BIOLOGY
LETTERS

royalsocietypublishing.org/journal/rsbl

Research

Cite this article: Borrero J, Mogollon Perez E, Wright DS, Lozano-Urrego D, Rueda-Muñoz G, Pardo-Diaz C, Salazar C, Montgomery SH, Merrill RM. 2024 Weighting of sensory cues reflect changing patterns of visual investment during ecological divergence in *Heliconius* butterflies. *Biol. Lett.* **20**: 20240377.
https://doi.org/10.1098/rsbl.2024.0377

Received: 3 April 2024
Accepted: 4 September 2024

Subject Category:
Evolutionary biology

Subject Areas:
behaviour, evolution

Keywords:
sensory weighting, vision, olfaction, Lepidoptera, ecological speciation

Authors for correspondence:
José Borrero
e-mail: jose.borrero@lmu.de
Richard M. Merrill
e-mail: merrill@bio.lmu.de

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.7465849.

THE ROYAL SOCIETY
PUBLISHING

Weighting of sensory cues reflect changing patterns of visual investment during ecological divergence in *Heliconius* butterflies

José Borrero[1], Elisa Mogollon Perez[1], Daniel Shane Wright[1], Daniela Lozano-Urrego[1,2], Geraldine Rueda-Muñoz[1,2], Carolina Pardo-Diaz[2], Camilo Salazar[2], Stephen H. Montgomery[3] and Richard M. Merrill[1]

[1]Division of Evolutionary Biology, Faculty of Biology, Ludwig Maximilian University of Munich, Munich, Germany
[2]Faculty of Natural Sciences, Universidad del Rosario, Bogotá, Colombia
[3]School of Biological Science, University of Bristol, Bristol, UK

JB, 0000-0003-0164-496X; EMP, 0009-0004-8874-4908; DSW, 0000-0002-4066-6718; CP-D, 0000-0002-7259-1183; CS, 0000-0001-9217-6588; SHM, 0000-0002-5474-5695; RMM, 0000-0003-4527-9298

Integrating information across sensory modalities enables animals to orchestrate a wide range of complex behaviours. The relative importance placed on one sensory modality over another reflects the reliability of cues in a particular environment and corresponding differences in neural investment. As populations diverge across environmental gradients, the reliability of sensory cues may shift, favouring divergence in neural investment and the weight given to different sensory modalities. During their divergence across closed-forest and forest-edge habitats, closely related butterflies *Heliconius cydno* and *Heliconius melpomene* evolved distinct brain morphologies, with the former investing more in vision. Quantitative genetic analyses suggest that selection drove these changes, but their behavioural consequences remain uncertain. We hypothesized that divergent neural investment may alter sensory weighting. We trained individuals in an associative learning experiment using multimodal colour and odour cues. When positively rewarded stimuli were presented in conflict, i.e. pairing positively trained colour with negatively trained odour and *vice versa*, *H. cydno* favoured visual cues more strongly than *H. melpomene*. Hence, differences in sensory weighting may evolve early during divergence and are predicted by patterns of neural investment. These findings, alongside other examples, imply that differences in sensory weighting stem from divergent investment as adaptations to local sensory environments.

1. Introduction

Animals rely on multiple sensory systems to perceive and navigate their environments. The integration of environmental information across different sensory modalities (e.g. vision and olfaction) facilitates complex behaviours such as foraging, predator avoidance and mate finding [1,2]. Cues perceived by these different sensory modalities can work in concert to enhance signal detection [3], but individuals may also prioritize one sensory modality over another [1,4]. This prioritization is likely shaped by divergent selection acting on heritable variation across different habitats [5,6], leading to evolutionary shifts in their relative importance between populations that exploit different habitats.

Evidence for divergence in sensory weighting, defined here as the relative emphasis given to different modalities during behavioural decision-making [4,7,8], has been observed across a handful of taxa where related species exploit different sensory environments. For instance, stingless bees rely less on visual cues, such as colour, than honeybees, possibly due to reduced investment in eye size [9]. In butterflies and moths, brain areas processing sensory information are among the most variable structures between species, and studies investigating multisensory integration emphasize associations between interspecific differences in neuroanatomy and behaviour [10,11]. For example, while hawkmoths broadly use both visual and olfactory cues during foraging [4,12], nocturnal species invest relatively more in brain regions associated with olfactory processing, whereas diurnal species are characterized by larger investments in visual neuropils. This shift in neural investment is mirrored in the relative importance given to olfactory and visual cues during foraging [7]. How such specializations in the weighting of sensory cues evolve over smaller evolutionary timescales and across more subtle ecological gradients at the early stages of divergence is less clear.

The neotropical *Heliconius* butterflies provide an excellent opportunity to study how sensory environments can shape multisensory integration during the early stages of divergence. Although olfaction also plays a role [8,13–17], these butterflies rely heavily on vision during foraging [18,19], mate recognition [20–22] and oviposition [23,24]. In *Heliconius*, speciation is often associated with ecological transitions across forest types [25–28] such that closely related species experience different sensory conditions [26,29,30]. Sister taxa are also known to differ in multiple aspects of both the peripheral and central sensory pathways, likely reflecting adaptation to local sensory conditions [31–33].

