# Variation in transparency degree: response to light availability or part of mimicry syndrome?

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**Abstract (247 words)**

Transparency is common in aquatic environments where it reduces detectability by predators. Transparency degree (the proportion of transmitted light) varies under water and is higher as light availability increases. Transparency is rare on land and poorly studied. Recent studies have shown that Lepidoptera – a group in which transparency has independently evolved multiple times – also display large variation in transparency degree. The large diversity encompasses different types of clearwing species, such as bee/wasp mimics and death leaf mimics. This study explores how the evolution of different transparency degree could be related to habitat openness, and to mimicry syndrome, through two complementary approaches. First, by exposing artificial moth-like prey to wild avian predators in open and closed habitats, our fieldwork experiment shows an increase in prey survival at higher transparency degrees in open compared to closed habitats. Second, by analysing the evolution of wing characteristics and ecological traits in 107 clearwing species, comparative analyses show that instead, open habitat species show in average

similar or slightly lower transparency degree than closed habitat species. We also find that bee/wasp mimics have highest and least varying transparency degrees and are more often diurnal. Compared to bees/wasp mimics, leaf mimics have lower but more varying transparency degree and are more often nocturnal. High transparency degree likely reduces detectability in open habitats, as shown by field experiments and comparative analyses of optical measurements on bee/wasp mimics. Our results suggest that habitat openness, and species interactions play a crucial role in determining transparency design.

**Keywords**: transparency, irradiance, mimicry, nocturnality, Lepidoptera

## Introduction

Transparency is common in aquatic environments where it reduces detectability and works as an antipredatory defence, especially in the pelagic environment where there is nowhere to hide [1]. However, the amount of light that is transmitted by transparent tissues (hereafter referred to as transparency degree) varies between organisms. Based on the observation of 29 zooplankton species, Johnsen and Widder [2], found that transparency degree could range from 50 to 90%, with the most transparent species living in lighter, shallower places. In a theoretical approach, they show that efficiency at detecting transparent species depends on visual performance (for instance, minimum contrast that predator can detect, also known as contrast threshold), which varies with ambient light conditions: in shallow places with bright light conditions, high visual performance (low contrast threshold values) selects for high transparency degree, and only highly transparent organisms are effectively undetected. Instead, at deeper areas with dimmer light conditions, visual systems are less performing (high contrast threshold values) and select for low transparency degree, thus organisms can be poorly transparent and be effectively undetected [3, Figure S1]. On land, studies in primates have also shown higher visual performance at brighter light conditions as the retinal cells increase their neural response more strongly when mean luminance is higher [4,5]. Similarly, visual performance increases with ambient light luminance in other terrestrial vertebrates such as birds [6–8]

Transparency is rather rare in terrestrial environments and is almost restricted to insect wings. Transparency also reduces detectability of prey on land [9–11]. Recently, Gomez et al [12] reported that transparency has evolved multiple times independently in Lepidoptera, and that it is produced by a large diversity of structures and configurations with different transparent degrees. This study does not investigate the links between transparency and habitat, but suggests in several aspects an important role of visual predators in driving the evolution of transparency (for instance diurnal species were reported to have higher transparency degree than nocturnal species). Hence, we expect that terrestrial transparent species living in more open habitats - with brighter light conditions - should present higher transparency degree compared to species living in more closed habitats - with dimmer light conditions -. In addition, closed-habitat species

78 might show larger variation in transparency degrees as many degrees are similarly
79 visually protective, thus high transparency degrees are not strongly selected.
80 Predation risk can be reduced not only by reducing detectability, but also by
81 mimicking inedible elements such as leaves [masquerade, 13] or unprofitable prey
82 [batesian mimicry, 14], and transparency might help achieving such mimicry.
83 Several clearwing Lepidoptera exhibit brownish colorations that combined with
84 transparent surfaces resembling "holes" can mimic dead leaves (examples of
85 potential dead-leaf mimics in Fig. 1a). Other small clearwing moths and butterflies
86 exhibit yellow and dark stripes on their bodies and highly transparent wings
87 (examples in Fig. 1b), resembling harmful species for predators, such as bees and
88 wasps. Mimicry syndrome could concur in driving transparency degrees to have
89 different optima in bee/wasp and leaf mimics, but whichever factor (habitat light
90 availability versus mimicry) is driving the evolution is still unknown. Bees and
91 wasps often live in open habitats [15,16], and their mimics should do as well. High
92 transparency degree should be selected in bees, wasps and their mimics because
93 their body sizes are rather small, a trait associated to higher transparency degrees in
94 butterflies [12], and light conditions are brighter in open habitats. Instead, dead-leaf
95 mimics might show a larger variation in body size, may more often live in closed
96 habitats, where more leaves fall down and should exhibit a broader diversity of
97 transparency degrees, as all of them can contribute to mimic leaves in
98 decomposition and confer similar protection against predators. The expected
99 differences in transparency degrees should also be associated to variations in
100 morphology and coloration of wing scales, with absent or transparent hair-like
101 scales for bee/wasp mimics and classically shaped scales for leaf mimics.
102 Here, we studied whether differences in transparency degree found in
103 Lepidoptera clearwing species are related to light availability and/or mimicry using
104 two complementary approaches. First, we tested whether the efficiency of
105 transparency at reducing detectability changed according to light availability on
106 terrestrial environments, by carrying out a fieldwork experiment with artificial prey.
107 Second, using real butterflies, we tested whether differences in transparency degree
108 could be explained by species habitat and by mimicry, by conducting comparative
109 analyses at broad interspecific level. We took advantage of the 107-species dataset of
110 Lepidopteran species analysed by Gomez et al [12] to add species ecological traits

(habitat and mimicry) and relate them to clearwing optical and structural traits. Together, these analyses help understanding the evolution of interspecific differences in transparency degree on land.

