Altitude and life-history shape the evolution of *Heliconius* wings

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Running title: "Evolution of *Heliconius* wings"

Author contributions

G.M.K., J.E.S., N.J.N and C.D.J. conceived the study. G.M.K., J.E.S. and C.N.B. conducted fieldwork. G.M.K., J.E.S., J.I.M., and E.W.R collated the dataset and conducted the analyses. All authors contributed to the writing of the manuscript.

Acknowledgements

We would like to thank all the *Heliconius* researchers and field assistants that have contributed to the wing collection used in this study. We also thank Simon H. Martin and Stephen H. Montgomery for useful comments on early versions of this manuscript. G.M.K. was supported by a Natural Environment Research Council Doctoral Training Partnership. J.E.S. was supported by the Scurfield Memorial Bursary (University of Sheffield). Funding was provided to C.B. by the Spanish Agency for International Development Cooperation (AECID, grant number 2018SPE0000400194). N.J.N. and C.D.J were supported by a Natural Environment Research Council (grant number: NE/R010331/1, NE/R153436/1).

Data accessibility

All images are now available in the public repository Zenodo (https://zenodo.org/communities/butterfly/)
All R and image analysis scripts are available on Zenodo (DOI:10.5281/zenodo.3491029)

Abstract

Phenotypic divergence between closely related species has long interested biologists. Taxa that inhabit a range of environments and have diverse natural histories can help understand how selection drives phenotypic divergence. In butterflies, wing colour patterns have been extensively studied but diversity in wing shape and size is less well understood. Here we assess the relative importance of phylogenetic relatedness, natural history and habitat on shaping wing morphology in a large dataset of over 3500 individuals, representing 13 *Heliconius* species from across the Neotropics. We find that both larval and adult behavioural ecology correlate with patterns of wing sexual dimorphism and adult size. Species with solitary larvae have larger adult males, in contrast to gregarious *Heliconius* species, and indeed most Lepidoptera, where females are larger. Species in the pupal-mating clade are smaller than those in the adult-mating clade. Interestingly, we find that high-altitude species tend to have rounder wings and, in one of the two major *Heliconius* clades, are also bigger than their lowland relatives. Furthermore, within two widespread species we find that high-altitude populations also have rounder wings. Thus, we reveal novel adaptive wing morphological divergence among *Heliconius* species beyond that imposed by natural selection on aposematic wing colouration.

Keywords: *Heliconius*, phenotypic divergence, wing morphology, Lepidoptera, sexual dimorphism, altitude

Introduction

Identifying the selective forces driving phenotypic divergence among closely related species lies at the core of evolutionary biology research. Adaptive radiations, in which descendants from a common ancestor rapidly fill a variety of niches, are ideal systems to investigate morphological divergence (Schluter 2000). The study of adaptive radiations has revealed that evolution often comes up with similar solutions for similar problems at the phenotypic and genetic levels (Losos 2010; Marques et al. 2019). Speciose groups that have repeatedly and independently evolved convergent adaptations to life-history strategies and environments are good systems in which study selection drivers (Schluter 2000). Nevertheless, adaptive phenotypic evolution is often complex and multifaceted, with more than a single selective force in action (Maia et al. 2016; Nosil et al. 2018). For example in birds, sex differences in plumage colouration are driven by intra-specific sexual selection, while natural selection drives sexes towards more similar colourations (Dunn et al. 2015). Integrative approaches that make use of tractable traits across well-resolved phylogenies are needed to explore the selective forces driving phenotypic evolution.

Butterfly wing colouration has been the focus of considerable research effort and major strides have been made towards understanding how and when evolution leads to complex wing colour patterns, conferring aposematism, camouflage, or a mating advantage (Merrill et al. 2012; Chazot et al. 2016; Nadeau et al. 2016). The dazzling diversity of butterfly colour patterns among species has perhaps obscured the less conspicuous phenotypic diversity of wing shapes and sizes, which are more often regarded as the result of sexual selection, flight trade-offs or developmental constraints (Singer 1982; Allen et al. 2011), rather than drivers of local adaptation and species diversification (Srygley 2004a; Cespedes et al. 2015; Chazot et al. 2016). A recent review assessing the ecology of butterfly flight, identified habitat, predators and sex-specific behaviours as the selection forces most likely driving wing morphology variation, but highlighted the need for further phylogenetic comparative

studies that identify the adaptive mechanisms shaping wings (Le Roy et al. 2019).

Differences in behaviour between sexes have been identified as one of the main drivers of wing aspect ratio and size sexual dimorphism in insects (Rossato et al. 2018a; Le Roy et al. 2019). In butterflies, males tend to spend more time looking for mates and patrolling territories, while females focus their energy on searching for suitable host plants for oviposition (Rossato et al. 2018b). The same wing trait can be associated with different life history traits in each sex, resulting in sex-specific selection pressures. For example, in the Nearctic butterfly *Melitaea cinxia*, wing aspect ratio only correlates with dispersal in females, as males experience additional selection pressures that counteract selection for dispersal wing phenotypes (Breuker et al. 2007). Sex-specific behaviours can impact wing aspect ratio and size, but differences in life histories, even across closely related species, could also have large impacts on the strength and direction of these effects (Cespedes et al. 2015; Chazot et al. 2016).

Another important source of phenotypic variation in insect wings is the physical environment they inhabit throughout their range. Air pressure decreases with altitude, which in turn reduces lift forces required for flight. To compensate for this, insects may increase wing area relative to body size to reduce the velocity necessary to sustain flight (Dudley 2002; Dillon et al. 2018). Wing aspect ratio in *Drosophila melanogaster* has been observed to vary adaptively across latitudes and altitudes, with wings getting rounder and larger in montane habitats, possibly to maintain flight function in lower air pressures (Stalker and Carson 1948; Pitchers et al. 2012; Klepsatel et al. 2014).

In butterflies, high aspect ratios, i.e. long and narrow wings, reduce drag caused by wing tip vortices, thus lowering the energy required for flight and promoting gliding for longer distances (Le Roy et al. 2019). Variation in wing phenotypes can occur at the microhabitat level, for example *Morpho* butterfly clades in the understory have rounder wings than

canopy-specialist clades, presumably for increased manoeuvrability (Chazot et al. 2016). An extreme case of environmental effects on wing morphology can be found in Lepidoptera inhabiting the windy, barren highlands of the Andes, where an interaction between behavioural sex differences and extreme climatic conditions have led to flightlessness in females of several species (Pyrcz et al. 2004).

Heliconius is a genus of Neotropical butterflies that has been studied for over two centuries with a well resolved phylogeny (Kozak et al. 2015, 2018). It represents a striking case of Müllerian mimicry, with co-occurring subspecies sharing warning wing colour patterns to avoid predators and leading to multi-species mimicry rings across South America (Merrill et al. 2015). Wing aspect ratio and size are part of the mimetic signal (Jones et al. 2013; Mérot et al. 2016; Rossato et al. 2018a). Wing morphology is involved in many aspects of *Heliconius* biology other than mimicry, such as mating or flight mode, but these have been less well studied (Rodrigues and Moreira 2004; Srygley 2004b; Mendoza-Cuenca and MacÍas-Ordóñez 2010). As the only butterflies that pollen-feed, their long life-spans and enlarged brains allow them to memorise foraging transects that are repeated daily following a short dispersal post-emergence phase of up to 1.5 km (Cook et al. 1976; Jiggins 2016).

Larval gregariousness has evolved independently three times across the phylogeny, with some species laying clutches of up to 200 eggs, while others lay eggs singly and larvae are often cannibalistic (Beltrán et al. 2007). Gregarious *Heliconius* species would be predicted to have larger-sized females to carry the enlarged egg load, as is the case with most Lepidoptera (Allen et al. 2011). Another striking life history trait is pupal-mating, which is only found in one of the two major clades (hereafter the “erato clade”), having arisen following the most basal split in the *Heliconius* phylogeny. This mating strategy involves males copulating with females as they emerge from the pupal case (Deinert et al. 1994; Beltrán et al. 2007). Pupal-mating leads to a whole suite of distinct selection pressures but these are hard to tease apart from the effects of phylogeny due to its single origin (Beltrán et al. 2007;

Thurman et al. 2018). Further ecological differences could arise from adaptation to altitude. Some species are relatively high-altitude specialists, such as *H. telesiphe* and *H. hierax* found above 1000m, whilst others range widely, such as *H. melpomene* and *H. erato*, which can be found from 0 to 1800 m above sea-level (Rosser et al. 2015; Jiggins 2016). Potential adaptations to altitude are yet to be explored.

The wide range of environments that *Heliconius* species inhabit, together with their diverse natural history and well-resolved phylogeny make them a good study system for teasing apart the selective forces driving wing phenotype (Merrill et al. 2015; Jiggins 2016). Here we examine variation in wing aspect ratio and size across 13 species that span most of the geographical range of the *Heliconius* genus. First, we photographed thousands of wings collected by many *Heliconius* researchers since the 1990s from wild populations across South and Central America, covering a 2100 m elevation range (Fig. 1 A). Wing dimensions for 3515 individuals, obtained with an automated pipeline and standardised images, were then used to address the following questions. (1) Are there size and aspect ratio sexual dimorphisms, and if so, do they correlate with known life-history traits? (2) To what extent are wing aspect ratio and size variation explained by shared ancestry? (3) Are wing aspect ratio and size affected by the elevations species inhabit?

Methods

STUDY COLLECTION

The wild specimens studied here were collected using hand nets between 1998 and 2018 in 313 localities across Panama, Colombia, Ecuador, French Guiana, Suriname, and Peru (Fig. 1 A), and stored in the Department of Zoology, University of Cambridge (Earthcape database). Collection altitudes ranged from sea level to 2100m above sea level (Fig 1 A). Detached wings were photographed dorsally and ventrally with a DSLR camera with a 100 mm macro lens in standardised conditions. All the images are available in the public repository Zenodo (https://zenodo.org/communities/butterfly/) and full records with data are stored in the EarthCape database (https://heliconius.ecdb.io).

WING MEASUREMENTS

Damage to wings was manually scored in all the images and damaged specimens were excluded from our analyses. To obtain wing measurements from the images, we developed custom scripts for Fiji (Schindelin et al., 2012), to automatically crop, extract the right or left forewing, and perform particle size analysis (Fig. 1 B). Butterflies predominantly use their forewings for flight (Wootton 2002; Le Roy et al. 2019) and hindwings tend to be more damaged in Heliconius due to in-flight predation and fragile structure, thus we only include forewings here. Forewing and hindwing areas are tightly correlated in this genus (Strauss, 1990). For wing area, we obtained total wing area (in mm^2, hereafter “size”).

