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2 **Clearwing butterflies challenge the thermal melanism hypothesis**

2

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19 CP defined the protocol. ME and DG collected the specimens. VO, CP and FP performed
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22

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25 **This PDF file includes:**

26 Main Text

27 Figures 1 to 4

28

29

30

31 **Abstract**

32

33 In contrast to most butterflies harboring opaque wing colorations, some species display large
34 transparent patches on their wings. Wing transparency, which entails a dramatic reduction of
35 pigmentation, raises the question of potential costs for vital functions, such as thermoregulation,
36 especially along climatic gradients. The thermal melanism hypothesis posits that darker colorations
37 should be favored in colder environments, which enables them to absorb more radiation and
38 maintain a body temperature compatible with activity. This prediction extends to the near infrared
39 (NIR) range, which represents a large proportion of solar radiation. Here we assess the implications
40 of wing transparency for light absorption and thermal properties in 42 butterfly species from the
41 neotropical tribe Ithomiini that range the extent of transparency, from fully opaque to highly
42 transparent, and we test whether those species conform to the prediction of the thermal melanism
43 hypothesis. We find that transparent wings are less efficient than opaque wings to absorb light
44 across UV, Visible and NIR wavelength ranges, and are also less efficient to collect heat. Moreover,
45 dark coloration occupies a lower proportion of wing area as altitude increases, and ithomiine
46 species harbor more transparency at higher altitudes, where climatic conditions are colder, going
47 strongly against the prediction of the thermal melanism hypothesis. We discuss these surprising
48 results in light of recent studies suggesting that factors other than adaptation to cold, such as
49 predation pressure, physiology or behavior, may have driven the evolution of wing patterns in
50 Ithomiini.

51

52 **Significance Statement**

53

54 The thermal melanism hypothesis predicts that organisms should be darker and absorb solar
55 radiation more efficiently in colder environments. The Neotropical butterflies Ithomiini are unusual
56 in that many species harbor large transparent patches on their wings, raising questions related to
57 their efficacy of solar radiation absorption and heating capacities. We investigate optical and
58 thermal properties of several ithomiine species along a climatic gradient. We find that transparent
59 wings are less efficient at absorbing radiation and collecting heat. Unexpectedly, the proportion of
60 transparent species increases with altitude, challenging the thermal melanism hypothesis and
61 suggesting that factors other than adaptation to cold, such as predation pressure, may have driven
62 the evolution of wing patterns in Ithomiini.

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66 **Main Text**

67

68 **Introduction**

69

70 Diurnal butterflies are known for the great variety of colors they display. The scales covering their
71 wings produce colorations through pigments (1) or structures (2, 3). Coloration is involved in
72 multiple functions, including intraspecific signaling (4); antipredator defenses such as crypsis and
73 camouflage (5), masquerade (6), aposematism and mimicry (7); as well as thermal adaptation (8).
74 The balance between these selective pressures drives the diversity of color patterns in butterfly
75 wings (9).

76 In ectotherms, coloration has been shown to play an important role in determining body
77 temperature and activity, since the degree of darkness is positively correlated with internal body
78 temperature (10). Darker butterfly wings absorb more light, and are associated with longer flight
79 activity and larger flight distances in cold conditions than lighter wings (11, 12).

80 The thermal melanism hypothesis (TMH) posits that darker colorations provide thermal
81 advantages in cold environments (13), especially for ectotherms, as dark surfaces are more
82 efficient to absorb heat from the environment than light surfaces. Darker species or individuals are
83 expected to live at high altitudes or latitudes, whereas lighter ones are more likely to be present at
84 lower altitudes and latitudes, which offer warmer conditions. The existence of such melanism
85 gradients has been shown in many case studies, across geographical regions and wide ranges of
86 taxa, from reptiles to insects (14–16). However, visible colors represent only a small part of the
87 incident solar radiation reaching the earth's surface. Over 50% of the solar energy lies in the near-
88 infrared wavelengths (NIR, 700 to 2500 nm). NIR should then be of particular importance in
89 ectotherm thermal capacities, more than ultraviolet to red (i. e. 300 to 700 nm, hereafter UV-Visible
90 range) (17), since they are not involved in other aspects of their ecology because they cannot be
91 seen by most organisms. Recent studies on communities of European (18) and Australian (19)
92 butterflies confirmed that reflectance in the NIR range is strongly correlated with climatic factors,
93 especially temperature, and more strongly compared to UV and Visible wavelengths. In colder
94 environments, butterflies tend to reflect less and therefore absorb more in the NIR. These findings
95 highlight that optical properties can be disconnected to a certain extent between NIR and UV-Visible
96 ranges. By contrast, no clear trends exist regarding humidity or precipitation and wing darkness.
97 Gloger's rule, which predicts that darker individual should be found in more humid climates, has
98 been evidenced mainly for endotherms with little clear support in ectotherms (18, 20).