If these shifts in neural investment and sensory weighting are the result of divergent selection imposed by differences in sensory ecology, we expect populations diverging across similar environmental gradients to show associated changes in behaviour [34–36]. The *Heliconius* species pair, *Heliconius melpomene* and *Heliconius cydno*, are a well-studied case of ecological speciation, which provides an opportunity to test this hypothesis. These species exist in 'mosaic sympatry', where *H. melpomene* inhabits open forest edges while *H. cydno* occupies more closed canopy, inner forest habitats (figure 1) [26,38,39]. *Heliconius cydno* has larger eyes and invests more heavily in brain structures associated with vision when compared with *H. melpomene*; however, these species do not differ in antennal lobe investment [32]. Multiple sources of data including common-garden-reared individuals strongly suggest that these differences have likely been shaped by adaptive processes [32,33,37]. In insects, visual information is relayed from the optic lobes to the mushroom bodies (the major sensory integration centres), which also receive input from the olfactory pathway [11]. Changes in investment in specific sensory pathways may, therefore, affect the strength of input received, consequently influencing the weight given to different sensory cues during behavioural decision-making processes.

Here, we test how *H. cydno* and *H. melpomene* prioritize visual and olfactory information during foraging, predicting that increased investment in the visual pathway may lead to *H. cydno* prioritizing visual cues. To this end, we trained butterflies to a combination of visual and olfactory stimuli using flower models and then quantified the relative weights given to visual and olfactory cues by presenting the rewarded stimuli in conflict.

2. Material and methods

(a) Butterfly rearing and maintenance

We maintained outbred stock populations of *H. cydno* and *H. melpomene* at the Experimental Station José Celestino Mutis—Universidad del Rosario, near La Vega, Colombia (5.0005° N, 74.3394° W), established from wild individuals caught in the local area. Outdoor cages (1 × 3 × 2 m) housed butterfly groups with access to approximately 20% sugar solution, *Gurania, Lantana* and *Psiguria* spp. flowers as pollen sources, and *Passiflora* vines for oviposition. All individuals used in the experiments were reared under common garden conditions.

(b) Experimental set-up and stimuli

To test for visual and olfactory preferences, learning and sensory weighting, we presented male and female butterflies with artificial feeders with both visual (red or blue) and olfactory (lavender or rose essential oils) stimuli on two 25 × 25 cm hard polyvinyl chloride (PVC) sheet 'arrays' separated by 200 cm in a 1 × 3 × 2 m insectary (figure 2*a*). Each array had four feeders made from a 5 ml Eppendorf tube surrounded by a 3 cm radius circle printed on waterproof paper. One array had all red circles, and on the other, all blue. Olfactory stimuli were presented in a 200 µl PCR tube below the paper circles, containing either lavender (*Lavandula angustifolia*) or rose (*Rosa damascena*) essential oils diluted with paraffin oil to 1% concentration (figure 2*b*). By keeping the olfactory cues separate underneath the waterproof paper and not mixing them with the feeding solution, we ensured that olfactory associations were not confounded by gustatory reception. All four feeders on the same array had the same olfactory stimuli to avoid odour mixing.

We trained and tested butterflies in same-sex groups of 20–24 uniquely marked individuals (10–12 per species). There is no evidence of social learning in *Heliconius* butterflies [40], and we did not see evidence of group effects. Butterflies were introduced to the experimental cages at least 12 h before the initial testing period to allow for acclimatization and were food-deprived overnight. Butterflies were trained to associate the combination of blue colour and rose odour with a positive reward [blue/rose] (20% sugar–water solution) and the combination of [red/lavender] with a negative stimulus (approx. 18% vitamin C solution; [8,41]).

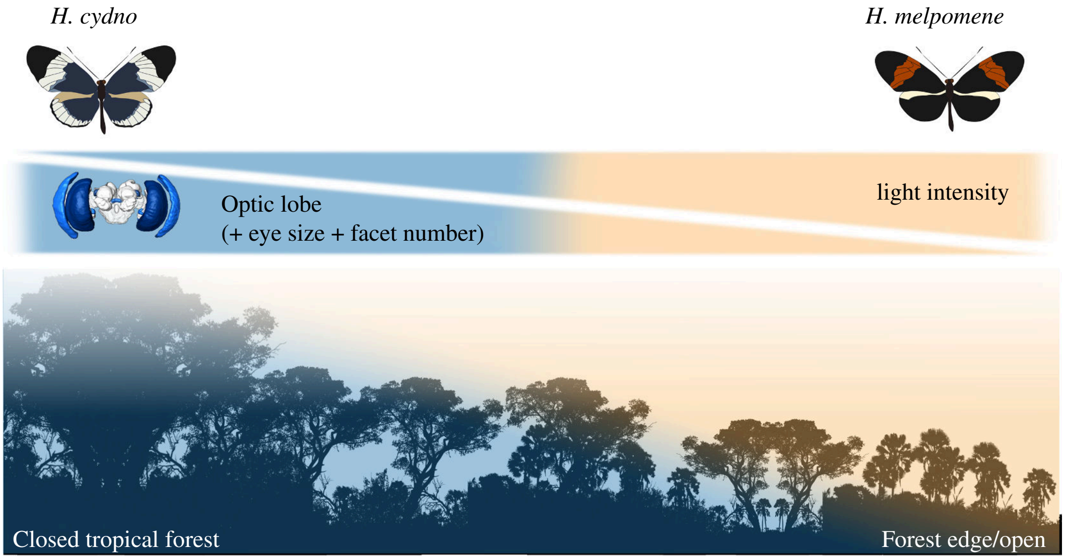

Figure 1. Habitat preference and the sensory environment are associated with differences in brain investment in *Heliconius* butterflies. *Heliconius cydno* invests more in visual neuropils [32] and visual periphery [33,37] and inhabits more-closed canopy forest habitats than its close relative. *Heliconius melpomene* is found in the forest edge [21,30].

