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# Behavioural changes in aposematic *Heliconius melpomene* butterflies in response to their predatory bird calls

Sushant Potdar <sup>a 1</sup>  , Madhuri Dinakar <sup>b 2</sup>, Erica L. Westerman <sup>a 3</sup>

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<https://doi.org/10.1016/j.beproc.2024.105071> ↗

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## Highlights

- We show that *Heliconius melpomene* change behaviour in response to jacamar calls.
- Males increased walking and fluttering, but did not alter courting behaviour.
- The butterflies returned to pre-call behaviours after the call ended.
- *H. melpomene* did not respond to predatory Eastern kingbird or tropical kingbird calls.
- This may suggest lower kingbird predation pressure or selective hearing capabilities.

## Abstract

Prey-predator interactions have resulted in the evolution of many anti-predatory traits. One of them is the ability for prey to listen to predators and avoid them. Although prey anti-predatory behavioural responses to predator auditory cues are well described in a wide range of taxa, studies on whether butterflies change their behaviours in response to their predatory calls are lacking. *Heliconius* butterflies are unpalatable and form Müllerian mimicry rings as morphological defence strategies against their avian predators. Like many other butterflies in the *Nymphalidae* family, some *Heliconius* butterflies possess auditory organs, which are hypothesized to assist with predator detection. Here we test whether *Heliconius melpomene* change their behaviour in response to their predatory bird calls by observing the behaviour of male and female *H. m. plessini* exposed to calls of *Heliconius* avian predators: rufous-tailed jacamar, migratory Eastern kingbird, and resident tropical kingbird. We also exposed them to the calls of the toco toucan, a frugivorous bird as a control bird call, and an amplified greenhouse background noise as a noise control. We found that individuals changed their behaviour in response to jacamar calls only. Males increased their walking and fluttering behaviour, while females did not change their behaviour during the playback of the jacamar call. Intersexual behaviours like courtship, copulation, and abdomen lifting did not change in response to bird calls. Our findings suggest that despite having primary predatory defences like toxicity and being in a mimicry ring, *H. m. plessini* butterflies changed their behaviour in response to predator calls. Furthermore, this response was predator specific, as *H. m. plesseni* did not respond to either the Eastern kingbird or the tropical kingbird calls. This suggests that *Heliconius* butterflies may be able to differentiate predatory calls, and potentially the birds associated with those calls.

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## Introduction

Predation is a ubiquitous interspecific interaction in almost all ecosystems and can be a strong evolutionary force for the emergence and selection of prey anti-predatory strategies that increase survival (Lind and Cresswell, 2005). Anti-predatory strategies are widespread in prey animals and often involve both morphological and behavioural adaptations. Morphological strategies include aposematism, chemical toxicity, and crypsis (Rojas et al., 2019, Vallin et al., 2006), while behavioural anti-predatory strategies include active evasion of predatory attacks, and behaviours that decrease detection (Palmer and Packer, 2021). Anti-predatory strategies can also be a combination of both morphological and behavioural

strategies such as the deimatic displays in mountain katydid *Acripeza reticulata* and swallowtail butterflies (Olofsson et al., 2012; Umbers and Mappes, 2015).

The most common anti-predatory strategies are behavioural responses to detection and active attacks by predators. These behavioural responses help prey escape predation, either in the absence of morphological defences, or as a combination with morphological defences, and can be highly variable across species, within species, and between sexes (Apfelbach et al., 2005, Lind and Cresswell, 2005). Some species, such as desert isopods (*Hemilepistus reaumuri*), freeze and retreat inside their burrows upon smelling their predator's scent (Zaguri and Hawlena, 2020); while others, such as male tree lizards (*Urosaurus ornatus*) actively escape by fleeing after detecting their predators (Thaker et al., 2009). Anti-predatory behaviours can also differ within species in response to different predators, as illustrated by red squirrels (*Tamiasciurus hudsonicus*), which have different alarm calls for avian predators and ground predators (Greene and Meagher, 1998).

