

This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Arias, Mónica; Mappes, Johanna; Desbois, Charlotte; Gordon, Swanne; McClure, Melanie; Elias, Marianne; Nokelainen, Ossi; Gomez, Doris

Title: Transparency reduces predator detection in mimetic clearwing butterflies

Year: 2019

Version: Accepted version (Final draft)

Copyright: © 2019 The Authors. Functional Ecology © 2019 British Ecological Society

Rights: In Copyright

Rights url: <http://rightsstatements.org/page/InC/1.0/?language=en>

Please cite the original version:

Arias, M., Mappes, J., Desbois, C., Gordon, S., McClure, M., Elias, M., Nokelainen, O., & Gomez, D. (2019). Transparency reduces predator detection in mimetic clearwing butterflies. *Functional Ecology*, 33(6), 1110-1119. <https://doi.org/10.1111/1365-2435.13315>

Functional Ecology

MÓNICA ARIAS (Orcid ID : 0000-0003-1331-2604)

DR MELANIE MCCLURE (Orcid ID : 0000-0003-3590-4002)

Article type : Research Article

Section: Animal Morphology and Colouration

Editor: Dr Caroline Isaksson

Transparency reduces predator detection in mimetic clearwing butterflies

Mónica Arias¹, Johanna Mappes², Charlotte Desbois³, Swanne Gordon², Melanie McClure³,
Marianne Elias^{3*}, Ossi Nokelainen^{2*}, Doris Gomez^{1,4*}

¹CEFE, Univ Montpellier, Univ Paul Valéry Montpellier 3, EPHE, IRD, Montpellier, France

²Department of Biological and Environmental Science, Centre of Excellence in Biological Interactions, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

³Institut de Systématique, Evolution, Biodiversité (ISYEB), CNRS, MNHN, Sorbonne Université, EPHE, Université des Antilles. 45 rue Buffon CP50, F-75005 Paris, France

⁴INSP, Sorbonne Université, CNRS, Paris, France

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2435.13315

This article is protected by copyright. All rights reserved.

Accepted Article

*co-last authors

Correspondence author email: moarias@gmail.com

Keywords: aposematic, bird, citizen science, crypsis, detectability, experiment, Ithomiini, vision modelling

Abstract

1. Predation is an important selective pressure and some prey have evolved conspicuous warning signals that advertise unpalatability (i.e. aposematism) as an antipredator defence. Conspicuous colour patterns have been shown effective as warning signals, by promoting predator learning and memory. Unexpectedly, some butterfly species from the unpalatable tribe Ithomiini possess transparent wings, a feature rare on land but common in water, known to reduce predator detection.
2. We tested if transparency of butterfly wings was associated with decreased detectability by predators, by comparing four butterfly species exhibiting different degrees of transparency, ranging from fully opaque to largely transparent. We tested our prediction using both wild birds and humans in behavioural experiments. Vision modelling predicted butterfly detectability to be similar for these two predator types.
3. In concordance with predictions, the most transparent species were almost never found first and were detected less often than the opaque species by both birds and humans, suggesting that transparency enhances crypsis. However, humans were able to learn to better detect the more transparent species over time.

4. Our study demonstrates for the first time that transparency on land likely decreases detectability by visual predators.

Introduction

Predation is an important selective pressure and a strong evolutionary force shaping prey colouration. Some prey have evolved colours and textures that mimic those of the background, hence rendering them cryptic (Endler, 1988) and reducing predator detection. In midwater environments, where there is nowhere to hide, crypsis can be achieved by different means, including transparency (Johnsen, 2014). Transparency is common in aquatic organisms where it has been shown to decrease detectability by visual predators, enabling prey to blend in with their environment (Kerfoot, 1982; Langsdale, 1993; Tsuda, Hiroaki, & Hirose, 1998; Zaret, 1972). By contrast, transparency is generally rare in terrestrial organisms, except for insect wings, which are made of chitin, a transparent material. However, Lepidoptera (named after ancient Greek words for scale – *lepis* – and wing – *pteron*) are an exception as their wings are generally covered with colourful scales that are involved in intraspecific communication (Jiggins, Estrada, & Rodrigues, 2004), thermoregulation (Miaoulis & Heilman, 1998), water repellence (Wanasekara & Chalivendra, 2011), flight enhancement (Davis, Chi, Bradley, & Altizer, 2012), and antipredator adaptations such as crypsis (Stevens & Cuthill, 2006), masquerade (Suzuki, Tomita, & Sezutsu, 2014) and aposematism (i.e. advertisement of unpalatability by the means of conspicuous colouration, Mallet & Singer, 1987).

Ithomiini (Nymphalidae: Danainae), also known as clearwing butterflies, are some of the most abundant butterflies in Neotropical forests (Willmott, Willmott, Elias, & Jiggins, 2017). Ithomiini species are considered to be unpalatable to some extent due to the accumulation of

Accepted Article

pyrrolizidine alkaloids collected from Asteraceae, Boraginaceae and Apocynaceae plants (Brown, 1984, 1985). Pyrrolizidine alkaloids, naturally present in Ithomiini butterflies, *Oreina* beetles, or artificially added to mealworms, have been reported to effectively deter predation by birds (Brown & Neto, 1976). Many Ithomiini represent classic examples of aposematic prey, whereby bright wing colour patterns – including orange, yellow and black - advertise their unprofitability to predators (Mappes, Marples, & Endler, 2005; Nokelainen, Hegna, Reudler, Lindstedt, & Mappes, 2011; Poulton, 1890). Ithomiini butterflies are also involved in mimicry with other aposematic species such as several *Heliconius* butterflies (Beccaloni, 1997). Bright contrasting and aposematic colouration is likely to be the ancestral state in the group, since most species in sister lineages (Tellerveni and Danaini) are opaque and aposematic (Freitas & Brown, 2004). However, transparency has evolved to some degree in approximately 80% of clearwing butterfly species, even though many retain minor opaque and colourful wing elements (Beccaloni, 1997; Elias, Gompert, Jiggins, & Willmott, 2008; Jiggins, Mallarino, Willmott, & Bermingham, 2006). Similarly to cicadas and damselflies, transparency in these butterfly wings is sometimes enhanced by anti-reflective nanostructures (Siddique, Gomard, & Hölscher, 2015; Watson, Myhra, Cribb, & Watson, 2008; Yoshida, Motoyama, Kosaku, & Miyamoto, 1997). Since transparency is often associated with crypsis, for example in aquatic organisms (Johnsen, 2014), transparency in these butterfly may decrease detectability by predators.

