Peer Community In Ecology

Intimidation or deflection: field experiments in a tropical forest to simultaneously test two competing hypotheses about how butterfly eyespots confer protection against predators

Doyle Mc Key *based on peer reviews by 2 anonymous reviewers*

Cristiano Agra Iserhard, Shimene Torve Malta, Carla Maria Penz, Brenda Barbon Fraga, Camila Abel da Costa, Taiane Schwantz, Kauane Maiara Bordin (2024) The large and central *Caligo martia* eyespot may reduce fatal attacks by birds: a case study supports the deflection hypothesis in nature. Zenodo, ver. 1, peer-reviewed and recommended by Peer Community in Ecology. https://doi.org/10.5281/zenodo.10980357

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Eyespots—round or oval spots, usually accompanied by one or more concentric rings, that together imitate vertebrate eyes—are found in insects of at least three orders and in some tropical fishes (Stevens 2005). They are particularly frequent in Lepidoptera, where they occur on wings of adults in many species (Monteiro et al. 2006), and in caterpillars of many others (Janzen et al. 2010). The resemblance of eyespots to vertebrate eyes often extends to details, such as fake « pupils » (round or slit-like) and « eye sparkle » (Blut et al. 2012). Larvae of one hawkmoth species even have fake eyes that appear to blink (Hossie et al. 2013). Eyespots have interested evolutionary biologists for well over a century. While they appear to play a role in mate choice in some adult Lepidoptera, their adaptive significance in adult Lepidoptera, as in caterpillars, is mainly as an anti-predator defense (Monteiro 2015). However, there are two competing hypotheses about the mechanism by which eyespots confer defense against predators. The « intimidation » hypothesis postulates that eyespots intimidate potential predators, startling them and reducing the probability of attack. The « deflection » hypothesis holds that eyespots deflect attacks to parts of the body where attack has relatively little effect on the animal's functioning and survival. In caterpillars, there is little scope for the deflection hypothesis, because attack on any part of a caterpillar's body is likely to be lethal. Much observational and some experimental evidence supports the intimidation hypothesis in caterpillars (Hossie & Sherratt 2012). In adult Lepidoptera, however, both mechanisms are plausible, and both have found support (Stevens 2005). The most spectacular examples of intimidation are in butterflies in which eyespots located centrally in hindwings and hidden in the natural resting position are suddenly exposed, startling the potential predator (e.g., Vallin et al. 2005). The most spectacular examples of deflection are seen in butterflies in which eyespots near the hindwing margin combined with other traits give the appearance of a false head (e.g., Chotard et al. 2022; Kodandaramaiah 2011).

Most studies have attempted to test for only one or the other of these mechanisms—usually the one that seems a priori more likely for the butterfly species being studied. But for many species, particularly those that have neither spectacular startle displays nor spectacular false heads, evidence for or against the two hypotheses is contradictory.

Iserhard et al. (2024) attempted to simultaneously test both hypotheses, using the neotropical nymphalid butterfly *Caligo martia*. This species has a large ventral hindwing eyespot, exposed in the insect's natural resting position, while the rest of the ventral hindwing surface is cryptically coloured. In a previous study of this species, De Bona et al. (2015) presented models with intact and disfigured eyespots on a computer monitor to a European bird species, the great tit (*Parus major*). The results favoured the intimidation hypothesis. Iserhard et al. (2024) devised experiments presenting more natural conditions, using fairly realistic dummy butterflies, with eyespots manipulated or unmanipulated, exposed to a diverse assemblage of insectivorous birds in nature, in a tropical forest. Using color-printed paper facsimiles of wings, with eyespots present, UV-enhanced, or absent, they compared the frequency of beakmarks on modeling clay applied to wing margins (frequent attacks would support the deflection hypothesis) and (in one of two experiments) on dummies with a modeling-clay body (eyespots should lead to reduced frequency of attack, to wings and body, if birds are intimidated). Their experiments also included dummies without eyespots whose wings were either cryptically coloured (as in unmanipulated butterflies) or not. Their results, although complex, indicate support for the deflection hypothesis: dummies with eyespots were mostly attacked on these less vital parts. Dummies lacking eyespots were less frequently attacked, especially when they were camouflaged. Camouflaged dummies without eyespots were in fact the least frequently attacked of all the models. However, when dummies lacking eyespots were attacked, attacks were usually directed to vital body parts. These results show some of the complexity of estimating costs and benefits of protective conspicuous signals vs. camouflage (Stevens et al. 2008).