99 In Lepidoptera, few species exhibit transparency on their wings, a feature that has evolved
100 multiple times independently (21). Transparency is achieved by a large diversity of wing
101 microstructures, including a combination of scale shape or insertion modification, reduced scale

102 size or density, as well as a reduction of scale pigments (21). In many cases, transparency is
103 enhanced by the presence of nanostructures on the wing membrane, which decreases reflection
104 (22–24). Transparency has attracted recent research effort regarding its now-established role in
105 crypsis in various species (25–27), and its involvement in predator-prey signaling in some clades
106 of mimetic Lepidoptera (22). Yet, transparency may come at the cost of other functions involving
107 wing coloration, notably thermoregulation. While thermoregulatory processes are well documented
108 in opaque species, they are largely unknown in clearwing butterflies. A study focusing on wing
109 transparency across the entire Lepidoptera order found that clearwing species tend to transmit less
110 light, and thus absorb more, in high latitudes than in tropical climates, suggesting a thermal cost
111 for butterflies (21). Yet, the actual impact of transparent wing patches on wing thermal properties
112 needs to be investigated.

113 Among Lepidoptera, Ithomiini is a remarkable neotropical tribe of ca. 400 species (28, 29),
114 80% of which exhibit transparency on their wings to some extent. Species are highly segregated
115 along the elevational gradient, from warm lowlands up to colder highlands, as high as 3,000 meters
116 (29, 30). Because wing transparency in Ithomiini is achieved by a reduction in membrane coverage
117 by scales (21, 22), and is therefore associated with a lower quantity of pigments, one might expect
118 those areas to poorly absorb solar light. Previous studies of geographical distribution of Ithomiini
119 wing patterns revealed that the proportion of clearwing species increases with altitude, to the extent
120 that all species above 2000 m have transparent wings (29, 30), contrary to the predictions of the
121 TMH. Yet, this observation is based on the UV-Visible range (22, 29, 30), and optical transparency
122 shown in that range may be dissociated from the absorption properties in the NIR.

123 Here, we investigate for the first time wing traits relevant for thermal adaptation in 42
124 transparent and opaque Ithomiini species that represent the diversity of wing colorations, by using
125 Infrared imaging and spectrophotometry. We assess the distribution of these wing traits in relation
126 to the climatic conditions the species are exposed to in a phylogenetic framework. Specifically, we
127 characterize and quantify the optical and thermal properties of transparent and opaque wing
128 patches to investigate two mutually exclusive scenarios:

129 (i) *Absorption compensation*: in this scenario, the higher transmission and lower absorption of
130 wavelengths in the UV-Visible range of transparent wing areas are compensated by a high
131 absorption in the NIR, and the thermal capacities of transparent wings may be similar to or even
132 better than those of opaque wings. Under this hypothesis, adaptation to climatic conditions may
133 drive the altitudinal distribution of wing patterns in Ithomiini.

134 (ii) *Thermal cost*: in this scenario, the disconnection between wavelength ranges is not significant
135 enough, with transparent wings transmitting more and absorbing less in the NIR range than opaque
136 wings, therefore, reflecting similar absorption patterns at shorter wavelengths. This results in
137 transparency being costly in terms of intrinsic heating capacities. Under the thermal cost scenario,

138 thermal properties may not be the main target of selection with regard to the altitudinal distribution
139 of wing patterns in Ithomiini.

140

141 In addition, assuming that opaque patches - even if not covering a high proportion of the wing
142 surface in transparent species – may follow the thermal melanism hypothesis, we tested whether
143 they represent a higher proportion of wing surface in species found in colder climates, a mechanism
144 that could also compensate for poorer thermal capacities of transparency under the *thermal cost*
145 scenario. The absence of evidence of compensation mechanisms (disconnection NIR from UV-
146 Visible range, or increased proportion of opaque patches with altitude) would imply that factors
147 other than wing color patterns, such as behavior or metabolism, explain adaptation to colder
148 highland habitats in Ithomiini.

149

150

151 **Results**

152

153 To assess the thermal adaptation of Ithomiini butterflies, we examined the thermal and optical
154 properties of the wings of 42 species in relation to climatic conditions. Unless specified otherwise,
155 all statistical tests implemented Bayesian mixed models with Markov Chain Monte Carlo correcting
156 for phylogenetic relatedness, with an associated Bayesian posterior P-value set to 0.05.

157 **Wing optical and thermal properties**

158 We first tested whether optical properties in the NIR wavelengths were correlated with those
159 properties in the UV-Visible range, and whether those properties differed between transparent and
160 opaque species, as defined hereafter. We measured transmission and reflection spectra on a 5mm-
161 diameter spot on one hindwing and one forewing for each specimen, spanning the UV-Visible (300-
162 700 nm) and NIR (700-2500 nm). We used the physical principle stating that Transmission (λ) +
163 Reflection (λ) + Absorption (λ) = 1 to compute the total absorption spectra over 300-2500 nm (Fig.
164 4C). The transmission of the patch in the UV-Visible range was set as a measure for the degree of
165 optical transparency. We split the species in two categories: transparent, for which wing
166 transmission was higher than 35%, and opaque for the others (Table S1, Fig.S1).

