator defences (Ruxton et al. 2018). Characteristics that can increase
201 conspicuousness for visual predators such as the presence of UV patterns (Lyytinen
202 et al. 2004) and large body sizes (Guevara and Avilés 2013) are more commonly
203 found in nocturnal than in diurnal insect species. Therefore, species that are active at
204 night are unlikely to face similar selective pressures as species that are actively
205 moving during the day although both could fly in similarly open habitats. For this
206 reason, daytime rhythm and habitat were coded as a single variable with 4 states
207 (diurnal open habitat, diurnal closed habitat, nocturnal open habitat and nocturnal
208 closed habitat). To include mimicry syndrome, whenever species were described as
209 bee and wasp mimics, they were assumed as so and they were grouped together in
210 the same mimicry category, as both exhibit the striped pattern with black and often
211 with yellow that advertise their unpalatability (Plowright and Owen 1980) and they
212 exhibit similar body sizes. Butterflies and moths mimicking bees and wasps have
213 forewings longer than their hindwings, and often strong delimitations of their wing
214 borders and veins by dark coloration, as the hornet moth *Sesia apiformis* (*api* - bee,

215 *formis* -shaped) or the broad-bordered bee hawk-moth *Hemaris fuciformis* (Fig 1b).
 216 Dead-leaf mimics were considered as so whenever they were described as death-leaf
 217 mimics on the literature and according to their general morphology. Dead-leaf
 218 mimics include brownish moths and butterflies with small or large transparent
 219 surfaces and that could touch or not the wing borders (Fig. 1a). *Rotschildia lebeau*
 220 has been proposed as a leaf mimic (Janzen 1984) but such mimicry is controversial
 221 (Hernández-Chavarría et al. 2004). *Rotschildia ericina* was therefore not considered
 222 as a leaf mimic for the analyses.

223

224 2. c. Wing structural and optical measurements

225 *Macrostructure*

226 Wing structural and optical measurements were taken from Gomez et al (2021),
 227 using the following methods. Museum specimens were photographed using a D800E
 228 Nikon camera equipped with a 60mm lens, placed on a stand with an annular light.
 229 Photos were then analysed using ImageJ (Schneider et al. 2012) to extract
 230 descriptors of wing macrostructure: wing length (mm), wing surface (mm²), and
 231 clearwing area (the surface of transparent area in mm²), for the forewing and
 232 hindwing separately. Proportion of clearwing area was considered by Gomez et al
 233 (2021) as the ratio clearwing area/wing surface, i.e. the proportion of the total wing
 234 area occupied by transparency. For optical measurements, Gomez et al (2021)
 235 followed the same procedure described above for measuring transmittance in
 236 artificial prey. For each species and wing, they took five measurements in the
 237 transparent zone, and analysed spectral shape using Avicol v6 (Gomez 2006) to
 238 extract the mean transmittance over [300-700] nm, which described the level of
 239 transparency.

240

241

242 *Microstructure*

243 Gomez et al (2021) described a large diversity in transparent wing surface
244 characteristics. Using binocular imaging (Zeiss Stereo Discovery V20) and digital
245 microscopic imaging (Keyence VHX-5000), these authors found that transparency
246 could be achieved through the presence or absence of scales that had different
247 morphologies, positions and colouration. Different combinations of structures and
248 configurations are related to different light transmission levels. Therefore, we also
249 explored the evolution of mimicry syndrome and of the different scale
250 characteristics, including scale presence (nude membranes or with membranes
251 covered by scales), type (lamellar scales, piliform scales or both), coloration
252 (coloured or transparent) and insertion in the membrane (erect or flat).

