Quantifying visual acuity in *Heliconius* butterflies

Daniel Shane Wright[1]*, Anupama Nayak Manel[1], Michelle Guachamin-Rosero[1,2], Pamela Chamba-Vaca[1], Caroline Nicole Baquet[2] & Richard M. Merrill[1]*

[1] *Division of Evolutionary Biology, Faculty of Biology, LMU Munich, Planegg-Martinsried, Germany*

[2] *Universidad Regional Amazónica IKIAM, Tena, Ecuador*

*Corresponding authors: dswright@bio.lmu.de (DSW); merrill@bio.lmu.de (RMM)

Running title: *Heliconius* visual acuity

Abstract

Heliconius butterflies are well-known for their colourful wing patterns, which advertise distastefulness to potential predators and are used during mate choice. However, the relative importance of different aspects of these signals will depend on the visual abilities of *Heliconius* and their predators. Previous studies have investigated colour sensitivity and neural anatomy, but visual acuity (the ability to perceive detail) has not been studied in these butterflies. Here, we provide the first estimate of visual acuity in *Heliconius*: from a behavioural optomotor assay, we found that mean visual acuity = 0.49 cycles-per-degree (cpd), with higher acuity in males than females. We also estimated visual acuity from eye morphology and reported slightly lower values (mean visual acuity = 0.38 cpd), but acuity was still higher in males. Finally, we estimated how visual acuity affects *Heliconius* visual perception compared to a potential avian predator. Whereas the bird predator maintained high resolving power, *Heliconius* lost the ability to resolve detail at greater distances, though colours may remain salient. These results will inform future studies of *Heliconius* wing pattern evolution, as well as other aspects in these highly visual butterflies, which have emerged as an important system in studies of adaptation and speciation.

Keywords: sensory perception, colour pattern, aposematism

1. Introduction

Since Bates (1) first described mimicry over 160 years ago, studies of *Heliconius* butterflies have made an important contribution to our understanding of adaptation and speciation (2). These Neotropical butterflies are well known for their diversity of bright colour patterns, which both advertise distastefulness to potential predators (3–8) and are used during mate choice (9). Because males distinguish between the warning colour patterns of con- and hetero-specific females (and to some extent against con-specific females from populations with different patterns), colour pattern contributes an important premating reproductive barrier, e.g. (10–12). *Heliconius* also use visual cues during foraging (13) and host plant selection (14,15). Vision, therefore, plays a crucial role in *Heliconius* behaviour, and studies of *Heliconius* have increasingly considered vision, especially with respect to colour perception (16–20) and neuroanatomy (21,22). However, a key element of *Heliconius* visual ecology has not yet been studied, specifically visual acuity.

Visual acuity is the ability to perceive detail in a visual scene and is one of the three fundamental parameters of visual systems (the others being spectral sensitivity and temporal resolution (23,24)). Visual acuity is typically reported as the number of black and white stripe pairs that an organism can discriminate within a single degree of visual angle (cycles-per-degree; cpd) (24). Across animal taxa, acuity varies greatly. For example, human visual acuity is 72 cpd (25), whereas fruit fly visual acuity is only 0.09 cpd (26). This discrepancy is particularly relevant for researchers designing studies of visual signals, whereby hypotheses about the form and function of a given trait may not consider the visual capacity of the receiver (27). Caves, Frank and Johnsen (28) demonstrated this principle empirically by showing that cleaner shrimp visual acuity is too poor to resolve colour patterns previously

believed to be used in intraspecific signalling. This result nicely illustrates the potential downfalls of biassing studies towards our own perceptual abilities.

A battery of studies have left little doubt that *Heliconius* males can distinguish between potential mates with divergent colour patterns (6,10–12,29–31). However, visual acuity has never been measured in these butterflies, so it is unclear how well the butterflies can perceive these signals at different distances, or what is the relative importance of colour vs. pattern at different points of courtship (e.g. long-range vs short-range attraction). For other diurnal butterflies, visual acuity has been reported from 0.66 cpd (*Colias eurytheme* (32)) to ~1.0 cpd (*Morpho peleides* (33)), and as body size scales with eye size, visual acuity tends to increase in larger butterflies (34). *M. peleides* is larger than *Heliconius*, so visual acuity values <1.0 cpd are expected for *Heliconius*. If this is true, the ability of *Heliconius* to resolve wing pattern details is questionable, particularly at greater distances. Avian predators, on the other hand, likely perceive the same colour patterns clearly - *Heliconius* bird predators are unknown, but visual acuity studies in other insectivorous birds suggest high visual acuity (e.g. *Acanthiza chrysorrhoa* = 25.6 cpd (35); *Zosterops lateralis* = 18.5 cpd (36)).

