# Does mimicry extend to flight in a mimetic damselfly?

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## Introduction

Mimicry has traditionally been studied regarding color, pattern and morphology. Motor mimicry occurs when a species mimics the motor functions of an aposematic species (e.g., flight[1]), adding another layer of complexity to aposematic signals. Multicomponent aposematic signals, and more specifically the motor component, have been previously overlooked. **The goal of this project was to expand our understanding of flight mimicry in multicomponent aposematic signals**.

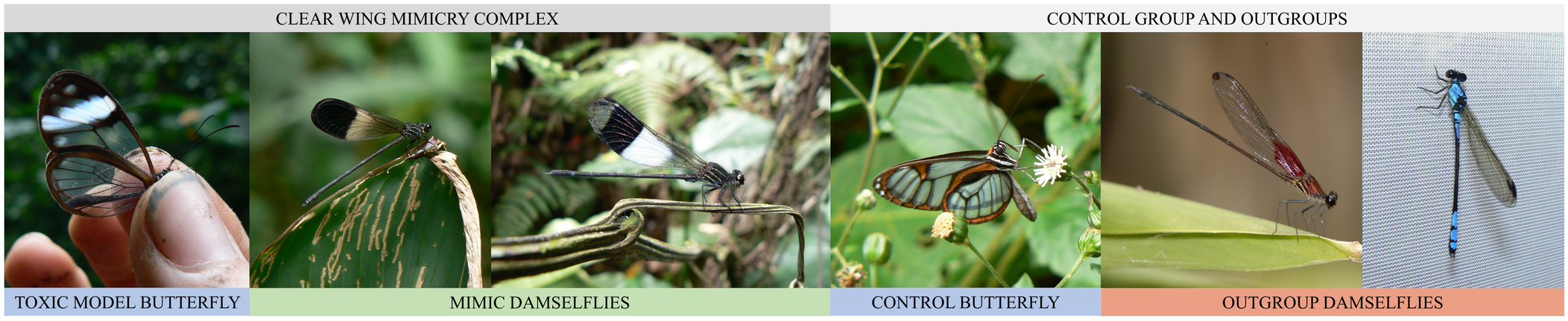



**Fig. 1.** The six species of butterflies and damselflies used in this study. From left to right: the toxic model Ithomiini butterflies (with white patches); two mimic Polythoridae damselflies, *Polythore procera* (male) and *Euthore fasciata* (male); a control group of Ithomiini butterflies without white patches; and two damselfly outgroups: *Hetaerina* sp. (male), from a closely related family to Polythoridae, and *Cora* sp. (male), a local non-mimic Polythoridae (image credits: D Outomuro, KDP Wilson).

## Material and Methods

*Study system*

The clear wing mimicry complex[2] was studied (Fig. 1). This mimicry complex is dominated by Ithomiini glasswing butterflies, and the aposematic color trait is an ultraviolet-reflective white patch on mostly transparent forewings (Fig. 1). Some species of the neotropical damselfly family Polythoridae seem to imperfectly mimic wing color, shape and certain characteristics of flight (wing-beat frequency and flight speed[3]). We predicted that motor mimicry is being exhibited by damselfly species that are also mimicking the aposematic coloration of the Ithomiini glasswing butterflies, but not by non-mimic species.

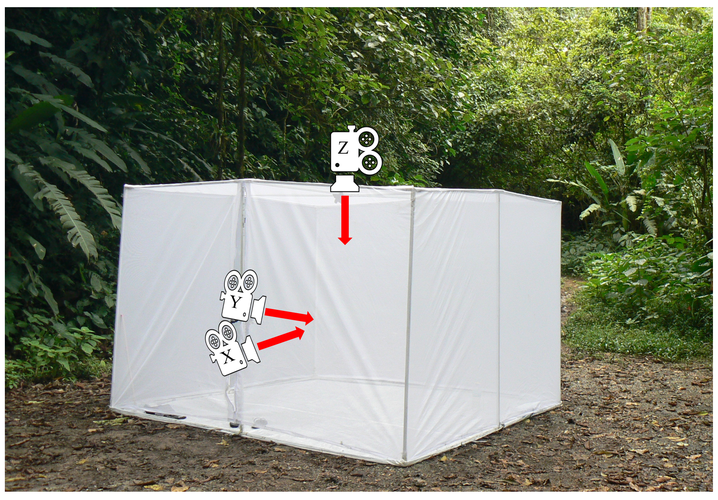



**Fig. 2.** Field insectarium (3x3x2m) installed in the natural habitat showing the placement of the three cameras.

*Experimental set-up and data collection*

The flight of specimens of each species (samples sizes in Fig. 3B) was filmed using three GoPro Hero5 cameras in a field insectarium (Fig. 2). The synchronized and calibrated videos from the three cameras were used to track the flight trajectory in the three-dimensional space using DLTdv8[4] for Matlab. The tracked dataset was analyzed as trajectories using the R package trajr[5]. For each flight, the following variables were calculated for each bi-dimensional plane: mean, maximum and minimum speed and acceleration, sinuosity of the flight path, and mean and standard deviation of directional change.