253

254 2. e. Analyses of transmittance differences between habitats and mimics

255 To explore whether differences in the proportion of transmitted light are related to
256 variation in habitat and mimicry we fitted both 1) a linear mixed model including
257 107 species for which we found data on habitat preference and 2) a Bayesian
258 phylogenetic mixed model, including the phylogeny of the 107 species. Comparisons
259 between the first and the second analysis allowed us to assess the influence, if any, of
260 phylogenetic relationships on the observed trends. Light availability, mimicry and
261 morphological traits were included as explanatory variables in our model. We
262 defined *ActHab*, a variable combining activity rhythm and habitat openness with four
263 levels (diurnal open, diurnal closed, nocturnal open, nocturnal closed) and three
264 contrasts (diurnal vs. nocturnal, open vs. closed habitats, and diurnal open habitat

265 vs. diurnal closed habitat). The variable mimicry has three levels (bee-wasp mimics,
266 leaf mimics and non-mimics) and we tested two contrasts (bee/wasp mimics vs. all
267 other species, and leaf mimics vs. non- mimics). Other explanatory variables
268 included: wing length (as a proxy of butterfly size), proportion of clearwing surface
269 and fore-/hindwing, reported by Gomez et al (2020) as correlated to light
270 transmittance. Wing transmittance, wing length and clearwing proportion were
271 obtained from Gomez *et al* (2021). As five measurements per wing (fore- and
272 hindwing), thus ten measurements per species were included in our dataset, wing
273 measurement replicates nested in species was considered as a random effect. We
274 fitted different models including different combinations of two-factor interactions
275 and we compared them using the AIC criterion to select the best model. As mimicry
276 levels show differences in the variance of their transparency degree (see results for
277 Fligner test and Fig. 4), we estimated different variances for the different factor
278 levels by specifying a variance structure into the model, using the function *varIdent*.
279 To better understand the effect of each of the included variables, we reported and
280 compared the full model without interactions and the best model including
281 interactions (Tables S1 and S3). The Bayesian phylogenetic mixed models with
282 Markov chain Monte Carlo analyses were performed using the ‘mulTree’ R package
283 (Guillerme and Healy 2014). Using the best formulated linear mixed model,
284 uninformative priors were used with an Inverse-Gamma distribution with shape =
285 0.001 and scale = 0.001 for both random effect and residual variances (Hadfield
286 2010). Models were run using two chains of 600,000 iterations, a thinning interval of
287 300 and a burn-in of 10,000. Fixed effects were considered statistically significant
288 when the probabilities in the 95% credible intervals did not include zero. As
289 Bayesian phylogenetic mixed models consider no interactions between factor levels,

290 several submodels were fitted to test the interactions from the best model obtained
291 according to AIC criterion.

292 We also explored the coevolution between other factors that are correlated to
293 conspicuousness, transparency degree and mimicry. At least in the tropics, insects
294 that are active during the day are smaller, on average, than insects active at night a
295 segregation probably related to the higher diurnal visual predation risk and the
296 higher detectability associated to larger bodies (Guevara and Avilés 2013).
297 Moreover, Gomez et al (2021) showed that transmittance was higher at smaller wing
298 sizes and larger proportions of clearwing surface. Therefore, we performed several
299 phylogenetic generalised least square (PGLS) analyses (1 observation per species) to
300 test whether small wing size and large clearwing surface proportion associate with
301 diurnality, open habitats and/or bee/wasp mimicry. Additionally, using also PGLS
302 we tested whether wing ratio (forewing length/hindwing length), which is usually
303 high in bees and wasps, is higher in bee/wasp mimics than dead-leaf mimics or in
304 non-mimics. Finally, using BayesTraits (Pagel and Meade 2013) we tested whether
305 bee/wasp mimics are more often diurnal and/or live in more open areas than non-
306 or dead-leaf mimics. For this last test, we calculated the likelihood of the dependent
307 (correlated) and independent (non-correlated) models of evolution mimicry
308 syndrome and *ActHab* levels using a Markov-Chain Monte-Carlo approach. Models
309 were compared using the likelihood ratio test with 4 degrees of freedom (Pagel
310 1994). As this approach can only be applied to binary characters, and mimicry has
311 three factors, models were run with different pairwise combinations of variables to
312 explore: a. the evolution of dead-leaf mimicry (including non-mimics and dead-leaf
313 mimics); b. evolution of bee/wasp mimics (including non-mimics and bee/wasp
314 mimics) and c. differences in the evolution of dead-leaf and bee/wasp- mimics

(including bee/wasp and dead-leaf mimics). Similarly, for the composed variable *ActHab* we compared (i) diurnal and nocturnal species flying in open habitats, and (ii) nocturnal species flying in open and in closed habitats.

