Doyle McKey

Recommender – PCI Ecology


Dear Dr. McKey,

Please find attached our manuscript entitled *"The large and central Caligo martia eyespot may reduce fatal attacks by birds: a case study supports the deflection hypothesis in nature"*. We greatly appreciated your comments and the revision made by the two referees on our manuscript, which was submitted for publication in *PCI - Ecology*.

We have taken into account all the comments made by the reviewers while preparing this new version of the ms for resubmission. We are most grateful for the valuable criticisms on the first version of the ms – we do think that this revised version is considerably improved, and hope that you find our study appropriate for *PCI - Ecology*.

Please see below the major comments from both reviewers, followed by our response to each of them.


Sincerely,

Cristiano A. Iserhard, on behalf of all authors

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**Recommenders' comments:**

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 useful 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.

**R:** We thank the recommender for the opportunity to revise the manuscript to make it more clear and publishable. We revised the manuscript to address the reviewers' suggestions. With regard to the reviewer and your suggestion that the intimidation and deflection hypotheses are not mutually exclusive, we tried to be as specific as possible in their predictions when applying alone (the simplest of possible scenarios, see last

paragraph of Introduction). We also limited our conclusions to the local community (see first paragraph of the Discussion) and discussed the possibility that some birds may find *Caligo* eyespots intimidating, while others use them as targets (see Discussion).

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.

**R:** Done. We changed the text to highlight this issue (lines 90-97).

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.

**R:** Agreed. We revised accordingly. Please, see the new figures four and five.

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?

**R:** Done. We provided images of both experiments in the field to address the sampling methodology as well as the facsimile in UV light (please, see the figures within the manuscript and the supplementary information figure one).

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.

**R:** We included all the statistical results within the main text, see table 2.

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.

**R:** Done. We included the work of the Bona et al. in the introduction and other sections of the manuscript, as well as other suggested references across the manuscript.

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

**Reviewer #1**

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.

**R:** We thank the reviewer for the positive revision, comments, and suggestions to improve the manuscript.

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.

**R:** We revised the manuscript and supplementary files to address the suggestions and improved the description of the points raised by the reviewer. Please see details below.

**Detailed comments:**

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

**R:** No, unfortunately, our methods do not allow us to associate any of the bird damage to particular species. The only way to discover what bird species made the mark would be by using video cameras.

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).

**R:** Agreed. We revised accordingly throughout the manuscript.

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.

**R:** Agreed. We revised accordingly and made changes throughout the manuscript.

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

**R:** Agreed. We revised accordingly.

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.

**R:** Done. Thanks a lot for the suggestion that improved the introduction given all possibilities of bird responses to the presence of eyespots (see lines 78-104).

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).

**R:** Done. We changed the text and included the suggested reference (see lines 90-97).

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.

**R:** Done. We agreed and downplayed the previous emphasis on "eye-mimicry" to focus more closely on deimatic display.

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).

**R:** Done. We included the reference Hill and Vaca (2004) and changed the text (see lines 92-95).

Line 96 – "no experimental study" instead of "not a single experimental study"

**R:** Done.

Line 111 – missing "of": "instead of the wings"

**R:** Done.

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

**R:** Agreed. We revised accordingly.


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?

**R:** Thanks for the comment. There was no evidence of bird attacks on the "base" of the facsimile (representing the body). We did not find any indication in the glue and the paper of the facsimiles.


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.

**R:** Agreed. We revised accordingly.


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.

**R:** Thanks for your suggestion. In this revised version, we corrected figure 4 indicating the panels accordingly. We also brought the statistical results from experiment 1 and experiment 2 to the main file, see table 2.


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

**R:** Done.


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

**R:** Done.

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

**R:** Done.


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.

**R:** Agreed. We revised accordingly.


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.

**R:** Done. We agree and included in the discussion (see line 304).


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.

**R:** We opted to not include the study of Sourakov 2013 because the deflection strategies of Lycaenidae (e.g., alighting head-down, movement of "false antennae") are quite distinct from the strategy of the eyespot in *Caligo* butterflies.


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.

**R:** Agreed. We revised accordingly.


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

**R:** Done.


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.

**R:** Agreed. We revised accordingly.


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.

**R:** Agreed. We revised accordingly.


**Reviewer #2**

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.

**R:** Thanks a lot for the revision and for your work to make our manuscript much better. We tried to include all issues and statements made in your comments. We think that this new version is much more appropriate for publication in PCI – Ecology.

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.
**R:** Done. Thanks to correcting this definition, we changed the text (see lines 54-55).

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.

**R:** Done. We modified this portion of the introduction by including a different approach explaining the real function of *Catocala* strategies as deimatic display following the comments and suggestions made by the first reviewer (please see lines 90-97).

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

**R:** We removed this part in the introduction and better explained our postulations (see lines 90-92).

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

**R:** Done. We included the references to the hypothesis of intimidation (see lines 83-89).

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

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/

**R:** We prefer to maintain the original text because we highlighted the novelty of our study and explain in details the work of De Bona et al. (2015) in the introduction, as suggested by the first reviewer.

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?

**R:** Thanks for the suggestions. We agree that we did not address our hypothesis and predictions well, thus we re-worded the paragraph including aspects suggested by both reviewers (see lines 114-134).

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

**R:** Agreed. We revised accordingly.

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

**R:** There is no evidence that mantids prey on butterflies as large as *C. martia*. Moreover, there are no records of other potential invertebrate predators that could forage on *Caligo* butterflies in our 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.
**R:** Done. We improved this part of the manuscript significantly and included additional photographs to elucidate the experiments in the field (please see the figure one on the supplementary information).

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

**R:** Done, please see figures in the methods section.

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

**R:** Done, please see figures in the methods section.


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

**R:** Done. We included all information in the methods section.


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.

**R:** Done. We included the references of Huq et al. 2019 and Robertson and Monteiro (2005) instead Oliver et al. (2009) (see line 320).


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.
**R:** We changed some parts of the discussion to address both intimidation and deflection strategies concerning experiments 1 and 2 (see lines 350-355). Moreover, we measured the diameter of *Caligo martia* and included it in the methods section (sampling design, see line 156-157). We change the hypotheses and predictions to better explain our expectations about the rate of predation in the distinct facsimile treatments too.


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

**R:** We opted to maintain the last paragraph of the discussion in its original form because we think that we properly discuss in a broad sense the possible explanations of the defence strategies of eyespot of *Caligo* with the support of the literature about the role of eyespots concerning avoiding predation (intimidation *vs* deflection). Moreover, this paragraph made a great conclusion about the work including possible limitations of the experiments in nature without being definitive in our findings.