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Transparency reduces predator detection in mimetic clearwing butterflies

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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

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 butteflies 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

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.

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+(3x11)=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

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. ninona* 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;

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.

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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).

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Figures

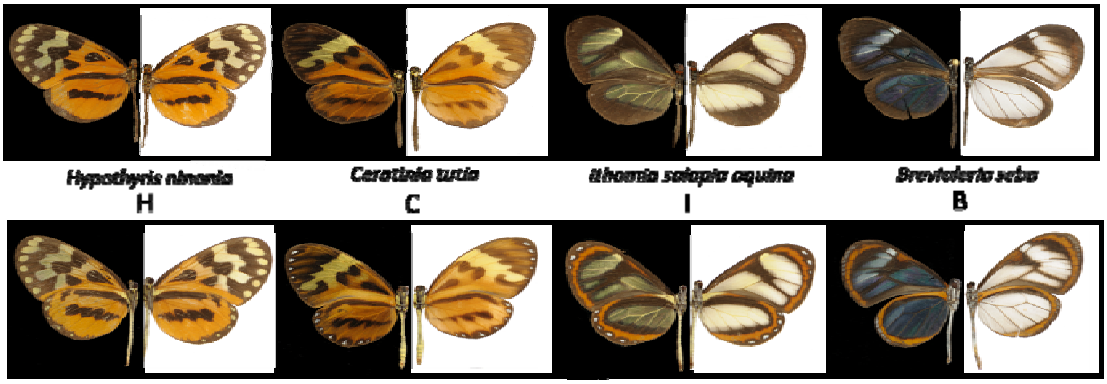

Figure 1. Dorsal (top row) and ventral (bottom row) view of butterfly species used in the study (photographed against a black and a white background to show the location and degree of transparency in the wings). Wing transparency (transmission and area occupied by transparent patches) increases from left (most opaque) to right (most transparent): *Hypothyris ninonia* (largely opaque), *Ceratinia tutia* (translucent but brightly coloured), *Ithomia salapia* (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

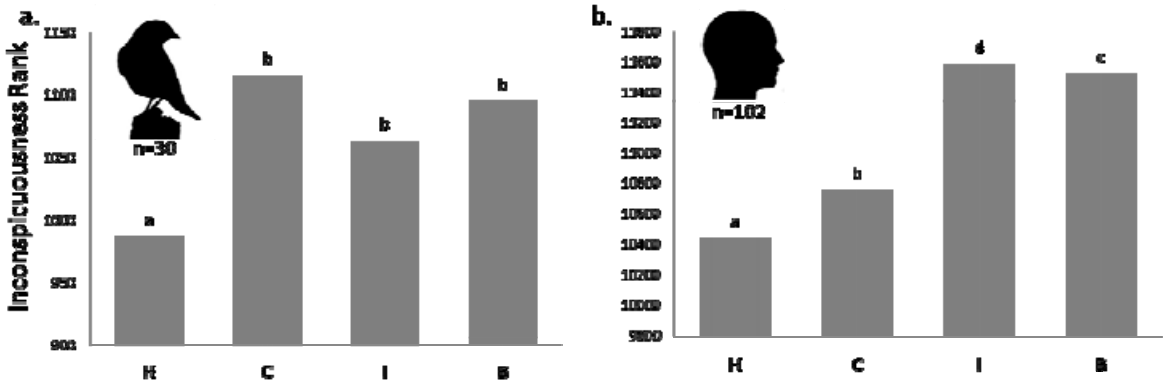

Figure 2. Sum of the inconspicuousness rank for each butterfly species calculated from the behavioural experiments using **a)** great tits and **b)** humans. Species for which butterflies were detected first and most often by birds or humans have lower values of "inconspicuousness rank". Butterfly transparency 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.