For examining wing aspect ratio, the custom scripts first fitted an ellipse to the forewings and measured the length of the longest axis and the length of the axis at 90 degrees to the former (Fig. 1 C). Aspect ratio corresponds to the length of the major axis divided by the length of the minor axis, hereafter “aspect ratio” (Fig. 1 C). The data were checked for visual outliers on scatter-plots, which were examined, and removed from the analyses if the wing extraction pipeline had failed.

STATISTICAL ANALYSES

All analyses were run in R V2.13 (R Development Core Team 2011) and graphics were generated with the package *ggplot2* (Ginestet 2011). Packages are specified below. All R scripts can be found in the public repository Zenodo (Zenodo: TBC), including custom Fiji scripts for wing image analysis. Species and sexes mean trait values were calculated for the 13 *Heliconius* species in our study. Each species had more than 30 individuals and all individuals had accurate locality and altitude data (S.I.: Table S1), resulting in a dataset of 3515 individuals.

Sexual dimorphism across species

Sexual dimorphism in wing area and aspect ratio was estimated as the female increase in mean wing area and aspect ratio with respect to males, thus negative values represent larger trait values in males, while positive values represent larger trait values in females. Pairwise t-tests were used to estimate the significance of sexual size/shape dimorphism in each species.

We modelled variation in wing area and aspect ratio sexual dimorphism across species with ordinary least squares (OLS) linear regressions, implemented in the 'lm' function. For models of sexual wing area and aspect ratio sexual dimorphism, predictor variables initially included larval gregariousness of the species (gregarious or solitary, as classified in Beltrán et al. 2007), mating strategy (pupal-mating vs. adult-mating clade), species mean wing aspect ratio and area, and species wing aspect ratio or size sexual dimorphism (respectively). Wing size sexual dimorphism had a marginally significant phylogenetic signal (Abouheif Cmean=0.25, p=0.05), so we present the sexual size dimorphism model incorporating phylogeny as correlation term in the Supplementary Information (S.I., Table S3 and Table S4). We used backward selection with Akaike Information Criterion corrected for small sample sizes (AICc, Hurvich and Tsai 1989) where the best models had the lowest

AICc values, implemented with the package MuMin (Bartón 2018). We report the overall variation explained by the fitted linear models (R^2) and the relative contributions of each explanatory variable (partial R^2), estimated with the package *relaimpo* (Grömping 2006).

Variation across species

To test whether variation in wing aspect ratio and area across species was constrained by shared ancestry, we calculated the phylogenetic signal index Abouheif's Cmean (Abouheif 1999) which is an autocorrelation metric suitable for datasets with a relatively low number of species and that does not infer an underlying evolutionary model (Münkemüller et al. 2012). Observed and expected distribution plots for phylogenetic signal estimates are shown in the Supplementary Information and were computed with the package *adephylo* (Jombart and Dray 2010). We used a pruned tree with the 13 species under study from the most recent molecular *Heliconius* phylogeny (Kozak et al., 2015). We plotted centred trait means across the phylogeny with the function barplot.phylo4d() from the package *phylosignal* (Keck et al. 2016). To test and visualise phylogenetic signal further, we built phylocorrelograms for each trait with the function phyloCorrelogram() of the same package, which estimates Moran's I autocorrelation across matrices with varying phylogenetic weights. Then, the degree of correlation (Morans' I) in species trait values can be assessed as phylogenetic distance increases (Keck et al. 2016).

To study variation in wing area and aspect ratio across species we took a phylogenetic comparative approach. These methods assume that species-specific mean trait values are a good representation of the true trait values of the species under study, in other words, that the within-species variation is negligible compared to the across-species variation (Garamszegi 2014). To test this, we first used an ANOVA approach, with species as a factor explaining the variation of mean trait values. We then estimated within-species trait repeatability, or intra-class correlation coefficient (ICC), with a linear mixed model approach.

This requires the grouping factor to be specified as a random effect, in this case species, with a Gaussian distribution and 1000 parametric bootstraps to quantify uncertainty, implemented with the function rptGaussian() in *rptR* package (Stoffel et al. 2017). By specifying species as a random effect, the latter approach estimates the proportion of total trait variance accounted for by differences between species. A trait with high repeatability indicates that species-specific trait means are reliable estimates for further analyses (Stoffel et al. 2017). We, nevertheless, accounted for within-species variation in the models described below.

To test the effect of altitude on wing aspect ratio and size across species, we used a phylogenetic generalised least squares (PGLS) approach. Species wing trait means may be correlated due to shared ancestry (Freckleton et al. 2002; Chazot et al. 2016). Therefore, to explore the effects of the environment on the traits under study, models that incorporate expected correlation between species are required, such as PGLS. Although often ignored, these models assume the presence of phylogenetic signal on the model residuals of the trait under study (here wing aspect ratio or size) controlling for covariates that affect the trait mean (allometry, sex ratio) , and not just phylogenetic signal on the species mean trait values (Revell 2010; Garamszegi 2014). Thus, to check if this assumption was met we estimated phylogenetic signal as described above (Keck et al. 2016) for the residuals of a generalised least squares (GLS) of models that had wing aspect ratio or size as response variables, and the size and aspect ratio (respectively) and sex ratio as explanatory variables, to ensure this assumption of PGLS model was met. To visually inspect phylogenetic sinal on the residuals we obtained phylogenetic correlograms for these and centred trait residuals for plotting across the phylogeny as detailed above for trait means (presented in the S.I., Fig. S3 and Fig. S4 (Keck et al. 2016).

Significant phylogenetic signal was detected in mean wing size and in the residuals of both traits, wing aspect ratio and area regression models (S.I., Fig. S4, Fig. S5), so we used

maximum log-likelihood PGLS regression models with the phylogenetic correlation fitted as a correlation term, implemented with the gls() function from the *nmle* package (Pinheiro et al. 2007). We assumed a Brownian motion model of trait evolution for both traits, by which variation across species accumulated along all the branches at a rate proportional to the length of the branches (Freckleton et al. 2002). To select the most supported model given the available data, i.e. one that improves model fit while penalising complexity, we used the Aikaike Information Criteria corrected for small sample sizes (AICc, Hurvich and Tsai 1989), where the best models had the lowest AICc values, implemented with the package MuMin (Bartón 2018). Maximal PGLS models included species mean altitude and distance from the Equator (to control for potential latitudinal clines), sex ratio in our samples interacting with either wing aspect ratio or wing size, to control for potential allometric and sexual dimorphism relationships, which could be different among closely-related taxa (Outomuro and Johansson 2017). Most species are found in the Andean mountains or the Amazonian region near the Equator, so we did not have much power to examine variation with latitude in wing aspect ratio and size across species, but we included distance from the Equator as an explanatory variable in the PGLS models to account for it. Minimal PGLS models consisted of the trait under study explained solely by its intercept, without any fixed effects. All model selection tables can be found in the S.I. (Table S3, S5). Finally, we weighted PGLS regressions to account for unequal trait variances and unbalanced sample sizes across species (for sample sizes and standard errors of species' trait means see S.I. Table S1). This was achieved by modifying the error structure of the model with combined variances obtained with the function varFixed() and specified with the argument "weights" (Pinheiro et al. 2007; Paradis 2012; Garamszegi 2014). In this study, 74.8% of the individuals were collected in the last 10 years, thus we did not have power to detect any changes in wing morphology across species potentially incurred by climate change (Fig. S1). Future studies could focus on temporal changes in wing morphology in areas and species that have been well sampled throughout the years.

296 *Variation within species*

297 We selected the two most abundant and geographically widespread species within our
298 dataset, *H. erato* (n=1685) and *H. melpomene* (n= 912) (S.I. Table S1), to examine variation
299 in wing area and aspect ratio within species. We modelled variation in size and aspect ratio
300 with ordinary least squares (OLS) linear regressions for each species, implemented in the
301 'lm' function. For all models, predictor variables initially included the terms altitude, distance
302 from the Equator, longitude, aspect ratio or wing area, and sex, as well as the plausible
303 interactions between them (Table S5). We then used step backward and forward selection
304 based on AIC with the function stepAIC(), from the *MASS* package (Ripley, 2011; Zhang,
305 2016) (full models and model selection tables in S.I. Table S5, S6).

306

Results

We obtained intact-wing measurements for 3515 individuals of 13 *Heliconius* species from across the phylogeny and from over 350 localities (Fig. 1, Table S1). We have made all of these wing images publicly available at the Zenodo repository.

SEXUAL DIMORPHISM

Sexual dimorphism in wing area was found throughout the phylogeny, but in opposing directions in different species (Fig. 2). Mean sizes were significantly or marginally significantly different among sexes in nine species, all of which were represented by more than 40 individuals (S.I., Table S2 for two sample T-test summary statistics), indicating that the non-significant trends in other species probably reflect a lack of power caused by low numbers of females typically collected in the wild (S.I., Table S1). The six species with trends toward larger females have gregarious larvae (pink, Fig. 2), whereas the seven species with trends toward larger males lay eggs singly (black, Fig. 2). Larval gregariousness alone explained 69% of the total natural variation in sexual size dimorphism across species (Table 1; Gaussian LM: $F_{1,11}$= 27.2, P<0.001, R^2=0.69). There was a marginally significant phylogenetic signal in sexual size dimorphism (Abouheif's Cmean=0.24, P=0.05; S.I., Fig. S3), so we repeated the analysis accounting for phylogeny and the results are presented in the Supplementary Information. This would be expected from the evolutionary history of gregariousness, as it is present in all species of three lineages that are well represented in our study (Beltrán et al. 2007). However, when accounting for phylogenetic correlation in the model larval gregariousness remained a significant predictor of size sexual dimorphism (S.I., Table S4).

Sexual dimorphism in wing aspect ratio was found in three species (Fig. S4), *H. erato* and *H. wallacei* had longer-winged males whereas the high-altitude specialist *H. eleuchia* had longer-winged females (Table S2, T-test, *H. erato*: t_{843}=10.4, P<0.0001, *H. eleuchia*: t_{49}=-2.3,

p<0.05, *H. wallacei*: t_{19}=2.2, P<0.05). Wing aspect ratio sexual dimorphism across species could not be explained with the variables here studied and had no phylogenetic signal (Abouheif's Cmean=-0.02, P=0.3; S.I., Fig. S3).