To determine if transparency in clearwing butterflies decreases detectability by visual predators, we compared predator detection of four Ithomiini species that differ in the amount of transparency of their wings (Fig 1): *Hypothyris ninonia* (largely opaque and brightly coloured), *Ceratinia tutia* (brightly coloured and translucent), *Ithomia salapia* (transparent with a pale yellow tint and an opaque contour) and *Brevioleria seba* (transparent without

Accepted Article

colouration other than a white band in the forewing and an opaque contour). Given the proportion of light that is transmitted through the butterfly wing of the different species (Fig S1), we predicted that the opaque species *Hypothyris ninonia* should be the easiest to detect, followed by the translucent species *Ceratinia tutia*. Finally, the more transparent butterfly species *Ithomia salapia* and *Brevioleria seba* should be the least detectable. However, it is also possible that the coloured opaque elements of the transparent species, such as the white band in *B. seba* and the opaque contour found in most of these species, enhance detection. We tested our predictions using two complementary behavioural experiments involving birds and humans, and further supported by a vision modelling approach.

Detectability of butterflies was first tested using wild great tits (*Parus major*) as model bird predators. Great tits are sensitive to UV wavelengths (UVS vision in Ödeen, Håstad, & Alström, 2011). Their vision is similar to that of naturally occurring Ithomiini predators such as the houtouc motmot (*Momotus momota*, Pinheiro et al., 2008), the fawn-breasted tanager (*Pipraeidea melanonota*, Brown & Neto, 1976) or the rufous-tailed tanager (*Ramphocelus carbo*, Brower et al., 1963). However, unlike Neotropical insectivorous birds, great tits are naïve to ithomiine butterflies and have not learned to associate their colour patterns to toxicity. As a bird's propensity to attack prey is the result of both prey detection and motivation to attack the prey, we also performed behavioural experiments using human participants, which can be useful in disentangling these two factors. Differences in colour perception between great tits and humans include the presence of a fourth single cone type receptor (instead of three cones in humans) that extend the great tits' sensitivity into the UV light spectrum (Hart, 2001), and oil droplets that refine colour discrimination in birds (Vorobyev, 2003). However, neither humans or birds are able to detect linear polarization, which excludes the use of polarization cues to detect and discriminate between butterfly

species (Foster et al., 2018; Greenwood, Smith, Church, & Partridge, 2003; Melgar, Lind, & Muheim, 2015; Montgomery & Heinemann, 1952). Moreover, humans have been found to be good predictors of insect prey survival in the wild (Penney, Hassall, Skevington, Abbott, & Sherratt, 2012). Finally, models of predator vision (both for birds and humans) were used to complement behavioural experiments and infer the relative detectability of each butterfly species based on their contrast against the background.

Materials and Methods

Butterflies used for the behavioural experiments

Specimens of the four Ithomiini species used in both experiments – which, in order of increasing transparency are *Hypothyris ninonia*, *Ceratinia tutia*, *Ithomia salapia aquina*, *Brevioleria seba* (see Figs 1 and S1) – were collected in Peru in 2016 and 2017, along the Yurimaguas - Moyobamba road (-6.45°, -76.30°). Butterflies were kept dry in glassine envelopes until use. In behavioural experiments, a single real hindwing and a single real forewing were assembled into artificial butterflies using glue and a thin copper wire to attach the artificial butterfly to a substrate (see Fig S2 for an example). These artificial butterflies mimicked real Ithomiini butterflies at rest, with wings closed and sitting on plant leaves (a typical posture for resting butterflies).

Behavioural experiments using wild birds

Behavioural experiments took place in August and September 2017 at the Konnevesi Research Station (Finland). Thirty wild-caught great tits (*Parus major*) were used. Birds were caught using spring-up-traps and mist-nets, individually marked with a leg band and used only once. Each bird was housed individually in an indoor cage (65x65x80 cm) and were fed

with seeds and water *ad libitum*, except during training and experiments. During training, birds were given mealworms attached to butterfly wings (see Training section). Birds were deprived of food for up to 2 hours before the experiment to increase their motivation to hunt.

Training. In indoor cages, birds were taught that all four species of butterflies were similarly palatable by offering them laminated wings of four butterflies (one of each species) with a mealworm attached to the copper wire. Wings were laminated during training only, using transparent thin plastic so as to minimize damage and enabling us to re-use the wings between trials. Butterflies were presented to the birds in the absence of vegetation during training so as to enhance the association between butterfly colour patterns and fully edible prey. When birds had eaten all four prey items (one of each species), a new set was presented. Training ended when birds had eaten 3 sets of butterflies. No time constraint was imposed for training and most birds completed it in less than 4 hours.

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

Experiments. The experimental set-up consisted of a 10m x 10m cage that had tarpaulin walls and a ceiling of whitish dense net that let in natural sunlight. Butterflies were disposed in a 5 x 5 grid, delimited by poles all around the borders and a rope defining rows and columns (see

Fig S3). Five specimens of each species (20 specimens in total) were placed in the grid, one per cell. Before each trial, butterflies were photographed over graph paper, used as a scale to measure butterfly size on Image J (Rueden et al., 2017). Butterflies were pinned on top of meadowsweet leaves (*Filipendula ulmaria*) that had naturally grown in the outdoor cages. Butterflies were always put in similar places within the cell and could be easily seen from a nearby pole. Butterfly position was randomized but care was taken in 1) leaving the 5 cells closest to the observer empty as birds tended to avoid this area, 2) avoiding having more than two specimens of the same species in the same row or column, and 3) having two specimens of the same species in neighbouring cells. This ensured that all species were evenly represented along the grid. This random configuration was reshuffled between trials.

For each trial, an observer, hidden to the birds, watched from outside the cage through a small window and took notes of which butterfly species were attacked and in which order. A GoPro camera also recorded the experiments. A butterfly was considered detected only if a bird directly approached to attack it, including when the attack failed. No bird was seen hesitating during an attack once it had initiated it. Experiments took place between 9 am and 5 pm. Before each trial, the radiance of ambient light (coming from the sun and sky) was taken by spectrophotometry in the same location. We computed the total radiance (TR) over the bird's spectral sensitivity, which range from 300-700 nm, to account for the intensity of ambient light associated to each experimental trial in the statistical analyses. Further information on weather conditions (cloudy, sunny, etc) was also recorded. Experiments ended when a bird had eaten half of the available butterflies (ie. 10 butterflies) or after 2 hours, whichever happened first. Wings were occasionally re-used if they had not been damaged.