We also tested whether scale characteristics associated to differences in light transmittance (as described by Gomez et al (2021)) have coevolved with mimicry syndrome and their specific optical properties, using again the test of correlated evolution implemented in BayesTraits (Pagel and Meade 2013). Scale characteristics included in this analyses were: absence/presence of scales, scale insertion (flat or erect), scale coloration (coloured or transparent) and scale type (piliform scales and lamellar scales). Independent analyses were performed for anterior and posterior wings, as differences between wings have been reported by Gomez et al (2021).

Finally, we predicted higher variance in light transmittance for those conditions where different levels of transparency might be equally efficient, as would be the case for butterflies and moths that are active during the night, that live in closed habitats and/or that are dead-leaf mimics. We applied Fligner-Killeen tests of homogeneity of variance in R to test for difference in variance between (i) diurnal and nocturnal species living in open and in close habitats, and (ii) bee/wasp mimics, leaf mimics and no mimic species. As Gomez et al (2021) reported that transmittance is closely related to wing size, we also tested for differences in the variation of wing length between habitat, activity rhythm and mimicry using the test mentioned above.

Results

Fieldwork experiment

339 A total of 243 out of the 1149 artificial butterflies were attacked (21.14 %). The best
340 model included artificial type and habitat openness without interaction (Delta AIC
341 2.79 between a model including and excluding the interaction). Artificial moth
342 survival was lower in more open habitats ($z = -2.67$, $p = 0.007$, Figure 2). Fully
343 opaque moths “C” and the least transparent moths (T6) show similar survival
344 (Figure 2) and together were more attacked than both T1 and T0 ($z = 3$, $p = 0.003$).
345 However, no differences are detected anymore when comparing survival of T6 and
346 either T0 ($z = 0.44$, $p = 0.66$) or T1 ($z = 1.25$, $p = 0.21$).

347

348 Comparative analyses

349 Relationships between light transmittance, habitat and activity rhythm

350 Activity rhythm and habitat openness partly explain the variation in light
351 transmission. Diurnal and nocturnal species transmit light in a similar way (Estimate
352 and confidence interval for Bayesian models without interaction: 0.46 [-10.07,
353 10.82]; with interaction -5.2[-18.52, 7.72], Figures 3a & 5, Table S1 & S2a). However,
354 diurnal (D) species transmit slightly more light than nocturnal (N) species at smaller
355 clearwing surfaces and at larger wing lengths (D>N: %clearwing surface: -0.18[-0.28,
356 -0.18]; D>N: wing length 0.46[0.13,0.77], Figures 5, S4a & S4b, Table S1 & S2a).
357 Moreover, forewings (F) transmit more light in diurnal than in nocturnal species
358 (D>N:F>H : 8.41[4.76, 12.12]). Wing length and proportion of clearwing surface is
359 similar in diurnal and nocturnal species (Table S2a). Variation in forewing light
360 transmission is lower for diurnal than for nocturnal species (Fligner-Killeen test for
361 forewings: 17.44, df=1, $p < 0.001$, Figure 3a). Similarly, wing size variation is lower in
362 diurnal than in nocturnal species and for both fore- and hindwings (Fligner-Killeen

test for forewings: 8.87, df=1, p= 0.003; for hindwings: 28.86, df=1, p < 0.001, Figure 3b).

Regarding habitat openness, we found that light transmittance is slightly higher for species living in closed than in open habitats (with interactions: -16.98[-30.04, -4.19], Table S2b). However, two characteristics associated to higher light transmission are more common in open habitats: the proportion of clearwing surface is larger for species flying in open than in closed habitats (PGLS forewing: t = 4.02, p < 0.001; hindwing: t= 2.01, p = 0.04, Table S3) and butterflies and moths flying in open habitats have smaller forewings than species flying in closed habitats (PGLS forewing: t = -2.12, p = 0.04, Table S3). When the proportion of clearwing surfaces is high, species flying in open habitats (O) transmit slightly more light than species living in closed habitats (C) ($O > C$: %clearwing surface: 0.44 [0.3, 0.59], Figure S4a). Variation in light transmission and wing length are similar between open and closed habitats (Fligner-Killeen test for light transmission in forewings: 3.23, df=1, p = 0.07; in hindwings: 1.66, df=1, p = 0.20. Fligner-Killeen test for length in forewings: 0.24, df=1, p = 0.62; in hindwings: 0.19, df=1, p = 0.66, Figures 3b & 3c)