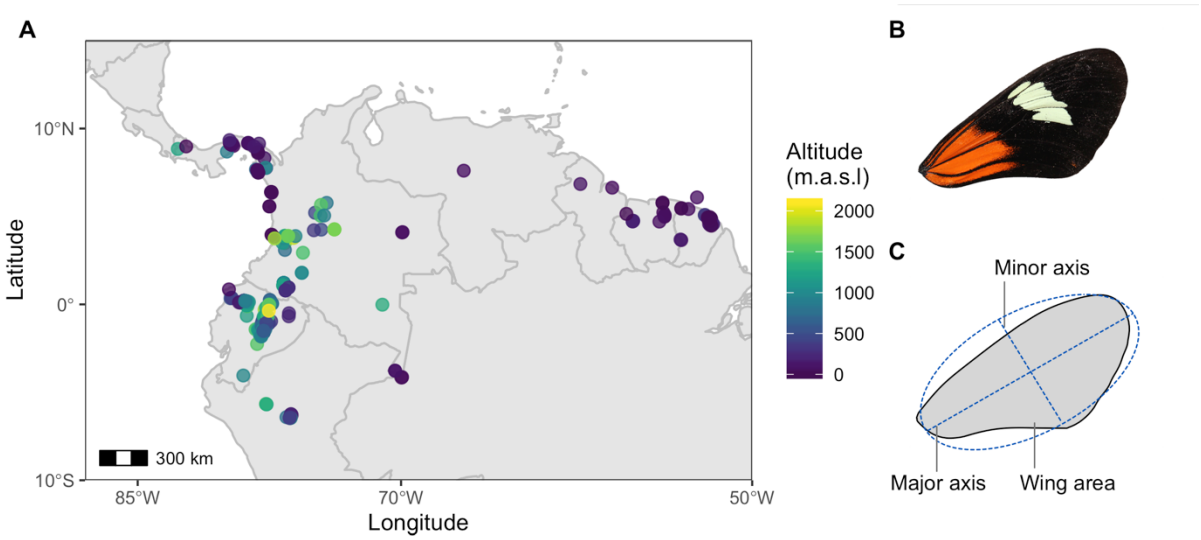

Figure 1. Localities and forewing measurements. (A) Map of exact locations (n=313) across South America from where the samples used for our analyses were collected. Points are coloured by altitude. (B) Representative of a right forewing image of *H. melpomene malleti*. (C) Measurements taken from each wing by fitting an ellipse with Fiji custom scripts.

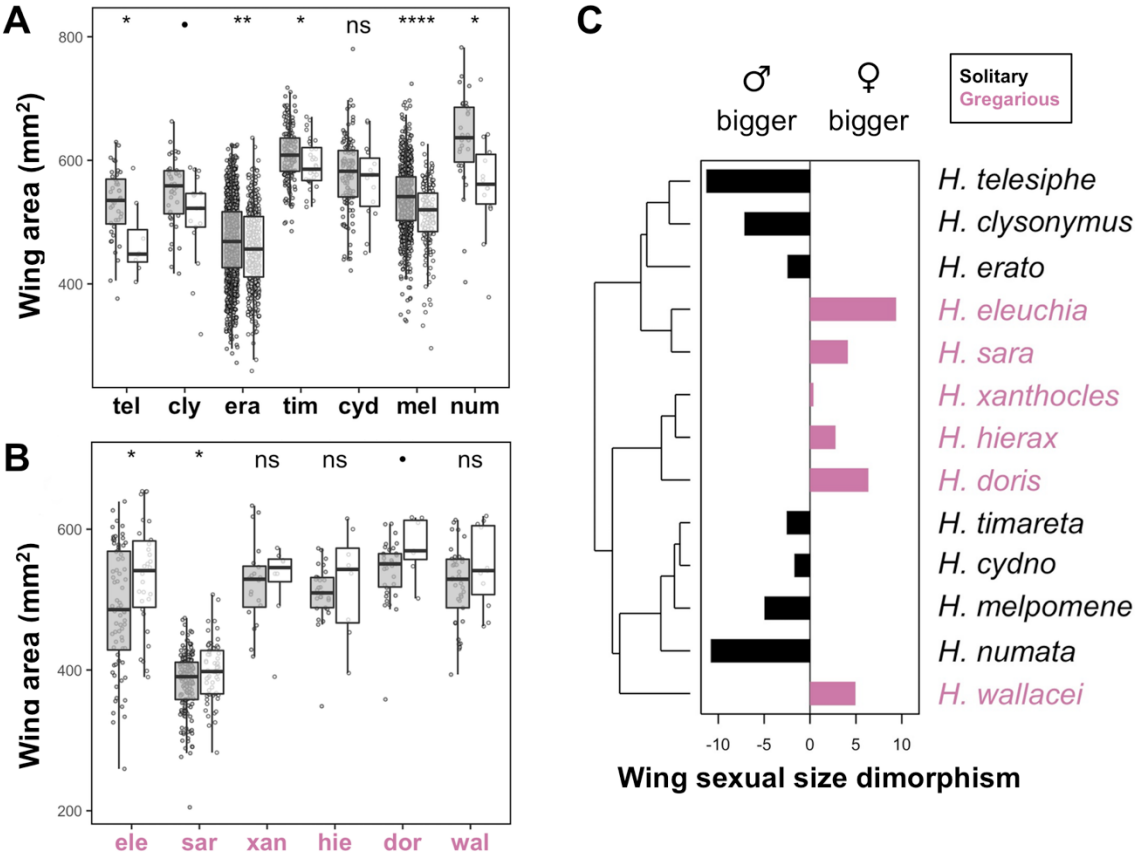

Figure 2. Sexual wing area dimorphism across species and the phylogeny. (A) Wing size differences between males (grey) and females (white) of the seven single egg-laying species and (B) the six gregarious species in this study. Error bars represent 95% confidence intervals of the means. Stars represent significance levels of two sample t-tests between female and male wing areas for each species (•<0.1, *< 0.05, **<0.01, ***<0.001), for full t-tests output see Table S1. (C) Bar plot represents sexual size dimorphism calculated as percentage difference in female vs. male size (positive means bigger females, right panel). Species with gregarious larvae are coloured in pink, and those with solitary larvae are coloured in black.

PHYLOGENETIC SIGNAL

The 13 *Heliconius* species studied differed significantly in wing area and aspect ratio (ANOVA, Aspect ratio: $F_{12, 3502} = 228.4$, $P < 0.0001$, Area: $F_{12, 3502} = 216.4$, $P < 0.0001$; Tukey-adjusted comparisons S.I. Fig. S2). We estimated within-species trait repeatability to assess their reliability as species mean estimates for phylogenetic analyses. Wing aspect ratio had higher intra-class repeatability than wing area, with 74% and 48% of the total aspect ratio and size variance explained by differences between species, respectively

(Aspect ratio: R=0.74, S.E.=0.09, P<0.0001; Size: R=0.48, S.E.=0.1, P<0.0001). We estimated intra-class repeatability for males and females separately to remove the potential effect of size sexual dimorphism on trait variation, and male size repeatability remained much lower than male wing aspect ratio repeatability (Male aspect ratio: R=0.75, S.E.=0.08, P<0.0001; Male Size: R=0.53, S.E.=0.1, P<0.0001). Females had the same wing aspect ratio repeatability as males, whereas wing size repeatability was lower for females probably due to smaller sample sizes (Female aspect ratio: R=0.75, S.E.=0.05 P<0.0001; Female Size: R=0.44, S.E.=0.1, P<0.0001).

Mean wing aspect ratio showed no phylogenetic signal (Abouheif's Cmean=0.15, P=0.1; S.I.: Fig. S3, Fig. S5 B), in other words closely-related species were not more similar to each other than to distant ones. In contrast, mean wing area showed a strong phylogenetic signal, by which phylogenetically closely-related species were more likely to have similar wing areas (Fig. 3, Abouheif's Cmean=0.33, P=0.01; S.I.: Fig. S3, Fig. S6 A, B). Wing areas of species in the melpomene clade were on average 14.8% larger than those of species in the erato clade, with *H. timareta* being 64% larger than *H. sara* (Fig. 3, *H. timareta*: mean=606.6 mm^2, s.e.=3.1; *H. sara*: mean=387 mm^2, s.e=2.9). Nevertheless, when controlling for sex ratios and allometry on the traits under study, wing aspect ratio and size, the residuals of both traits show a strong phylogenetic signal (S.I.: Fig. S5/6 AC; Aspect ratio residuals: Abouheif's Cmean=0.42, P<0.001; Fig. S3 A, C- Size residuals: Abouheif's Cmean=0.44, P<0.001). These results support the use of phylogenetic models to study variation in wing aspect ratio and size across species.

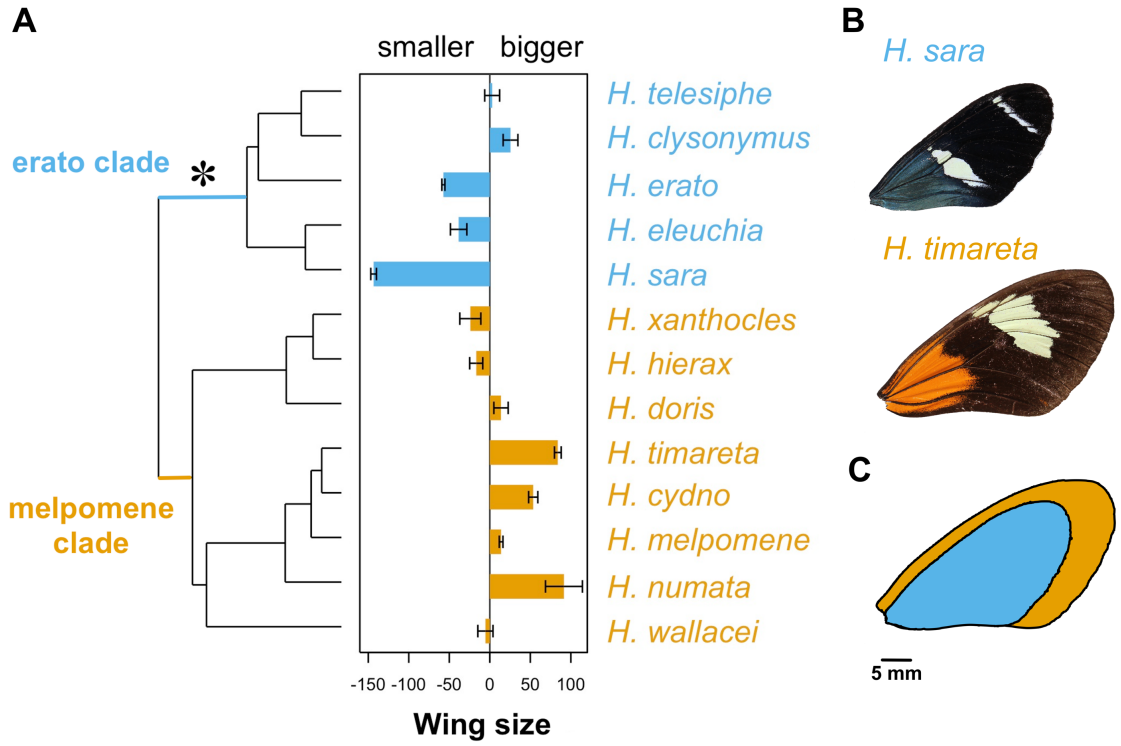

Figure 3. Male wing area differences across the phylogeny. (A) Bar plot represents centred mean wing area per species (positive values represent species with bigger wings than the average *Heliconius* wing). Wing area, x-axis, is the difference in wing area from the mean (in mm^2). Error bars represent standard errors. The star represents the origin of pupal-mating. Species from the erato clade are in blue, and those from the melpomene clade are in orange. (B) Representatives of *H. timareta* and *H. sara* closest to the mean wing area of the species are shown (606.25 mm^2 and 386.6 mm^2, respectively). (C) Images from (B) superimposed to compare visually the mean size difference between the two species.