Here, we measure visual acuity in *Heliconius* butterflies for the first time. We took two complementary approaches. First, we used a behavioural assay to measure optomotor responses, where animals turn in the direction of a rotating stimulus so as to minimise displacement of the moving image (26). Second, we used an anatomical approach to quantify the number of photoreceptors, allowing estimates of visual acuity from eye morphology. By more thoroughly investigating the potential mismatch between the visual abilities of *Heliconius* and their predators, our results shed light on the evolution and function of the warning patterns.

2. Methods

(a) Study species

We established a stock of *Heliconius erato cyrbia* from wild individuals caught in forests near Balsas (3°43′60″S, 79°50′45″W) in Southern Ecuador. These were maintained at the Universidad Regional Amazónica IKIAM in Tena, Ecuador, and replenished with wild individuals intermittently over the course of the experiment. All butterflies were maintained under common garden conditions in outdoor insectaries in 2 x 2 x 2.3 m cages, where they were provided with 20% sugar solution, and *Lantana* sp. and *Psiguria sp*. flowers as a source of pollen. Eggs were collected regularly from the hostplants, *Passiflora punctata*, provided in the insectaries. The larvae were reared individually in pots and fed with fresh leaves from the host plants. All butterflies were marked with unique identification codes on their wings after eclosion.

(b) Behavioural estimates of visual acuity

An optomotor device (Fig. 1) was built following Caves et al. (37), which permits a non-invasive and reliable behavioural assay for studying visual acuity across taxa. Briefly, the device consisted of interchangeable visual stimuli of alternating vertical black and white stripes printed on waterproof paper (145 µm, Premium NeverTear, Xerox, CT, USA) on a rotating wheel around a fixed white PVC base (17 cm radius). The width of one cycle (a set of alternating black and white stripes) was calculated as *cycle width (mm) = [(C/360) / a]*, where '*C*' is the circumference of the experimental arena and '*a*' is the intended visual acuity in cycles-per-degree (cpd) (37). We used stimuli with spatial frequencies of 0.3 cpd to 1 cpd (in pilot trials, butterflies consistently responded to cpd levels >0.3). As visual acuity depends on the distance between the stimulus and the perceiver, all butterflies were restrained in a clear PLEXIGLAS cylinder (4 cm radius; 15 cm height) at the centre of the base. A blank

stimulus was used to confirm that responsiveness was due the moving stripes and no other external cues.

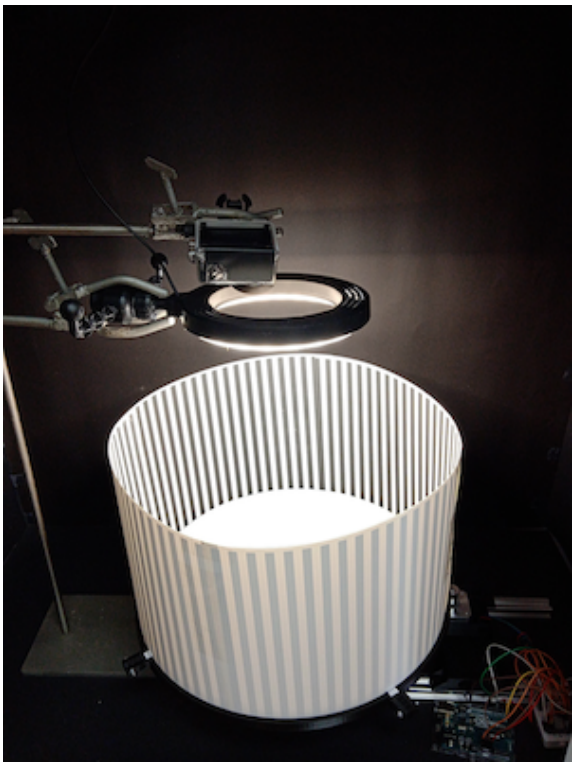

Figure 1. The optomotor set up. Butterflies were contained at the centre of the fixed white PVC base in a clear PLEXIGLAS cylinder (not pictured) while the visual stimulus (alternating black and white lines) rotated (in both directions for 10 seconds at 3 rpm). All trials were illuminated with a LED ring lamp and video recorded from above.