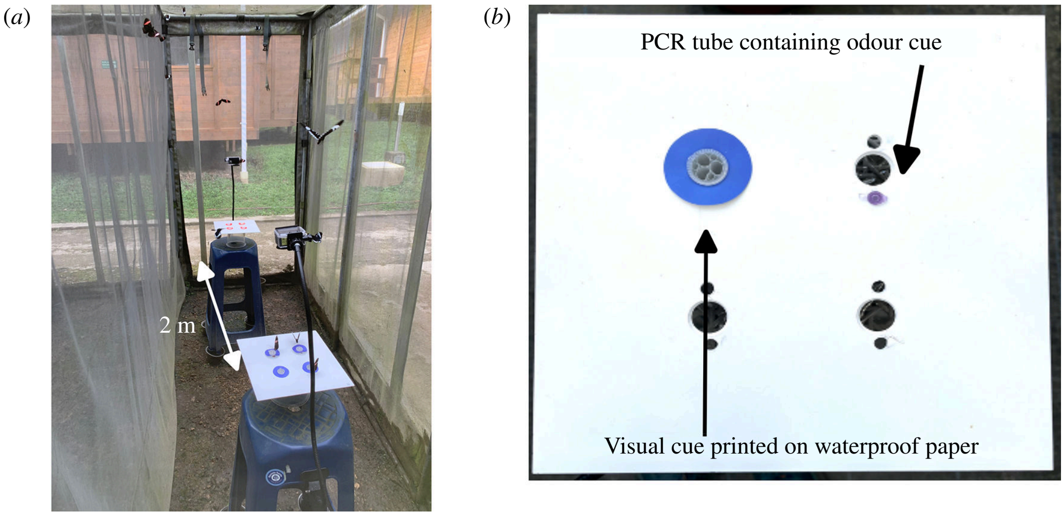

Figure 2. Experimental set-up. (*a*) Groups of *H. cydno* and *H. melpomene* were presented with artificial flower arrays at the end of the cages and were trained to a certain colour/odour combination. (*b*) Artificial flower array containing the multimodal visual cues (coloured circles) and olfactory cue (PCR tube containing essential oil).

We chose red as the negatively rewarded colour because *Heliconius* often feed on red flowers, which are perceived through filtering pigments, to which they have a naive preference [18,42]. This allowed us to show that butterflies can learn to associate novel colours with a food reward. Butterflies were tested with the visual and olfactory cues in the trained test (i.e. [blue/rose] versus [red/lavender]: days 1 and 5), in 'conflict' (i.e. [blue/lavender] versus [red/rose]: day 6) or with colour alone (i.e. [blue] versus [red]: day 7; figure 3*a*). In contrast to the training phases, during testing, clean Eppendorf tubes were only filled with water (i.e. with no positive or negative 'reward'), and fresh visual stimuli were used.

(c) Training and testing

(i) *Naive test (day 1)*. We recorded the naive preferences toward combinations of the visual and olfactory stimuli [blue/rose] versus [red/lavender] for 1 h (10.00–11.00) using only water feeders. Afterwards, each butterfly was then hand-fed once on a positive [blue/rose (+)] and once on a negative rewarded feeder [red/lavender (−)] and then allowed to feed freely for 2 h from the positive and negative rewarded feeders (11.00–13.00).

(ii) *Training (days 2–4)*. Training was reinforced on experimental days 2–4. First, each individual was hand-fed once on a positive [blue/rose (+)] and once on a negative rewarded feeder [red/lavender (−)]. Afterwards, butterflies were allowed to feed freely for 3 h (10.00–13.00) from the positive or negative rewarded feeders. The feeders' position was randomized (coin toss) every day to prevent positional learning.