PATTERNS ACROSS SPECIES AND ALTITUDES

Species mean altitude had an effect on wing area and aspect ratio (Table 1). Species wings got rounder, i.e. lower aspect ratios, with increasing altitudes both when accounting for fixed effects and the phylogeny (Table 1, full model Table S4). These patterns were also evident when examining raw mean wing aspect ratios (Fig. 4A, Gaussian LM: $F_{1,9} = 5.37$, $P < 0.05$, R^2=0.30), except in the *H. telesiphe* and *H. clysonymus* highlands clade, which showed significant phylogenetic autocorrelation (Moran's I index: *H. clysonymus* 0.53, *H. telesiphe* 0.49). Species wings got larger with elevation (Table 1, full model Table S4). Without accounting for phylogeny or any fixed effect this is only evident in the erato clade, where

high altitude species were bigger than their lowland sister species (Fig. 4B, blue, Gaussian LM: $F_{1,10}$= 17.1, R^2=0.80, p=0.03). However, when assessing individuals from all species together, it becomes clear that larger individuals of both clades tend to be found at higher altitudes (Fig. S8). Both wing size and wing aspect ratio were also significantly correlated with distance from the Equator, and wing aspect ratio was affected by species sex ratio too (S.I. Table S4).

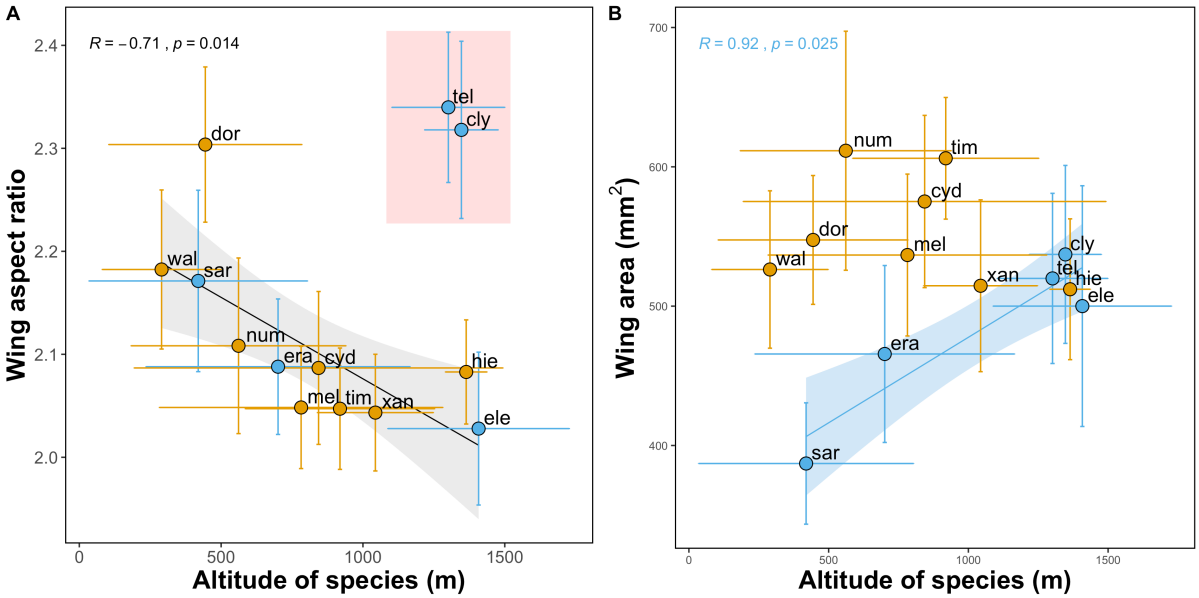

Figure 4. Species variation in wing aspect ratio (A) and wing area (B). Plots show the effect of altitude (meters above sea level) on wing aspect ratio (major axis/minor axis, higher values represent longer wings) and wing area (mm2). Points represent species mean raw values per species. Horizontal and vertical lines show standard error for species mean altitude and mean trait, respectively. Lines show best linear fit and are coloured by clade when clade was a significant predictor (blue: erato clade, orange: melpomene clade). Shaded areas show confidence bands at 1 standard error. The point labels correspond to the first three characters of the following *Heliconius* species: *H. telesiphe*, *H. clysonymus*, *H. erato*, *H. eleuchia*, *H. sara*, *H. doris*, *H. xanthocles*, *H. hierax*, am*H. wallacei, H. numata*, *H. melpomene*, *H. timareta*, *H. cydno.* Two species, *H. telesiphe* and *H. clysonymus*, showed high levels of phylogenetic autocorrelation (Fig. S7) and were thus excluded from the linear model plotted (but not from the main analyses where phylogeny is accounted for).

PATTERNS WITHIN SPECIES AND ACROSS ALTITUDES

Wings got rounder (lower aspect ratio) with increasing altitude in *H. erato* and *H. melpomene* (Fig. 5. *H. erato*: Gaussian LM: $F_{6, 1296}$ = 32.7, P < 0.001, R^2=0.13; *H. melpomene*: Gaussian

LM: $F_{6, 673} = 20.1$, $P < 0.001$, $R^2=0.14$). Individual altitude was the strongest predictor of wing aspect ratio for both species, with sex and wing area being second best in *H. erato* and *H. melpomene*, respectively (Table S6, Fig. S13 A and B, Fig. 5). Conversely, the relative importance of explanatory variables of wing area varied for each species (Table S6, Fig. S13 A and B, Fig. 5), and the *H. erato* model explained less of the overall variation in wing area (Fig. S11, *H. erato*: Gaussian LM: $F_{7,1295} = 9.36$, $P < 0.001$, $R^2=0.04$, *H. melpomene*: Gaussian LM: $F_{7, 672} = 23.06$, $P < 0.001$, $R^2=0.18$). Wing area in *H. erato* was correlated with allometric factors interacting with altitude, whereas wing area in *H. melpomene* was correlated with distance from the Equator (Table S6, Fig. S10 and S13 C and D). Wing area and aspect ratio differed among co-mimicking races of *H. erato* and *H. melpomene*, despite inhabiting the same geographic areas (Fig. S12).

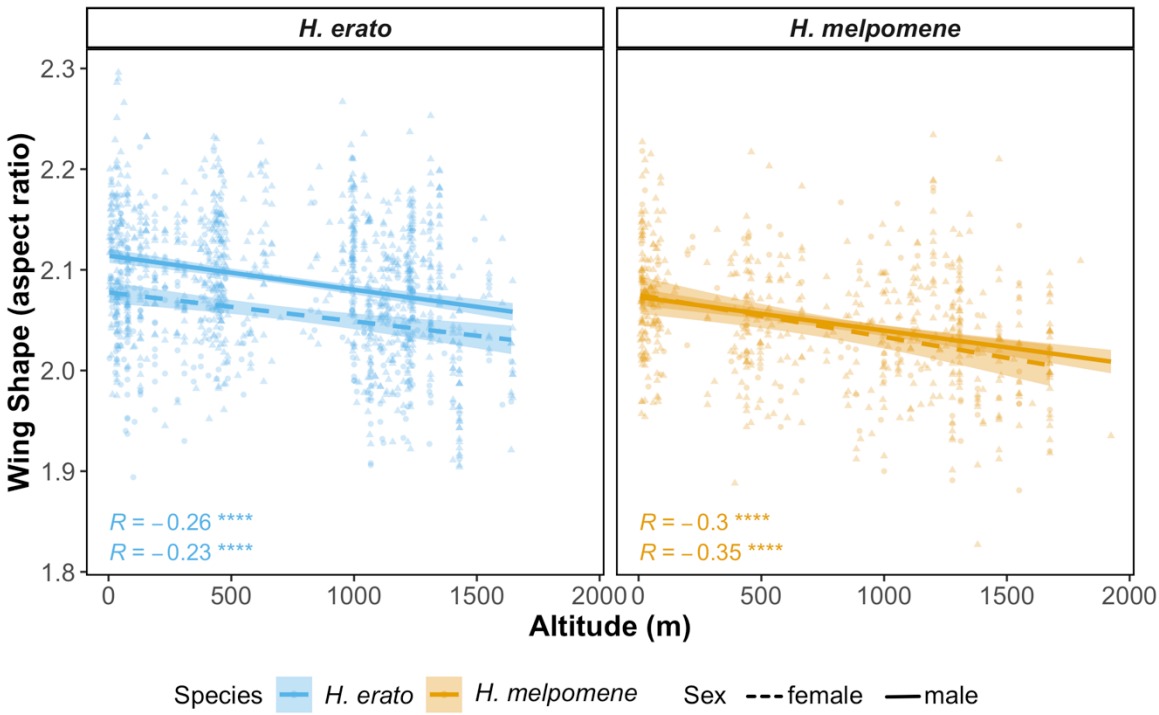

Figure 5. Within-species variation in wing aspect ratio across altitudes in *H. erato* (blue) and *H. melpomene* (orange), females (triangles, dotted line) and males (circles, solid line). Lines show best linear fit and are colored by species. Shaded areas show confidence bands at 1 standard error. Pearson correlation coefficients and p-values are shown for each regression plotted.