## Material and Methods

Artificial butterfly Elaboration

To test for the efficiency of transparency degree at reducing detectability in habitats with different light availability, we elaborated plain grey artificial butterflies with paper wings and a malleable edible body. Following the general methodology described in Arias *et al* [9], butterflies did not replicate any real local butterfly, but mimicked a general grey moth with closed wings (i. e., a triangular shape). The rather cryptic butterfly colour was chosen based on 120 reflectance measures of green oak *Quercus ilex* trunks. Reflectance measurements were taken using a spectrophotometer (Starline Avaspec-2048 L, Avantes) and a deuterium halogen lamp (Avalight DHS, Avantes) emitting in the 300-700 nm range, including UV, to which some predators of butterfly and moths, such as birds, are sensitive [17]. Measurements were done relative to a white reference (lights turned on with no sample) and a dark reference (light turned off with no sample). For reflectance measurements, we used an optic probe (FC-UV200-2-1.5 x 100, Avantes) merging illumination and collection angles. Grey wings (R=155, G=155, B=155) were printed on sketch paper Canson using a HP Officejet Pro 6230 printer. Paper wings were laminated with a Polyester Opale Mat 75µm poach. Four forms of artificial butterflies we used that in order of transparency were: completely opaque butterfly "C", poorly transparent butterfly with 6 layers of transparent film "T6", highly transparent butterfly with a single layer of transparent film "T1", and fully transparent butterfly with no film in the transparent zones "T0". For the butterflies that included transparent elements, triangular windows of 234 $mm^2$ were cut down from the grey triangle and the remaining part was put on top of one or six layers of transparency film 3M for inkjet printing. This transparent film was chosen as it is highly transparent, even in the UV range of the spectrum, and going from 1 to 6 transparent layers permitted a reduction of 50% of the transmittance between treatments (Fig. S2). Such transparent layer was coated with a transparent mate varnish to reduce its shininess. For transparency measurements, we measured specular transmittance

144 from 300 to 1100 nm, using a deuterium-halogen lamp (Avalight DHS, Avantes),
145 optical fibres (FC-UV200-2-1.5 x 100, Avantes) and a spectrometer (Starline
146 Avaspec-2048 L, Avantes). Fibres were separated, aligned and 5mm apart and the
147 wing sample was placed perpendicular between them at equal distance (light spot of
148 1mm diameter). Spectra were taken relative to a dark (light off) and to a white
149 reference (no sample between the fibres) measurement. On top of butterfly wings,
150 we added an artificial grey body. Butterfly bodies were prepared with flour (428 g),
151 lard (250 g) and water (36 g), as reported on Carrol & Sherratt [18]. Yellow, red and
152 blue food edible colourings were used to dye the pastry in grey, imitating artificial
153 wing colour. Such malleable mix permits to register and distinguish marks made by
154 bird beaks from insect jaws. Both paper wings and artificial bodies were measured
155 in spectrometry, similarly to how trunk reflectance was measured (Fig. S2). Bodies
156 could attract predator attention, but it has been previously shown that wingless
157 bodies had the same attractiveness as that of butterflies with large transparent
158 elements [9]. Artificial butterflies and bodies were pinned to green oak *Quercus ilex*
159 trunks. To avoid ant attacks, Vaseline and a piece of highly sticky double-faced
160 transparent tape were stuck in between the wings and the trunk.
161
162 Experimental set up
163 Predation experiments were performed in May and June 2018 at the zoological Park
164 of Montpellier (43.64°, 3.87°) and at La Rouviere forest (close to Montarnaud city
165 43.65°, 3.64°). This experiment overlapped with breeding season of local great
166 (*Parus major*) and blue tit (*Cyanistes caeruleus*) populations, when birds are looking
167 for food in an intensive way, what can maximise the number of attacks on our
168 artificial prey. Artificial prey items were pinned every 10 metres on oak trunks of at
169 least 10 cm of diameter and with few or no moose cover. Prey order was randomized
170 by blocks of eight items, two per treatment, before starting the experiment. Initially,
171 prey was evenly disposed in closed and open areas. However, throughout the
172 experiment a stronger predation rate was detected in open areas (see Results
173 section). Therefore, we increased the number of artificial prey released on closed
174 areas to compensate for the differential predation pressure and get more similar
175 numbers of attacked prey items in closed and open habitats. We analysed results for
176 both original (larger sample size in closed habitats) and reduced datasets (same

177 number of butterflies placed in both closed and open habitats). Prey were mostly
178 disposed facing north and as perpendicular to the floor as possible, to reduce direct
179 sunlight reflection on them. Prey was checked every 24h. After 96h all prey were
180 removed. When V- or U- marks were detected, or when the body was missing
181 without signals of invertebrate attacks (i.e. no body scraps left on the wings or
182 around the butterfly on the trunk) prey were considered as attacked and wings or
183 pins where removed. Instead, if invertebrate attacks were detected, or when prey
184 was missing, it was replaced. Non-attacked prey were considered as censored data.
185 Canopy cover was used to classify places in open and closed habitat. We took
186 pictures each 20 meters in the trails (at forest edges for the open habitat, inside the
187 forest for the closed habitat, examples Fig. S3) that were used for the experiment,
188 using a Canon EOS 550D camera and a fisheye lens. Canopy cover was further
189 quantified using Fiji software [19]. After verifying unimodality in canopy cover
190 distribution (Hartigans' dip test: D = 0.01, p-value = 0.6), we used a discrete
191 classification, assuming that places with cover larger than the mean where closed
192 habitats and places with lower covers where open habitats (see Fig. S3 for the
193 distribution of canopy cover in the experimental sites).
194 To test how different treatments survive along the experiment on both open and
195 closed habitats, Cox proportional hazard regression [20] were applied, first for all
196 data points including form, habitat openness and their interaction as explanatory
197 variables, and then, Cox regression were independently applied for each habitat
198 using the different treatments as factors, to further explore the effect of form within
199 each habitat. Overall significance was measured using a Wald test. Statistical
200 analyses were performed in R [21] using the *survival* package [22].
201
202 Species selection and phylogeny
203 We used a dataset of 107 clearwing species extracted from that used by Gomez *et al*
204 [12], and including those species for which information about the openness of their
205 habitat was available. These species spanned 31 of the 124 existing Lepidoptera
206 families and were selected based on the presence of transparent or translucent
207 zones in their wings. For each species, we selected one specimen from the
208 Lepidoptera collection of National Museum of Natural History (Paris, France). For
209 comparative analyses, we used the phylogenetic tree built by Gomez *et al* [12]. In