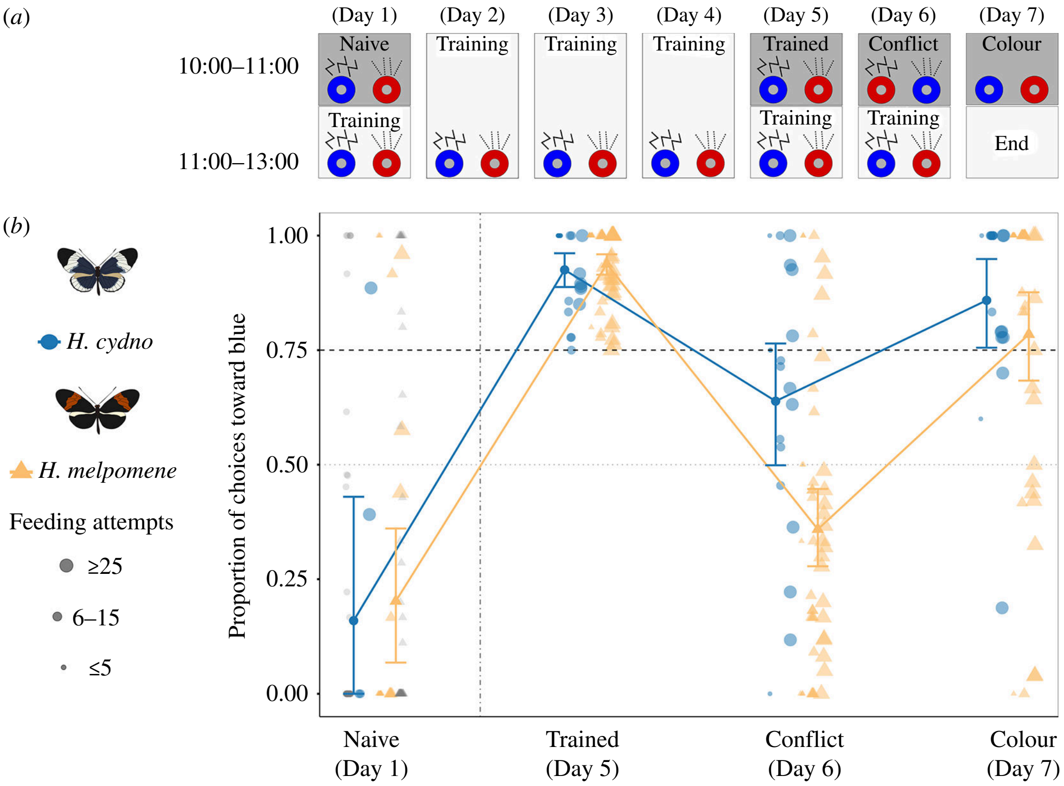

Figure 3. *Heliconius cydno* and *H. melpomene* weight visual and olfactory cues differently while foraging. (*a*) Butterflies were trained to feed on blue feeders with a rose odour (zigzag lines) and negatively reinforced red feeders with a lavender odour (dotted lines). In the conflict test, the positively trained feeders were presented in combination with the negatively trained odours and *vice versa*. During training, positive stimuli were rewarded with sugar–water solution and negative stimuli with approximately 18% vitamin C solution, but during all tests (naive, trained, conflict and colour), feeders were only filled with water (i.e. with no positive or negative reward). Training and reinforcement training periods are delineated by white rectangles, while behavioural tests are indicated by grey rectangles (water only). (*b*) Proportion of feeding attempts toward blue feeder for *H. cydno* and *H. melpomene*. Each point represents the feeding choices of an individual; solid dots and error bars represent the mean choices per species ± 95% confidence intervals. Blue circles represent *H. cydno*, and orange triangles represent *H. melpomene*. Shapes in grey represent naive individuals that did not learn. On naive and trained tests, feeder combinations were blue/rose and red/lavender; in the conflict test, the positively trained colour (blue) was presented in conflict with the negatively rewarded odour (lavender) and vice versa. In the colour tests, individuals were presented only with red or blue feeders without an odour. Only individuals that passed our training criteria (≥ 75% on the trained day) are shown (see §2).

(iii) *Trained test (day 5)*. On day 5, we recorded the trained preferences for 1 h (between 10.00 and 11.00) using only water in the feeders with the stimuli in trained conditions (i.e. [blue/rose] versus [red/lavender]). This was immediately followed by 2 h of reinforcement training with rewarded feeders, i.e. [blue/rose (+)] and [red/lavender (−)].

(iv) *Conflict test (day 6)*. To test for differences in sensory weighting, we presented the visual and olfactory cues in conflict (i.e. [blue/lavender] versus [red/rose]; figure 3). As for the previous tests, feeding behaviour was recorded for 1 h (between 10.00 and 11.00) using only water in the feeders. This was then followed by reinforcement training for 2 h, i.e. [blue/rose (+)] and [red/lavender (−)].

(v) *Colour test (day 7)*. We tested visual preferences in isolation for 1 h (between 10.00 and 11.00; i.e. blue versus red, without olfactory stimuli and using water only).

(d) Behavioural tests

All feeding test assays ((i) naive, (ii) trained, (iv) conflict and (v) colour) were filmed from above with an AKASO Brave 4 Pro action camera. We scored the videos in BORIS [43], recording the total number of feeding attempts directed toward each stimulus per individual. Feeding attempts were defined as a proboscis extension within the coloured circle feeders. We scored instances where an individual moved outside the circle and subsequently returned as a new feeding attempt. In contrast, we scored instances where an individual remained within a circle and probed continuously as a single attempt unless the proboscis was retracted for 3 s or longer.

(e) Training criterion

We established a stringent training criterion to incorporate only individuals that had successfully learned the correct stimulus combination, following similar learning studies in lizards [44,45] and butterflies [19]. Out of 260 individuals, 73 were excluded from the analysis for not attempting to feed on the trained test (day 5; electronic supplementary material, table S1). To investigate species differences in sensory weighting, we included only those individuals that demonstrated robust associative learning of the trained cue combinations (i.e. ≥ 75% of feeding choices toward the positive multimodal stimuli [blue/rose] on the

Table 1. Pairwise *p*-value comparison matrix of *post hoc* test with Bonferroni correction for the minimal adequate model for the effects of the interaction between species and treatment (trained, conflict and colour) on choices toward blue feeders. Only individuals that passed our training criteria (≥ 75% on the trained day) are included. Results are from a binomial GLMM (see §2 for statistical details; Proportion blue ~ Species × Level + (1|Group/ID)).

	H. cydno trained	*H. melpomene* trained	*H. cydno* conflict	*H. melpomene* conflict	*H. cydno* colour	*H. melpomene* colour
H. cydno trained	[2.329]	1	<0.0001	<0.0001	0.3041	0.008
H. melpomene trained		[2.874]	<0.0001	<0.0001	0.0116	<0.0001
H. cydno conflict			[0.465]	0.0199	<0.0001	1
H. melpomene conflict				[−0.601]	<0.0001	<0.0001
H. cydno colour					[1.639]	1
H. melpomene colour						[0.989]

trained test (day 5), resulting in a reduced sample size from 187 to 73 individuals. Although biased toward *H. melpomene* (52 individuals versus 21 *H. cydno* individuals), this rigorous criterion ensured that our analysis focused solely on individuals who had successfully learned. However, our results did not quantitatively change when including all 117 individuals with a lower threshold of learning (i.e. > 50% correct; see electronic supplementary material, tables S2, S5 and S6).