Discussion

The fascination for butterfly wing colouration has stimulated many generations of research and *Heliconius* wing patterns have proven to be excellent study systems for understanding evolution and speciation. Here we have extended this research by examining wing shape and size variation among more than 3500 individual butterflies, across sexes, clades, and altitudes in 13 species of *Heliconius* butterflies. We have shown that a large proportion of female biased sexual size dimorphism can be explained by the evolution of larval gregariousness, and that male biased sexual size dimorphism is present only in species that lay eggs singly, regardless of their mating strategy. For the first time in this system, we describe wing morphological variation across environmental clines, with species and populations found at higher altitudes consistently having rounder wings. Here we demonstrate that *Heliconius* wing area and aspect ratio are potentially shaped by a plethora of behavioural and environmental selection pressures, in addition to those imposed by Müllerian mimicry.

WING ASPECT RATIO VARIATION

Wing aspect ratio in butterflies and other flying animals determines flight mode and speed (Farney and Fleharty 1969; Buler et al. 2017), and is therefore predicted to vary with life-history requirements across sexes and species. Despite being a simple descriptor of wing shape, aspect ratio has been demonstrated to correlate functionally with gliding efficiency in butterflies by increasing lift-to-drag ratios (Ortega Ancel et al. 2017; Le Roy et al. 2019). Long wings are generally associated with faster gliding flying, whereas round wings with low aspect ratio values favour slow but more manoeuvrable flight motions (Betts and Wootton 1988; Chai and Srygley 1990; Chazot et al. 2016; Le Roy et al. 2019). For instance, monarch butterfly populations with longer migrations have more elongated wings than resident populations (Satterfield and Davis 2014), and males of *Morpho* species that dwell in the canopy also have higher aspect ratios to glide faster through open areas (DeVries et al.

2010). In contrast, female *Morpho* butterflies tend to have rounder wings, and aspect ratio sex differences are stronger in species with colour dimorphism, as varying crypsis may require specific flight behaviours (Chazot et al. 2016).

Heliconius are not notoriously sexually dimorphic especially when compared to other butterflies such as *Morpho* (Chazot et al. 2016; Jiggins 2016). However, there are important behavioural differences between the sexes. Females are thought to have different flight habits, as they spend much of their time looking for specific host plants for oviposition (Dell'Aglio et al. 2016), or precisely laying eggs on suitable plants, while males tend to patrol open areas searching for receptive females and visit flowers more often (Joron 2005; Jiggins 2016). Thus, it might be predicted that females should have lower aspect ratios, i.e. rounder wings, than males (Jones et al. 2013). However, we only found three species with significant, but opposing, sexually dimorphic wing aspect ratios. The wings of males in *H. erato* were longer than the wings in females, whereas male *H. eleuchia* and *H. wallacei* had rounder wings than those of females (S.I. Fig. S3). *Heliconius* wing shape sex differences may require multivariate descriptors of wing morphology and/or analysis of the hindwings, which possess the pheromone-dispersing androconial patch in males (Jones et al. 2013; Mérot et al. 2013, 2016). In addition, the relatively low collection numbers of female *Heliconius* could hinder the detection of subtle wing aspect ratio differences across the sexes.

Sexual selection has long been known to affect wing colour pattern in *Heliconius*, as it is used for mate recognition and choice (Merrill et al. 2012). More recently, wing aspect ratio has been shown to be part of the mimetic warning signal in *Heliconius* and their co-mimics (Jones et al. 2013), as it determines flight motion and defines the overall appearance of the butterfly (Srygley 1994, 2004a). For instance, wing aspect ratios between two different morphs of *H. numata* differed consistently across their overlapping ranges, in parallel with their respective and distantly related *Melinea* co-mimics (Jones et al. 2013). Within-morph

wing aspect ratio variation was observed across the altitudinal range of *H. timareta* in Peru (Mérot et al. 2016), and in the *Heliconius* postman mimicry ring in Brazil significant across-species wing aspect ratio differences were also found (Rossato et al. 2018a). These studies highlight that while it is clear that colour pattern and, to some extent, flight are important for mimicry in *Heliconius*, wing aspect ratio is also subject to other selection pressures (Mérot et al. 2016; Rossato et al. 2018b).

We found that species inhabiting higher altitudes tend have rounder wings, after accounting for phylogeny, sample size and intra-specific variance (Fig. 4 A), except in the *H. telesiphe* – *H. clysonymus* clade. The latter species may require morphometric analyses of wing tip shape alone, as the overall wing morphology differs significantly from the rest of the *Heliconius* species here studied (Fig. S7). Interestingly, these patterns were maintained within-species, with high-altitude populations of *H. erato* and *H*. melpomene having lower aspect ratios (Fig. 5). Furthermore, altitude was the best predictor of wing aspect ratio in both species (Fig. S13). Rounder wings aid manoeuvrability and are associated with slower flight in butterflies (Berwaerts et al. 2002; Le Roy et al. 2019) and slower flights are generally associated with a decrease in ambient temperature (Gilchrist et al. 2000). In addition, air pressure, which directly reduces lift forces required to offset body weight during flight (Dillon 2006), decreases approximately 12% across the mean altitudinal range of the species here studied. Thus, the rounder wings in high altitude *Heliconius* species and populations may aid flying in dense cloud forests, where increased manoeuvrability could be beneficial, or compensating for lower air pressure at higher altitude.

WING AREA VARIATION

Wing area showed significant sexual dimorphism in more than half of the species studied here, but some species had larger males and others larger females (Fig. 2). In most butterflies, females are overall larger than males, presumably because fecundity gains of increased body size are greater for females (Allen et al. 2011). Larger wings are required to

carry larger and heavier bodies, and so Lepidoptera females also tend to have larger wings (Allen et al. 2011; Le Roy et al. 2019). Indeed, in this study the *Heliconius* species with larger-winged females were those that lay eggs in large clutches and that have highly gregarious larvae (Fig. 2 , Beltrán et al. 2007). A recent study on two species not included here reported wing size dimorphism with larger females in the gregarious *H. eratosignis ucayalensis* and larger males in the single-egg layer *H. demeter joroni* (Rosser et al. 2019). Thus, females of these species are likely investing more resources in fecundity than males, which leads to larger body and wing sizes that allow them to carry and lay eggs in clutches throughout adulthood. Larval development time correlates with adult size in *H. erato* (Rodrigues and Moreira 2002) and growth rates seem to be the same across sexes, at least in the gregarious *H. charithonia* (Kemp 2019), so we hypothesize that females take longer to develop in gregarious species. Selection for larger females is generally constrained by a trade-off between the benefits of increased fecundity at the adult stage and the higher predation risk at the larval stage associated with longer development times (Allen et al. 2011). This constraint might be alleviated in the unpalatable larvae of *Heliconius*, as bigger larval and adult size could increase the strength of the warning toxic signal to predators (Jiggins 2016).

An extensive survey identified that only six percent of lepidopteran species exhibit male-biased sexual size dimorphism, and that these patterns were generally explained by male-male competition (i.e. intrasexual selection), in which larger males had a competitive advantage (Stillwell et al. 2010; Allen et al. 2011). In contrast, nearly half of the *Heliconius* species studied here have male-biased sexual size dimorphism, and all of these lay eggs singly and have solitary larvae (Fig. 2). Male-male competition is high for *Heliconius* species, as females rarely re-mate despite their very long reproductive life-spans (Merrill et al. 2015). In addition, large reproductive investments in the form of nuptial gifts from males can, in principle, explain male-biased sexual size dimorphisms, as is the case in the polyandrous butterfly *Pieris napi* whose male spermatophore contains the amount of nitrogen equivalent

561 to 70 eggs (Karlsson 1998; Allen et al. 2011). Male *Heliconius* spermatophores are not only
562 nutrient-rich, but also loaded with anti-aphrodisiac pheromones that prevent re-mating of
563 fertilised females (Schulz et al. 2008; Merrill et al. 2015). Therefore, it seems likely that in
564 species that lay eggs singly, sexual selection favouring larger males exceeds selection
565 pressures for the large female size needed to carry multiple mature eggs. To our knowledge,
566 *Heliconius* is the first example of a butterfly genus in which both female- and male-biased
567 size dimorphism are found and can be explained by contrasting reproductive strategies.
568
569 We found a strong phylogenetic signal for wing area, with species from the erato clade being
570 on average 12% smaller than those in the melpomene clade (Fig. 3). There are many
571 ecological factors that could explain this pattern, and all could have contributing effects that
572 are hard to disentangle (Fig. 3). Firstly, the erato clade is characterised by facultative pupal-
573 mating (Beltrán et al. 2007; Jiggins 2016), by which males fight for pupae, guard them, and
574 mate with females as they are emerging from the pupal case (Deinert et al. 1994; Jiggins
575 2016). Smaller males have been shown to outcompete others for a spot on the female pupal
576 case and more successfully inseminate emerging females compared to larger, less agile
577 males (Deinert et al. 1994), which would remove the potential choice of females for larger
578 males. Secondly, pupal-mating seems to have far-reaching impacts on species life-histories
579 (Boggs 1981). Species in the melpomene or adult-mating clade are polyandrous, which
580 leads to selection favouring large spermatophores (Boggs 1981) to provide mated females
581 with abundant nutritional resources and defences that prevent them from re-mating with
582 other males (Cardoso et al. 2009; Cardoso and Silva 2015). This could decrease selection
583 pressure for larger males in the pupal-mating clade, as nuptial gifts need not be so large or
584 nutrient/defence rich, leading to smaller male and female offspring. However, the single
585 origin of pupal-mating in *Heliconius* (Fig. 2) makes it challenging to infer the impacts of this
586 mating strategy on wing morphology, as the behaviour is confounded by phylogeny.
587

Wing area across species positively correlated with altitude in the erato clade (Fig. 4B), but no clear pattern was found for the melpomene clade species here studied. In contrast, wing area variation within-species (*H. erato* and *H. melpomene*) was more correlated with geography (distance to Equator, longitude) and allometry than with altitude (Fig. S10). Nevertheless, high-altitude populations of *H. melpomene* were slightly bigger than their lowland conspecifics, whereas *H.* erato did not change (S.I., Fig. S13). Two major environmental factors are known to affect insect size across altitudinal clines. One is temperature, such that at lower temperatures, development times are longer and insects grow larger (Chown and Gaston 2010). This perhaps explains cases of Bergmann's rule among ectotherms, where larger species are found in colder climates (Shelomi 2012; Classen et al. 2017). In the geographical range here studied (Fig. 1), we predict temperatures to vary more dramatically along elevational gradients than latitudinal gradients (García-Robledo et al. 2016). We found some evidence for species being bigger with increasing latitudes when accounting for phylogeny and allometry (Table S4), in accordance with Bergmann's rule, but more species at the extremes of the ranges are needed to clarify this (Fig. S7).