210 brief, 9 nuclear and mitochondrial DNA sequences were taken from GenBank or
211 BOLD for the clearwing species or a surrogate species from the same genus, tribe,
212 subfamily or family. 60 additional species were incorporated to improve tree
213 topology. After sequence alignment and partitioning, tree Bayesian inference using
214 BEAST 1.8.4 [23] by constraining family topology and node ages to follow [24] was
215 performed. After combining several runs with appropriate burn-in periods, the
216 maximum clade credibility tree (MCCT) was computed with median node ages,
217 which was then used for our analyses.
218
219 Ecological data on species habitat, daytime activity and mimicry type
220 Ecological information was gathered consulting papers and experts for a total of 107
221 species for activity rhythm (58% of species are diurnal species), habitat (56% of
222 species living in open habitats), and mimicry status (14 species were bee/wasp
223 mimics, 13 species were leaf mimics, while the remaining 80 did not fall in either of
224 those categories. Table S1 and Fig S4). We excluded from the analyses species that
225 were reported to live in open and closed habitats, or to fly at day and night. Bee and
226 wasp mimics were grouped together in the same mimicry category, as both exhibit
227 the striped pattern with black and yellow that advertise their unpalatability [25].
228 Butterflies and moths mimicking bees and wasps have forewings longer than their
229 hindwings, and often strong delimitations of their wing borders and veins by dark
230 coloration, as the hornet moth *Sesia apiformis* (*api* - bee, *formis* -shaped) or the
231 broad-bordered bee hawk-moth *Hemaris fuciformis* (Fig 1b). Fallen leaf mimics
232 include brownish moths and butterflies with small or large transparent surfaces and
233 that could touch or not the wing borders (Fig. 1a). *Rotschildia lebeau* has been
234 proposed as a leaf mimic [26] but such mimicry is controversial [27]. *Rotschildia*
235 *ericina* was therefore not considered as a leaf mimic for the analyses.
236
237 Wing structural and optical measurements
238 Museum specimens were photographed using a D800E Nikon camera equipped with
239 a 60mm lens, placed on a stand with an annular light. Photos were then analysed
240 using ImageJ [28] to extract descriptors of wing macrostructure: wing length (mm),
241 wing surface ($mm^2$), and clearwing area (the surface of transparent area in $mm^2$), for
242 the forewing and hindwing separately. We defined and computed proportion of

243 clearwing area as the ratio clearwing area/wing surface, i.e. the proportion of the
244 total wing area occupied by transparency.
245 For transparency measurements, we followed the same procedure described
246 above for measuring transparency in artificial prey. For each species and wing, we
247 took five measurements in the transparent zone. We analysed spectral shape using
248 Avicol v6 [29] to extract the mean transmittance over [300-700] nm, which
249 described the level of transparency.
250
251 Physical properties of transparent wings
252 Gomez et al [12] described a large diversity in transparent wing surface
253 characteristics. Using binocular imaging (Zeiss Stereo Discovery V20) and digital
254 microscopic imaging (Keyence VHX-5000), these authors found that transparency
255 could be achieved either with nude membranes or with membranes covered by
256 scales of various types (either classical scales, hair-like scales or both) that could be
257 coloured or transparent, and erected of flat according to their insertion on the wing
258 membrane. Therefore, we also explored the evolution of mimicry according to these
259 characteristics.
260
261 Analyses of transparency degree differences between habitats and mimics
262 To explore whether differences in transparency degree (percentage of transmitted
263 light) are related to variation in habitat and mimicry we fitted both 1) a linear mixed
264 model including 107 species for which we found data on habitat preference and 2) a
265 Bayesian phylogenetic mixed model, using the phylogenetic reconstructions
266 obtained by Gomez et al including 183 species from 31 families. Comparisons
267 between the first and the second analysis allowed us to assess the influence, if any, of
268 phylogenetic relationships on the observed trends. We included habitat
269 (open/closed) and mimicry (bee-wasp mimics/leaf mimics/no mimicry with the
270 contrasts bee/wasp mimics more transparent than all other species, and leaf mimics
271 less transparent than other types of species) as explanatory variables, together with
272 variables reported by Gomez et al (2020) as correlated to transparency degree: wing
273 length (as a proxy of butterfly size), proportion of clearwing surface, fore-/hindwing
274 and daytime activity (diurnal/nocturnal). Butterfly transparency, wing length and
275 clearwing proportion were obtained from Gomez *et al* [12]. As five measurements

276 per wing (fore- and hindwing), thus ten measurements per species were included in
277 our dataset, wing measures nested in species was considered as a random effect. We
278 fitted different models including different combinations of two- and three-factor
279 interactions and we compared them using the AIC criterion to select the best model.
280 We reported and compared the model without interactions and the best model
281 including interactions. Additionally, we fitted Bayesian phylogenetic mixed models
282 with Markov chain Monte Carlo analyses using the 'mulTree' R package [30]. Using
283 the best formulated linear mixed model, uninformative priors were chosen, with
284 variance and belief parameter set to 1 for random effects and 0.002 for residual
285 variances [31]. Models were run using two chains of 600,000 iterations with and a
286 thinning interval of 300 and a burn-in of 10,000. Fixed effects were considered
287 statistically significant when the probabilities in the 95% credible intervals did not
288 include zero.
289 Additionally, we performed several phylogenetic generalised least square (PGLS)
290 analyses (1 observation per species) to test for potential correlations between size,
291 proportion of clearwing surface, daytime activity, habitat and mimicry, and between
292 wing ratio (forewing length/hindwing length, usually high in bees and wasps) and
293 mimicry. Using BayesTraits [32] we additionally explored character coevolution
294 between mimicry/habitat, mimicry/daytime activity and habitat/daytime as well as
295 between mimicry and scale characteristics: absence/presence of scales, scale
296 insertion (flat or erected), scale coloration (coloured or transparent) as well as scale
297 type (hair-like scales and classic scales). When analysing physical wing properties
298 and mimicry evolution, independent analyses were performed for anterior and
299 posterior wings, as differences between wings have been reported by Gomez et al
300 [12]. We calculated the likelihood of the dependent and independent models of
301 evolution of each pair of binary characters, using a Markov-Chain Monte-Carlo
302 approach. We then estimated the likelihood ratio test between them and compared it
303 to a chi-square distribution with 4 degrees of freedom to obtain their statistical
304 significance [33]. As this approach can only be applied to binary characters, and
305 mimicry has three factors, models were run including the different possible pairwise
306 combinations to explore: a. evolution of leaf mimicry (including non-mimics and leaf
307 mimics); b. evolution of bee/wasp mimics (including non-mimics and bee/wasp
308 mimics) and c. differences in the evolution of leaf and bee/wasp- mimics (including