All assays were conducted inside (at room temperature) at IKIAM University, illuminated by an overhead LED ring lamp, and video recorded from above. Butterflies (10+ days post eclosion) were tested only when they stopped crawling on the cylinder, followed by 3-4 rotations of the stimulus (alternating between clockwise and counter-clockwise for 10 seconds each, at 3 rpm). A positive response was scored only if the butterfly changed the orientation of its head/antenna in the direction of the moving stimulus in two consecutive stimuli rotation reversals. All positive responses were confirmed from videos, and butterflies with unclear responses were excluded from the experiment.

121

122 Male butterflies tend to have larger eyes than females (34), so we explored how visual acuity
123 differs between male vs. female *H. e. cyrbia*, while accounting for the time-of-day butterflies
124 were tested (10:00~16:00) and potential observer effects using generalised linear mixed
125 models implemented in the lme4 package in R (38). The generalised linear model (family =
126 Gamma) was as follows: *glmer(visual acuity) ~ sex + time + (1|observer).* The significance
127 of fixed effect parameters (*sex* & *time*) was determined by likelihood ratio tests (LRT) via the
128 *drop1* function and minimum adequate models (MAM) were selected using statistical
129 significance (39,40). Model assumptions were confirmed via visual inspection (residual vs.
130 fitted and normal Q-Q plots). We used the *Anova* function in the car package (41) to estimate
131 the parameters of significant fixed effects based on LRT.

132

133 **(c) Morphological estimates of visual acuity**

134 The insect compound eye consists of numerous independent photosensitive units, ommatidia,
135 each of which receives visual information and transfers it to the brain. Variation in
136 ommatidial number/density directly affects visual acuity (26,42,43), which can be estimated
137 from ommatidia counts (see below).

138

139 Specimens from the stock populations were preserved in DMSO/EDTA/NaCl (44) and stored
140 at -20° C. Following previously published methods (45), frozen specimens were thawed at
141 room temperature and both eyes were removed and placed in 20% sodium hydroxide (NaOH)
142 for 18-24 hours to loosen the tissues behind the cuticular cornea. The following day, the
143 cuticle was cleaned of excess tissue, mounted on a microscope slide in Euparal (Carl Roth
144 GmbH, Germany), and left to dry overnight.

145

We used ImageJ/Fiji (46) to analyse each mounted cornea for the total number of ommatidia. All slides were imaged at 7.5x on a Leica M80 stereomicroscope fitted with a Leica Flexacam C1 camera and the Leica Application Suite X (LAS X) software. Each image contained a 1 mm scale bar to calibrate the measurements. Ommatidia counts were measured via image thresholding and the *Analyze particles* function (methods provided as supplementary information). To account for differences in body size, the hind legs of each butterfly were also removed and imaged; hind tibia length was measured using the *Straight line* and *Measure* options.

In a compound eye hemisphere covering 180° of space, the number of ommatidia 'n' that can cover the hemisphere without overlap is $23818/(\Delta\Phi^2)$, where $\Delta\Phi$ is the interommatidial angle (26). Thus, the number of ommatidia (n) can be used to calculate the interommatidial angle as: $\Delta\Phi = (23818/n)^{1/2}$, and from this, visual acuity can be estimated as: $acuity = 1 / (2\Delta\Phi)$ (equation 1 from (26)). Based on the strong correlation between the left and right eye ommatidial counts ($r = 0.979$, $t = 8.46$, $df = 3$, $p = 0.0034$), only one eye from each individual was used for calculating visual acuity. For uniformity, we always used the left eye unless it was damaged or imaged poorly, in which case the right eye was substituted.

We used linear models (lm) to explore how the morphological estimates of visual acuity differs between the sexes while accounting for body size (hind tibia length) as *lm(visual acuity ~ sex + tibia length)*. We also investigated sex-specific differences in eye morphology as l*m(log10(ommatidia count ~ sex + log10(tibia length))*. We used log10-transformations to normalise the residuals around the allometric relationship between ommatidia count and tibia length (47). Model simplification and parameter estimates were as detailed above.