Accepted Article

To control for any positional effect on overall species detection, we computed the probability of a bird being present in a given grid area. To do so, a 10-minute interval of each recorded trial was selected and revised to calculate the proportion of time birds spent on the different poles. The time intervals were possible for all trials as they all lasted at least 10 minutes and were selected either as a result of the birds actively attacking prey or actively exploring the cage during that time, based on notes taken by the observer. These probabilities were later used to divide the grid into four main areas according to bird occupancy: furthest and closest corner to the observer, grid border and grid centre (Fig S4a). Most birds fed willingly on all butterflies located on the borders of the grid. Given that butterfly species distribution was random and reshuffled between trials, the four species were similarly represented throughout the grid (Fig S4b), so no bias was expected. For more details about permits, husbandry conditions, training and experiments, see Supplementary Material.

Behavioural experiments using human participants

Between mid-November and early December 2017, visitors of the Montpellier botanical garden (France) were invited to take part in an experiment where they searched for artificial butterflies. Before each trial, participants were shown pictures of various ithomiine butterfly species, both transparent and opaque, different from those used in the experiments to familiarize them with what they would be searching for. Anonymous personal data was collected from each participant, including gender, age group (A1: <10 years, A2: 11-20 y, A3: 21-30 y, A4: 31-40 y, A5: 41-50 y and A6: >51 years), and vision problems. Each participant attempted the experiment only once.

Experimental set-up. As with the behavioural bird experiments, artificial butterflies (N=10 of each of the four species, for a total of 40 butterflies) consisted of one real forewing and one real hindwing assembled with copper wire and placed on leaves, but without the mealworm used in the bird experiments. These butterflies were set-up along two corridors in a forest-like understory habitat of similar vegetation and light conditions. Butterfly order followed a block randomisation, with five blocks each consisting of eight butterflies (i.e. two of each species; see Fig S5). This ensured that observers were similarly exposed to the four species all throughout the experimental transect. Whether a butterfly was placed on the left or right side of the corridor was also randomised and both order and corridor side were changed daily. Participants could start the path from either end of the set-up and were given unlimited time to complete the trial. However, they could only move forward on the path. Only one participant was allowed in the path at any given time, and they were accompanied by an observer who recorded which butterflies were found. Trials ended when the participant had completed both corridors.

Statistical analyses.

Experiments using birds and humans were analysed independently. Differences in the total number of butterflies of each species that were attacked by predators (for the sake of simplicity we use ‘attacked’ hereafter for both birds and humans) were compared by fitting generalised linear mixed effect models (GLMM), with bird/human identity as a random factor. A binomial distribution was used for the response variable (attacked or not). For the experiments using birds, butterfly species, butterfly size, trial duration, age and sex of the bird, time to first attack, first butterfly species attacked, butterfly position on the grid (corner –furthest or closest to the observer-, grid border, grid centre), weather (as a qualitative

variable), and total radiance (TR), as well as their interactions, were all included as explanatory variables. For human trials, butterfly species, first species attacked, butterfly position, corridor, left or right side of the path, time of day, gender and age of the participant, duration of the experiment, and their interactions, were all used as explanatory variables. In each case, the best fitting model was selected based on minimization of Akaike's Information Criteria (AIC), assuming that models differing by two units or less were statistically indistinguishable (Anderson, Burnham, & White, 1998). Coefficients and standard errors were computed using a restricted maximum likelihood approach and a Wald z test was used to test for factor significance.

In addition to the total number of butterflies attacked per species, an "inconspicuousness" rank was calculated for each butterfly species, as done in a previous study (Ihalainen, Rowland, Speed, Ruxton, & Mappes, 2012). This ranking takes into consideration both the specimens that were attacked and those that were not for each species. Lower values are assigned to those specimens that were attacked (from 1 to 10, according to the sequence of overall prey discovery), and higher values are given to those specimens that were not attacked (all unnoticed specimens are given a value of 11: the maximum number of butterflies that could be attacked before the experiment ended + 1). For example, if a bird captures two *H. ninonia* second and fifth in the sequence of captured prey, leaving three specimens unnoticed (out of a total of 5 placed in the cage), this species gets a rank value of $2+5+(3 \times 11)=40$ for that trial. This inconspicuousness rank distinguishes species attacked first and in higher numbers (lower values of inconspicuousness) from those attacked last and in lower numbers (higher values of inconspicuousness). We fitted a linear mixed effect model to test for differences in rank for each species, assuming a normal distribution, with rank as the response variable. We fitted independent models for birds and human experiments. For bird

Accepted Article

experiments, bird individual was considered a random factor, and butterfly species, age and sex of the bird, date, time until first attack, first butterfly species attacked, weather as a qualitative variable, and total radiance (TR) were explanatory variables. For humans, participant identity was a random factor, and butterfly species, first species attacked, time of day, gender and age of the participant, duration of the experiment, and their interactions, were all explanatory variables. Again, the best fitting model was selected using AIC minimization. GLMMs were fitted using *nlme* (Pinheiro, Bates, DebRoy, Sarkar, & R Core team, 2009) and *lme4* (Bates, Maechler, Bolker, & Walker, 2015, p. 4) packages for R. Moreover, whether specific species were more frequently detected first by either birds or humans was tested using a χ^2 test.

Additionally for birds, we tested whether butterfly location in the grid could explain differences in the overall species' detection, i.e. whether species more likely to be attacked were more often placed on areas more likely to be visited. To do so, the frequency per species on the four different grid zones was compared using a χ^2 .

Finally, we tested whether birds and humans created a “search image” (i.e. improved ability in finding butterflies of a given species after encountering a similar one) by counting the number of butterflies of each species attacked consecutively. Results were compared among butterfly species using a χ^2 test. Additionally, whether finding some species improved a bird's or a human's ability to find others was also tested. For each combination of two species, we calculated how many times a butterfly of species 1 was found after a butterfly of species 2. Differences between combinations of butterfly species found by birds were tested using a χ^2 test. For humans, observed results and the frequency at which each possible pair of

species was placed consecutively in the original experimental setup were compared using a χ^2 test. All analyses were performed in R (R Foundation for Statistical Computing, 2014).