309 bee/wasp and leaf mimics). We calculated δ, a new metric inspired in the
310 phylogenetic analog of the Shannon entropy to measure phylogenetic signal in
311 categorical non-ordered data [34], for testing for phylogenetic signal in mimicry
312 evolution. δ is useful to detect whether the coevolution between mimicry and other
313 traits are homologies (mimicry and the other characteristics are shared within a
314 clade) or convergences (mimicry and the other characteristics evolved
315 independently and several time along the phylogeny).
316 Finally, we predicted more variance in transparency degree for those
317 conditions where different levels of transparency might be equally efficient as would
318 be the case for butterflies and moths that are active during the night, that live in
319 closed habitats and/or that are leaf mimics. We applied Fligner-Killeen tests of
320 homogeneity of variance in R to test for difference in variance between a) diurnal
321 and nocturnal species, b) species living in open and in close habitats and c)
322 bee/wasp mimics, leaf mimics and no mimic species. As Gomez et al (2020) reported
323 that transparency degree is closely related to wing size, we also tested for
324 differences in the variation of wing length between habitat, daytime activity and
325 mimicry using the test mentioned above.
326
327 Results
328 Fieldwork experiment
329 In total, 1149 artificial butterflies were used during the experiment and 243 prey
330 were attacked (21.14 %). When data from both open and closed habitats were
331 analysed together (same numbers of attacked prey items), survival was lower in
332 open than in closed areas. In closed areas 651 prey were released, 110 attacked, with
333 an attack rate of 16.89% while in open areas, 498 prey were released, 133 attacked,
334 with an attack rate of 26.71% (Wald test = 34.39, df = 7, $p<0.001$). Results are similar
335 when including the "reduced" dataset (a random subsample of the closed habitat set
336 and of the same sample size as the set released in open habitats): 15.66% of attacks
337 in closed habitats (92 attacks). Wald test = 24.67, df = 7, $p<0.001$). Additionally, in
338 open habitats the opaque form was more attacked than the other forms that had
339 transparency ($z= 2.15$, $p=0.031$), but when including the "reduced" dataset, such
340 difference could not be detected anymore ($z= 1.52$, $p=0.127$). When data was
341 independently analysed by habitat closeness, all prey treatments were similarly

342 attacked in closed areas (Fig. 2a, Wald test = 0.9, df = 3, p = 0.825, and for the
343 reduced dataset Wald test = 0.5, df = 3, p = 0.5), in contrast to open areas (Fig. 2b,
344 Wald test = 9.86, df = 3, p = 0.02). In open areas, opaque artificial butterflies were
345 more attacked than all the other treatments (z = 2.68, p = 0.007); artificial butterflies
346 with six layers of transparency were marginally more attacked than those with a
347 single transparency layer (z = 1.839, p = 0.066), while artificial butterflies without or
348 with a single transparent layer where similarly attacked and reported the lowest
349 number of attacks (z = -1.61, p = 0.11).
350
351 Comparative analyses
352 Variations in transparency degree, and relationship to habitat and daytime activity
353 Habitat explains a small part of the variation found in transparency degree. When
354 interactions between factors are excluded from the model, species have in general
355 the same level of transparency for closed and open habitats (t=-1.58, df=102, p
356 =0.117, Table 1). When interactions are included, species living in closed habitats
357 transmit slightly more light than species living in open habitats (t=-2.09, df=102, p
358 =0.04, Table 1, Fig. 3). In open habitats transmittance is slightly higher in most
359 species for the forewing and in species showing smaller hindwing sizes, but
360 transparency degree decreases steeply for hindwings with increase in wing size
361 (triple interaction wing, habitat and size: t=30,2, df=488, p=0.003, Table 1, Fig 3b.).
362 Regarding correlations between habitat and other physical characteristics of species
363 wings, species living in closed habitats are not different in size (PGLS forewing: t=-
364 1.8, p= 0.07; hindwing: t=-1.32, p= 0.19) or proportion of clearwing surface from
365 those living in open habitats (PGLS forewing: t=0.27, p = 0.78; hindwing: t=0.13,
366 p=0.9). No relationship was found between habitat and daytime activity (BayesTraits
367 LRT= 6.08, df=4, p=0.193, Table 2). Daytime activity has no significant contribution
368 to explaining variations in transparency degree in models including mimicry and
369 habitat as explanatory variables (Table 1). Diurnal species have larger hindwings
370 than nocturnal species (PGLS t=-2.33, p=0.02) but no differences in forewing sizes
371 were detected (PGLS t = -1.45, p =0.15). No differences in the proportion of
372 clearwing area were found between diurnal and nocturnal species (PGLS forewing: t
373 = -1.58, p =0.12; hindwing: t=0.16, p=0.87). No differences in the variance of
374 transparence degree were found between habitats (Fligner-Killeen test for forewing:

375 2.02, df=1, p=0.155; for hindwing: 1.48, df = 1, p=0.224). However, nocturnal species
376 vary more in transparency degree than diurnal species for their forewing (Fligner-
377 Killeen test for forewing: 15.47, df=1, p<0.001; for hindwing: 2.87, df = 1, p=0.09, Fig.
378 S8). Diurnal species vary more on their wing size than nocturnal species (Fligner-
379 Killeen test for all forewing: 8.79, df=1, p=0.003; for all hindwing: 30.51, df = 1,
380 p<0.001, Fligner-Killeen test for wings smaller than 60 mm, forewing: 22.22, df=1,
381 p<0.001; hindwing: 39.66, df=1, p<0.001, Fig. S8). No difference in wing size
382 variation was found for species from different habitat openness (Fligner-Killeen test
383 for forewing: 0.26, df=1, p=0.610; for hindwing: 0.196, df = 1, p=0.657).
384
385 Variations in transparency degree, transparency structural basis and mimicry
386 Mimicry explains a large portion of the variation found in transparency degree.
387 When fitting a model without interactions, bee/wasp mimics had a higher
388 transparency degree than other clearwing species (t=2, df = 102, p = 0.05, Table 1,
389 Fig. 4). When interactions were included, the best model additionally shows that
390 within bee/wasp mimics, small-sized species have higher transparency degrees than
391 large-sized species (t=-3.04, df=488, p=0.003), in contrast to leaf mimics that
392 transmit more light at larger wing sizes (t=-4, df=488, p<0.001, Fig. 4d). Additionally,
393 light transmittance differences between mimics are larger for hindwing than for
394 forewing (t=2.69, df=488, p=0.007, Figure 4a). Regarding the relationship between
395 mimicry and wing characteristics, leaf mimics have smaller hindwings than non-
396 mimics (PGLS forewing: t=1.66, p = 0.1; hindwing: t=1.99, p = 0.05, Figure 4b), and
397 the length ratio forewing/hindwing is larger for bee/wasp mimics than for other
398 clearwing species (PGLS t=4.51, p <0.001, Fig. 4e). Additionally, proportion of
399 clearwing surface is larger for no mimics and for leaf mimics (PGLS forewing:
400 t=4.005, p < 0.001; hindwing: t=2.86, p=0.005, Fig. 4c). Most bee/wasp mimics are
401 diurnal while most leaf mimics are nocturnal (BayesTraits LRT=13.56, df = 4,
402 p=0.009, Table 2, Fig S4 & Fig S6).
403 We found that transparence degree varies less in bee/wasp mimics than in
404 leaf-mimics (Fligner-Killeen test for forewing: 15.6, df= 1, p<0.001, for hindwing:
405 36.14, df = 1, p<0.001), or in other clearwing species (Fligner-Killeen test for
406 forewing: 16.38, df=1, p<0.001; for hindwing: 36.34, df = 1, p<0.001), but there is no
407 difference in the transparence degree variation in leaf mimics and non-mimic