(d) Bird-butterfly comparison

We used the AcuityView package in R (27) to estimate how the visual acuity values reported here influence *H. e. cyrbia* perception. We also modelled how bird predators may view the same visual scene. The exact bird species that prey upon *Heliconius* are unknown, as are their visual acuity values, but prior studies (e.g. (48)) have used insectivorous Passerines as representative predators. Thus, we used the mean acuity value (22.05 cpd) of two insectivorous Passerines (*Acanthiza chrysorrhoa and Zosterops lateralis* (35,36)) as a proxy.

3. Results

(a) Behavioural estimates of visual acuity

We tested 26 males and 21 females in the optomotor assay. Overall, the mean behavioural visual acuity (± standard error) for *H. e. cyrbia* was 0.494 ± 0.028 cpd. Mean male visual acuity was 0.547 ± 0.043 cpd, which was significantly higher than the mean value for females (0.428 ± 0.026; $\chi 2 = 4.99$, df = 1, p = 0.025; Fig. 2A, Table 1). The time of day the butterflies were tested did not influence the results ($p > 0.4$)

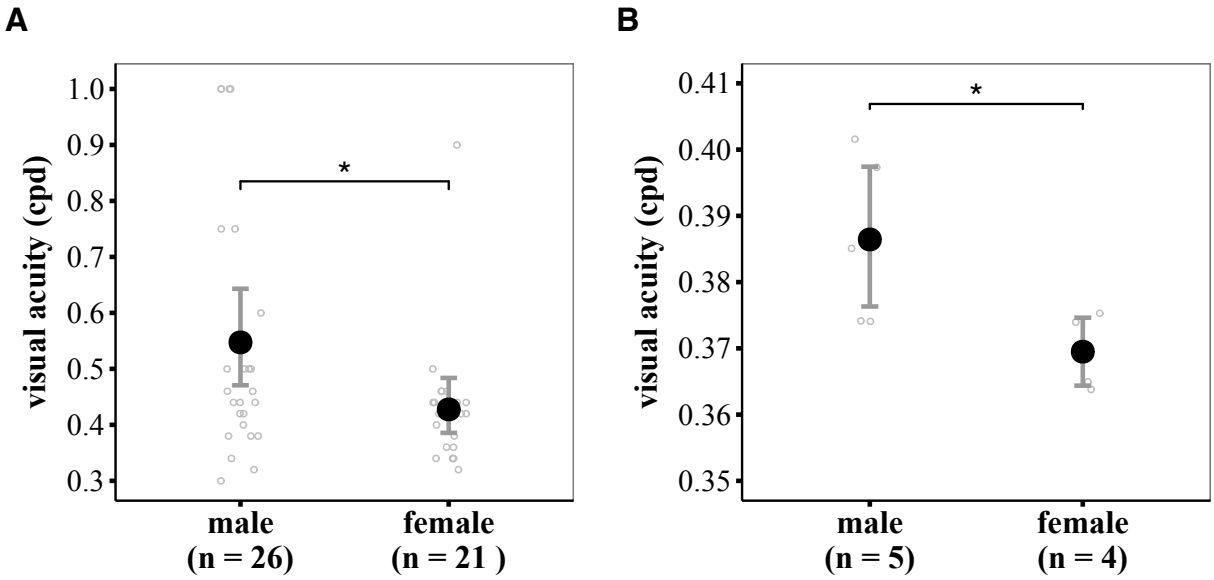

Figure 2. Estimates of visual acuity in the **(A)** behavioural optomotor assay and from **(B)** morphological analyses both indicate that male *H. e. cyrbia* have higher visual acuity (in cycles-per-degree, cpd) than females. Solid black circles represent the mean values, and grey error bars show 95% confidence intervals. * indicates $p < 0.05$.

(b) Morphological estimates of visual acuity

We analysed the eye morphology of 5 male and 4 female butterflies. The morphological estimates of visual acuity were lower than the behavioural results presented above (see Table 1); the overall mean was 0.378 ± 0.004 cpd, compared to 0.494 cpd in the behavioural assay. Nonetheless, patterns were similar, and males had significantly higher visual acuity than females ($F_1 = 5.87$, $p = 0.045$, Fig. 2B, Table 1). This can be attributed to the fact that males had more ommatidia than females ($F_1 = 5.98$, $p = 0.044$), despite no significant differences in body size (using hind tibia length as a proxy; $p > 0.5$).