167 Overall, UV-Visible transmission (300-700 nm), i.e., transparency as perceivable by
168 butterflies and predators, was a good predictor of both overall absorption (300-2500 nm) and NIR
169 absorption (700-2500 nm, Table S2, Fig. 1). NIR and UV-Visible absorption were highly correlated
170 (Table S2, Fig. 1), be the species transparent or opaque. More transparent patches absorbed less
171 light than opaque patches whatever the wavelength considered, supporting the *thermal cost*
172 scenario for transparency (Fig. S2).

173 To investigate the thermal capacities of transparent wings and test whether they differ from
174 those of opaque wings, we heated up wings with a flash illumination, and imaged them with an
175 infrared camera to record temperature across the wing and its variation through time (Fig. 4A-B).
176 Image analysis showed that the average heating capacity of the wing spot, represented by excess
177 temperature ΔT (maximum temperature reached after illumination - initial temperature), was closely
178 related to its transparency, as estimated by the transmission in the UV-Visible range (Table S3,
179 Fig. 2). Hence, the more transparent the spot, the less it heated up, in agreement with the *thermal*
180 *cost* scenario. ΔT of wing spots was also positively predicted by the mean total absorption of the
181 spot and by its NIR absorption (Table S3, Fig. 2). Therefore, wings that absorbed more in the UV-
182 Visible-NIR range or in the NIR range heated up more. In an in-depth exploration of the intra-wing
183 variation in heating capacity, we extracted ΔT on various points on different parts of the wings
184 (veins and cells, i.e., the parts between veins), either in darkly colored patches DP (black to brown
185 colors), lightly colored patches LP (for opaque species only, yellow to orange colors), or transparent
186 patches TP, and on light LV or dark veins DV. A close look at the different areas and structures of
187 the wings revealed that dark patches heated up significantly more than any other coloration on the
188 wings, transparent or light (DP>(LP,TP) in Table S4, Fig. S3). Moreover, light patches heated up
189 significantly more than transparent patches (LP>TP in Table S5, Fig. S3). Dark patches heated up
190 more than dark veins (DP>DV in Table S6, Fig. S3), but on the other hand, light veins absorbed
191 more heat than light patches (LV>LP in Table S7, Fig. S3). In all cases, the position of the patch
192 with respect to the thorax, proximal or distal, played a role in predicting ΔT , but this varied according
193 to the color of the patch considered, showing no clear-cut trend (Table S4-7).

194 **Wing trait distribution along climatic gradients**

195 We tested whether climatic variables predicted the proportion of dark patches on the wings of all
196 the species, as posited by the TMH. We extracted 19 published average climatic variables for each
197 species (29) that included temperature and precipitation descriptors and performed a PCA. PC1
198 and PC2 explained respectively 49 % and 27.1 % of the total variation. Higher PC1 values
199 corresponded to warmer climates, while higher PC2 values corresponded mainly to wetter and
200 more time-stable climates (tables S8, S9, Fig. S4). Pearson's product-moment correlation tests
201 showed that PC1 was strongly negatively correlated with altitude ($R=-0.95$, $N=42$, $p < .0001$),
202 meaning that species inhabiting higher altitude habitats had a lower PC1 value. PC2 did not
203 correlate with altitude ($R=0.24$, $N=42$, $p=0.12$). To test if opaque and transparent species had
204 similar climatic ranges we used the Levene's heteroscedasticity test on climatic variables. We found
205 that opaque species were restricted to warmer environments, while transparent species were
206 ubiquitous and not associated to particular altitudes ($F = 110.71$, $p < .005$) (transparent species:
207 mean=-0.27, var=10.56, opaque species: mean=1.08, var=1.09), in contradiction with the TMH.
208 Opaque and transparent species did not significantly differ in their variance for PC2 ($F=1.76$,

209 $p=0.19$) (transparent species: mean=0.29, var= 3.88, opaque species: mean=-1.89, var=3.45),
210 showing that they did not segregate according to humidity or environmental stability gradients.
211 Overall, climate did not predict wing optical properties. The degree of transmission of the wing spots
212 over the range UV-Visible were not predicted by PC1, with a negligible positive effect for PC2 which
213 goes against Gloger's rule (Table S10, Fig. S5). There was no significant association of spot
214 absorption in the UV-Visible-NIR (table S11) or NIR ranges (table S12) with PC1 or PC2. By
215 contrast, the average ΔT of the whole wing surfaces was positively correlated to PC1 (Table S13,
216 Fig. 3). In other words, species living in warm environments heated up more than butterflies living
217 in colder environments.