Two complementary experiments were conducted. The first used facsimiles with « wings » in a natural resting position (folded, ventral surfaces exposed), but without a modeling-clay « body ». In the second experiment, facsimiles had a modeling-clay « body », placed between the two unfolded wings to make it as accessible to birds as the wings. However, these dummies displayed the ventral surfaces of unfolded wings, an unnatural resting position. The study was thus not able to compare bird attacks to the body vs. wings in a natural resting position. One can understand the reason for this methodological choice, but it is a limitation of the study.

The naturalness of the conditions under which these field experiments were conducted is a strong argument for the biological significance of their results. However, the uncontrolled conditions naturally result in many questions being left open. The butterfly dummies were exposed to at least nine insectivorous bird species. Do bird species differ in their behavioral response to eyespots? Do responses depend on the distance at which a bird first detects the butterfly? Do eyespots and camouflage markings present on the same animal both function, but at different distances (Tullberg et al. 2005)? Do bird responses vary depending on the particular light environment in the places and at the times when they encounter the butterfly (Kodandaramaiah 2011)? Answering these questions under natural, uncontrolled conditions will be challenging, requiring onerous methods, (e.g., video recording in multiple locations over time). The study indicates the interest of pursuing these questions.

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Reviews

Evaluation round #3

DOI or URL of the preprint: http://doi.org/10.22541/au.170000972.25951252/v2
Version of the preprint: 2

Authors' reply, 25 March 2024

Download author's reply
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Decision by Doyle Mc Key, posted 25 March 2024, validated 25 March 2024

revision– a few minor queries on format

The authors have integrated all my corrections and suggestions.

I found two further minor corrections concerning format of references and have two minor queries on the text. These can be very quickly addressed (a matter of a few minutes). I attach the document with these few comments.

Sincerely,

Doyle McKey **Download recommender's annotations**

Evaluation round #2

DOI or URL of the preprint: http://doi.org/10.22541/au.170000972.25951252/v2
Version of the preprint: 2

Authors' reply, 22 March 2024

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Decision by Doyle Mc Key, posted 20 March 2024, validated 20 March 2024

Minor revision

The authors have responded to the reviewers' comments in a satisfactory way, making the needed changes. In cases where they disagreed with the reviewers' recommendations, they justified their choice.

There are still some technical corrections that need to be made. There are also a few places where the text (or the illustrations) need to be a bit clearer. I have attached my marked-up copy of the Word document of the manuscript, which includes all my suggestions.

After attaching this document, I saw that I had not read the Supplementary. The following corrections to Figure S2 need to be added:

In the first line of the box "Dataset", change "models" to "facsimiles"—also in the sixth line

Same correction in the second line of the box "Issues". Also, on this line write "predation on the facsimiles" (not "in" the facsimiles)

Once these issues are addressed, the manuscript will be in good shape.

Sincerely,

Doyle McKey **Download recommender's annotations**

Evaluation round #1

DOI or URL of the preprint: http://doi.org/10.22541/au.170000972.25951252/v1
Version of the preprint: 1

Authors' reply, 18 March 2024

Download author's reply
Download tracked changes file

Decision by Doyle Mc Key, posted 10 January 2024, validated 11 January 2024

Dear Dr. Iserhard,

I have received comments from two anonymous reviewers, both of whom carefully read your manuscript. I have examined their comments and found them pertinent and constructive. I agree with their conclusions and recommendations. Both reviewers found the manuscript interesting and usefu and believe it is worthy of publication. Both also pointed out important issues that need your attention and do not permit acceptance of the manuscript in its present form. I Invite you to resubmit the manuscript after making major revisions that address the reviewers' comments.