Colour measures and vision modelling

Finally, models of predator vision (both for birds and humans) were used to complement behavioural experiments and infer the relative detectability of each butterfly species based on their contrast against the background. First, we measured colour (i.e. reflectance) and transmission properties (i.e. transmittance of transparent wing areas) using spectrophotometry. Vorobyev & Osorio's discriminability model (1998) was then used to calculate the contrast between butterfly and background for birds and humans. Detailed methods for measurements and vision modelling can be found in the electronic supplementary material (additional materials and methods).

Results

Behavioural experiments using wild birds

The model that best explained whether butterflies were attacked or not included only the time required before the first attack and the cage area in which the butterfly was located (Table S1). Butterflies were most likely to be attacked when located in the furthest corners and in the borders than in the rest of the cage ($z = 9.13$, $p < 0.001$). By contrast, the inconspicuousness rank of a butterfly species was best explained by a model including butterfly species as an explanatory variable (Table S2). Which species was attacked first closely matched wing transmission properties: *H. ninonia*, the fully opaque species, followed by the translucent *C. tutia*, the transparent and yellow-tinted *I. salapia* and the most transparent species in our

study, *B.seba* ($X^2 = 11.07$, $df = 3$, $p = 0.011$; Table S3). *Hypothyris ninonia*, which was the most colourful species, was usually the first species attacked ($t = -3.15$, $p = 0.002$, Fig 2a; Tables S2 and S3). Species distribution along the four different grid zones was similar ($X^2 = 6.19$, $df = 9$, $p = 0.72$; Fig. S4b).

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

Behavioural experiments using human participants

Younger participants found more butterflies than older ones (number of butterflies: $z = -2.34$, $p = 0.019$; Fig S7a). Additionally, participants found more butterflies earlier than later in the afternoon (number of butterflies: $z = -2.80$, $p = 0.005$; Fig S7a). Generally, the more time participants spent on the experiment, the more butterflies they found (number of butterflies: $z = 5.21$, $p < 0.001$), although this was most significant for women (number of butterflies: $z = -2.96$, $p = 0.003$), Fig S7b). Participants found more butterflies on the corridor that had slightly larger vegetation cover (number of butterflies: $z = 3.14$, $p = 0.002$). Participants also found more butterflies at the end rather than at the start of the experiment (number of butterflies: $z = 3.70$, $p < 0.001$, Tables S4), most likely because they became accustomed to the set-up and what they were searching for.

Participants were more likely to find opaque butterflies than transparent ones, following the order *H. ninonia* (H), *C. tutia* (C), *B. seba* (B) and *I. salapia* (I) (H>C, I, B: number of butterflies: $z = 5.73$, $p < 0.001$; inconspicuousness rank: $t = -3.96$, $p < 0.001$; C>B: inconspicuousness rank: $t = -4.81$, $p < 0.001$; B>I: inconspicuousness rank: $t = -1.325$, $p < 0.001$; Tables S4 and S5; Fig 2b). However, the gain in detection with increasing time spent searching was highest for the most transparent species ($z = -2.75$, $p = 0.006$, Fig S7c). *Hypothyris ninonia* was also the species most frequently found first, followed by *C. tutia*, *B. seba* and *I. salapia* ($X^2 = 19.5$, $df = 3$, $p < 0.001$, Table S3). More butterflies of each species were found when *C. tutia* was found first ($t = -3.96$, $p < 0.001$).

There were also differences in the consecutive order in which butterflies were found. Participants were more likely to find two consecutive butterflies of the same species when they were colourful (*H. ninonia* -50 times- and *C. tutia* -58 times) than when they were transparent (*B. seba* -32 times- or *I. salapia* -18 times; $X^2 = 29.14$, $df = 3$, $p < 0.001$). *Brevioleria seba* and *H. ninonia* were found consecutively up to four times in a single trial. Some species were also more likely to be found consecutively after another species. The two most opaque butterflies *H. ninonia* and *C. tutia* (found 278 times consecutively), and the two transparent species *B. seba* and *I. salapia* (found 186 times consecutively), were found consecutively more frequently than any of the other possible combinations after correcting for the number of butterflies found for each species ($X^2 = 170.95$, $df = 5$, $p < 0.001$). These observed frequencies differed significantly from expected as a result of their physical position along the path ($X^2 = 79.12$, $df = 11$, $p < 0.001$, Fig S6b).

Models of bird and human vision

The achromatic weighted contrast between butterfly colour patches and green-leaf background were similar for both birds and humans (mean achromatic contrast for birds: H=3.81, C= 3.15, I=2.31, B=2.11; for humans: H=5.25, C=4.35, I=3.58, B=3.86; Fig S8). For both observers, *H. ninonia* (the most colourful species) followed by *C. tutia* (colourful but translucent species) contrasted the most against the leaves, while the transparent butterflies (*I. salapia* for humans and *B. seba* for birds) were the least contrasting. Butterflies seem to be more chromatically detectable by birds than for humans (mean chromatic contrast for humans: H = 0.44, C = 0.37, I = 0.25, B = 0.22). For the chromatic contrast seen by birds, *C. tutia*, followed by *H. ninonia* were the most contrasting, whereas *B. seba* and *I. salapia* were the least contrasting (mean chromatic contrast for birds: H = 2.02, C = 2.05, I = 1.30, B = 1.38). For further details of the experiment results, see the Electronic Supplementary Material.

Discussion

Transparency reduces detectability

As initially predicted based on wing transmittance, and as demonstrated by our behavioural experiments and visual modelling results, transparency decreases butterfly detectability. Interestingly, detection by human participants was similar to that of naïve birds, as shown in other studies (Beatty, Bain, & Sherratt, 2005; Sherratt, Whissell, Webster, & Kikuchi, 2015), providing further support for using human participants to measure predator detection. Surprisingly, experimental results from the bird experiments differed slightly from predictions based on the measures of transmittance of transparent patches and results obtained from the vision models. For instance, according to the transmittance and the

chromatic contrast measured between butterflies and their background, birds should have detected *C. tutia* more easily than the two more transparent species. Indeed, semi-transparent objects should be more easily detected than fully transparent objects at short distances and when more light is available (Johnsen & Widder, 1998), such as conditions present during our experiments. Yet this transparent but brightly coloured species was detected at rates similar to those of the most transparent species, perhaps because transparent butterflies were more easily detected and attacked by birds than we predicted (e. g., if an opaque contour enhances detectability of otherwise transparent prey). Alternatively, the semi-transparent *C. tutia* could have been less detectable by birds, because it shows less strongly delimited contours than those of the most opaque species *H. ninonia*. Perhaps this hampered its detection as occurs in disruptively coloured prey (Honma, Mappes, & Valkonen, 2015; Stevens & Cuthill, 2006). These contradicting results highlight the importance of combining both modelling and behavioural experiments to better understand the evolution of transparency and other prey defences.