When analysing only diurnal species, we found that light transmittance is similar between species flying in open and closed habitats (without interactions: 1.64 [-8.88, 12.88], with interactions: -17.24 [-37.39, 2.68], Table S2c). However, at larger proportions of clearwing surfaces, diurnal species flying in open habitats transmit slightly more light than diurnal species flying in closed habitats ($DO > DC$: %clearwing surface: 0.44 [0.3, 0.58], Figure 3c & 5, Table S1 & S2c). Nevertheless, diurnal species flying in closed habitats exhibit larger proportions of clearwing surface than do species flying in open habitats (PGLS t=-4.34, p<0.001, Table S3). Transmittance in the hindwing of diurnal species flying in closed habitats

varies less than for species flying in open habitats (Fligner-Killeen test for forewing: 0.06, df=1, p= 0.80, hindwing: 6.45, df=1, p=0.01, Figure 3a). In diurnal species, open habitat species exhibit larger variation in wing length than closed habitat species (Fligner-Killeen test for forewing: 5.9, df=1, p=0.015, hindwing: 43.91, df=1, p<0.001, Figure 3b).

Relationships between light transmittance, transparency structural basis and mimicry

Mimicry also explains part of the variation found in light transmittance. Bee/wasp mimics transmit more light than dead-leaf mimics and other clearwing species (without interactions: 26.38 [15.16, 37.12]; with interactions: 36.97 [23.3, 50.28], Figures 4a & 5, Tables S1 & S2a). Light transmittance in bee/wasp mimics is slightly lower at larger wing lengths (bee/wasp mimics>others species: wing length: -0.73 [-1.04, -0.41]; Figure 5, Tables S1 & S2a). Additionally, non-mimics transmit slightly less light than dead-leaf mimics at larger proportions of transparent wing surface (leaf mimics<non-mimics: %clearwing surface: -0.37 [-0.57, -0.19]; Figures 5 & S5b, Tables S1 & S2d).

We found that transmittance varies more in dead-leaf mimics than in bee/wasp mimics (Fligner-Killeen test for forewing: 31.38, df= 1, p<0.001, for hindwing: 50.43, df = 1, p<0.001), or in other clearwing species (Fligner-Killeen test for forewing: 37.95, df=1, p<0.001; for hindwing: 57.51, df = 1, p<0.001), but we found no difference in transmittance variation between leaf mimics and non-mimic species (Fligner-Killeen test for forewing: 2.02, df=1, p= 0.155; for hindwing: 0.00005, df = 1, p=0.995). Likewise, wing length varies less in bee/wasp mimics than in leaf mimics (Fligner-Killeen test for forewing: 16.96, df=1, p<0.001; for hindwing: 37.98, df = 1, p<0.001, Figure 4b) or in other clearwing species (Fligner-Killeen test

for forewing: 37.87, df=1, $p < 0.001$; for hindwing: 57.39, df = 1, $p < 0.001$). Leaf and non-mimics show similar variations in wing length (Fligner-Killeen test for forewing: 0.68, df=1, $p = 0.40$; for hindwing: 2.52, df = 1, $p = 0.11$).

Regarding the relationship between mimicry and wing characteristics, non-mimics have smaller hindwings than dead-leaf mimics (PGLS forewing: $t = 1.82$, $p = 0.07$; hindwing: $t = 1.99$, $p = 0.05$, Table S3), and the length ratio forewing/hindwing is larger for bee/wasp mimics than for any other clearwing species group (PGLS $t = 4.14$, $p < 0.001$, Fig. 4c). Additionally, the proportion of clearwing surface in forewings and hindwings is larger for non-mimics and for dead-leaf mimics (PGLS forewing: $t = 2.74$, $p = 0.007$; hindwing: $t = 2.29$, $p = 0.02$, Figure S5a). Mimicry syndrome and activity rhythm have likely coevolved: most bee/wasp mimics are diurnal while most leaf mimics are nocturnal (BayesTraits activity rhythm and bee/wasp or leaf mimics: LRT=11.43, df = 4, $p = 0.02$, Table 2, Figure S3 & S6).