Anti-predatory behavioural responses can also be sex-specific, either due to the inherent sex-specific differences in physiology and behaviour, or due to the increased vulnerability of predation during intraspecific sexual behaviours (Curlis et al., 2016, Edomwande and Barbosa, 2020, Lea and Blumstein, 2011, Sitvarin and Rypstra, 2012, Wormington and Juliano, 2014). Both males and females of some species are known to alter their courtship and mating behaviours under predation risk (Acharya and McNeil, 1998, Torsekar et al., 2019). In wolf spiders (*Schizocosa ocreata*), males cease courtship behaviour after detecting predatory birds' calls and take longer to return to courting compared to non-threatening control sounds (Lohrey et al., 2009), while female túngara frogs (*Physalaemus pustulosus*) approach calling males more cautiously when exposed to bat wingbeat sounds (Bernal et al., 2007). The cost of predation during intraspecific sexual behaviours such as courtship and copulation is high, forcing individuals to switch from sexually oriented behaviours to survival behaviours.

Detecting and recognizing predatory cues are necessary for active predator avoidance behaviours, and these cues can include visual, chemical, vibrational, or auditory elements. For many species, auditory cues play a vital role in predator avoidance by prey animals, from invertebrates (Faure and Hoy, 2000, Jacobs et al., 2008, Lohrey et al., 2009, Prakash et al., 2021, Rosen et al., 2009, Triplehorn et al., 2008) to vertebrates (Bernal et al., 2007, Cantwell and Forrest, 2013, Deecke et al., 2002). In Lepidoptera (moths and butterflies), anti-predatory behaviours in moths to predators' auditory cues have been extensively studied under various ecological contexts. Some moths can detect bat echolocation calls and avoid bat predation by performing aerial manoeuvres, and tiger moths (*Bertholdia trigona*) can

jam bat echolocation calls (Conner and Corcoran, 2012). Some non-flying moths have been shown to freeze and not move when exposed to simulated bat echolocation calls (Werner, 1981). Both males and females of some species of *noctuidae* and *pyralidae* moths also reduce sexual activity under bat predation pressure (Acharya and McNeil, 1998, Edomwande and Barbosa, 2020).

While moths are particularly well known for their hearing ability and anti-predator behaviours, butterflies, their day-flying relatives, are also known to have auditory organs, which may be sensitive to predator sounds (Lane et al., 2008). In particular, many species in the family *Nymphalidae* possess auditory organs on their wings, such as the blue morpho *Morpho peleides* (Lane et al., 2008, Lucas et al., 2009, Mikhail et al., 2018), common wood nymph *Cercyonis pegala* (Sun et al., 2018), the owl butterfly *Caligo eurilochus* (Lucas et al., 2014), butterflies from the genus *Erebia* (Ribarič and Gogala, 1996), and *Heliconius erato* butterflies (Swihart, 1967). However, unlike moths, it is generally unknown whether butterflies that possess auditory organs change their behaviour in response to their predator's vocalizations. In this study, we used the butterfly *Heliconius melpomene* to test whether these butterflies change their behaviour in response to their predators' vocalizations.

*Heliconius* butterflies (Family *Nymphalidae*), found in North, Central, and South America, are toxic, unpalatable, display aposematic colouration, form Müllerian mimicry rings, and roost communally to avoid bird and bat predation (Engler-Chaouat and Gilbert, 2007, Finkbeiner et al., 2012, Mallet and Gilbert, 1995, Pinheiro De Castro et al., 2019). Despite these anti-predatory strategies, *Heliconius* butterflies are vulnerable to predation by specialist avian predators, as well as by naïve generalist predatory birds; and their mortality is higher when young birds are learning which butterfly species are toxic and should be avoided (Chai, 1986, Langham, 2004, Langham, 2006, Pinheiro, 1996, Pinheiro and Cintra, 2017). Hence, it may be evolutionarily advantageous for *Heliconius* butterflies to detect the presence of their avian predators and change their behaviours to reduce detection, despite having multiple anti-predatory strategies. One possible way these butterflies could detect the presence of their avian predators is by using avian vocalization cues, which are often species specific (Lane et al., 2008, Lucas et al., 2009, Mikhail et al., 2018). In *Heliconius erato* butterflies, hearing organs located at the base of the hindwing with peak sensitivity between 0.5 and 4 KHz at 70–90 dB SPL (sound pressure level) have been described (Swihart, 1967). However, the hypothesis that *Heliconius* butterflies change their behaviour in response to their predatory birds' vocalizations has never been tested.