Both reviewers felt that the presentation of the two hypotheses, deflection and intimidation, could be improved, for example, with clear predictions about what results would support each. Doing so in the introduction would help improve presentation and discussion of the results. Both reviewers felt that from the outset you gave much more credence (and attention) to the deflection hypothesis. They recommend, and I agree with this recommendation, that you give equal attention to both hypotheses at the start. Another recommendation is that you consider a third alternative, in which both intimidation and deflection are supported. The two hypotheses are not mutually exclusive. The reviewers make many suggestions about how to revise the introduction, the results, and the discussion to implement this shift in focus.

Both reviewers also point out that *Catocala* moths do not have true eyespots, and that while *Catocala* is a good model for 'startle', it is not a good model for eye-mimicry.

The reviewers make several important recommendations for improving the figures, in terms of both form and content. Importantly, one reviewer points to the need for better visual documentation of some of the main points of the paper. More information is needed on what the paper « models » (or « facsimiles », a change in wording that, as one reviewer points out, would reduce confusion) look like in the field (for both experiments), and photographic documentation of the beak marks on the paper wings. We need to know how you scored beak marks. Could you also provide images of the paper models photographed in UV light ?

One reviewer called for presentation of the statistical results in the main text, not solely in the supplemental material. I agree that at least summary results of statistical tests on main points should be in the main text.

Finally, both reviewers pointed to additional relevant literature that should be cited in the manuscript. They also indicated some references that are cited (e.g. de Bona et al.) but that should also be cited in other places in the text.

I trust that you will find the reviewers' comments helpful as you prepare a revised version of the manuscript. If you do plan to resubmit a revision, please send the revised manuscript within **x weeks.** Please send along with the revised manuscript a letter showing line-by-line how you have responded to the each of the reviewers' comments. Please send the manuscript not only in pdf, but also as a Word document, as this will facilitate making minor editorial corrections.

Sincerely,
Doyle McKey

Reviewed by anonymous reviewer 2, 08 January 2024

This is an interesting paper reporting results of field experiments with facsimile butterflies in Brazil testing whether eyespots work in intimidation or deflection. The experiments are generally well-designed, statistics appropriate, and conclusions robust. As the authors indicate, it is an important contribution given it was done with predators in the field and their discussion is careful to not over-generalize their results. With this last point in mind I recommend editing in several places to be more general about “wings” vs “hind wings” since no test was done of forewings vs hind wings. For example, use “wings” rather than “hind wings” in the question in supplementary doc 1 and methods and results text and discuss how wings is interpreted to mean attack at hindwings if needed. In addition, I suggest editing the introduction paragraph reviewing previous work on deflection, taking care in the section on Catocala (see detailed comments below), and including statistics in the main document as well as making several edits to Figure 4. Overall, I enjoyed the manuscript and think it should be published. Please see my additional detailed comments below.

Detailed comments:

Out of curiosity, is it possible to associate any of the bird damage with particular species listed on lines 126-130?

The main question is about hind wing but no test was focused on forewing vs hind wing so I recommend revising it to be more general and say “wings”(supplemental doc 1) or just “eyespots”(line 99).

line 32 – “model butterfly” would be better than “paper model”. Also, consider “facsimile” as a potential alternate term for “model” here, as it is used in studies of mimicry to avoid confusion with model and mimic.

line 35 – missing “a” or “s”: “in natural resting positions” or “in a natural resting position”

line 74 – I think this paragraph could be improved by editing it to emphasize evidence of deflection, evidence of intimidation and evidence of both occurring in concert. The paragraph treats deflection and then intimidation, setting up the focal topic of the experiments in the paper, but loses steam when transitioning to intimidation (line 79 see next comment), and risks confusing things when it brings up an example where both intimidation and deflection may be working in line 81. The example with Catocala is relevant for showing how deflection and intimidation may work in concert, but it needs to be emphasized as a third element, rather than placed between the other two. It might work to do two separate paragraphs to treat evidence of deflection and intimidation in other species, and one for Catocala where they work in concert. Given the emphasis on this I was expecting it to come up more in the discussion.

line 79 – The section of this paragraph on Catocala needs to be edited since Catocala do not have “eyespots” or “eye-mimicry” but are a good model for deimatic (startle) displays. Bringing up Catocala as an example of startle is fine, but not eye-mimicry, and the paragraph would be strengthened by staying focused here on “how” eyespots work in intimidation and including other examples of deimatic displays in Lepidoptera (for example Olofsson et al 2012, PLoS vol 7, but note the swallowtail in this paper also does not have eyespots).