Some scale characteristics are correlated to mimicry evolution. Scales are more often absent and less often coloured and flat when present in hindwings of bee/wasp mimics in comparison to dead-leaf mimics (BT LRT presence = 10.45, df=4, $p = 0.033$; BT LRT insertion= 11.06, df=4, $p = 0.03$) and non-mimics wings (BT LRT presence = 29.99, df=4, $p = 0.001$; BT LRT colour= 21.34, df=4, $p = 0.001$; BT LRT insertion = 11.17, df=4, $p = 0.025$, Table S4 & Fig S7). Similarly, dead-leaf mimics have less often coloured scales in comparison to non-mimics both in forewing (BT LRT=11.62, df=4, $p = 0.02$) and hindwing (BT LRT=12.87, df=4, $p = 0.012$, Table S4 & Fig S7). Moreover, leaf mimics only exhibit lamellar scales in contrast to non-mimics (BT LRT=9.33, df=4, $p = 0.053$, Table S4 & Fig S7).

Discussion

438 Fieldwork experiments using artificial prey that differed in their proportion of
 439 transmitted light suggest that variation in transparency is similarly detected in
 440 bright and dim light conditions, contrasting with what has been suggested for
 441 aquatic environments (Johnsen and Widder 1998). Opaque prey were more
 442 attacked than highly transparent prey in both open and closed habitats, suggesting
 443 that transparency enhances crypsis in terrestrial environments, independently of
 444 light conditions. Moreover, T6 survival is marginally the lowest in comparison to the
 445 other transparent types (T1 and T0), suggesting that terrestrial predators probably
 446 detect differences in transparency degree as well as aquatic predators do. The lack of
 447 larger differences between artificial prey types might be related to the continuum
 448 created by our moths that could have elicited only slightly different reactions
 449 towards each of them as in other similar studies (Arias et al 2021). An experiment
 450 where higher number of responses towards each type is be obtained, can contribute
 451 to the understanding of this trend. Moreover, whether factors such as movement can
 452 enhance differences between transparency degrees remains to be tested.
 453 Additionally, fewer artificial moths were attacked in closed habitats, in accordance to
 454 the higher predation rate often reported for open habitats such as forest edges
 455 (Barbaro et al. 2014; Seymoure et al. 2018). Such higher predation rate can be
 456 related to a higher detectability of prey in such exposed areas (Stankowich and
 457 Campbell 2016) and in the case of forest edges, to their use as travel lanes by
 458 predators, increasing their presence in those places (Andrn 1995; Saab 1999).

459 Our comparative analyses show some evidence supporting that variation in
 460 transparency degree is related to light availability. Diurnal species transmit more
 461 light on their forewings and exhibit a higher transparency degree at lower
 462 percentages of clearwing surface and at larger wing length sizes than nocturnal

species. Moreover, diurnal species vary less in the light they transmit than nocturnal species, suggesting a strong selection for high levels of transmittance. Although both diurnal and nocturnal species are exposed to visual predators during the day, species that are active during the day, independently of the habitat where they fly, are at higher predation risk as they can be more easily detected by their movement (Lyytinen et al. 2004; Galloway et al. 2020). Therefore, traits reducing this detectability, such as high transparency levels will be strongly selected.

However and in accordance to our experimental results, no differences in transmittance were detected between diurnal species flying in open and closed habitats. Additionally, butterflies and moths living in closed habitats (including both diurnal and nocturnal) transmit similar to slightly more light than species living in open habitats. Gomez et al (2021) reported that poor light transmittance is associated with small proportions of clearwing surface and large wing sizes. Diurnal species living in closed habitats show higher proportion of clearwing surface than diurnal species living in open habitats (Table 1, Fig. 3), probably contributing to the similar/slightly higher values of light transmittance in closed habitats in comparison to the open habitats. Selective pressures related to abiotic conditions might also help understanding this pattern. Species living in open habitats undergo higher risks of tissue damage by higher exposure to UV light, more wind or higher exposure to direct rainfall, conditions that have been reported as important at shaping moth and butterfly segregation in different habitats (Brown Jr and Hutchings 1997). In case transparency entails costs related to one or several of these factors, selection may promote lower proportions of clearwing surfaces in open habitats, decreasing the vulnerability of clearwing Lepidoptera species to these abiotic effects, and light transmittance by default.