	Behavioural	Morphological
male	0.547 ± 0.043	0.386 ± 0.006
female	0.428 ± 0.026	0.369 ± 0.003

Table 1. Visual acuity estimates (mean cycles-per-degree ± standard error) for *H. e. cyrbia* males and females from the behavioural optomotor assay and morphological analyses.

(c) Bird-butterfly comparison

Using the mean visual acuity values from the optomotor assay, we found that *H. e. cyrbia* has relatively poor visual resolution, particularly at larger distances (Fig. 3). In contrast, our proxy bird predator maintains high resolution at all distances.

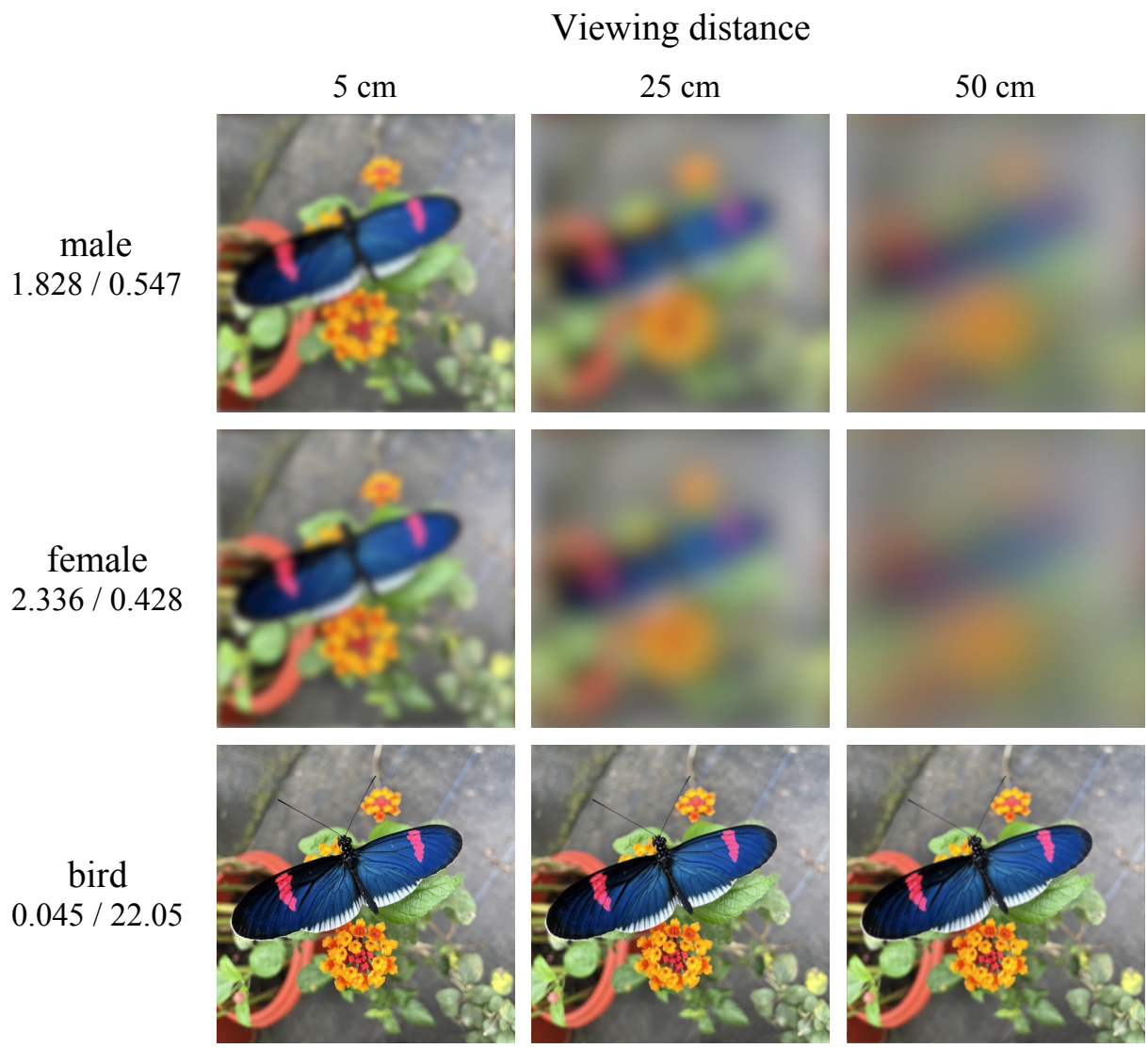

Figure 3. Perceptual estimates of *H. e. cyrbia* males (top) and females (middle) using the mean visual acuity values from the optomotor assay (generated with the AcuityView R package (27)). For comparison, the bottom row presents the perceptual estimate of a hypothetical bird predator viewing the same visual scene. *Heliconius* bird predators and their visual acuity values are unknown, so we used the mean visual acuity value of two insectivorous Passerines (*Acanthiza chrysorrhoa and Zosterops lateralis*; (35,36)). Values on the left side represent the minimum resolvable angle in degrees / visual acuity in cycles-per-degree.