(f) Statistical analysis

We used general linear mixed models (GLMM) with a binomial distribution using the *lme4* package [46] in R [47] to assess if species differed in their feeding attempts when the positively rewarded stimuli agreed or were in conflict. The dependent variable was the proportion of choices toward the blue feeders, and we included species and treatment (trained test (day 5), conflict test (day 6) or colour test (day 7)) and their interaction as fixed factors. Sex was also included as a fixed factor in our initial model but was later dropped during model selection. To account for the varying number of choices made by each butterfly, individual observations are weighted by their total number of feeding attempts [48]. Individual ID nested within group ID was included as random effects to account for repeated measures (table 1). We applied a stepwise model reduction approach to identify the minimum adequate statistical models, using likelihood ratio tests (LRTs) via the *drop1* function to determine significance. When fixed effects or their interactions were significant, we conducted pairwise comparison tests using *post hoc* Bonferroni corrections with the *emmeans* package [49]. Plots were made using the *ggplot2* package [50].

3. Results

Among the 73 individuals (52 *H. melpomene* and 21 *H. cydno*) that passed our stringent training criterion (i.e. > 75% correct choices on day 5), we conducted tests to quantify the species' reliance on visual and olfactory cues. Importantly, during these tests, all feeders were unrewarded. *Heliconius cydno* did not differ from *H. melpomene* in the trained test (table 1; *post hoc*: *H. cydno* trained versus *H. melpomene* trained, $Z = -1.375$, $p = 1.0$). In contrast, *H. melpomene* and *H. cydno* responded differently when presented with the olfactory and visual stimuli in conflict (figure 3*b*). Importantly, the interaction between species and trial type was retained in our model, confirming that this was an effect of how the stimuli were presented (species × treatment LRT: $2\Delta lnL = 22.876$, d.f. = 2, $p < 0.001$; electronic supplementary material, table S3). In the conflict test, *H. cydno* fed mostly on the blue feeder, while *H. melpomene* primarily chose the feeder with the rose scent, suggesting a difference between these species in how olfactory and visual cues are weighed during behavioural decisions (figure 3*b*; table 1; post hoc: *H. cydno* conflict versus *H. melpomene* conflict, $Z = 3.210$, $p = 0.019$). We found no effect of sex, which was excluded from our model (sex LRT: $2\Delta lnL = 3.067$, d.f. = 1, $p = 0.079$; electronic supplementary material, table S3).

Our *post hoc* analysis revealed that individuals of both species made fewer feeding attempts on the blue feeder when the stimuli were presented in conflict, suggesting that both species use both visual and olfactory stimuli when making foraging decisions (table 1; *post hoc*: *H. cydno* trained versus *H. cydno* conflict, $Z = 6.932$, $p \leq 0.0001$; *H. melpomene* trained versus *H. melpomene* conflict, $Z = 20.614$, $p \leq 0.0001$). *Heliconius cydno* made fewer feeding attempts on the blue feeder when the positively trained olfactory and visual stimuli were in conflict than when they were in the trained combinations, confirming the use of both cues when present. However, there was no distinction between the trained test and the colour-only test (table 1; *post hoc*: *H. cydno* trained versus *H. cydno* colour, $Z = 2.321$, $p = 0.304$), further emphasizing that *H. cydno* was more attentive to the visual cue in our assay. Conversely, *H. melpomene* differed between the trained test and the colour-only test, indicating greater use of the olfactory stimulus (table 1; *post hoc*: *H. melpomene* trained versus *H. melpomene* colour, $Z = 10.920$, $p \leq 0.0001$).

4. Discussion

We explored how two closely related butterfly species, *H. cydno* and *H. melpomene*, weigh visual and olfactory information in a foraging context. Our findings indicate a significant divergence in behaviour between these two species, with *H. cydno* favouring visual cues over olfactory cues compared with *H. melpomene*. Importantly, our results mirror differences in brain morphology and suggest that divergence in the sensory environment may have led to changes in brain composition and how these species process, integrate and prioritize information across sensory modalities.

To assess how *H. cydno* and *H. melpomene* prioritize visual and olfactory information, we presented trained butterflies with conflicting stimuli—pairing positively trained colours with negatively trained odours and *vice versa*. Both species chose the 'correct' colour less frequently in these conflict scenarios compared with their trained preferences or when presented with colours alone, indicating that both visual and olfactory cues influence foraging decisions. However, when presented with conflicting cues, the species differed in their reliance on each sensory modality. *Heliconius cydno* predominantly fed on the positively rewarded visual cue (the blue feeder). In contrast, *H. melpomene* showed a stronger preference for the feeder containing the positive trained odour stimulus (rose), indicating a difference between the two species in the weight given to the two cues during behavioural decision-making. Additionally, *H. melpomene* differed between the trained and colour-only test, making fewer correct choices when only colours were presented, again suggesting a larger reliance on the olfaction stimulus compared to *H. cydno*. Given that the behavioural variation we observe is from common-garden-reared individuals, these interspecific shifts are likely to have a genetic basis. In addition, our results align with divergent patterns of investment in sensory pathways between the two species, whereby *H. cydno* exhibits greater relative investment in the visual system [32,33,37]. Therefore, heritable shifts in neural investment and sensory weighting may result from divergent selection imposed by differences in sensory ecology.

One potential limitation of our study is that we managed to successfully train a larger number of *H. melpomene* than *H. cydno* individuals. This discrepancy in training could be due to *H. melpomene* being more active during the trials, possibly due to the outdoor insectaries being found in the forest edge, which closely resembles the *H. melpomene* habitat [26,30]. Indeed, in contrast to *H. cydno*, *H. melpomene* is frequently observed flying next to the insectaries (J Borrero, personal observation). Despite this difference, individuals of both species successfully learned the rewarded combination under our rigorous training criteria (≥ 75% correct choices), and our main conclusions hold even under less rigorous training criteria (see electronic supplementary material, results).