218 Across all species, the proportion of dark surface on the wings increased with PC1 and
219 was therefore higher in warmer environments (Table S14, Fig. S6). We found a similar trend when
220 restricting the analysis to only transparent species (Table S15, Fig. S7). We examined whether this
221 proportion varied with climate within the proximal zones alone, because wing conduction could be
222 limited, and a patch was expected to have a greater thermal effect when closer to the body. Again,
223 the proportion of dark surface in the proximal zones increased with PC1 (Table S16, Fig. S8), and
224 was therefore higher in warmer environments, in contradiction with the TMH. The proportion of dark
225 surface did not correlate with PC2, except when considering only proximal zones, indicating that
226 proximally darker wings were associated with more humid habitats. Finally, we found no effect of
227 climatic variables on total wing area, a proxy for butterfly size (Table S17).

228
229

230 Discussion

231

232 Thermal properties correlate with optical properties in clearwing butterflies

233 The thermal imaging of *Ithomiini* wings reveals that opaque wings are on average more efficient to
234 heat up than transparent wings. Hence, transparency entails thermal costs, a conclusion supported
235 by the close-up study of the different wing patches, in agreement with the *thermal cost* scenario.
236 The darker the color, the more the patch heats up. Therefore, transparent patches contribute little
237 to wing heat absorption, in line with the long studied link between melanization and heating
238 capacities (31). Consistent with this finding, the optical examination of the transparent wing spots
239 reveals that the low absorption in the UV-Visible wavelength range is not compensated by a higher
240 absorption in the invisible NIR range, and therefore wing transparency comes at the cost of light
241 absorption. This result departs from findings in other organisms where visible reflectance tends to
242 be a poor predictor of NIR reflectance (32). Moreover, transparent spots always absorb less light
243 than opaque ones, regardless of the wavelength considered. This is in agreement with the
244 correlation of the UV-Visible and NIR spectra that has been previously described for transmission

245 (21) or reflection (18, 19) in interspecific studies of butterflies taxa. Yet, veins in transparent
246 Ithomiini species are always covered with melanized scales. Those veins heat up significantly more
247 than transparent and even lightly colored patches, and likely represent a major part of the overall
248 wing thermal capacities by providing heat to the dynamic hemolymph flow system to the thorax
249 (33).

250 In Ithomiini, absorption decreases in long wavelengths, and is quite low in the NIR for all
251 the studied species. An interspecific comparison of the absorption of longer wavelengths (~8-12
252 μm), especially infrared radiated by the environment, remains to be carried out as this wavelength
253 range is supposed to have greater thermal impact. However, the thermal imaging results clearly
254 show that transparent patches are poorer at accumulating heat than opaque patches, suggesting
255 an absence of compensation in the far infrared range.

256 **The altitudinal distribution of transparent species contradicts the thermal melanism** 257 **hypothesis**

258 This study confirms the counterintuitive altitudinal distribution of Ithomiini wing patterns (30). While
259 the opaque species are only found in warm climates, clearwing species are ubiquitous and widely
260 distributed, from hot lowlands to the cold Andean highlands. Moreover, among clearwing species,
261 the proportion of opaque patches on the wings decreases in colder environments, i.e. at higher
262 altitudes, challenging the long history of studies supporting the TMH in butterflies and insects
263 e.g.(15, 16, 34, 35), but adding to the observation that TMH does not apply to all taxa or
264 communities. For instance a recent study found support for TMH in geometrids but not in noctuids
265 found in the same study sites (36). Yet, these studies often focus on temperate climates.
266 Neotropical case studies are scarce and show mixed support to the TMH. Two closely related
267 neotropical butterfly genera, *Catastica* and *Leptophobia* have a divergent coloration distribution
268 along altitudinal gradients (37), possibly explained by different behaviors and elevation ranges.
269 While body and wing colorations become darker with increasing altitude in *Catastica*, *Leptophobia*
270 shows a reversed pattern, similar to that observed in Ithomiini. In the case of Ithomiini, not only the
271 non-conformity to TMH is outstanding, but there is no high NIR absorbance to compensate the poor
272 UV-Visible absorption that would make highland butterflies more adapted to cooler conditions.

273 The NIR range is expected to be only subjected to thermoregulatory pressures, unlike UV-
274 Visible wavelengths involved in vision. Indeed, NIR reflection spectra of Australian and European
275 butterflies communities are well predicted by climatic conditions (18, 19). In contrast to their
276 conclusions, our study of the absorption properties of wing spots showed that in general, climatic
277 conditions do not predict wing absorption in any radiation range. Moreover, the exploration of wing
278 heating capacities shows that Ithomiini species living in colder environments are less efficient to
279 heat up than species living in warmer climates, adding even more to the thermal cost of

280 transparency. Finally, there is hardly any link between thermal or optical properties and humidity,
281 and as such this study does not support Gloger's rule in Ithomiini.