4. Discussion

There are three fundamental parameters of visual systems: spectral sensitivity, temporal resolution, and visual acuity (23,27). For *Heliconius* butterflies, spectral sensitivity is well

222 characterised (16–20), but temporal resolution and visual acuity are undocumented. Here, we
223 report the first measures of visual acuity in *Heliconius* using two approaches: mean
224 behavioural visual acuity was 0.494 cpd, whereas morphological estimates were somewhat
225 lower (0.378 cpd). Our use of two experimental approaches provides insight into the
226 morphological correlates of visual acuity in *Heliconius*, while also accounting for post-retinal
227 processing involved in visual perception and behavioural output. We report lower
228 morphological estimates of visual acuity than in the behavioural assay, but the morphological
229 estimates do not account for higher-order visual system processing. Despite this discrepancy,
230 both approaches revealed higher visual acuity in males than females, as has been observed in
231 other butterflies (34). Morphological and ecological differences between the sexes might
232 explain this sexual dimorphism; males have more ommatidia (larger eyes) than females,
233 despite similar body size (see results above), and males actively search for and identify
234 conspecific mates (49,50).
235
236 *Heliconius* wing patterns warn potential predators that these butterflies are unprofitable prey
237 and are also used during mate choice. Previous studies have repeatedly demonstrated that
238 *Heliconius* males can distinguish between potential mates with divergent colour patterns both
239 between (10,11,30), and to a lesser extent, within species (6,12,29,31). However, our results
240 suggest that these signals may not be viewed equally by conspecifics and predators. Although
241 visual acuity will always limit what information can and cannot be perceived (27), our visual
242 representations (Fig. 3) are of course only approximations informed by acuity measurements
243 and cannot depict what the animals actually see. Other factors, such as neural image
244 enhancement and motion detection are involved, but these processes cannot add information
245 to the visual image (27). Nevertheless, our recreation of how the butterflies and a potential
246 avian predator may view the same visual scene highlight that while potential predators

maintain high visual resolving power at all tested distances, *Heliconius* will quickly lose the ability to resolve pattern detail as distances increase.

In contrast to specific finer scale patterns, colour elements may remain salient for the butterflies across a broader range of distances, suggesting that colour may be more useful for e.g. long-range attraction. This is consistent with experimental work showing that *Heliconius* colours likely have a greater influence than patterning for both predators and conspecifics (6), and that more prominent shifts in male preference are often associated with major colour differences (e.g. white to yellow, or white to red shifts in forewing colouration (51)). Such differences between avian and *Heliconius* perceptive abilities may help to inform future studies of *Heliconius* wing pattern evolution. While we can never fully appreciate the perceptive abilities of our study animals, our results highlight differences that our own perceptual biases may have otherwise missed in an important model of adaptation and speciation, which relies heavily on visual information to survive and reproduce.

Acknowledgements

We are grateful to the Ministerio del Ambiente, Agua y Transición Ecológica for permission to collect butterflies in Ecuador (MAATE-DBI-CM-2021-0176; MAATE-DNB-CM-2021-0176). Lucie Queste, Gladis Grefa, José Borreo Malo, Sophie Smith, Whitney Wright, Tamia Chimba, Esteban Arévalo-Morocho and Samantha Vasco Viteri contributed to plant and butterfly stock maintenance. This research was supported by an ERC starting grant (Grant number: 851040) to RMM.

References

1. Bates H. Contributions to an Insect Fauna of the Amazon Valley (Lepidoptera: Heliconidae). Zool J Linn Soc. 1862;6(22):73–7.

2. Merrill R, Dasmahapatra KK, Davey JW, Dell'Aglio DD, Hanly JJ, Huber B, et al. The diversification of Heliconius butterflies: what have we learned in 150 years? J Evol Biol. 2015;28(8):1417–38.