If the authors want to maintain emphasis on the hindwings of Catocala working by combining deflection and intimidation, then “eye-mimicry” should be edited, and as mentioned above this would be a third category in the paragraph.

In addition, since Catocala do not have eyespots, but are of general interest to the topic of deflection and deimatic displays, it also makes sense to include Pierella butterflies (Nymphalidae, Satyrinae) in the first part of the paragraph listing studies with strong evidence of marks acting as deflection and cite Hill and Vaca 2004 paper in Biotropica (Vol 36).

Line 96 – “no experimental study” instead of “not a single experimental study”

Line 111 – missing “of”: “instead of the wings”

Line 161 – a and b indicated in legend but not in the figure

Line 164-166 – omission of the body in exp 1 does not necessarily mean the birds will not attack the base of the wings. Was there any damage indicating they attacked the glue where the facsimiles were glued?

Line 167 – it specifies hindwing here but the results say “wings” (i.e. line 200) so I recommend removing hindwing here because it does not appear you distinguished attacks on the FW vs HW. If the observation was that most attacks were on the hind wings but it was not recorded, this point could be brought up in the discussion or clarified as such here.

Line 218 and Figure 4 – This may be an editorial issue with the specific journal, but I would like to see the statistical results in the main paper without having to go to supplemental information to find them. Doing this adds tables if kept in present format, but the statistical results could be added in the text for specific results or comparisons at least and preferably for all. For example, readers without the online materials may look at the Figure 4 legend where it says the “a” and “b” indicate significant differences. Are the a’s indicating significant difference between body in model WE and NC, and b’s indicating significant difference between wing in model WE and NC? If so, what about differences between body and wing in WE and NC? Based on the text and Table S2 it is the latter, and body and wing are significantly different in WE and NC. Having the stats in the main manuscript would help with this. Given the result and test conducted shouldn’t “a” be for the body vs wing comparison of WE, and “b” for the body vs wing comparison in NC? The letters in each panel could be combined with a bracket or lines highlighting the comparison being made.

Line 239 – omit “hind wing” so it reads “eyespots of Caligo…”

Line 249 – I suggest removing the parenthetical description of De Bona’s setup and just using a comma.

Line 250 – remove “preferably” since no test was done of FW vs HW

Line 251 – remove “in the hind wing” since no test was done of FW vs HW; removing emphasis on where the eyespot is does not detract from the result of deflection being more important than intimidation.

Line 262 – Hill and Vaca’s 2004 (Biotropica vol 36) study on Pierella deflection marks and wing strength is very relevant to cite here with the DeVries papers.

Line 264 - Hill and Vaca 2004, as well as Sourakov 2013 (Journal of Natural History vol 47), are also very relevant to cite here with the Olofsson, Stevens, and Vallin papers.

Line 282 – Since body vs wings was compared in Experiment 2 I think it makes sense to clarify the end of this sentence by adding “in a natural resting position.” Otherwise, it would contradict your experiment 2.

Figure 4 should have “number of attacks” on y-axis instead of “number of predations”

Figure 4 – panel b is very small and the points are hard to see and what looks like text by the points is not legible.

Figure 4 – the CM panel in b is on a different scale (4% vs 100%) making it look like something is going on with this model type when in fact it is a very low attack rate. Perhaps draw attention to this in the legend, or put it on the 100% scale like the others.