282 Adaptation to cooler conditions is not the only thermal constraint faced by organisms, as
283 high temperature may also be challenging. Here we found that opaque species, which absorb more
284 radiation and have higher thermal capacities, live in environments where they are most exposed to
285 heat. For ectotherms, avoiding overheating (i.e. increasing body temperature above a critical
286 temperature threshold) can be challenging. Butterflies may have physiological adaptations (38) or
287 behavioral adjustments, such as changing the position of their wings according to environmental
288 radiations (33). Information on the metabolism and behavior of Ithomiini *in natura* would shed light
289 on their altitudinal adaptation, but are currently lacking. In addition, microhabitat variability can
290 result in individuals sharing the same overall environment being exposed to contrasting light and
291 temperature conditions. Microhabitat preferences thus likely play a role in adjusting body
292 temperature (38, 39). However, in lowland ithomiine communities, butterflies with transparent wing
293 patterns mostly fly in the understory, close to the vegetation and out of direct sunlight, while the
294 proportion of species with opaque wings increases with height in the canopy (40). The latter are
295 therefore more exposed to solar radiation, another counterintuitive distribution in relationship to
296 thermoregulation.

297 **Transparency under multiple selection pressures**

298 Colorations in butterflies, and therefore wing optical properties, are under multiple selection
299 pressures as they are involved in anti-predator defenses, signaling to conspecifics and thermal
300 adaptation, with potential trade-offs (9). Consequently, selection for crypsis and aposematism could
301 be detrimental for thermal capacities (41). Wood tiger moths are a good example of the
302 phenomenon: larvae with large black patches are at a thermal advantage compared to larvae with
303 equally black and orange patches, but the latter display a more efficient aposematic signal (42).
304 Imagoes display latitudinal variation in wing pattern, consistent with the TMH, which incurs cost in
305 the efficiency of warning signals as melanic individuals experience more predation (34). In Ithomiini,
306 the existence and pervasiveness of transparency is surprising given that those butterflies are toxic
307 and harbor aposematic signals. Previous work has shown that transparency in Ithomiini reduces
308 detection and attacks by naïve predators (27). Even though transparent patches are likely part of
309 the aposematic signal (22), transparency may reduce predator learning speed and memorization
310 of prey, as indirectly suggested by the observation that unpalatability tends to be stronger in
311 clearwing than in opaque species (43). Thus, transparency may incur a cost on aposematism, with
312 a trade-off between crypsis and aposematism. A possible explanation for the unexpected altitudinal
313 distribution of transparency in Ithomiini species may be different predation pressures at different
314 altitudes in tropical mountains (44), which may favor crypsis over aposematism at higher altitudes,
315 as observed in Andean arctiine moths or which conspicuousness decreases with height (45).

316 Therefore, the altitudinal distribution of color patterns in ithomiine may be primarily driven by
317 predation rather than climatic conditions.

318 **Conclusion**

319 In Ithomiini, the altitudinal distribution of wing patterns is unlikely driven by thermal constraints, and
320 goes against the predictions of the thermal melanism hypothesis. Transparent wings have a low
321 absorption of wavelengths in the UV-Visible-NIR range and this comes at a cost regarding heating
322 capacities. Conversely, transparency in these butterflies has probably evolved in response to
323 selection incurred by predation. The results question the critical significance of wing color pattern
324 for thermal adaptation in tropical ectotherms and lead the way to further investigations about
325 metabolic and behavioral thermoregulation, and their relative contributions to climatic adaptation in
326 these species.

327

328

329 **Materials and Methods**

330

331 **Specimen sampling, climatic data and phylogeny**

332 We studied wings of dried Ithomiini butterfly specimens, preserved in glassine envelopes, collected
333 between 2005 and 2018 in south America. We focused on 42 species represented by two or more
334 females to reduce the potential variance due to sexual differences (Table S1). Measurements were
335 taken on one hindwing and one forewing of each specimen. The selected species spanned a large
336 range of wing coloration, from fully opaque to highly transparent, and are evenly distributed along
337 the altitudinal gradient and across the phylogeny. GPS data on species distributions were obtained
338 from a database that contains more than 3500 occurrences of specimens of the species studied
339 here (29). We extracted the corresponding 19 climate variables (Table S8-9., Fig. S4) from
340 WorldClim2 historical climatic data (46) with a resolution of 2.5 arc-minutes. Variables were
341 averaged for each species. For the phylogenetic comparative analyses, we used the most
342 comprehensive phylogeny of the Ithomiini to date (28), which comprises 340 out of 393 extant taxa.