3. Mallet J, Barton NH. Strong Natural Selection in a Warning-Color Hybrid Zone. Evolution. 1989;43(2):421–31.

4. Kapan DD. Three-butterfly system provides a field test of Müllerian mimicry. Nature. 2001;409(6818):338–40.

5. Merrill R, Wallbank RWR, Bull V, Salazar PCA, Mallet J, Stevens M, et al. Disruptive ecological selection on a mating cue. Proc R Soc B Biol Sci. 2012;279(1749):4907–13.

6. Finkbeiner SD, Briscoe AD, Reed RD. Warning signals are seductive: relative contributions of color and pattern to predator avoidance and mate attraction in Heliconius butterflies. Evolution. 2014;68(12):3410–20.

7. Benson WW. Natural selection for Müllerian mimicry in Heliconius erato in Costa Rica. Science. 1972;176(4037):936–9.

8. Langham GM. Specialized avian predators repeatedly attack novel color morphs of Heliconius butterflies. Evolution. 2004;58(12):2783–7.

9. Crane. Imaginal behaviour of a Trinidad butterfly, Heliconius erato hydara Hewitson, with special reference to the social use of color. Zoologica. 1955;40:167–96.

10. Jiggins CD, Naisbit RE, Coe RL, Mallet J. Reproductive isolation caused by colour pattern mimicry. Nature. 2001;411(6835):302–5.

11. Merrill R, Chia A, Nadeau NJ. Divergent warning patterns contribute to assortative mating between incipient Heliconius species. Ecol Evol. 2014;4(7):911–7.

12. Estrada C, Jiggins CD. Interspecific sexual attraction because of convergence in warning colouration: is there a conflict between natural and sexual selection in mimetic species? J Evol Biol. 2008;21(3):749–60.

13. Toure MW, Young FJ, McMillan WO, Montgomery SH. Heliconiini butterflies can learn time-dependent reward associations. Biol Lett. 2020;16(9):20200424.

14. Gilbert LE. The coevolution of a butterfly and a vine. Sci Am. 1982;247(2):110–21.

15. Dell'Aglio DD, Losada ME, Jiggins CD. Butterfly Learning and the Diversification of Plant Leaf Shape. Front Ecol Evol. 2016;4.

16. Zaccardi G, Kelber A, Sison-Mangus MP, Briscoe AD. Color discrimination in the red range with only one long-wavelength sensitive opsin. J Exp Biol. 2006;209(Pt 10):1944–55.

17. McCulloch KJ, Osorio D, Briscoe AD. Sexual dimorphism in the compound eye of Heliconius erato: a nymphalid butterfly with at least five spectral classes of photoreceptor. J Exp Biol. 2016;219(Pt 15):2377–87.

18. McCulloch KJ, Yuan F, Zhen Y, Aardema ML, Smith G, Llorente-Bousquets J, et al. Sexual Dimorphism and Retinal Mosaic Diversification following the Evolution of a Violet Receptor in Butterflies. Mol Biol Evol. 2017;34(9):2271–84.

19. McCulloch KJ, Macias-Muñoz A, Mortazavi A, Briscoe AD. Multiple Mechanisms of Photoreceptor Spectral Tuning in Heliconius Butterflies. Mol Biol Evol. 2022;39(4):msac067.

20. Finkbeiner SD, Briscoe AD. True UV color vision in a female butterfly with two UV opsins. J Exp Biol. 2021;224(18):jeb242802.

21. Montgomery SH, Merrill R. Divergence in brain composition during the early stages of ecological specialization in Heliconius butterflies. J Evol Biol. 2017;30(3):571–82.

22. Montgomery SH, Rossi M, McMillan WO, Merrill RM. Neural divergence and hybrid disruption between ecologically isolated Heliconius butterflies. Proc Natl Acad Sci. 2021 Feb 9;118(6).

23. Cronin TW, Johnsen S, Marshall NJ, Warrant EJ. Visual Ecology. Princeton University Press; 2014.

24. Caves EM, Brandley NC, Johnsen S. Visual Acuity and the Evolution of Signals. Trends Ecol Evol. 2018;33(5):358–72.

25. Land MF, Nilsson DE. Animal eyes. 2nd ed. Oxford ; New York: Oxford University Press; 2012. (Oxford animal biology series).