species (Fligneer-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: 18.91, df=1, p<0.001; for hindwing: 39.86, df = 1, p<0.001) or in other clearwing species (Fligner-Killeen test for forewing: 39.06, df=1, p<0.001; for hindwing: 59.15, df = 1, p<0.001). Leaf and non-mimics show a similar variation in wing length (Fligner-Killeen test for forewing: 0.58, df=1, p=0.447; for hindwing: 2.46, df = 1, p=0.117).

Some scale characteristics are correlated to mimicry evolution. Scales are more often absent and less often coloured when present in hindwings of bee/wasp mimics in comparison to non-mimics wings (BT LRT presence = 24.55, df=4, p<0.001, Table S2 & Fig S7; BT LRT colour= 16.14, df=4, p=0.003, Table S2 & Fig S7). Similarly, 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 (LRT=12.87, df=4, p=0.012, Table S2 & Fig S7). Moreover, leaf mimics only exhibit classic scales in contrast to bee/wasp mimics and non-mimics (LRT=9.33, df=4, p=0.053, Table S2 & Fig S7). More than evolutionary convergences, these similarities are likely to be homologies as mimicry has a strong phylogenetic signal ($\delta$ =4.471).

Some characteristics were common for the general set of species that we studied here. Transparency degree is higher on forewings (t=9.45, df=502, p<0.001), wings with large proportion of clearwing areas (t=10.88, df=502, p<0.001, Fig. S5a) and smaller species (t=-6.61, df=502, p<0.001, Fig. S5b). Regarding general wing characteristics, smaller species have larger proportion of transparent surface in hindwings than do larger species (PGLS forewing: t=-1.42, p = 0.16; hindwing: t=-2.58, p = 0.01).

## Discussion

Fieldwork experiments using artificial prey that differed on their transparency degree suggest that variation in transparency can be detected only in brighter light conditions, similar to what has been suggested for aquatic environments [2]. Poorly transparent prey types survived less than highly transparent ones in open environments, while they performed similarly in closed areas. Additionally, less artificial moths were attacked in closed habitats, even after increasing the number of artificial prey in closed habitats to compensate for the higher predation rate often

441 reported for open habitats such as forest edges [35]. By controlling for differences in
442 predation rate between habitats by increasing the number of prey placed in closed
443 habitat our results suggest that selective pressure towards an increase in
444 transmittance is higher in open than in closed habitats.

445 Our experimental results suggest that if detectability reduction is an
446 important trigger for the evolution of transparency degree variation, butterflies and
447 moths with poorer transparency degree should be mostly restricted to closed
448 habitats. However, we found that when excluding factor interactions, overall
449 butterflies and moths living in closed habitats have a similar transparency degree,
450 and that there is a slightly higher mean transmittance in species living in open
451 habitats, only revealed when interactions with other factors were included in the
452 analyses. Gomez et al [12] reported that poor light transmittance is associated to
453 small proportions of clearwing surfaces and large wing sizes, characteristics that
454 were similarly segregated in open and closed habitats (LMM estimates for the
455 interactions wing size: habitat = 0.31±0.05; and clearwing surface: habitat = -
456 0.28±0.14 , Fig. 3), probably explaining the small differences found in transparency
457 degree between different light conditions. However, we also found that forewings
458 and small hindwings transmitted more light in open than in closed habitat species, in
459 accordance to what would be selected according to the high visual performance of
460 visual predators under bright conditions [2]. This suggests that differences detected
461 in light transmittance between habitats are closely related to selective pressures
462 acting in size as well as in fore- and hindwing in an independent manner. As Gomez
463 *et al* [12] suggested, transparency might be highly effective at small body sizes, but
464 its efficiency likely decreases with size, as larger wings and bodies imply larger
465 opaque elements, such as the butterfly body itself. Our study confirms this trend and
466 sheds more light on the evolution of these morphological traits. For cryptic Erebidae
467 moths, Kang et al [36] have suggested that the increase of conspicuousness related
468 to larger body sizes might have triggered the evolution of hidden contrasting colour
469 signals on the hindwing that are probably involved in startle displays. Selective
470 pressure towards decreased detectability might promote different character
471 evolutions according to prey size, especially in open habitats. Species living in open
472 habitats incur in higher risks of tissue damage by higher exposure to UV light, more
473 wind or higher exposition to direct rainfall, conditions that have been reported as

important at shaping moth and butterfly segregation in different habitats [37]. In case transparency entails costs related to one or several of these factors, they will promote the presence of opaque scales in open habitats, as it might be the case for large species, where additionally large opaque elements will be difficult to hide even with the highest transparency degree.

Transparency can decrease prey detectability but it is also involved in mimicry evolution, and differences in transparency degree seem to be intimately associated to mimicry evolution. When mimicry is included in the analysis, we found that bee/wasp mimics have higher transparency degrees and are more often restricted to open habitats, in agreement to our fieldwork results and confirming that high transparency in open habitats can be a visual optimum in terrestrial environments. 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.

Other characteristics of bee/wasp mimics include the absence of scales in their wings, their diurnal activity [38], their smaller wing sizes and their smaller hindwings in comparison to their forewings [39,40]. Insects that mimic bees or wasps resemble their harmful counterparts in body colour, body shape and flight pattern (for butterflies: [41]) but also in activity patterns (for diptera: [42]). Convergence in high transparency degree along with reduction in variation in wing size and increase in fore/wing ratio can contribute to such multicomponent mimicry, that improves prey chances at deceiving a larger set of predators, in case several traits are similarly salient for them [43] or in case different predator species find different cues as salient [44]. The evolution of highly transparent wings in pollinators is likely triggered by their higher mobility and the openness of the habitat where the majority of nectar sources occurred [45]. Studies in other non-insect transparent organisms have shown that transparent forms of *Hippolyte obliquimanus* shrimps are more mobile and exploit a larger diversity of habitats [46]. Whether transparency is also related to higher mobility in insects remains to be properly tested.