343 **Spectrophotometry measurements**

344 To quantify the extent to which wings transmit and absorb light, we performed spectrophotometry
345 measurements on a spot of 5 mm of diameter on the hindwing and forewing of each specimen. For
346 transparent species, the spot was in the proximal transparent patch. For opaque species, the spot
347 was located on the center of the proximal zone of the wing. Since a wing can reflect and transmit
348 a large proportion of the incident light, we measured both total (specular + diffuse) reflection and
349 total (specular + diffuse) transmission spectra of each patch, with an Agilent CARY 5000 UV-
350 Visible-NIR spectrophotometer, equipped with an integrating sphere, over the range 300-2500 nm,
351 which includes ultraviolet (UV), Visible, and near-infrared (NIR) wavelengths. Wings were

352 maintained in a black paper mask with an opening of 5 mm of diameter. Spectra were measured
353 relative to a dark (paper mask without the wings, light off) and to a white reference (Spectralon
354 white diffuse reflectance standard), with the paper mask for reflection, nothing for transmission,
355 light on. The resulting total absorption spectra were calculated for each wavelength λ as Absorption
356 (λ) = 1 - (Transmission (λ) + Reflection (λ)) according to the law of conservation of energy (Fig. 4).
357 The negative values due to the noise of the signal were fixed to zero with the function `fixneg` of the
358 package `Pavo` in R (47), and each spectrum was smoothed with the `loess` function in R. We
359 quantified mean absorption at different wavelength ranges, in the UV-Visible between 300-700 nm,
360 in the NIR between 700-2500 nm, and the total 300-2500 nm. Mean transmission in the UV-Visible
361 range 300-700 nm was measured as a proxy for animal-visible transparency. We divided the
362 species into two categories, transparent and opaque, based on a threshold of mean transmission
363 at 35% in the UV-Visible, matching butterflies and predators' visual perception (Table S16).

364 **Thermal measurements**

365 To characterize and compare the wing thermal characteristics, we measured wing temperature
366 kinetics following instant exposure to a flash light (Fig. 4B). We conducted stimulated heating
367 measurements on one hindwing and one forewing for each specimen, with a Neewer professional
368 photo system flash lamp N-300W set at maximum power, which emits over the UV-Visible-IR range.
369 Wings were recorded by an infrared camera T650sc 2019 FLIR Systems equipped with a FLIR lens
370 T197-915. Emissivity was set at 0.95. Wings were kept at a constant ambient temperature of 23°C
371 prior to the experiment. We placed each single wing horizontally on a custom grid made of 3 human
372 hair in a frame to minimize contact with any surface, 6 cm below the camera and the flash and 30
373 cm above a table covered with white paper, to avoid background interference with the measure.
374 The flash lasted for 1/800 seconds. We started the recording a few seconds before activating the
375 flash, and let the record run for 10 seconds. Each movie was recorded at 30 images.second⁻¹. From
376 the movies, we extracted the thermal kinetics - temperature as a function of time - of different
377 regions of interest on the wings with FLIR ResearchIR Max software, and more specifically the
378 excess temperature ΔT as the difference between the maximal temperature attained by the region
379 of interest after the flash hits minus the temperature prior to the flash. We extracted mean ΔT of
380 the overall wing surface, mean ΔT of the spot measured in spectrophotometry and finally ΔT of
381 precise points of interest on each wings as follows: 6 points in the dark colored patches DP (black
382 to brown colors), 6 points in light colored patches LP (yellow to orange colors and for opaque
383 species only), 6 points in the transparent patches TP (for transparent species only), and 8 points
384 on veins, assigned to a category depending on their color: dark veins DV (black to brown) and light
385 veins LV (yellow to orange).

386 **Image analysis**

387 We used digital photography to quantify the proportion of dark pigmented surface on the full wings
388 of all the specimens. Melanization in proximal zones of the wing can be of primary importance in
389 thermoregulation as the zone is closer to the thoracic muscles (8). We also quantified the color
390 proportion separately on the proximal and distal zone of each wing. We set the limit between the
391 zones as the perpendicular line to the middle of the transect line from the wing insertion to its apex.
392 We cropped the photos for each zone considered and the background was filled with green, a color
393 ignored when calculating the color proportions. In R, we used the `getKMeanColors` function from
394 `colordistance` package (48) to sort the pixels in clusters according to their RGB values. We set the
395 number of clusters to two, corresponding to mainly dark and brown, versus transparent, or any
396 other light colors in opaque wings. With ImageJ 1.52 (49) we computed the total surface of each
397 wing in mm².

398 **Statistical analyses**

399 We explored various scenarios linking absorption, transmission, thermal capacities and climatic
400 variables across the 42 species of the dataset (see supplementary materials for model details and
401 results). Because specimens are not independent and the species are related through the
402 phylogeny, we implemented for each test Bayesian mixed models with Markov Chain Monte Carlo,
403 correcting for phylogenetic relatedness, using the R package `MCMCglmm` (50) and the phylogeny
404 from (28). We controlled for chain convergence visually and with the Heidelberg stationarity test
405 diagnostic function of convergence `heidel.diag` from the R package `coda` (51). To get accurate
406 estimations of posterior distributions and avoid chain autocorrelation, we adjusted the iterations,
407 burn-in and thinning for each model to ensure an effective sample size across all parameters of at
408 least 1000. Models were run with a weakly informative prior for random effect and residual
409 variances ($V = 1$, $\nu = 0.002$) not to constrain the exploration of parameter values. We selected the
410 best model with a backward selection of fixed parameters based on Bayesian P-value of 0.05,
411 within the 95% confidence interval excluding zero. In all the models, species identity was
412 implemented as a random factor. We included all individuals in the models unless specified
413 otherwise.