26. Land MF. Visual acuity in insects. Annu Rev Entomol. 1997;42(1):147–77.

27. Caves EM, Johnsen S. AcuityView: An r package for portraying the effects of visual acuity on scenes observed by an animal. Methods Ecol Evol. 2018;9(3):793–7.

28. Caves EM, Frank TM, Johnsen S. Spectral sensitivity, spatial resolution, and temporal resolution and their implications for conspecific signalling in cleaner shrimp. J Exp Biol. 2016;jeb.122275.

29. Jiggins CD, Estrada C, Rodrigues A. Mimicry and the evolution of premating isolation in Heliconius melpomene Linnaeus. J Evol Biol. 2004;17(3):680–91.

30. Kronforst MR, Kapan DD, McNeely C, O'Neill RJ, Gilbert LE. Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. Proc Natl Acad Sci. 2006;103(17):6575–80.

31. Hausmann AE, Kuo CY, Freire M, Rueda-M N, Linares M, Pardo-Diaz C, et al. Light environment influences mating behaviours during the early stages of divergence in tropical butterflies. Proc R Soc B Biol Sci. 2021;288(1947):20210157.

32. Merry JW, Morehouse NI, Yturralde K, Rutowski RL. The eyes of a patrolling butterfly: Visual field and eye structure in the Orange Sulphur, Colias eurytheme (Lepidoptera, Pieridae). J Insect Physiol. 2006;52(3):240–8.

33. Frederiksen R, Warrant EJ. Visual sensitivity in the crepuscular owl butterfly *Caligo memnon* and the diurnal blue morpho *Morpho peleides* : a clue to explain the evolution of nocturnal apposition eyes? J Exp Biol. 2008;211(6):844–51.

34. Rutowski R, Gislén L, Warrant EJ. Visual acuity and sensitivity increase allometrically with body size in butterflies. Arthropod Struct Dev. 2009;38(2):91–100.

35. Coimbra JP, Collin SP, Hart NS. Variations in retinal photoreceptor topography and the organization of the rod-free zone reflect behavioral diversity in Australian passerines. J Comp Neurol. 2015;523(7):1073–94.

36. Martin GR. The Sensory Ecology of Birds. Oxford University Press; 2017.

37. Caves EM, Troscianko J, Kelley LA. A customizable, low-cost optomotor apparatus: A powerful tool for behaviourally measuring visual capability. Methods Ecol Evol. 2020;11(10):1319–24.

38. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw. 2015;67(1):1–48.

39. Crawley MJ. The R book. Second edition. Chichester, West Sussex, UK: Wiley; 2013.

40. Nakagawa S, Cuthill IC. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev. 2007;82(4):591–605.

41. Fox J, Weisberg S. An R Companion to Applied Regression. SAGE Publications; 2018.

42. Warrant E. Vision in the dimmest habitats on earth. J Comp Physiol A. 2004;190:765–89.

43. Greiner B. Adaptations for nocturnal vision in insect apposition eyes. Int Rev Cytol. 2006;250:1–46.

44. Kilpatrick CW. Noncryogenic Preservation of Mammalian Tissues for DNA Extraction: An Assessment of Storage Methods. Biochem Genet. 2002;40(1):53–62.

45. Seymoure BM, Mcmillan WO, Rutowski R. Peripheral eye dimensions in Longwing (Heliconius) butterflies vary with body size and sex but not light environment nor mimicry ring. J Res Lepidoptera. 2015;48:83–92.

46. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9(7):676–82.

47. Thorpe R. Quantitative handling of characters useful in snake systematics with particular reference to intraspecific variation in the ringed snake Natrix natrix (L.). Biol J Linn Soc. 1975;7(1):27–43.

48. Dell'Aglio DD, Troscianko J, McMillan WO, Stevens M, Jiggins CD. The appearance of mimetic Heliconius butterflies to predators and conspecifics. Evolution. 2018;72(10):2156–66.

49. Yagi N, Koyama N. The Compound Eye of Lepidoptera. Shinkyo Press, Tokyo; 1963.

50. Rutowski RL. Variation of eye size in butterflies: inter-and intraspecific patterns. J Zool. 2000;252(2):187–95.

51. Merrill R, Gompert Z, Dembeck LM, Kronforst MR, McMillan WO, Jiggins CD. Mate preference across the speciation continuum in a clade of mimetic butterflies. Evolution. 2011;65(5):1489–500.