On the other hand, we found that leaf mimics are larger, are more often found in closed habitats and in contrast to the trends for all clearwing species, have a

507 higher transparency degree at larger wing sizes and lower proportions of clearwing
508 surfaces. Some fully opaque fallen leaf mimic species, as the comma butterfly
509 (*Polygonia c-album*) exhibit a distinctive pale mark that might decrease their
510 predation [47,48], although it is controversial [49]. Either way, reduced and highly
511 transparent zones as those found in *Orthogonioptilum violascens* might produce a
512 similar appearance to those markings given their high transparency degree that
513 might be highly reflective and be perceived as a white mark, conferring thus the
514 potential benefits of the "comma mark" when seen for a given angle, as well as the
515 full crypsis, when no reflections can be perceived. Whether highly transparent
516 windows work as other highly visible elements displayed in cryptic species such as
517 eyespots that deter predators [50,51] and that are also important in mate choice
518 [52] remains to be tested.
519 Other fallen leaf mimics include large transparent surfaces that can be large
520 and can touch moth wings edges as for *Bertholdia* species or not touching them as
521 for *Pseudasellodes fenestraria*. In both cases, transparency surfaces are likely
522 disrupting prey shape (as in the first case) and/or prey surface (as in the second
523 example [53]), thus reducing their detectability. Disruptive coloration, especially
524 when touching prey borders has been broadly studied as it is common in fully
525 opaque cryptic species [54–57]. Therefore, in case transparent surfaces touch wing
526 edges, they might work as disruptive marks regardless of their transparency degree.
527
528 **Conclusion**
529 Similar to aquatic environments, differences in transparency degree keeping
530 butterfly size constant seems more easily detectable in lighter environments, and
531 higher transparency degrees can indeed decrease detectability of prey by predators,
532 as our field experiment suggests. In real species, transparency degree between
533 habitats is rather similar. Yet, bee/wasp mimics are more often diurnal and
534 associated to open habitats, and have higher and less varying transparency degrees,
535 while leaf-mimics have lower and more varying transparency degree. Both habitat
536 light availability and mimicry can concur to fostering variations in transparency
537 degree. Further studies exploring the eventual coast of transparency regarding
538 irradiance, wind and direct rainfall, will help to better understand the evolution and
539 the constraints imposed on transparency on land.

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**Figures and tables**

# Variation in transparency degree: response to light availability or part of mimicry syndrome?

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a. b.

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

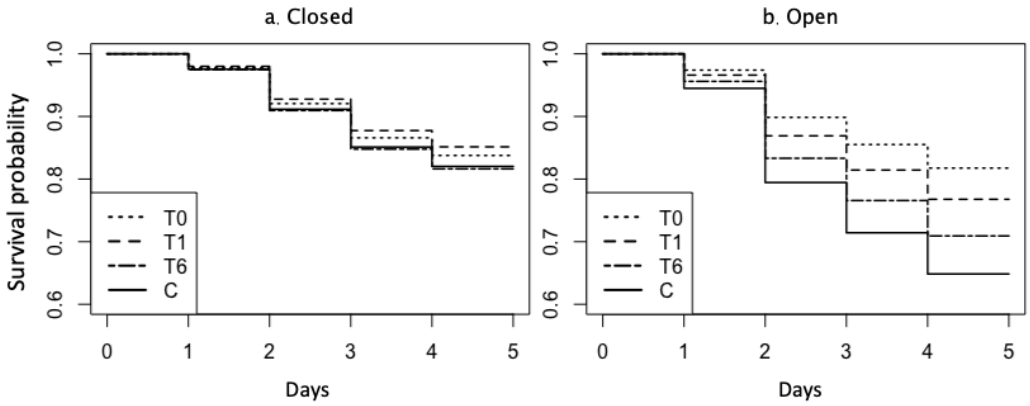


Figure 2. Survival of artificial prey in closed (a.) and open (b.) areas, without any transparent area (C), and with transparent elements covered by 6 (T6), 1 (T1) or non (T0) transparent film layers. Prey placed on tree trunks were monitored for 'survival' every day for 4 days.