Wing beat frequency tends to be lower at low temperatures, so larger wings are required to compensate and gain the extra lift required for flight, as seen in *Drosophila robusta* (Azevedo et al. 2006; Dillon 2006). A second factor likely to contribute to altitude related differences in wing area is air pressure changes and the correlated lower oxygen availability, which affects flight motion and kinematics as well as many physiological processes. High-altitude insects can minimise the impacts of lower air pressure by having larger wings, because this lowers the velocity required to induce flight (Dudley 2002).

HERITABILITY

Our study demonstrates that multiple selective forces may be affecting *Heliconius* wing area and aspect ratio. However, this raises the question of how plastic these traits are in the wild.

In *Drosophila*, the genetic architecture of wing aspect ratio appears to be complex (Gilchrist and Partridge 2001), and is independent of that of wing area (Carreira et al. 2011). Within-species variability of wing area halved when flies were reared in controlled conditions compared to wild populations whereas wing shape variability remained the same, but both traits had a detectable and strong heritable component (Bitner-Mathé and Klaczko 1999; Klaczko 1999). In this study we found that 74% of the variation in wing aspect ratio could be explained by species identity, in contrast to 48% of the variation in wing area. This high and moderate intra-class repeatability is indicative of heritable traits (Nakagawa and Schielzeth 2013). The fact that closely related species are more likely to have similar wing morphologies, i.e. phylogenetic signal, is also indicative of species-level heritability (Queiroz and Ashton 2004).

In insects wing shape is functionally more constrained than wing size. For example, genetic manipulations of wing shape in *Drosophila melanogaster* have shown that even subtle changes can have huge biomechanic impacts (Ray et al. 2016), whereas wing/body size differences may impact fecundity more than survival. Here we find size differences between sexes that can be explained by reproductive strategy, and are likely to be genetically controlled as most sexual dimorphisms are (Allen et al. 2011).The patterns of variation in size across altitudes or latitudes are often not due to phenotypic plasticity alone, as many studies have shown their retention when populations are reared in common-garden conditions (Chown and Gaston 2010). In Monarch butterflies, for example, common-garden reared individuals from wild populations that had different migratory habits showed a strong genetic component for both wing aspect ratio and size (Altizer and Davis 2010).

We have shown that different selection pressures may be shaping the evolution of wing morphology in *Heliconius* and that the strength of these varies across sexes and environmental clines. Interestingly some of these patterns are maintained at the intra-specific level, with high-altitude populations of *H. erato* and *H.* melpomene having rounder

wings (Fig. 5), thus potentially adapting locally to the environment in the same way that species of this genus have adapted to altitude over longer evolutionary timescales (Fig. 4). Future work should assess the adaptive significance, plasticity, and heritability of these traits with common-garden rearing and physiological assays in controlled conditions.

CONCLUSIONS

Here we have demonstrated how an understanding of natural and evolutionary history can help to disentangle the putative agents of selection on an adaptive trait. Wing trait differences across sexes, clades and environments give insight into the selective forces driving phenotypic divergence in *Heliconius*, beyond the effects of natural selection imposed by Müllerian mimicry. Our study highlights the complexity of selection pressures affecting seemingly simple traits and the need for a thorough understanding of life history differences amongst species.

References

Allen, C. E., B. J. Zwaan, and P. M. Brakefield. 2011. Evolution of Sexual Dimorphism in the Lepidoptera. Annual Review of Entomology, doi: 10.1146/annurev-ento-120709-144828.

Altizer, S., and A. K. Davis. 2010. Populations of monarch butterflies with different migratory behaviors show divergence in wing morphology. Evolution, doi: 10.1111/j.1558-5646.2009.00946.x.

Azevedo, R. B. R., A. C. James, J. McCabe, and L. Partridge. 2006. Latitudinal Variation of Wing: Thorax Size Ratio and Wing-Aspect Ratio in Drosophila melanogaster. Evolution, doi: 10.2307/2411305.

Bartón, K. 2018. MuMIn: Multi-Model Inference.

Beltrán, M., C. Jiggins, A. Brower, E. Bermingham, and J. Mallet. 2007. Do pollen feeding and pupal-mating have a single origin in *Heliconius* butterflies? Inferences from multilocus sequence data. Biological Journal of the Linnean Society.

Berwaerts, K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly Pararge aegeria. Functional Ecology, doi: 10.1046/j.1365-2435.2002.00650.x.

Betts, C. R., and R. J. Wootton. 1988. Wing shape and flight behaviour in butterflies (Lepidoptera: Papilionoidea and Hesperioidea): a preliminary analysis. Journal of Experimental Biology.

Bitner-Mathé, B. C., and L. B. Klaczko. 1999. Size and shape heritability in natural populations of Drosophila mediopunctata: temporal and microgeographical variation. Heredity 35–42.

Boggs, C. L. 1981. Selection Pressures Affecting Male Nutrient Investment at Mating in Heliconiine Butterflies. Evolution, doi: 10.2307/2407864.

Breuker, C. J., P. M. Brakefield, and M. Gibbs. 2007. The association between wing morphology and dispersal is sex-specific in the glanville fritillary butterfly Melitae

cinxia (Lepidoptera: Nymphalidae). European Journal of Entomology, doi: 10.14411/eje.2007.064.

Buler, J. J., R. J. Lyon, J. A. Smolinsky, T. J. Zenzal, and F. R. Moore. 2017. Body mass and wing shape explain variability in broad-scale bird species distributions of migratory passerines along an ecological barrier during stopover. Oecologia, doi: 10.1007/s00442-017-3936-y.

Cardoso, M. Z., J. J. Roper, and L. E. Gilbert. 2009. Prenuptial agreements: Mating frequency predicts gift-giving in Heliconius species. Entomologia Experimentalis et Applicata, doi: 10.1111/j.1570-7458.2009.00837.x.

Cardoso, M. Z., and E. S. Silva. 2015. Spermatophore Quality and Production in two Heliconius Butterflies with Contrasting Mating Systems. Journal of Insect Behavior, doi: 10.1007/s10905-015-9536-y.

Carreira, V. P., I. M. Soto, J. Mensch, and J. J. Fanara. 2011. Genetic basis of wing morphogenesis in Drosophila: Sexual dimorphism and non-allometric effects of shape variation. BMC Developmental Biology, doi: 10.1186/1471-213X-11-32.

Cespedes, A., C. M. Penz, and P. J. Devries. 2015. Cruising the rain forest floor: Butterfly wing shape evolution and gliding in ground effect. Journal of Animal Ecology, doi: 10.1111/1365-2656.12325.

Chai, P., and R. B. Srygley. 1990. Predation and the Flight, Morphology, and Temperature of Neotropical Rain-Forest Butterflies. The American Naturalist 135:748–765.

Chazot, N., S. Panara, N. Zilbermann, P. Blandin, Y. Le Poul, R. Cornette, M. Elias, and V. Debat. 2016. Morpho morphometrics: Shared ancestry and selection drive the evolution of wing size and shape in Morpho butterflies.

Chown, S. L., and K. J. Gaston. 2010. Body size variation in insects: A macroecological perspective. Biological Reviews 85:139–169.

Classen, A., I. Steffan-Dewenter, W. J. Kindeketa, and M. K. Peters. 2017. Integrating intraspecific variation in community ecology unifies theories on body size shifts along climatic gradients. Functional Ecology 31:768–777.

Cook, L. M., E. W. Thomason, and A. M. Young. 1976. Population Structure, Dynamics and Dispersal of the Tropical Butterfly Heliconius charitonius. Journal of Animal Ecology 45:851–863.

Deinert, E. I., J. T. Longino, and L. E. Gilbert. 1994. Mate competition in butterflies [5]. Nature, doi: 10.1038/370023a0.

Dell'Aglio, D. D., M. E. Losada, and C. D. Jiggins. 2016. Butterfly Learning and the Diversification of Plant Leaf Shape. Frontiers in Ecology and Evolution, doi: 10.3389/fevo.2016.00081.

DeVries, P. J., C. M. Penz, and R. I. Hill. 2010. Vertical distribution, flight behaviour and evolution of wing morphology in Morpho butterflies. Journal of Animal Ecology, doi: 10.1111/j.1365-2656.2010.01710.x.

Dillon, M. E. 2006. Into thin air: Physiology and evolution of alpine insects. Integrative and Comparative Biology 46:49–61.

Dillon, M. E., M. R. Frazier, R. Dudley, S. Integrative, C. Biology, N. Feb, M. E. Dillon, M. R. Frazier, and R. Dudleyt. 2018. Into Thin Air : Physiology and Evolution of Alpine Insects Stable URL : http://www.jstor.org/stable/3884976 REFERENCES Linked references are available on JSTOR for this article : Into thin air : Physiology and evolution of alpine insects. 46:49–61.

Dudley, R. 2002. The Biomechanics of Insect Flight: Form, Function, Evolution. Annals of the Entomological Society of America, doi: 10.1093/aesa/93.5.1195f.

Dunn, P. O., J. K. Armenta, and L. A. Whittingham. 2015. Natural and sexual selection act on different axes of variation in avian plumage color. Science Advances, doi: 10.1126/sciadv.1400155.

Farney, J., and E. D. Fleharty. 1969. Aspect Ratio, Loading, Wing Span, and Membrane Areas of Bats. Journal of Mammalogy, doi: 10.2307/1378361.

Freckleton, R. P., P. H. Harvey, and M. Pagel. 2002. Phylogenetic Analysis and Comparative Data: A Test and Review of Evidence. The American Naturalist, doi: 10.1086/343873.

Garamszegi, L. Z. 2014. Modern phylogenetic comparative methods and their application in evolutionary biology.

García-Robledo, C., E. K. Kuprewicz, C. L. Staines, T. L. Erwin, and W. J. Kress. 2016. Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction. Proceedings of the National Academy of Sciences 113:680–685.

Gilchrist, A. S., R. B. R. Azevedo, L. Partridge, and P. O'Higgins. 2000. Adaptation and constraint in the evolution of Drosophila melanogaster wing shape. Evolution and Development 2:114–124.

Gilchrist, A. S., and L. Partridge. 2001. The contrasting genetic architecture of wing size and shape in Drosophila melanogaster. Heredity, doi: 10.1046/j.1365-2540.2001.00779.x.

Ginestet, C. 2011. ggplot2: Elegant Graphics for Data Analysis. Journal of the Royal Statistical Society: Series A (Statistics in Society), doi: 10.1111/j.1467-985X.2010.00676_9.x.

Grömping, U. 2006. Relative Importance for Linear Regression in *R*: The Package **relaimpo**. Journal of Statistical Software, doi: 10.18637/jss.v017.i01.