Transparency in potentially unpalatable butterflies?

Our results demonstrate that transparency can effectively reduce prey detectability in ithomiine butterflies, where several species have been experimentally demonstrated to be chemically-protected (Brown, 1985; Trigo et al., 1996). This is surprising as aposematic colour patterns, rather than inconspicuousness, are more common in toxic and unpalatable prey (Mappes et al., 2005; Poulton, 1890; Ruxton, Sherratt, & Speed, 2004). In fact, conspicuousness is positively correlated with toxicity or unpalatability in some species and can thus be an honest indicator of prey defences (Arenas, Walter, & Stevens, 2015; Blount, Speed, Ruxton, & Stephens, 2009; Maan & Cummings, 2012; Prudic, Skemp, & Papaj, 2007;

Accepted Article

Sherratt & Beatty, 2003). Moreover, predators learn more quickly to avoid unpalatable prey when colours are more conspicuous (Gittleman & Harvey, 1980; Lindstrom, Alatalo, Mappes, Riipi, & Vertainen, 1999). This might suggest that the evolution of transparency in these butterflies is the result of a loss or a reduction in unpalatability. If this is the case, the existence of mimicry rings of transparent clearwing butterflies remains unexplained, as this is usually the result of convergence of warning signals promoted by the positive frequency-dependent selection exerted by predators (Willmott et al., 2017). Alternatively, if defences are costly, prey may invest in either visual or chemical defences (Darst, Cummings, & Cannatella, 2006; Speed & Ruxton, 2007; Wang, 2011), as such options have been shown to afford equivalent avoidance by predators (Darst et al., 2006). In which case, transparency should instead be associated with an increase in unpalatability. This relationship between transparency and chemical defences in clearwing butterflies remains to be explored.

Alternatively, transparency may lower detection and function as a primary defence, with aposematism taking over as a secondary defence if the prey is detected. Indeed, transparent butterflies were not completely cryptic for either birds or humans. In fact, although birds detected the most colourful species first, in total they found a similar number of both colourful and transparent butterflies. Moreover, humans appear to learn to detect and perhaps remember common elements between the more transparent species, which might be the result of a search image. As such, Ithomiini butterflies may be cryptic from afar, but perceived as conspicuous from up close. The combination of crypsis and conspicuousness has also been shown for other defended prey (Järvi, Sillén-Tullberg, & Wiklund, 1981; Sillén-Tullberg, 1985). For example, toxic salamanders of the genus *Taricha* are generally cryptic, only revealing their warning coloured underbelly when threatened (Johnson & Brodie, 1975). In Ithomiini, conspicuous elements such as opaque areas that delineate the edges and contrast

with the background likely increase detection, as has been shown for artificial moths (Stevens & Cuthill, 2006). Furthermore, pigmentary or structurally produced opaque colours, such as the white band in *B. seba*, may also enhance butterfly detection. This suggests, as do our results and the occurrence of co-mimics in natural habitats, that these butterflies may reduce the cost of conspicuousness using transparency in addition to maintaining the benefits of detectable warning signals. Further behavioural experiments testing the distance at which Ithomiini butterflies are detected are needed to shed further light on the function of aposematism in less conspicuous prey.

Finally, transparency may have evolved as an additional protection against birds such as adult kingbirds (*Tyrannus melancholicus*, Pinheiro, 1996) which are able to tolerate their chemical defences. Indeed, both theoretical (Endler & Mappes, 2004) and experimental (Mappes, Kokko, Ojala, & Lindström, 2014; Valkonen et al., 2012) studies have shown that weak warning signals (not overtly conspicuous) can evolve and be maintained in communities where predators vary in their probability of attacking defended prey. Larvae of *Dryas iulia* butterflies, pine sawfly larvae (*Neodiprion sertifer* for example), and shield bugs (Acanthosomatidae, Heteroptera) are only a few of the examples that exist of unpalatable species that display weak visual warning signals (see Endler & Mappes, 2004). As in the polymorphic poison frog *Oophaga granulifera*, clearwing species may reflect a continuum between aposematism and crypsis, possibly shaped by differences in the strength of predator selection as a result of the frequency of naïve predators and/or the variation in predator sensitivities to chemical compounds (Willink, Brenes-Mora, Bolaños, & Pröhl, 2013). A thorough characterization of unpalatability, microhabitat and predator communities would be useful in better understanding conditions that promote the evolution of transparency.

Conclusions

Our study, which combines behavioural experiments with different predators and vision modelling, provides important insights into the complex role transparency may play in predator defences of terrestrial aposematic organisms. We show for the first time that transparency results in the reduction of detectability of terrestrial prey. We also demonstrate that Ithomiini butterflies may in fact be decreasing the costs of conspicuousness, while still retaining visual elements that are recognised as warning signals. Future studies exploring the efficiency of combining transparency and warning signals in decreasing predation risk will further contribute to our understanding of the evolution of cryptic elements in aposematic prey.

Acknowledgments

We thank Tuuli Salmi and Tiffanie Kortenhoff for their invaluable help with behavioural experiments, Helinä Nisu for her advice on bird care, SERFOR, Proyecto Huallaga and Gerardo Lamas for providing research permits in Peru (collecting and exportation permit 002-2015-SERFOR-DGGSPFFS), as well as Corentin Clerc, Monica Monllor, Alexandre Toporov and Marc Toporov-Elias for help with collecting butterflies used in this study, Céline Houssin for calculations of wing surfaces for each butterfly colour pattern patch and for Ithomiini pictures, Konnevesi Research Station, which provided the facilities used for bird experiments, and visitors to Montpellier botanical garden for their enthusiastic contribution. We thank Marcio Cardoso and another anonymous reviewer for their helpful comments and suggestions. The study was funded by the Academy of Finland (Grants 2100000256 and 21000038821), the Clearwing ANR program (ANR-16-CE02-0012) and the Human Frontier Science Program grant (RGP 0014/2016).