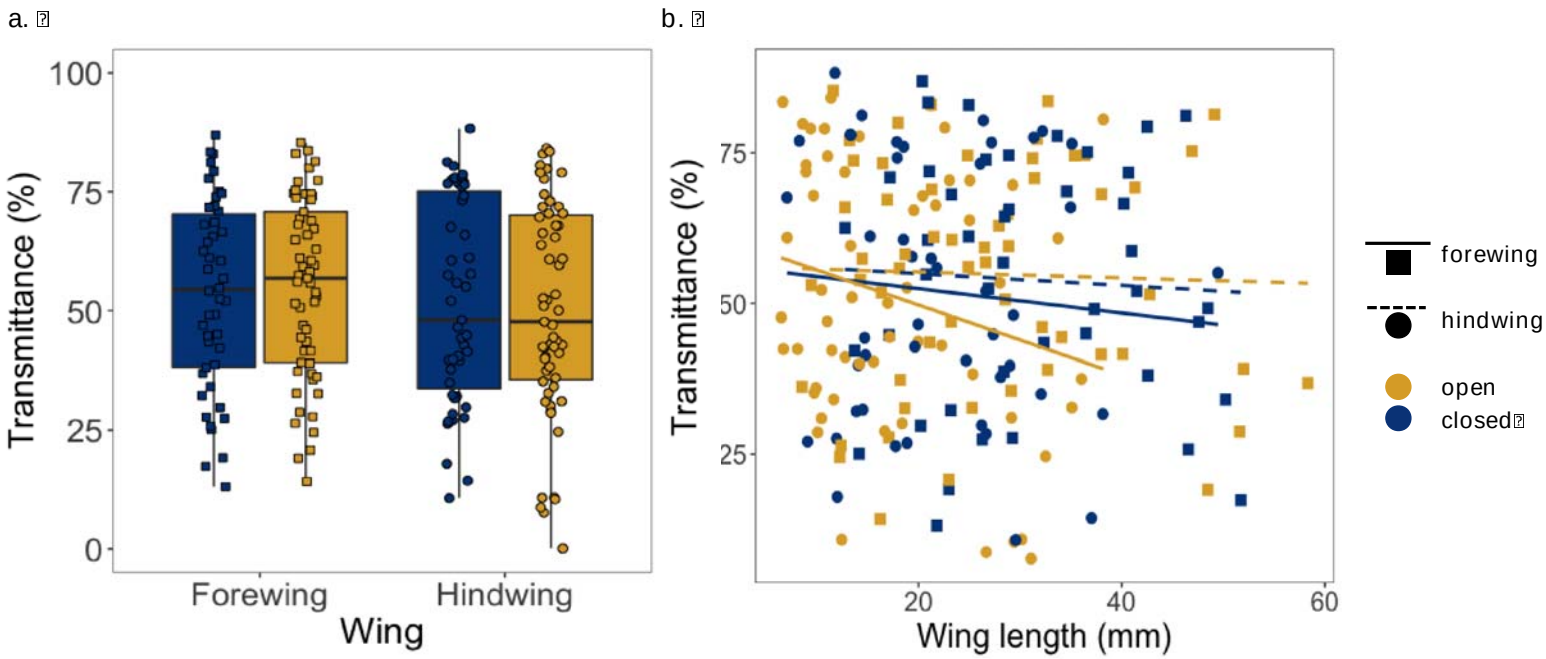


Figure 3: Variation between open (yellow) and closed habitats (dark blue) in a) transparency degree and b) the interaction between wings (forewing: fw, square/dashed and hindwing: hw, circle/plain), wing length and transparency degree. Values larger than 60 mm were not plotted for clarity reasons but were included in the analyses.

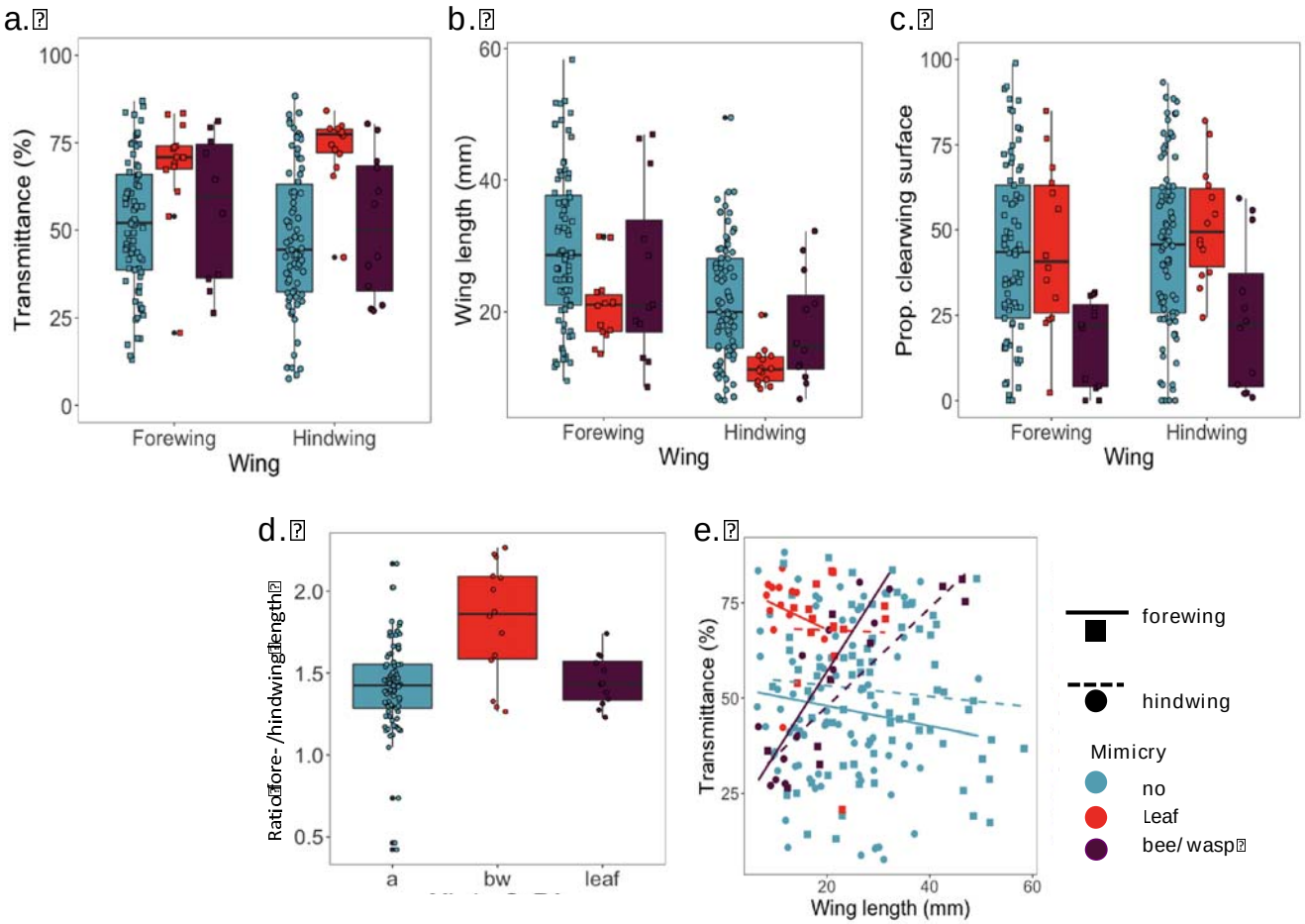
a.
Transmittance (%)
100
75
50
25
0
Forewing
Hindwing
Wing
b.
Wing length (mm)
60
40
20
Forewing
Hindwing
Wing
c.
Prop. clearwing surface
100
75
50
25
0
Forewing
Hindwing
Wing
d.
Ratio fore- /hindwing length
2.0
1.5
1.0
0.5
a
bw
leaf
e.
Transmittance (%)
75
50
25
20
40
60
Wing length (mm)
forewing
hindwing
Mimicry
no
Leaf
bee/ wasp

Figure 4: Variation between bee/wasp mimics (red), leaf mimics (purple) and no mimics (blue) in a) transparency degree b) wing length c) proportion of clearwing surface, d) Ratio between length of forewing and hindwing per mimicry group and e) in the interaction wing size and transparency.