Hurvich, C. M., and C.-L. Tsai. 1989. Regression and time series model selection in small samples. Biometrika 76:297–307.

Jiggins, C. D. 2016. The Ecology and Evolution of Heliconius Butterflies. Oxford University Press.

Jombart, T., and S. Dray. 2010. Adephylo: Exploratory Analyses for the Phylogenetic Comparative Method. Bioinformatics, doi: 10.1093/bioinformatics/btq292.

Jones, R. T., Y. L. Poul, A. C. Whibley, C. Mérot, R. H. ffrench-Constant, and M. Joron. 2013. Wing Shape Variation Associated With Mimicry In Butterflies. Evolution 67:2323–2334.

Joron, M. 2005. Polymorphic mimicry, microhabitat use, and sex-specific behaviour. Journal of Evolutionary Biology, doi: 10.1111/j.1420-9101.2005.00880.x.

Karlsson, B. 1998. Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. Ecology, doi: 10.1890/0012-9658(1998)079[2931:NGRBAR]2.0.CO;2.

Keck, F., F. Rimet, A. Bouchez, and A. Franc. 2016. Phylosignal: An R package to measure, test, and explore the phylogenetic signal. Ecology and Evolution, doi: 10.1002/ece3.2051.

Kemp, D. J. 2019. Manipulation of natal host modifies adult reproductive behaviour in the butterfly Heliconius charithonia. Proceedings of the Royal Society B: Biological Sciences 286:20191225.

Klaczko, L. B. 1999. Size and shape heritability in natural populations of. Heredity 35–42.

Klepsatel, P., M. Gáliková, C. D. Huber, and T. Flatt. 2014. Similarities and differences in altitudinal versus latitudinal variation for morphological traits in drosophila melanogaster. Evolution 68:1385–1398.

Kozak, K. M., W. O. McMillan, M. Joron, and C. D. Jiggins. 2018. Genome-wide admixture is common across the *Heliconius* radiation. Evolutionary Biology.

Kozak, K. M., N. Wahlberg, A. F. E. Neild, K. K. Dasmahapatra, J. Mallet, and C. D. Jiggins. 2015. Multilocus species trees show the recent adaptive radiation of the mimetic heliconius butterflies. Systematic Biology 64:505–524.

Le Roy, C., V. Debat, and V. Llaurens. 2019. Adaptive evolution of butterfly wing shape: from morphology to behaviour. Biological Reviews, doi: 10.1111/brv.12500.

Losos, J. B. 2010. Adaptive Radiation, Ecological Opportunity, and Evolutionary Determinism. The American Naturalist, doi: 10.1086/652433.

Maia, R., D. R. Rubenstein, and M. D. Shawkey. 2016. Selection, constraint, and the evolution of coloration in African starlings. Evolution, doi: 10.1111/evo.12912.

Marques, D. A., J. I. Meier, and O. Seehausen. 2019. A Combinatorial View on Speciation and Adaptive Radiation.

Mendoza-Cuenca, L., and R. MacÍas-Ordóñez. 2010. Female asynchrony may drive disruptive sexual selection on male mating phenotypes in a Heliconius butterfly. Behavioral Ecology 21:144–152.

Mérot, C., Y. Le Poul, M. Théry, and M. Joron. 2016. Refining mimicry: phenotypic variation tracks the local optimum. The Journal of animal ecology, doi: 10.1111/1365-2656.12521.

Mérot, C., J. Mavárez, A. Evin, K. K. Dasmahapatra, J. Mallet, G. Lamas, and M. Joron. 2013. Genetic differentiation without mimicry shift in a pair of hybridizing Heliconius species (Lepidoptera: Nymphalidae). Biological Journal of the Linnean Society 109:830–847.

Merrill, R. M., K. K. Dasmahapatra, J. W. Davey, D. D. Dell'Aglio, J. J. Hanly, B. Huber, C. D. Jiggins, M. Joron, K. M. Kozak, V. Llaurens, S. H. Martin, S. H. Montgomery, J. Morris, N. J. Nadeau, A. L. Pinharanda, N. Rosser, M. J. Thompson, S. Vanjari, R. W. R. Wallbank, and Q. Yu. 2015. The diversification of Heliconius butterflies: What have we learned in 150 years? Journal of Evolutionary Biology 28:1417–1438.

Merrill, R. M., R. W. R. Wallbank, V. Bull, P. C. A. Salazar, J. Mallet, M. Stevens, and C. D. Jiggins. 2012. Disruptive ecological selection on a mating cue. Proceedings of the Royal Society B: Biological Sciences, doi: 10.1098/rspb.2012.1968.

Münkemüller, T., S. Lavergne, B. Bzeznik, S. Dray, T. Jombart, K. Schiffers, and W. Thuiller. 2012. How to measure and test phylogenetic signal. Methods in Ecology and Evolution 3:743–756.

Nadeau, N. J., C. Pardo-Diaz, A. Whibley, M. A. Supple, S. V. Saenko, R. W. R. Wallbank, G. C. Wu, L. Maroja, L. Ferguson, J. J. Hanly, H. Hines, C. Salazar, R. M. Merrill, A. J. Dowling, R. H. Ffrench-Constant, V. Llaurens, M. Joron, W. O. McMillan, and C. D. Jiggins. 2016. The gene cortex controls mimicry and crypsis in butterflies and moths. Nature, doi: 10.1038/nature17961.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142.

Nosil, P., R. Villoutreix, C. F. De Carvalho, T. E. Farkas, V. Soria-Carrasco, J. L. Feder, B. J. Crespi, and Z. Gompert. 2018. Natural selection and the predictability of evolution in timema stick insects. Science, doi: 10.1126/science.aap9125.

Ortega Ancel, A., R. Eastwood, D. Vogt, C. Ithier, M. Smith, R. Wood, and M. Kovač. 2017. Aerodynamic evaluation of wing shape and wing orientation in four butterfly species using numerical simulations and a low-speed wind tunnel, and its implications for the design of flying micro-robots. Interface Focus 7:20160087.

Outomuro, D., and F. Johansson. 2017. A potential pitfall in studies of biological shape: Does size matter? Journal of Animal Ecology, doi: 10.1111/1365-2656.12732.

Paradis, E. 2012. Analysis of phylogenetics and evolution with R: Second edition.

Pinheiro, J., D. Bates, S. DebRoy, and D. Sarkar. 2007. nlme: Linear and Nonlinear Mixed Effects Models. R Development Core Team, doi: Doi 10.1038/Ncb1288.

Pitchers, W., J. E. Pool, and I. Dworkin. 2012. ALTITUDINAL CLINAL VARIATION IN WING SIZE AND SHAPE IN AFRICAN DROSOPHILA MELANOGASTER : ONE CLINE OR MANY ? , doi: 10.5061/dryad.r43k1.

Pyrcz, T. W., K. Sattler, D. C. Lees, G. W. Beccaloni, J. R. Ferrer-Paris, J. Wojtusiak, and A. L. Viloria. 2004. A brachypterous butterfly? Proceedings of the Royal Society of London. Series B: Biological Sciences, doi: 10.1098/rsbl.2003.0015.

Queiroz, A. de, and K. G. Ashton. 2004. The phylogeny of a species-level tendency: species heritability and possible deep origins of Bergmann's rule in tetrapods. evol 58:1674–1684.

Ray, R. P., T. Nakata, P. Henningsson, and R. J. Bomphrey. 2016. Enhanced flight performance by genetic manipulation of wing shape in Drosophila. Nat Commun 7:1–8.

Revell, L. J. 2010. Phylogenetic signal and linear regression on species data. Methods in Ecology and Evolution, doi: 10.1111/j.2041-210X.2010.00044.x.

Rodrigues, D., and G. R. P. Moreira. 2002. Geographical variation in larval host-plant use by Heliconius erato (Lepidoptera: Nymphalidae) and consequences for adult life history. Brazilian Journal of Biology 62:321–32.

Rodrigues, D., and G. R. P. Moreira. 2004. Seasonal variation in larval host plants and consequences for Heliconius erato (Lepidoptera: Nymphalidae) adult body size. Austral Ecology 29:437–445.

Rossato, D. O., D. Boligon, R. Fornel, M. R. Kronforst, G. L. Gonçalves, and G. R. P. Moreira. 2018a. Subtle variation in size and shape of the whole forewing and the red band among co-mimics revealed by geometric morphometric analysis in Heliconius butterflies. Ecology and Evolution, doi: 10.1002/ece3.3916.

Rossato, D. O., L. A. Kaminski, C. A. Iserhard, and L. Duarte. 2018b. More Than Colours: An Eco-Evolutionary Framework for Wing Shape Diversity in Butterflies. P. *in* Advances in Insect Physiology.

Rosser, N., A. V. L. Freitas, B. Huertas, M. Joron, G. Lamas, C. Mérot, F. Simpson, K. R. Willmott, J. Mallet, and K. K. Dasmahapatra. 2019. Cryptic speciation associated with geographic and ecological divergence in two Amazonian Heliconius butterflies. Zool J Linn Soc 186:233–249.

Rosser, N., K. M. Kozak, A. B. Phillimore, and J. Mallet. 2015. Extensive range overlap between heliconiine sister species: evidence for sympatric speciation in butterflies? BMC Evolutionary Biology 15:125. BMC Evolutionary Biology.

Satterfield, D. A., and A. K. Davis. 2014. Variation in wing characteristics of monarch butterflies during migration: Earlier migrants have redder and more elongated wings. Animal Migration, doi: 10.2478/ami-2014-0001.

Schluter, D. 2000. The Ecology of Adaptive Radiation. Oxford Series in Ecology and Evolution, doi: 10.2307/3558417.

Schulz, S., C. Estrada, S. Yildizhan, M. Boppré, and L. E. Gilbert. 2008. An antiaphrodisiac in Heliconius melpomene butterflies. Journal of Chemical Ecology, doi: 10.1007/s10886-007-9393-z.

Shelomi, M. 2012. Where Are We Now? Bergmann's Rule Sensu Lato in Insects. The American Naturalist 180:511–519.

Singer, M. C. 1982. Sexual Selection for Small Size in Male Butterflies. The American Naturalist, doi: 10.1086/283924.

Srygley, R. B. 1994. Locomotor Mimicry in Butterflies? The Associations of Positions of Centres of Mass among Groups of Mimetic, Unprofitable Prey. Philosophical Transactions of the Royal Society B: Biological Sciences, doi: 10.1098/rstb.1994.0017.

Srygley, R. B. 2004a. The aerodynamic costs of warning signals in palatable mimetic butterflies and their distasteful models. Proceedings of the Royal Society B: Biological Sciences, doi: 10.1098/rspb.2003.2627.