Reviewed by anonymous reviewer 1, 03 December 2023

Review of Iserhard et al. 2023

This is an interesting study that sets out to test two hypotheses for eyespot function in the wild using paper models of an Owl butterfly, and natural predators. The two hypotheses are deflection and intimidation. The authors, however, fail to make unbiased predictions of what they should find (in terms of predation risk) under each of these hypotheses, and bias their study, from the very beginning towards the deflection hypothesis alone. The results, in my opinion, support both hypotheses. The study also needs more descriptive photos of the models used (photographed both in visible light and in UV light), as well as better documentation of the mechanism that captured "beak" marks in their paper models. It is important to show photos of these marks. I have no idea of what these marks look like, and how they are captured on the paper wings. Below are more specific comments.

Page 3, line 50: Monteiro 2008 defines eyespots as a "roughly circular pattern on the wing, with at least two concentric rings or with a single color disc and a central pupil", not as the authors describe it here.

Page 4, line 79. Why are Catocala moths, which don't have eyespots, be considered an excellent model to study eye mimicry? I am confused here. I suggest the authors remove this section.

Line 82, what wing pattern are the authors referring to? There is no explicit mention of any pattern on the hindwings.

Line 86 – There is more evidence than the one the authors point to supporting the intimidation effect of eyespots. For instance – look up Ho et al. 2015 (cited by the authors) as well as :
https://academic.oup.com/beheco/article/22/6/1326/220549?login=true
https://academic.oup.com/cz/article/61/4/749/1803186?login=false
https://www.frontiersin.org/articles/10.3389/fevo.2022.951967/full

Figure 4 – Usually the a) and b) are placed to the left of the figure.

Line 97: There is a study that examined the function of these eyespots (not with local predators) but still should perhaps be mentioned at this stage.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4426626/

Line 99 – In this paragraph the authors say that their study aims to assess which of the two functions (intimidation versus deflection) are the eyespots of Caligo butterflies serving. Yet, in the sentences below they hypothesize a single outcome for these experiments: that eyespots serve in deflection and models with eyespots will be attacked more than models without eyespots. I don't understand the logic of this set-up. Why not be open to both hypotheses in the first place and say something like – If the deflection hypothesis is correct, then we expect X, but if the intimidation hypothesis is correct, they we expect Y?

Line 102: "Its natural…" Perhaps change to "their natural…"?

Line 125: Are there mantids and other large invertebrate predators that could prey on Caligo butterflies in the study area?

Line 136: Detail the brand of the UV ink used and produce a UV-photograph of all the models, as well as the WT Caligo martia butterflies. The authors mention that "the lighter ring of the eyespots (sparkle) enhanced with ultraviolet ink (UV, Figure 1b)". However, the image in Figure 1b shows the outer ring of the eyespots (the orange ring manipulated with a yellow color), not the sparkle. Note that the sparkle is the bit of white crescent in the center of the eyespot. It is important to show that the manipulated patterns had in fact enhanced UV, and it is important to show how that enhancement compares to the natural UV patterns of the Wt butterflies.

Line 163: Please provide a photo of what the models set in the field looked like, and what the modelling clay looked like.

Line 172: Please provide a photo of the models in experiment 2 as well, when placed in the field.

In the results section, please provide photos of what a beak mark looks like in both types of models in wings and in bodies.

Line 277 – Oliver et al. 2009 does not show how UV light matters in the context of mate selection. Other studies such as Robertson and Monteiro (2005; Proc Roy Soc Lond B) or Prudic et al. (2012; Science) or Huq et al. (2019; Journal of Insect Science) do.

Line 304 – In my mind, these data clearly support the intimidation function of Caligo eyespots. Two eyespots being displayed has double the intimidation value as a single eyespot. Were the beak marks counted per wing? Or per model? The role of large eyespots (> 6mm in diameter) in intimidation has also been shown in Ho et al. 2015, and as the authors point out, also in De Bona et al. 2015. What is the size of the eyespot in Caligo? The authors should discuss their data relative to the findings of these previous studies. In addition, setting clear hypothesis for predation rates at the start of the experiment (relative to the two hypotheses under investigation) would also help interpret their results.

Line 329 – the results of this study do support the intimidation hypothesis as well, with the comparison of experiments 1 and 2.