Table 1. Variation of transparency degree (mean percentage of transmitted light) according to mimicry, habitat, daytime activity, wing size, wing and proportion of clearwing surface excluding (in the left) or including interactions between them (in the right).

| | Bayesian | Linear mixed model | | | | Bayesian | Linear mixed model | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate [CI95%] | Estimate ± se | DF | *t* | | Estimate [CI95%] | Estimate ± se | DF | *t* | |
| (Intercept) | 60.09[31.06;88.73] | 60.61±3.92 | 502 | 15.48 | *** | 62.24[30.79;93.17] | 64.94±5.6 | 488 | 11.59 | *** |
| Activity (D>N) | 10.34[21.66;-0.51] | 1.99±2 | 102 | 0.99 | | -11.43[-22.67;-0.12] | 2.98±2.01 | 102 | 1.48 | |
| Wing (F>H) | 7.92[9.57;6.28] | 3.67±0.39 | 502 | 9.45 | *** | -7.7[-12.43;-2.83] | 1.92±1.31 | 488 | 1.47 | |
| Clearwing proportion | 0.27[0.22;0.32] | 0.26±0.02 | 502 | 10.88 | *** | 0.04[-0.12;0.2] | -0.11±0.07 | 488 | -1.59 | |
| Mimicry:bw>all | 21.45[9.4;33.57] | 3.97±1.98 | 102 | 2 | * | 41.29[19.43;62.83] | 9.9±2.84 | 102 | 3.49 | *** |
| Mimicry:leaf<non-mimics | 5.57[-9.84;20.81] | -5.05±3.07 | 102 | -1.64 | | -3.17[-25.14;19.52] | 1.62±4.21 | 102 | 0.39 | |
| Habitat (Open>Closed) | -7.47[-17.14;1.92] | -5.96±3.77 | 102 | -1.58 | | -17.21[-31.38;-3.18] | -12.07±5.77 | 102 | -2.09 | * |
| Wing length | -0.5[-0.64;-0.36] | -0.43±0.07 | 502 | -6.61 | *** | -0.45[-0.71;-0.2] | -0.3±0.21 | 488 | -1.4 | |
| Activity (D>N):Wing Wing(F>H) | - | - | - | - | | 3.1[0.54;5.49] | 0.82±0.28 | 488 | 2.95 | |
| Mimicry (bw>all):Clearwing prop. | - | - | - | - | | 0.04[-0.15;0.22] | 0.09±0.03 | 488 | 2.99 | ** |
| Mimicry (leaf<n.m):Clearwing prop. | - | - | - | - | | -0.46[-0.68;-0.24] | 0.24±0.05 | 488 | 4.8 | *** |
| Habitat(O>C):Wing length | - | - | - | - | | -0.04[-0.31;0.24] | -0.28±0.14 | 488 | -2.05 | * |
| Habitat(O>C):Wing(F>H) | - | - | - | - | | 1.88[-3.24;7.02] | -0.64±1.17 | 488 | -0.55 | |
| Wing length:Wing (F>H) | - | - | - | - | | -0.02[-0.14;0.1] | 0±0.03 | 488 | -0.09 | |
| W.length:Clearwing prop. | - | - | - | - | | 0[0;0.01] | 0±0 | 488 | 0.89 | |
| Mimicry (bw>all):W.length | - | - | - | - | | -1.08[-1.99;-0.14] | -0.46±0.15 | 488 | -3.03 | ** |
| Mimicry (leaf<n.m):W.length | - | - | - | - | | 0.82[0.2;1.44] | -0.55±0.14 | 488 | -4 | *** |
| Clearwing prop:Habitat (O>C) | - | - | - | - | | 0.34[0.24;0.44] | 0.31±0.05 | 488 | 6.51 | *** |
| Clearwing prop:Wing (F>H) | - | - | - | - | | 0.03[-0.04;0.1] | -0.02±0.02 | 488 | -1.31 | |
| Mimicry (bw>all):Wing(F>H) | - | - | - | - | | -4.6[-14.17;5.06] | 1.02±0.79 | 488 | 1.28 | |
| Mimicry (leaf<n.m):Wing(F>H) | - | - | - | - | | 6.04[-1.19;13.2] | 2.19±0.81 | 488 | 2.69 | ** |
| Wing (F>H): Habitat (O>C):Wing length | - | - | - | - | | -0.26[-0.46;-0.06] | 0.14±0.05 | 488 | 3.02 | ** |
| Phylogenetic variance | 951.7[702.02;1276.09] | - | - | - | | 968.02[725.74;1287.29] | - | - | - | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Residual variance | 951.7[702.02;1276.09] | - | - | - | 67.24[61.24;73.71] | - | - | - |

Bayesian regression and linear mixed model to explain transparency degree include daytime activity (nocturnal/diurnal), wing length in mm, proportion of clearwing area (%Clearwing), type of habitat (open/closed), mimicry syndrome (bee/wasp -, leaf - or no 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. Linear mixed models take into account contrast between factors that are not considered by Bayesian estimations, explaining differences in the sign of the estimates between analyses. Bayesian estimates whose confidence interval did not include 0 and LMM p values below 0.05 are statistically significant. *** stands for $p<0.001$, ** for $p<0.01$ and * for $p<0.05$.

Table 2. Results of coevolution tests between habitat, mimicry and daytime activity

| Tested characters | Mimicry levels included | L.Dep. | L.Indep. | LRT | df | p | |
|---|---|---|---|---|---|---|---|
| Daytime activity/ mimicry | Bee/wasp & leaf mimics | -30 | -36.78 | 13.56 | 4 | 0.009 | ** |
| | No- & leaf mimics | -101.82 | -101.82 | 0.01 | 4 | 1 | |
| | No- & bee/wasp mimics | -97.75 | -97.88 | 0.26 | 4 | 0.993 | |
| Habitat / mimicry | bee/wasp & leaf mimics | -34.86 | -36.78 | 3.85 | 4 | 0.427 | |
| | No- & leaf mimics | -102.27 | -102.34 | 0.13 | 4 | 0.998 | |
| | No- and bee/wasp mimics | -99.73 | -101.26 | 3.05 | 4 | 0.549 | |
| Habitat/daytime activity | No-, bee/wasp- & leaf mimics | -144.8 | -141.76 | 6.08 | 4 | 0.193 | |

Likelihood of dependent (L. Dep. Coevolution, 4 parameters estimated) and independent (L. Indep. 8 parameters estimated) evolution between pairs of binary characters obtained from BayesTraits. As mimicry has three levels, subsets including two of them were used and reported in the second column. A significant p-value indicated that the dependent model did significantly better than the independent model, indicating a correlated evolution between the traits. ** stands for statistical significance below 0.01