Transparency can decrease prey detectability but it is also involved in mimicry of bees or wasps, and differences in light transmittance seem to be closely associated to mimicry evolution. We found that bee/wasp mimics are diurnal (similar to most bees, Somanathan et al. 2009), and have smaller wing sizes and smaller hindwings in comparison to their forewings (as has been reported for wasps and bees, Wootton 1992; Lindauer 2019). Additionally, they have higher transmittance levels, frequently associated to the absence of scales in their wings, and are more often restricted to open habitats suggesting that high transmittance in open habitats can be a visual optimum in terrestrial environments under certain circumstances. As this is not the case for all clearwing species, this highlights the strong effect of biotic and abiotic interactions in shaping adaptive peaks, thus the segregation of species under different conditions and the evolution of different features. However, the correlation between open habitats, diurnal activity, small sizes and higher transmittance levels, suggests that transparency in bees, wasps and their mimics probably reduces their detectability, a question that remains open.

On the other hand, we found that leaf mimics are more often found in closed habitats, as expected given to the higher presence of dead leaves in closed habitats. Additionally, they are larger and in contrast to the trends for all other clearwing species, transmit more light at lower proportions of clearwing surfaces. Some fully opaque fallen leaf mimic species, such as the comma butterfly (*Polygonia c-album*) exhibit a distinctive pale mark that might decrease their predation by being perceived as ‘distractive marks’ preventing predators from recognising prey, thus enhancing background matching (Dimitrova et al. 2009; Olofsson et al. 2013), although it is controversial (Troschianko et al. 2018). Either way, reduced and highly transparent zones as those found in *Orthogonioptilum violascens* females might

513 produce a similar appearance to those markings; their high transmittance might be
 514 highly reflective and be perceived as a white mark, thus conferring the potential
 515 benefits of the “comma mark” when seen for a given angle, as well as the full crypsis,
 516 when no reflections can be perceived. Whether highly transparent windows work as
 517 other highly visible elements displayed in cryptic species such as eyespots that deter
 518 predators (Stevens et al. 2008b, 2009) and that are also important in mate choice
 519 (Robertson and Monteiro 2005) remains to be tested.

520 Other dead-leaf mimics include large transparent surfaces that can expand to
 521 the wings edges, as for *Bertholdia* species, or not, as for *Pseudasellodes fenestraria*. In
 522 both cases, transparent surfaces likely disrupt prey shape (as in the first case)
 523 and/or prey surface (as in the second example (Costello et al. 2020)), thereby
 524 reducing their detectability. Disruptive coloration, especially when touching wing
 525 margins, is common in fully opaque species with cryptic patterns/colours and has
 526 been broadly studied as it is common in fully opaque cryptic species (Cuthill et al.
 527 2005; Schaefer and Stobbe 2006; Stevens and Cuthill 2006; Fraser et al. 2007).
 528 Whether transparent surfaces touching wing margins, work as disruptive marks
 529 regardless of their transmittance, thus further decreasing prey detectability remains
 530 to be tested.

531

532 **Conclusion**

533 Both light availability (associated to activity rhythm and habitat openness) and
 534 mimicry can concur to fostering variations in light transmittance on land. Our results
 535 opens up new questions that need to be investigated in the future, in particular the
 536 effect of both biotic (such as mimicry interactions with different models) and abiotic
 537 (such as the possible cost associated to the physical conditions of each habitat), to

538 better understand the evolution and the constraints imposed on transparency on
539 land.

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Figures and tables - Ambient light and mimicry as drivers of wing

transparency in Lepidoptera

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Figure 1. Examples of a bee/wasp mimic (a. adult female *Hemaris fuciformis* *fuciformis*, Catalonia, Spain. Photo: © Tony Pittaway) and a potential dead leaf mimic (b. *Pseudasellodes fenestraria*, Rio Kosnipata, Peru. Photo: © Adrian Hoskins). Forewing length is ~24mm in *Hemaris fuciformis fuciformis* and ~18mm in *Pseudasellodes fenestraria*. Each tick in the scale represents one mm.