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437 **References**

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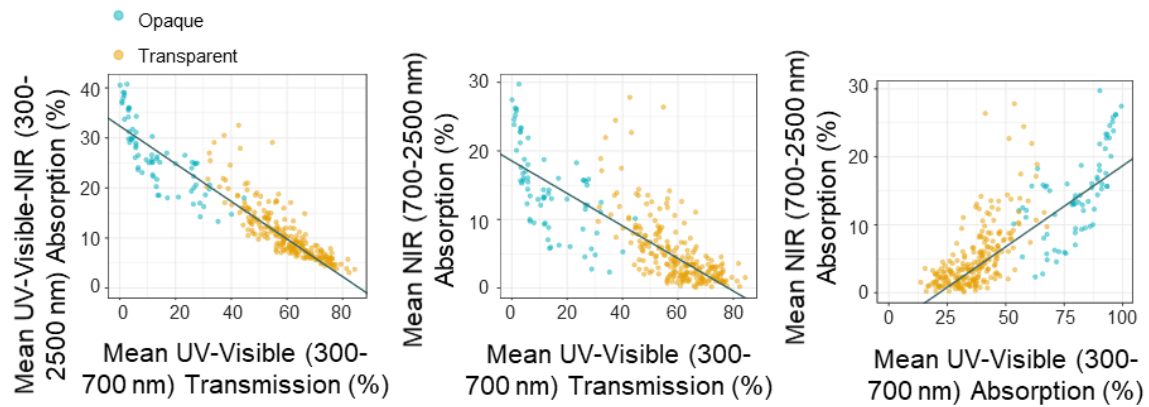
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589 **Figure 1.** Relationship between absorption and transmission spectra at different ranges: overall
590 UV-Visible-NIR (300-2500 nm) absorption against UV-Visible (300-700 nm) transmission, NIR
591 (700-2500 nm) absorption against UV-Visible transmission, NIR (700-2500 nm) absorption
592 against UV-Visible (300-700 nm) absorption. All relationships are significant and the solid line is
593 drawn from the prediction of the Bayesian phylogenetic mixed model (see Table S2) (N opaque =
594 72, N transparent = 235).

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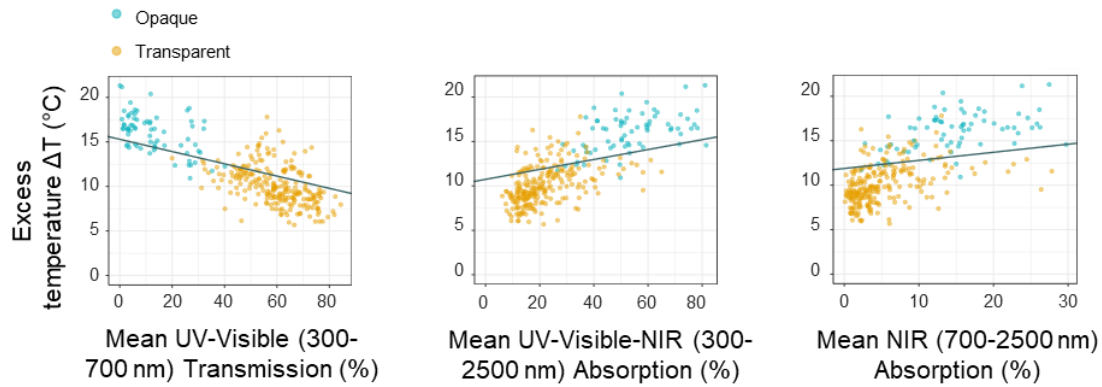
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608 **Figure 2.** The thermal properties of wing spots represented by the Excess Temperature ΔT
609 ($T^{\circ}\text{final} - T^{\circ}\text{initial}$) under a flash illumination, extracted from the thermal imaging, against their
610 optical properties at different ranges: UV-Visible (300-700 nm) transmission, UV-Visible-NIR (300-
611 2500 nm) absorption, and NIR (700-2500 nm) absorption. All relationships are significant and the
612 solid line is drawn from the prediction of the Bayesian phylogenetic mixed model (see Table S3) (N
613 opaque = 72, N transparent = 235).