In this study, we tested whether *Heliconius melpomene plessini* butterflies change their behaviour in response to the vocalizations of their known avian predators. We first determined that *H. m. plessini* butterflies have putative hearing organs similar in appearance and location to those described in *H. erato* (Swihart, 1967). We then tested butterfly response to the vocalizations of two predatory birds with disparate calls as well as the vocalization of a frugivorous bird, to assess whether *H. m. plesseni* butterflies respond to both predator bird calls and calls of non-predatory birds. After answering that question, we then tested the response of *H. m. plessini* butterflies to predators that differ in annual patterns of predation (year-round resident or migratory), to assess whether strength of *H. m. plesseni* response is associated with degree of annual avian predator exposure. During both these experiments, we also tested whether intraspecific sexual behaviours like male courtship and female acceptance/rejection behaviours changed in response to *H. m. plessini*'s bird predatory calls.

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## Section snippets

### Study species husbandry

*Heliconius melpomene* (Order: *Lepidoptera*, Family: *Nymphalidae*), is native to Central and South America with an elevational range of 0–1800 m above sea level (Montejo-Kovacevich et al., 2019). The subspecies *H. m. plessini* is found in the mountainous forests of Ecuador and Peru in South America (Hines et al., 2011). Live pupae of *H. m. plessini* were shipped from Ecodecision Heliconius Works in Quito, Ecuador to the University of Arkansas Biology greenhouse facility in Fayetteville AR, USA,...

### Ethical note

All butterflies used in this study were maintained in climate-controlled greenhouse conditions similar to those of their natural habitat, as stated in the U.S. Department of Agriculture, Animal and Plant Health Inspection Service permits P526P-17–00343 and P526P-20–00417. Before and after the assays, all butterflies were maintained in cages with *ad libitum* food (artificial nectar and flowering *Lantana spp.* plants for pollen). After the assays, they were moved to breeding cages with *ad libitum*...

*H. m. plessini* males and females possess putative hearing organs at the base of their hindwing

After dissecting and removing the scales from the base of the hindwing, we found a brown bulge in the veins of both male and female *H. m. plessini*, very similar in structure to those described and tested by Swihart (1967) to be hearing organs in *H. erato* (Supplementary figure 3), which is a putative auditory organ in male and female *H. m. plessini*...

## *H. m. plessini* immediately changed their behavioural state in response to the rufous-tailed jacamar call

*H. m. plessini* butterflies immediately changed their behavioural state (behaviour at a specific time) when the rufous-tailed jacamar call started ( $\chi^2$ ...

## Discussion

*Heliconius melpomene plessini* butterflies changed their behaviour in response to predatory rufous-tailed jacamar calls but did not change their behaviour in response to predatory Eastern kingbird or tropical kingbird calls. We found a sex-specific difference in behaviour, where males, but not females, increased their PC 2 levels (predominantly fluttering and walking behaviours for males) during the playback of the rufous-tailed jacamar calls. The observed behavioural changes in response to...

## Conclusions

We found that unpalatable and brightly coloured *Heliconius melpomene plessini* butterflies respond and change their behaviour during the playback of the rufous-tailed jacamar call. This change in behaviour is sex-specific, where males, but not females, increase their walking and fluttering behaviour over a short time-frame, although both sexes were found to have putative hearing organs. Males reverted back to their original behaviour after the call ended. *H. m. plessini* did not change their...

## Funding

This work was supported by an Arkansas Biosciences Institute grant to ELW, a Lepidopterists' Society Ron Leuschner Memorial Fund grant to SP, and the University of Arkansas....

## CRedit authorship contribution statement

**Sushant Potdar:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

**Madhuri Dinakar:** Writing – review & editing, Software, Data curation. **Erica L Westerman:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization....

## Acknowledgments

We thank David A. Ernst, Deonna N. Robertson, Grace Hirzel, Matthew Murphy, Yi Ting Ter, Kiana Kasmali, and Keity Farfán Pira for their contribution towards *Heliconius* butterfly husbandry and reviewing the manuscript. We thank Brian Counterman for providing valuable inputs and reviewing this manuscript, as well as anonymous reviewers. We also thank Pooja Panwar for fruitful discussions during the conceptualization of this project....

## Supplementary materials

Supplementary material 1: a PDF file containing all supplementary ...

[Recommended articles](#)

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1 ORCID: 0000-0002-6924-4826

2 ORCID: 0000-0002-9713-6320

3 ORCID: 0000-0002-3575-8298

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