Figure 2. Survival rate of artificial prey per form according to the canopy coverage. Prey forms include: prey without any transparent area (C), and with transparent elements covered by 6 (T6), 1 (T1) or no (T0) transparent film layers. Open habitats exhibit low canopy coverage (towards the left of the plot) while closed habitats will have larger canopy cover (towards the right of the plot).

Figure 3: Variation between diurnal/open (yellow), diurnal/closed (green), nocturnal/open (blue) and nocturnal/closed (purple) in a) mean light transmittance, b) wing length and c) proportion of clearwing surface. Values larger than 60 mm were not plotted for clarity reasons but were included in the analyses.

Figure 4: Variation between bee/wasp mimics (red), leaf mimics (purple) and no mimics (blue) in a) mean light transmittance, b) wing length and c) ratio between length of forewing and hindwing per mimicry group:

Figure 5. Bayesian regression estimates and 95% confidence intervals for each variable and their interactions in the best fitted model. Such model included transmittance as dependent variable and the following explanatory variables: the combination between daytime activity and habitat type (ActHab: nocturnal-open/nocturnal-closed/diurnal-open/diurnal-closed), wing length in mm, proportion of clearwing area (% clearwing), mimicry syndrome (bee/wasp -, leaf - or non-mimics), wing (fore-/hindwing) and the interactions: mimicry syndrome and proportion of clearwing area, mimicry syndrome and wing size, proportion of clearwing area and habitat type, wing size and habitat type, wing size and proportion of clearing area, wing and wing length and proportion of clearwing area, wing length and habitat as explanatory variables. Wing measurements nested in species was considered as random effect. Names of variables and interactions whose confidence interval excludes zero are in bold. Model results are found in Tables S2.

Table 1. Results of coevolution tests between habitat, mimicry and daytime activity

Tested characters	Mimicry levels included	L.Dep.	L.Indep.	LR	df	p	
daytime activity/ mimicry	bee/wasp & leaf mimics	-24.48	-30.19	11.43	4	0.02	*
	No- & leaf mimics	-84.5	-90.31	11.62	4	0.02	*
	No- & bee/wasp mimics	-98.5	-98.5	0	4	1	
habitat / mimicry	bee/wasp & leaf mimics	-29.12	-30.19	2.14	4	0.71	
	No- & leaf mimics	-91.52	-91.53	0.01	4	0.99	
	No- & bee/wasp mimics	-99.99	-101.82	3.67	4	0.45	
habitat/daytime activity	No-, bee/wasp- & leaf mimics	-142.49	-145.1	5.22	4	0.26	
day.open	bee/wasp & leaf mimics	-17.57	-21.17	7.2	4	0.12	
vs night.open	No- & leaf mimics	-45.89	-49.05	6.32	4	0.18	
/mimicry	No- & bee/wasp mimics	-57.48	-57.82	0.68	4	0.95	
night.open	bee/wasp & leaf mimics	-19.5	-19.52	0.04	4	1	
vs night.closed	No- & leaf mimics	-50.56	-50.57	0.02	4	1	
/mimicry	No- & bee/wasp mimics	-36.86	-36.87	0.02	4	1	

Log-likelihood of dependent (L. Dep. Coevolution, 4 parameters estimated) and independent (L. Indep. 8 parameters estimated) evolution models between pairs of binary characters obtained from BayesTraits, log-likelihood ratio (LR), associated degree of freedom (df: difference in number of parameters) and corresponding p-value of the Likelihood Ratio Test. Because BayesTraits only take binary characters, when factors have more than 2 levels (mimicry and the composed character ActHab), we implemented pairwise comparisons of level. Coevolution between mimicry and diurnal species flying either in open or in closed habitats was not tested as no bee/wasp mimic is simultaneously diurnal and closed habitat dweller (Fig S4). A significant p-value means that the dependent model is significantly better than the independent model, indicating a correlated evolution between the traits. ** stands for statistical significance below 0.01 and * for significance values below 0.05.