Author contribution

DG, ME, JM and MA designed the study; ME, MM and DG collected the butterfly samples; MA, SG, ON, ME and JM performed the experiments; DG and CD did the optical measurements; MA, DG and ME analysed the data; MA, DG, MM, ME, ON, SG and JM wrote the manuscript. Authors have none conflict of interest to declare.

Data available from the Dryad Digital Repository doi:10.5061/dryad.17pk7v8 (Arias *et al.* 2019).

References

- Anderson, D., Burnham, K., & White, G. (1998). Comparison of Akaike information criterion and consistent Akaike information criterion for model selection and statistical inference from capture-recapture studies. *Journal of Applied Statistics*, 25(2), 263–282.
- Arenas, L. M., Walter, D., & Stevens, M. (2015). Signal honesty and predation risk among a closely related group of aposematic species. *Scientific Reports*, 5.
- Arias, M., Mappes, J., Desbois, C., Gordon, S., McClure, M., Elias, M., ... Gomez, D. (2019). Data from: Transparency reduces predator detection in mimetic clearwing butterflies. Dryad Digital Repository. doi:10.5061/dryad.17pk7v8

- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1–7. 2014.
- Beatty, C. D., Bain, R. S., & Sherratt, T. N. (2005). The evolution of aggregation in profitable and unprofitable prey. *Animal Behaviour*, 70(1), 199–208.
- Beccaloni, G. (1997). Ecology, natural history and behaviour of the Ithomiinae Butterflies and their mimics in Ecuador. *Tropical Lepidoptera*, 8, 103–124.
- Blount, J. D., Speed, M. P., Ruxton, G. D., & Stephens, P. A. (2009). Warning displays may function as honest signals of toxicity. *Proceedings of the Royal Society of London B: Biological Sciences*, 276(1658), 871–877. doi:10.1098/rspb.2008.1407
- Brower, L. P., Brower, J. V. Z., & Collins, C. T. (1963). *Experimental studies of mimicry: Relative palatability and Müllerian mimicry among Neotropical butterflies of the subfamily Heliconiinae*. New York Zoological Society.
- Brown, K. S. J. (1985). Chemical ecology of dehydropyrrolizidine alkaloids in adult Ithomiinae (Lepidoptera: Nymphalidae). *Rev. Bras. Biol.*, 44, 453–460.
- Brown, K. S. J. (1984). Chemical ecology of dehydropyrrolizidine alkaloids in adult Ithomiinae (Lepidoptera: Nymphalidae). *Revista Brasileira de Biologia*, 44(4), 435–460.
- Brown, K. S. J., & Neto, J. V. (1976). Predation on aposematic ithomiine butterflies by tanagers (Pipraeidea melanonota). *Biotropica*, 136–141.
- Brown, K. S. J. (1985). Chemical ecology of dehydropyrrolizidine alkaloids in adult Ithomiinae (Lepidoptera: Nymphalidae). *Rev. Bras. Biol.*, 44, 453–460.

- Darst, C. R., Cummings, M. E., & Cannatella, D. C. (2006). A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. *Proceedings of the National Academy of Sciences*, *103*(15), 5852–5857.
- Davis, A. K., Chi, J., Bradley, C., & Altizer, S. (2012). The redder the better: wing color predicts flight performance in monarch butterflies. *PloS One*, *7*(7), e41323.
- Elias, M., Gompert, Z., Jiggins, C., & Willmott, K. (2008). Mutualistic interactions drive ecological niche convergence in a diverse butterfly community. *PLoS Biology*, *6*(12), e300.
- Endler, J. A. (1988). Frequency-dependent predation, crypsis and aposematic coloration. *Phil. Trans. R. Soc. Lond. B*, *319*(1196), 505–523.
- Endler, J. A., & Mappes, J. (2004). Predator mixes and the conspicuousness of aposematic signals. *The American Naturalist*, *163*(4), 532–547.
- Foster, J. J., Temple, S. E., How, M. J., Daly, I. M., Sharkey, C. R., Wilby, D., & Roberts, N. W. (2018). Polarisation vision: overcoming challenges of working with a property of light we barely see. *The Science of Nature*, *105*(3), 27. doi:10.1007/s00114-018-1551-3
- Freitas, A. V. L., & Brown, K. S. J. (2004). Phylogeny of the Nymphalidae (Lepidoptera). *Systematic Biology*, *53*(3), 363–383.
- Gittleman, J. L., & Harvey, P. H. (1980). Why are distasteful prey not cryptic? *Nature*, *286*(5769), 149–150. doi:10.1038/286149a0
- Greenwood, V. J., Smith, E. L., Church, S. C., & Partridge, J. C. (2003). Behavioural investigation of polarisation sensitivity in the Japanese quail (*Coturnix coturnix*)

japonica) and the European starling (*Sturnus vulgaris*). *Journal of Experimental Biology*, 206(18), 3201–3210.

Hart, N. S. (2001). The visual ecology of avian photoreceptors. *Progress in Retinal and Eye Research*, 20(5), 675–703.

Honma, A., Mappes, J., & Valkonen, J. K. (2015). Warning coloration can be disruptive: aposematic marginal wing patterning in the wood tiger moth. *Ecology and Evolution*, 5(21), 4863–4874.

Ihalainen, E., Rowland, H. M., Speed, M. P., Ruxton, G. D., & Mappes, J. (2012). Prey community structure affects how predators select for Mullerian mimicry. *Proceedings of the Royal Society B-Biological Sciences*, 279(1736), 2099–2105.
doi:10.1098/rspb.2011.2360

Järvi, T., Sillén-Tullberg, B., & Wiklund, C. (1981). The cost of being aposematic. An experimental study of predation on larvae of *Papilio machaon* by the great tit *Parus major*. *Oikos*, 267–272.

Jiggins, C. D., Estrada, C., & Rodrigues, A. (2004). Mimicry and the evolution of premating isolation in *Heliconius melpomene* Linnaeus. *Journal of Evolutionary Biology*, 17(3), 680–691. doi:10.1111/j.1420-9101.2004.00675.x

Jiggins, C. D., Mallarino, R., Willmott, K. R., & Bermingham, E. (2006). The phylogenetic pattern of speciation and wing pattern change in neotropical *Ithomia* butterflies (Lepidoptera: Nymphalidae). *Evolution*, 60(7), 1454–1466.