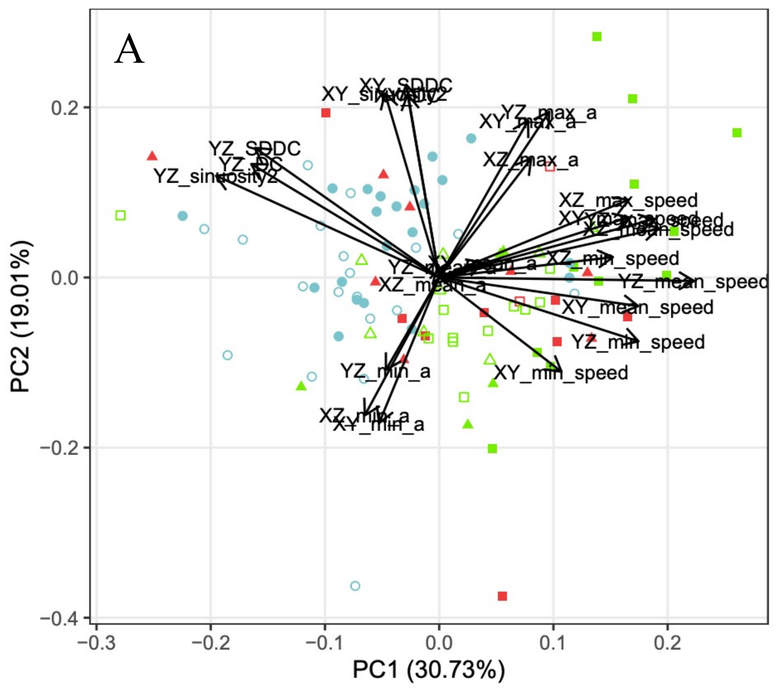
*Data analysis*

All the variables obtained for each plane were combined and a Principal Component Analysis (PCA) was performed. To test for differences in flight among the study groups (species and sex when known) a Multivariate Analysis of Variance (MANOVA) was run on the PC scores, using study group as a fixed factor. Finally, pairwise Mahalanobis distances were computed among all group means from the pooled within-group covariance matrix using the package Morpho[6] for R. The Mahalanobis distances were tested by permutation. All statistical analyses were run in R version 4.0.3[7].

## Results

Butterflies occupied the areas of the flight space with higher sinuosity and lower speeds, while damselflies were mostly associated to lower sinuosity and higher speeds (Fig. 3A). Butterflies only showed a more sinuous flight than damselflies when observed from the sides, but not from above (figure not shown). Interestingly, the male damselflies had a higher average velocity than their female counterparts (figure not shown).

The MANOVA showed significant differences among the study groups (p-value < 0.001). The pairwise Mahalanobis distances ranked the mimic species closer to the butterfly model than the non-mimics species, with the exception of *E. fasciata* males (Fig. 3B). Moreover, *Hetaerina* sp. females were not significantly separated in the flight space from the model butterflies (Fig. 3B).




B

| Species/Sex (N) | Mahalanobis D (p -value) |
|---|---|
| *Cora* sp. male (2) | 7.446 (0.004) |
| *Hetaerina* sp. male (8) | 4.445 (0.004) |
| *E. fasciata* male (11) | 3.907 (0.004) |
| *Hetaerina* sp. female (8) | 3.205 (0.087) |
| *P. procera* male (12) | 3.177 (0.004) |
| *E. fasciata* female (5) | 3.175 (n.s.) |
| *P. procera* female (8) | 3.171 (n.s.) |
| Ithomiini control (19) | 2.388 (0.065) |

**Fig. 3. A.** PCA depicting the PC loadings for each variable. The species and sexes are coded as: Ithomiini model (●), Ithomiini control (○), *E. fasciata* male (■), *E. fasciata* female (▲), *P. procera* male (□), *P. procera* female (△), *Hetaerina* sp. male (■), *Hetaerina* sp. female (▲), *Cora* sp. male (□). **B.** Mahalanobis distances from each study group to the model Ithomiini butterflies (N = 21).

## Discussion

Damselflies showed similar flight characteristics to other damselflies, and the butterfly groups shared similar flight characteristics with each other. However, our results also suggested that mimic damselflies *tended* to show more similar flight characteristics to the model butterflies than the non-mimic damselflies, with differences between the sexes. Interestingly, *E. fasciata* males are better color mimics than *E. fasciata* females or than *P. procera* males and females[3,8]. Flight mimicry, if present, might be compensating for poorer color aposematic signals in the damselflies: species considered to be poorer color mimics showed better flight mimicry than the species considered to be better color mimics. Our study highlights the potential role of motion in the communication of complex aposematic signals. Future studies should focus on free-flying insects outside of insectary conditions.

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