Adaptation to the local sensory environment may play an important role during the early stages of divergence. The gradient observed in the preferred environments of *H. cydno* and *H. melpomene*, from closed forest canopy to open forest edge [26,30], resembles the transition across an altitudinal gradient, from complex broadleaf forest environments to dry Andean forests seen in another distantly related *Heliconius* species pair, *Heliconius erato* and *Heliconius himera* [51]. *Heliconius erato cyrbia* and *H. himera* exhibit similar patterns of neural divergence, with *H. erato cyrbia* showing increased, heritable investment in the visual pathway, but with *H. himera* also showing some increased investment in the olfactory pathway [8,31]. Despite methodological differences (group versus individual testing and the exact visual and olfactory stimuli used), our results qualitatively reassemble a previous study in *H. erato* and *H. himera* [8]. Together, these findings demonstrate similar shifts in sensory weighting among *Heliconius* butterflies, with species inhabiting more closed habitats investing more in perceiving and processing visual information and favouring visual over olfactory cues. This suggests that changes in the light environment are a common source of divergent selection across the two species pairs.

More broadly, changes in sensory weighting have been observed across different taxa and across different evolutionary timescales. For instance, urban great tits prefer olfactory cues, while forest birds rely more on visual cues [52]. Nine-spined sticklebacks show plasticity in their sensory reliance; for instance, compared to fish reared in visually unrestricted habitats, individuals raised in visually restricted environments display a larger reliance on chemical over visual cues. This suggests that neural plasticity may underpin initial behavioural responses to novel environments that are later consolidated by selection [53]. In three spine stickleback fish, populations in different lakes segregate into limnetic and benthic ecomorphs along an environmental gradient [54]. These stickleback populations show repeated evolutionary shifts in brain regions that are correlated with differences in their sensory environments; limnetic species have larger structures for visual processing, and benthic ectomorphs possess larger olfactory bulbs. Comparing across larger evolutionary timescales, studies have found that nocturnal hawkmoths prioritize olfactory cues while foraging, as evidenced by their increased investment in olfactory processing. Conversely, diurnal hawkmoths show a larger investment in visual neuropils and place a greater emphasis on visual cues. These changes in the relative weight given to different sensory modalities and the accompanying changes in brain investment are likely driven by the transition from a nocturnal to a diurnal habitat and the associated alterations in their sensory environment [7]. Combined with our findings, these studies underscore the role of environmental factors in driving changes in neuroanatomy, sensory integration and behaviour across species.

In conclusion, our results highlight shifts in sensory weighting between species associated with neural investment and ecological divergence, consistent with patterns across *Heliconius* butterflies. We demonstrated that *H. cydno*, from a lower light habitat [30], which is characterized with increased visual investment [32,33], favours visual cues more strongly than *H. melpomene*. Alongside previous results in hawkmoths and *Heliconius* butterflies [7,8], our research reveals repeated shifts in sensory weighting associated with changes in neural investment and habitat use. This highlights the potential role of the sensory environment to shape differences in brain structure, which subsequently may influence key sensory behaviours crucial during the initial stages of species divergence.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. All behavioural data and analysis are available from the Dryad Digital Repository [55].

Supplementary material is available online [56].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. J.B.: conceptualization, data curation, formal analysis, investigation, visualization, writing—original draft, writing—review and editing; E.M.P.: data curation, writing—review and editing; D.S.W.: conceptualization, methodology, supervision, writing—review and editing; D.L.-U.: investigation, writing—review and editing; G.R.-M.: investigation, writing—review and editing; C.P.-D.: resources,

supervision, writing—review and editing; C.S.: resources, supervision, writing—review and editing; S.H.M.: conceptualization, supervision, writing—review and editing; R.M.M.: conceptualization, resources, supervision, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. Research funded by ERC starter grant 851040 to R.M.M.

Acknowledgements. We thank Universidad del Rosario for insectary access at JCM and Isabel León for assistance, as well as Amulya Hosur and Alejandro Mancino for assistance with scoring the videos.