Johnsen, S. (2014). Hide and seek in the open sea: pelagic camouflage and visual countermeasures. *Annual Review of Marine Science*, 6, 369–392.

- Johnsen, S., & Widder, E. A. (1998). Transparency and visibility of gelatinous zooplankton from the northwestern Atlantic and Gulf of Mexico. *The Biological Bulletin*, 195(3), 337–348.
- Johnson, J. A., & Brodie Jr, E. D. (1975). The selective advantage of the defensive posture of the newt, *Taricha granulosa*. *American Midland Naturalist*, 139–148.
- Kerfoot, W. C. (1982). A question of taste: crypsis and warning coloration in freshwater zooplankton communities. *Ecology*, 63(2), 538–554.
- Langsdale, J. (1993). Developmental changes in the opacity of larval herring, *Clupea harengus*, and their implications for vulnerability to predation. *Journal of the Marine Biological Association of the United Kingdom*, 73(1), 225–232.
- Lindstrom, L., Alatalo, R. V., Mappes, J., Riipi, M., & Vertainen, L. (1999). Can aposematic signals evolve by gradual change? *Nature*, 397(6716), 249–251. doi:10.1038/16692
- Maan, M. E., & Cummings, M. E. (2012). Poison frog colors are honest signals of toxicity, particularly for bird predators. *The American Naturalist*, 179(1), E1–E14. doi:10.1086/663197
- Mallet, J., & Singer, M. C. (1987). Individual selection, kin selection, and the shifting balance in the evolution of warning colours: the evidence from butterflies. *Biological Journal of the Linnean Society*, 32(4), 337–350.
- Mappes, J., Kokko, H., Ojala, K., & Lindström, L. (2014). Seasonal changes in predator community switch the direction of selection for prey defences. *Nature Communications*, 5.

- Mappes, J., Marples, N., & Endler, J. A. (2005). The complex business of survival by aposematism. *Trends in Ecology & Evolution*, *20*(11), 598–603.
doi:10.1016/j.tree.2005.07.011
- Melgar, J., Lind, O., & Muheim, R. (2015). No response to linear polarization cues in operant conditioning experiments with zebra finches. *Journal of Experimental Biology*, *218*(13), 2049–2054.
- Miaoulis, I. N., & Heilman, B. D. (1998). Butterfly thin films serve as solar collectors. *Annals of the Entomological Society of America*, *91*(1), 122–127.
- Montgomery, K., & Heinemann, E. G. (1952). Concerning the ability of homing pigeons to discriminate patterns of polarized light. *Science*.
- Nokelainen, O., Hegna, R. H., Reudler, J. H., Lindstedt, C., & Mappes, J. (2011). Trade-off between warning signal efficacy and mating success in the wood tiger moth. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20110880.
- Ödeen, A., Håstad, O., & Alström, P. (2011). Evolution of ultraviolet vision in the largest avian radiation - the passerines. *BMC Evolutionary Biology*, *11*(1), 313.
doi:10.1186/1471-2148-11-313
- Penney, H. D., Hassall, C., Skevington, J. H., Abbott, K. R., & Sherratt, T. N. (2012). A comparative analysis of the evolution of imperfect mimicry. *Nature*, *483*(7390), 461–464.
- Pinheiro, C. E. G. (1996). Palatability and escaping ability in neotropical butterflies: Tests with wild kingbirds (*Tyrannus melancholicus*, Tyrannidae). *Biological Journal of the Linnean Society*, *59*(4), 351–365. doi:10.1111/j.1095-8312.1996.tb01471.x

- Pinheiro, C. E., Medri, Í. M., & Salcedo, A. K. M. (2008). Why do the ithomiines (Lepidoptera, Nymphalidae) aggregate? Notes on a butterfly pocket in central Brazil. *Revista Brasileira de Entomologia*, 52(4), 610–614.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core team. (2009). nlme: Linear and nonlinear mixed effects models. R package version 3.1-93. Retrieved from <http://cran.r-project.org/web/packages/nlme/>
- Poulton, E. B. (1890). *The colours of animals: their meaning and use, especially considered in the case of insects*. D. Appleton.
- Prudic, K. L., Skemp, A. K., & Papaj, D. R. (2007). Aposematic coloration, luminance contrast, and the benefits of conspicuousness. *Behavioral Ecology*, 18(1), 41–46. doi:10.1093/beheco/arl046
- R Foundation for Statistical Computing, R. C. (2014). *R: A language and environment for statistical computing*. Vienna, Austria.
- Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, 18(1), 529.
- Ruxton, G. D., Sherratt, T. N., & Speed, M. P. (2004). *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry*. Oxford University Press.
- Sherratt, T. N., & Beatty, C. D. (2003). The evolution of warning signals as reliable indicators of prey defense. *The American Naturalist*, 162(4), 377–389.

- Accepted Article
- Sherratt, T. N., Whissell, E., Webster, R., & Kikuchi, D. W. (2015). Hierarchical overshadowing of stimuli and its role in mimicry evolution. *Animal Behaviour*, *108*, 73–79.
- Siddique, R. H., Gomard, G., & Hölscher, H. (2015). The role of random nanostructures for the omnidirectional anti-reflection properties of the glasswing butterfly. *Nature Communications*, *6*, 6909.
- Sillén-Tullberg, B. (1985). Higher survival of an aposematic than of a cryptic form of a distasteful bug. *Oecologia*, *67*(3), 411–415. doi:10.1007/BF00384948
- Speed, M. P., & Ruxton, G. D. (2007). How bright and how nasty: explaining diversity in warning signal strength. *Evolution*, *61*(3), 623–635.
- Stevens, M., & Cuthill, I. C. (2006). Disruptive coloration, crypsis and edge detection in early visual processing. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1598), 2141. doi:10.1098/rspb.2006.3556
- Suzuki, T. K., Tomita, S., & Sezutsu, H. (2014). Gradual and contingent evolutionary emergence of leaf mimicry in butterfly wing patterns. *BMC Evolutionary Biology*, *14*(1), 229. doi:10.1186/s12862-014-0229-5
- Trigo, J. R., Brown Jr, K. S., Witte, L., Hartmann, T., Ernst, L., & Barata, L. E. S. (1996). Pyrrolizidine alkaloids: different acquisition and use patterns in Apocynaceae and Solanaceae feeding ithomiine butterflies (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society*, *58*(1), 99–123.
- Tsuda, A., Hiroaki, S., & Hirose, T. (1998). Effect of gut content on the vulnerability of copepods to visual predation. *Limnology and Oceanography*, *43*(8), 1944–1947.