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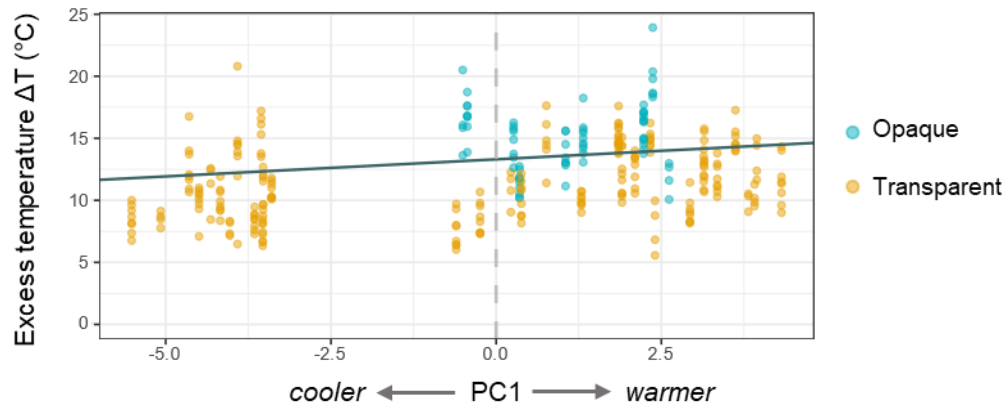
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626 **Figure 3.** Wings mean Excess Temperature ΔT ($T^{\circ}\text{final} - T^{\circ}\text{initial}$) under a flash illumination,
627 extracted from the thermal imaging, as a function of climatic principal component PC1. The
628 relationship is significant and the solid line is drawn from the prediction of the Bayesian phylogenetic
629 mixed model (see Table S13) (N opaque = 72, N transparent = 235).

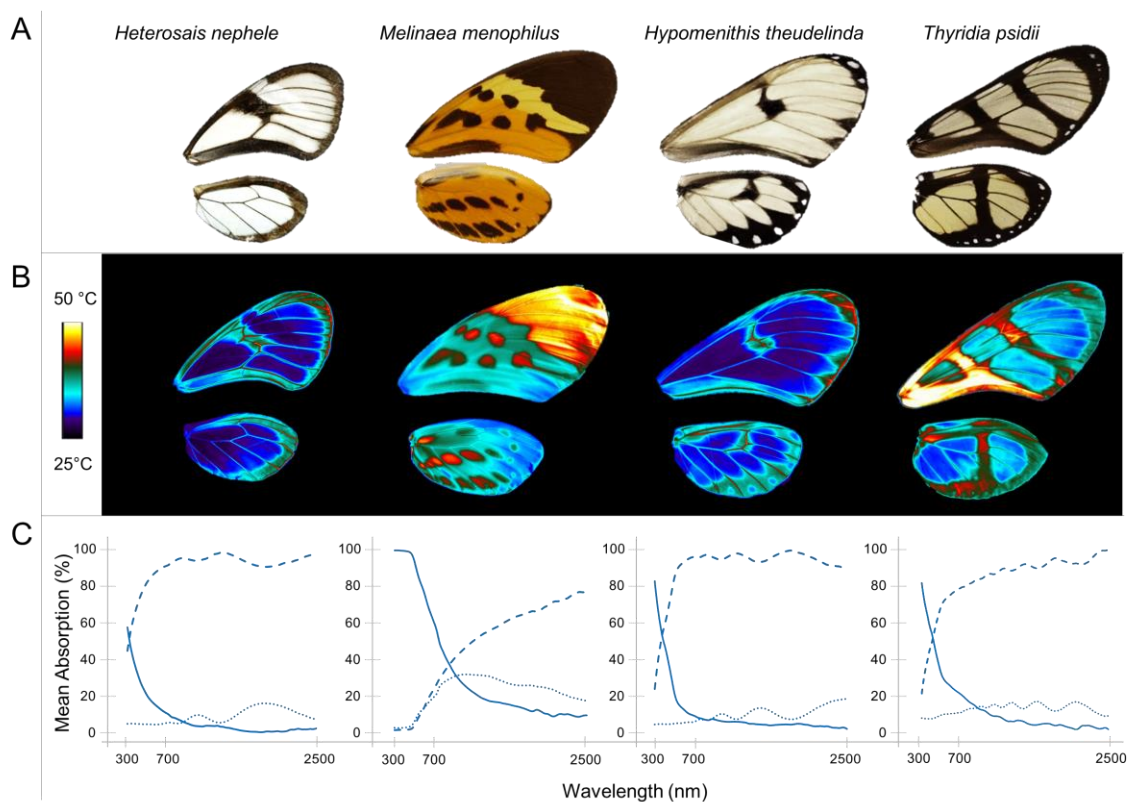
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636 **Figure 4.** Examples of optical and thermal imaging on the wings of four species of different opacity.
637 A: Photos under standard illumination of the hindwings and forewings of four of the species studied,
638 *Heterosais nephele*, *Melinaea menophilus*, *Hypomenithis theudelinda*, *Thyridia psidii*. B:
639 Visualization of the thermal imaging in false colors of the same wings under a flash light. C:
640 Corresponding measured transmission (dashed lines) and reflection (dotted lines) spectra in
641 percentage, for a spot of 5 mm of diameter on the proximal forewing of each butterfly, from 300 to
642 2500 nm, and the resulting computed absorption spectra (solid lines) for each wavelength λ as
643 $Absorption(\lambda) + Transmission(\lambda) + Reflection(\lambda) = 1$.

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