References

1. Talsma D, Senkowski D, Soto-Faraco S, Woldorff MG. 2010 The multifaceted interplay between attention and multisensory integration. *Trends Cogn. Sci.* **14**, 400–410. (doi:10.1016/j.tics.2010.06.008)
2. Stevens M. 2013 *Sensory ecology, behaviour, and evolution*, 1st edn. Oxford, UK: Oxford University Press.
3. Leonard AS, Dornhaus A, Papaj DR. 2011 Flowers help bees cope with uncertainty: signal detection and the function of floral complexity. *J. Exp. Biol*. **214**, 113–121. (doi:10.1242/jeb.047407)
4. Balkenius A, Rosén W, Kelber A. 2006 The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *J. Comp. Physiol. A* **192**, 431–437. (doi:10.1007/s00359-005-0081-6)
5. Dalbosco Dell'Aglio D, Rivas-Sánchez DF, Wright DS, Merrill RM, Montgomery SH. 2024 The sensory ecology of speciation. *Cold Spring Harb. Perspect. Biol.* **16**, a041428. (doi:10.1101/cshperspect.a041428)
6. Raine NE, Chittka L. 2007 The adaptive significance of sensory bias in a foraging context: floral colour preferences in the bumblebee *Bombus terrestris*. *PLoS One* **2**, e556. (doi:10.1371/journal.pone.0000556)
7. Stöckl A, Heinze S, Charalabidis A, El Jundi B, Warrant E, Kelber A. 2016 Differential investment in visual and olfactory brain areas reflects behavioural choices in hawk moths. *Sci. Rep*. **6**, 26041. (doi:10.1038/srep26041)
8. Dell'Aglio DD, McMillan WO, Montgomery SH. 2022 Shifting balances in the weighting of sensory modalities are predicted by divergence in brain morphology in incipient species of *Heliconius* butterflies. *Anim. Behav*. **185**, 83–90. (doi:10.1016/j.anbehav.2022.01.003)
9. Koethe S, Fischbach V, Banysch S, Reinartz L, Hrncir M, Lunau K. 2020 A comparative study of food source selection in stingless bees and honeybees: scent marks, location, or color. *Front. Plant Sci*. **11**, 516. (doi:10.3389/fpls.2020.00516)
10. Couto A, Wainwright JB, Morris BJ, Montgomery SH. 2020 Linking ecological specialisation to adaptations in butterfly brains and sensory systems. *Curr. Opin. Insect Sci*. **42**, 55–60. (doi:10.1016/j.cois.2020.09.002)
11. Couto A *et al*. 2023 Rapid expansion and visual specialisation of learning and memory centres in the brains of Heliconiini butterflies. *Nat. Commun*. **14**, 4024. (doi:10.1038/s41467-023-39618-8)
12. Balkenius A, Kelber A. 2006 Colour preferences influences odour learning in the hawkmoth, *Macroglossum stellatarum*. *Naturwissenschaften* **93**, 255–258. (doi:10.1007/s00114-006-0099-9)
13. Andersson S, Dobson HEM. 2003 Behavioral foraging responses by the butterfly *Heliconius melpomene* to *Lantana camara* floral scent. *J. Chem. Ecol*. **29**, 2303–2318. (doi:10.1023/a:1026226514968)
14. Thiele SC, Rodrigues D, Moreira GRP. 2016 Oviposition in *Heliconius erato* (Lepidoptera, Nymphalidae): how essential is drumming behavior for host-plant selection? *J. Insect Behav*. **29**, 283–300. (doi:10.1007/s10905-016-9559-z)
15. Darragh K *et al*. 2017 Male sex pheromone components in *Heliconius* butterflies released by the androconia affect female choice. *PeerJ* **5**, e3953. (doi:10.7717/peerj.3953)
16. Silva DS, Barp EA, Kucharski LCR, Moreira GRP. 2018 Sensing the plant surface prior to feeding and oviposition: differences in external ultrastructure and function among tarsi of *Heliconius erato*. *Neotrop. Entomol*. **47**, 85–95. (doi:10.1007/s13744-017-0508-0)
17. van Schooten B, Meléndez-Rosa J, Van Belleghem SM, Jiggins CD, Tan JD, McMillan WO, Papa R. 2020 Divergence of chemosensing during the early stages of speciation. *Proc. Natl Acad. Sci. USA* **117**, 16438–16447. (doi:10.1073/pnas.1921318117)
18. Zaccardi G, Kelber A, Sison-Mangus MP, Briscoe AD. 2006 Color discrimination in the red range with only one long-wavelength sensitive opsin. *J. Exp. Biol*. **209**, 1944–1955. (doi:10.1242/jeb.02207)
19. Toure MW, Young FJ, McMillan WO, Montgomery SH. 2020 Heliconiini butterflies can learn time-dependent reward associations. *Biol. Lett*. **16**, 20200424. (doi:10.1098/rsbl.2020.0424)
20. Jiggins CD, Naisbit RE, Coe RL, Mallet J. 2001 Reproductive isolation caused by colour pattern mimicry. *Nature* **411**, 302–305. (doi:10.1038/35077075)
21. Estrada C, Jiggins CD. 2008 Interspecific sexual attraction because of convergence in warning colouration: is there a conflict between natural and sexual selection in mimetic species? *J. Evol. Biol*. **21**, 749–760. (doi:10.1111/j.1420-9101.2008.01517.x)
22. Merrill RM, Chia A, Nadeau NJ. 2014 Divergent warning patterns contribute to assortative mating between incipient *Heliconius* species. *Ecol. Evol*. **4**, 911–917. (doi:10.1002/ece3.996)
23. Gilbert LE. 1982 The coevolution of a butterfly and a vine. *Sci. Am*. **247**, 110–121. (doi:10.1038/scientificamerican0882-110)
24. Dell'Aglio DD, Losada ME, Jiggins CD. 2016 Butterfly learning and the diversification of plant leaf shape. *Front. Ecol. Evol*. **4**, 1–7. (doi:10.3389/fevo.2016.00081)
25. Jiggins CD, Mallet J. 2000 Bimodal hybrid zones and speciation. *Trends Ecol. Evol.* **15**, 250–255. (doi:10.1016/S0169-5347(00)01873-5)
26. Estrada C, Jiggins CD. 2002 Patterns of pollen feeding and habitat preference among *Heliconius* species . *Ecol. Entomol*. **27**, 448–456. (doi:10.1046/j.1365-2311.2002.00434.x)
27. Jiggins CD. 2008 Ecological speciation in mimetic butterflies. *Bioscience* **58**, 541–548. (doi:10.1641/B580610)
28. Merrill RM *et al*. 2015 The diversification of *Heliconius* butterflies: what have we learned in 150 years? *J. Evol. Biol*. **28**, 1417–1438. (doi:10.1111/jeb.12672)
29. Endler JA. 1993 The color of light in forests and its implications. *Ecol. Monogr*. **63**, 1–27. (doi:10.2307/2937121)
30. Seymoure BM. 2016 Heliconius in a new light: the effects of light environments on mimetic coloration, behavior, and visual systems. PhD thesis, Arizona State University, Tempe, AZ. https://hdl.handle.net/2286/R.I.39452.
31. Montgomery SH, Merrill RM. 2017 Divergence in brain composition during the early stages of ecological specialization in *Heliconius* butterflies. *J. Evol. Biol*. **30**, 571–582. (doi:10.1111/jeb.13027)