- Valkonen, J. K., Nokelainen, O., Niskanen, M., Kilpimaa, J., Björklund, M., & Mappes, J. (2012). Variation in predator species abundance can cause variable selection pressure on warning signaling prey. *Ecology and Evolution*, 2(8), 1971–1976.
- Vorobyev, M. (2003). Coloured oil droplets enhance colour discrimination. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1521), 1255–1261.
- Vorobyev, M., & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society B-Biological Sciences*, 265(1394), 351–8.
- Wanasekara, N. D., & Chalivendra, V. B. (2011). Role of surface roughness on wettability and coefficient of restitution in butterfly wings. *Soft Matter*, 7(2), 373–379.
- Wang, I. J. (2011). Inversely related aposematic traits: reduced conspicuousness evolves with increased toxicity in a polymorphic poison \square dart frog. *Evolution: International Journal of Organic Evolution*, 65(6), 1637–1649.
- Watson, G. S., Myhra, S., Cribb, B. W., & Watson, J. A. (2008). Putative functions and functional efficiency of ordered cuticular nanoarrays on insect wings. *Biophysical Journal*, 94(8), 3352–3360.
- Willink, B., Brenes \square Mora, E., Bolaños, F., & Pröhl, H. (2013). Not everything is black and white: color and behavioral variation reveal a continuum between cryptic and aposematic strategies in a polymorphic poison frog. *Evolution*, 67(10), 2783–2794.
- Willmott, K. R., Willmott, J. C. R., Elias, M., & Jiggins, C. D. (2017). Maintaining mimicry diversity: optimal warning colour patterns differ among microhabitats in Amazonian clearwing butterflies (Vol. 284, p. 20170744). Presented at the Proc. R. Soc. B, The Royal Society.

Accepted Article

Yoshida, A., Motoyama, M., Kosaku, A., & Miyamoto, K. (1997). Antireflective nanoprotuberance array in the transparent wing of a hawkmoth, *Cephonodes hylas*. *Zoological Science*, *14*(5), 737–741.

Zaret, T. M. (1972). Predators, invisible prey, and the nature of polymorphism in the cladocera (class Crustacea). *Limnology and Oceanography*, *17*(2), 171–184.
doi:10.4319/lo.1972.17.2.0171

Figures

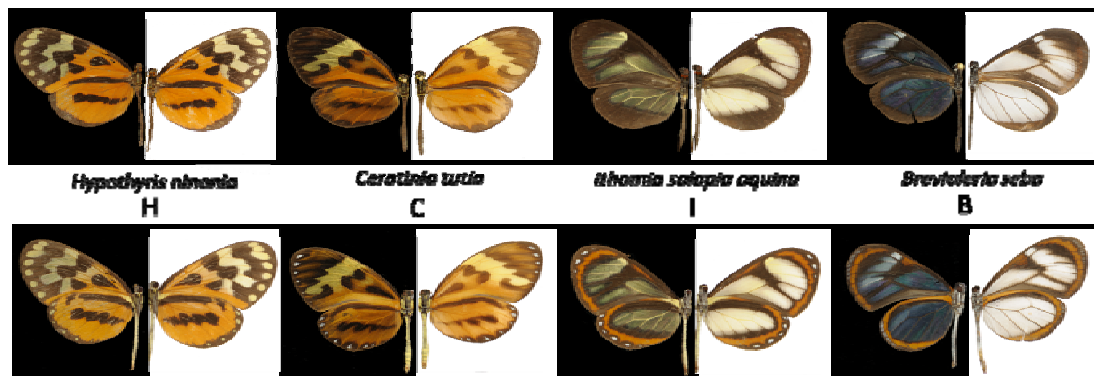


Figure 1. Dorsal (top row) and ventral (bottom row) view of butterfly species used in the study (photographed against a black and a white background to show the location and degree of transparency in the wings). Wing transparency (transmission and area occupied by transparent patches) increases from left (most opaque) to right (most transparent): *Hypothyris ninonia* (largely opaque), *Ceratonia tutia* (translucent but brightly coloured), *Ithomia salapia aquina* (transparent with a pale yellow tint and black wing contour), *Brevioleria seba* (transparent without colouration other than a white band in the forewing and a black wing contour). ©

Céline Houssin

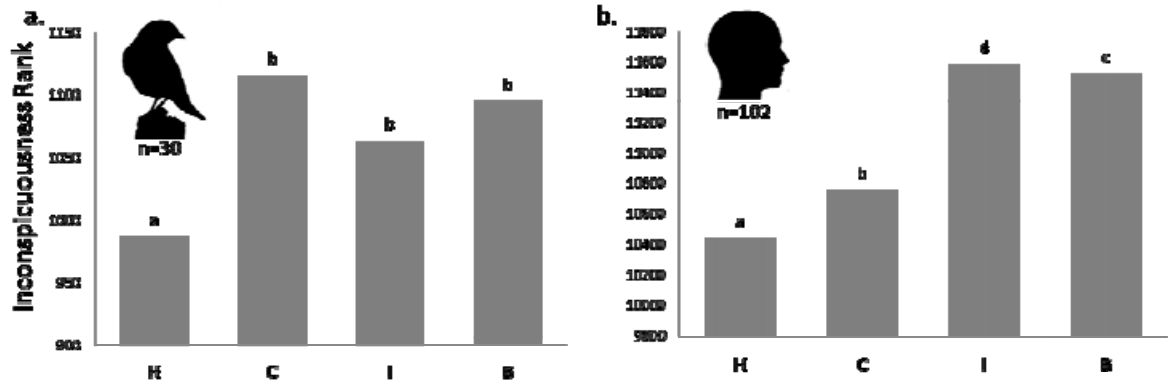


Figure 2. Sum of the inconspicuousness rank for each butterfly species calculated from the behavioural experiments using **a)** great tits and **b)** humans. Species for which butterflies were detected first and most often by birds or humans have lower values of “inconspicuousness rank”. Butterfly transparency increases from left to right: *H. ninonia* (H), *C. tutia* (C), *I. salapia* (I), and *B. seba* (B). Letters above the bars mean significant differences below 0.05.