32. Montgomery SH, Rossi M, McMillan WO, Merrill RM. 2021 Neural divergence and hybrid disruption between ecologically isolated *Heliconius* butterflies. *Proc. Natl Acad. Sci. USA* **118**, e2015102118. (doi:10.1073/pnas.2015102118)
33. Wright DS, Rodriguez-Fuentes J, Ammer L, Darragh K, Kuo CY, McMillan WO, Jiggins CD, Montgomery SH, Merrill RM. 2024 Selection drives divergence of eye morphology in sympatric *Heliconius* butterflies. *Evolution (N Y)* **78**, 1338–1346. (doi:10.1093/evolut/qpae073)
34. Johnson MA, Revell LJ, Losos JB. Behavioral convergence and adaptive radiation: effects of habitat use on territorial behavior in *Anolis* lizards. *Evolution* **64**, 1151–1159. (doi:10.1111/j.1558-5646.2009.00881.x)
35. Henry CS, Brooks SJ, Duelli P, Johnson JB, Wells MM, Mochizuki A. 2012 Parallel evolution in courtship songs of North American and European green lacewings (Neuroptera: Chrysopidae): parallel evolution in lacewing songs. *Biol. J. Linn. Soc*. **105**, 776–796. (doi:10.1111/j.1095-8312.2011.01845.x)
36. Bolnick DI, Barrett RDH, Oke KB, Rennison DJ, Stuart YE. 2018 (Non)Parallel evolution. *Annu. Rev. Ecol. Evol. Syst*. **49**, 303–330. (doi:10.1146/annurev-ecolsys-110617-062240)
37. Seymoure BM, Mcmillan WO, Rutowski R. 2015 Peripheral eye dimensions in longwing (*Heliconius*) butterflies vary with body size and sex but not light environment nor mimicry ring. *J. Res. Lepid*. **48**, 83–92. (doi:10.5962/p.266475)
38. Smiley J. 1978 *The host plant ecology of* Heliconius *butterflies in northeastern Costa Rica*. Austin, TX: Universtiy of Texas at Austin.
39. Mérot C, Salazar C, Merrill RM, Jiggins CD, Joron M. 2017 What shapes the continuum of reproductive isolation? Lessons from *Heliconius* butterflies. *Proc. R. Soc. B* **284**, 20170335. (doi:10.1098/rspb.2017.0335)
40. Moura PA, Cardoso MZ, Montgomery SH. 2023 No evidence of social learning in a socially roosting butterfly in an associative learning task. *Biol. Lett*. **19**, 20220490. (doi:10.1098/rsbl.2022.0490)
41. Bernays EA, Glendinning JI, Chapman RF. 1998 Plant acids modulate chemosensory responses in *Manduca sexta* larvae . *Physiol. Entomol*. **23**, 193–201. (doi:10.1046/j.1365-3032.1998.233079.x)
42. Jiggins CD. 2017 *The ecology and evolution of* Heliconius *butterflies*. Oxford, UK: Oxford University Press. (doi:10.1093/acprof:oso/9780199566570.001.0001)
43. Friard O, Gamba M. 2016 BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations . *Methods Ecol. Evol*. **7**, 1325–1330. (doi:10.1111/2041-210X.12584)
44. Damas-Moreira I, Oliveira D, Santos JL, Riley JL, Harris DJ, Whiting MJ. 2018 Learning from others: an invasive lizard uses social information from both conspecifics and heterospecifics. *Biol. Lett*. **14**, 20180532. (doi:10.1098/rsbl.2018.0532)
45. Batabyal A, Thaker M. 2019 Lizards from suburban areas learn faster to stay safe. *Biol. Lett*. **15**, 20190009. (doi:10.1098/rsbl.2019.0009)
46. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Soft.* **67**, 1–48. (doi:10.18637/jss.v067.i01)
47. R Core Team. 2023 R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org/.
48. Crawley MJ. 2013 *The R book*, 2nd edn. Chichester, UK: Wiley.
49. Lenth R, Singmann H, Love J, Buerkner P, Herve M. 2019 Package 'emmeans'. See http://cran.r-project.org/package=emmeans.
50. Wickham H. 2011 ggplot2. *WIREs Comput. Stats.* **3**, 180–185. (doi:10.1002/wics.147)
51. Jiggins CD, McMillan WO, Neukirchen W, Mallet J. 1996 What can hybrid zones tell us about speciation? The case of *Heliconius erato* and *H. himera* (Lepidoptera: Nymphalidae). *Biol. J. Linnean Soc.* **59**, 221–242. (doi:10.1111/j.1095-8312.1996.tb01464.x)
52. Rubene D, Low M, Brodin A. 2023 Birds differentially prioritize visual and olfactory foraging cues depending on habitat of origin and sex. *R. Soc. Open Sci*. **10**, 221336. (doi:10.1098/rsos.221336)
53. Pike TW, Ramsey M, Wilkinson A. 2018 Environmentally induced changes to brain morphology predict cognitive performance. *Phil. Trans. R. Soc. B* **373**, 20170287. (doi:10.1098/rstb.2017.0287)
54. Keagy J, Braithwaite VA, Boughman JW. 2018 Brain differences in ecologically differentiated sticklebacks. *Curr. Zool.* **64**, 243–250. (doi:10.1093/cz/zox074)
55. Borrero M, Antonio J. 2024 Weighting of sensory cues reflects changing patterns of visual investment during ecological divergence in Heliconius butterflies. Dryad Digital Repository. (doi:10.5061/dryad.s1rn8pkgk)
56. Borrero J, Perez M, Wright E, Shane D, Lozano D, Rueda-Muñoz G, Pardo-Diaz C *et al*. 2024 Data from: Weighting of sensory cues reflect changing patterns of visual investment during ecological divergence in Heliconius butterflies. Figshare. (doi:10.6084/m9.figshare.c.7465849)