Srygley, R. B. 2004b. The aerodynamic costs of warning signals in palatable mimetic butterflies and their distasteful models. Proceedings of the Royal Society B: Biological Sciences, doi: 10.1098/rspb.2003.2627.

Stalker, H. D., and H. L. Carson. 1948. An Altitudinal Transect of Drosophila robusta Sturtevant. Evolution 2:295–305.

Stillwell, R. C., W. U. Blanckenhorn, T. Teder, G. Davidowitz, and C. W. Fox. 2010. Sex Differences in Phenotypic Plasticity Affect Variation in Sexual Size Dimorphism in Insects: From Physiology to Evolution. Annual Review of Entomology, doi: 10.1146/annurev-ento-112408-085500.

Stoffel, M. A., S. Nakagawa, and H. Schielzeth. 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models.

Thurman, T. J., E. Brodie, E. Evans, and W. O. McMillan. 2018. Facultative pupal mating in Heliconius erato: Implications for mate choice, female preference, and speciation. Ecology and Evolution 8:1882–1889.

Wootton, R. 2002. Functional Morphology Of Insect Wings. Annual Review of Entomology, doi: 10.1146/annurev.ento.37.1.113.

Supplementary Materials

Table S1 Study species summary data. Sample sizes (N) and wing parameters for the 13 study species, ordered phylogenetically based on the most recent *Heliconius* phylogeny (Kozak et al. 2015). Male ratio refers to proportion of males in the sample.

Species	N	Area mean (mm^2)	Area S.E.	Aspect ratio mean	Aspect ratio S.E.	Alt. mean (m.a.s.l.)	N_{male}	N_{female}	Male ratio
H. telesiphe	48	519.4	8.9	2.35	0.009	1302	40	8	0.83
H. clysonymus	57	537.3	8.4	2.31	0.012	1346	40	17	0.70
H. erato	1687	465.8	1.5	2.09	0.002	700	1202	447	0.73
H. eleuchia	102	500.6	8.6	2.03	0.007	1408	72	30	0.71
H. sara	225	387.2	2.9	2.17	0.006	420	164	61	0.73
H. xanthocles	36	514.6	10.3	2.04	0.009	1044	20	8	0.71
H. hierax	37	512.1	8.3	2.08	0.008	1364	29	8	0.78
H. doris	42	547.5	7.1	2.30	0.012	444	34	7	0.83
H. timareta	195	606.7	3.1	2.05	0.004	883	163	32	0.84
H. cydno	127	575.1	5.5	2.09	0.007	844	112	15	0.88
H. melpomene	867	533.3	1.9	2.05	0.002	789	683	159	0.81
H. numata	44	611.6	12.9	2.11	0.013	561	30	14	0.68
H. wallacei	48	526.3	8.2	2.18	0.011	290	37	11	0.77

Table S2. Study species sexual dimorphism. Sexual size dimorphism (SSD) and sexual shape dimorphism (SShD) two-sample t-tests summary statistics. Positive t-values indicate smaller or longer-winged (higher aspect ratio) males (Fig. 2, main text).

Species	SSD t-value	SSD d.f.	SSD p-value	SShD t-value	SShD d.f.	SShD p-value
H. telesiphe	2.57	10	<0.05*	-0.5	10	ns
H. clysonymus	1.98	24	0.06•	-1.5	39	ns
H. erato	3.30	802	<0.001***	10.4	843	<0.001***
H. eleuchia	-2.61	61	<0.01**	-2.3	48	<0.05*
H. sara	-2.45	108	<0.05*	-0.6	100	ns
H. xanthocles	-0.08	13	ns	0.5	13	ns
H. hierax	-0.50	8	ns	0.5	16	ns
H. doris	-1.92	9	0.08 •	1.4	11	ns
H. timareta	2.03	49	0.05•	-0.2	46	ns
H. cydno	0.57	18	ns	0.1	16	ns
H. melpomene	5.54	240	<0.001***	1.6	230	ns
H. numata	2.57	24	<0.05*	-0.9	33	ns
H. wallacei	-1.31	16	ns	2.2	19	<0.05*

Table S3. Weighted PGLS model selection table for species sexual size dimorphism (SSD), mean wing aspect ratio and mean wing area based on AICc. All models have the species phylogeny as correlation structure and are weighted for mean trait/fixed effects variance and sample size.

Size sexual dimorphism (SSD)			
Minimal model	sisd.raw ~ 1		
Maximal model	sisd.raw ~ larva + shape.mean + shsd.raw + size.mean + clade		
Final model	sisd.raw ~ larva + shsd.raw + shape.mean		
Step	Df	Resid. Dev.	AICc
Initial model	6	73.7	109.8
-clade	7	73.46	99.5
-size.mean	8	75.6	93.7

Wing area (size)			
Minimal model	area.mean ~ 1		
Maximal model	area.mean ~ shape.mean * sex.ratio + alt.mean * dist.Eq.		
Final model	area.mean ~ sex.ratio + alt.mean + dist.Eq + alt.mean*lat.mean		
Step	Df	Resid. Dev.	AICc
Initial model	6	129.5	165.9
-shape.mean*sex.ratio	6	126.0	162.4
-shape.mean	7	126.0	152.0

Wing aspect ratio (shape)			
Minimal model	shape.mean ~ 1		
Maximal model	shape.mean ~ size.mean * sex.ratio + alt.mean * dist.Eq		
Final model	shape.mean ~ sex.ratio + alt.mean + dist.Eq		
Step	Df	Resid. Dev.	AICc
Initial model	5	-20.4	31.6
alt.mean*lat.mean	6	-19.3	17.1
- alt.mean*size.mean	7	-17.8	8.2
- size.mean	8	-17.3	1.3

Table S4. Phylogenetic Generalised Least Squares full model summaries for sexual size dimorphism, wing shape and wing size. Correlation structures of the models are shown in the third column. Dist. Eq.= distance from Equator, SD= sexual dimorphism.

Response variable (wing trait)	Model type	Corr. structure	Fixed effects	Estimate	SE	t-value	p-value	d.f. (d.f. res.)
Size Sexual Dimorphism	PGLS (nmle)	Phylogeny, sample size	(Intercept)	-31.8	21.4	-1.5	0.08	13 (9)
			Solitary larvae	-15.8	3.0	-5.3	0.0004***	
			Shape sex dim.	1.8	1.3	1.5	0.2	
			Shape mean	17.8	10.3	1.8	0.1	
Aspect ratio	PGLS (nmle)	Phylogeny, intra-sp variance, sample size	(Intercept)	1.93	0.23	8.36	0.00	13 (9)
			Altitude	-1.5E-4	6.3E-5	-2.4	0.04*	
			Dist. Eq	-4.6E-2	1.7E-2	-2.7	0.02*	
			Sex ratio	0.70	0.28	2.5	0.03*	
Area	PGLS (nmle)	Phylogeny, intra-sp variance, sample size	(Intercept)	474.52	75.37	6.30	0.00	13 (8)
			Altitude	0.16	0.04	3.47	0.008**	
			Sex ratio	-161.75	73.97	-2.19	0.06	
			Dist. Eq.	77.21	16.91	4.57	0.002**	
			Altitude* Dist. Eq	-0.07	0.02	-4.66	0.002**	

Table S5. Model selection based on AIC of within species variation in wing aspect ratio and wing area of *H. erato* and *H. melpomene*.

A) Aspect ratio, *H. erato*

Minimal model	aspect.ratio ~ 1
Maximal model	aspect.ratio ~ area.mm2 * altitude + dist.Eq. + longitude + sex
Final model	aspect.ratio ~ area.mm2 * altitude + longitude + sex

Step	Res. Df	Res. Dev.	AIC
Initial model	1294	4.92	-7246
- dist.Eq.	1295	4.92	-7248

B) Aspect ratio, *H. melpomene*

Minimal model	aspect.ratio ~ 1
Maximal model	aspect.ratio ~ area.mm2 * altitude + dist.Eq. + longitude + sex
Final model	aspect.ratio ~ area.mm2 * altitude + longitude + sex

Step	Res. Df	Res. Dev.	AIC
Initial model	704	2.3	-4070
- dist.Eq.	705	2.3	-4072

C) Wing area, *H. erato*

Minimal model	area ~ 1
Maximal model	area ~ aspect.ratio * altitude + dist.Eq + longitude + sex
Final model	area ~ aspect.ratio * altitude + dist.Eq + longitude + sex

Step	Res. Df	Res. Dev.	AIC
Initial model	1294	4841609	10720

D) Wing area, *H. melpomene*

Minimal model	area ~ 1
Maximal model	area ~ aspect.ratio * altitude + dist.Eq + longitude + sex
Final model	area ~ aspect.ratio * altitude + dist.Eq + longitude + sex

Step	Res. Df	Res. Dev.	AIC
Initial model	704	2092210	5701

Table S6. Full model output table for within-species (*H. erato* and *H. melpomene*) analyses of wing aspect ratio and wing area. Relative R^2 per fixed effect estimated with the package *relaimpo* (Grömping 2006) and the lmg statistic.

Trait (response)	Sp.	Fixed effects	Res. d.f.	Estimate	SE	t-value	p-value	Rel. R^2
Aspect ratio	*H. era.*	(intercept)	1295	2.2	0.03	75.57	0.00	
		altitude		-1.6E-04	0.00	-6.50	0.00***	0.43
		sex(female)		-3.4E-02	0.01	-2.87	0.004**	0.37
		area		-1.8E-04	0.00	-3.73	0.00***	0.14
		longitude		4.9E-04	0.00	2.00	0.05	0.05
		area*alt.		2.9E-07	0.00	5.30	0.00***	0.02
	H. melp.	(intercept)	705	2.3E+00	4.2E-02	55.08	0.00	
		altitude		-1.4E-04	3.4E-05	-4.17	0.00***	0.50
		area		-3.4E-04	6.1E-05	-5.63	0.00***	0.23
		area*alt.		2.2E-07	6.4E-08	3.40	0.001**	0.10
		longitude		6.6E-04	2.6E-04	2.53	0.012*	0.09
		sex(female)		-4.0E-02	1.3E-02	-3.16	0.001**	0.08
		sex(male)		-3.1E-02	1.2E-02	-2.65	0.008**	(0.08)
Wing area	*H. era.*	(intercept)	1294	879	102.27	8.60	0.00	
		AR*alt.		0.25	0.05	4.80	0.00***	0.38
		longitude		0.87	0.25	3.54	0.00***	0.18
		sex(female)		-19.3	11.90	-1.62	0.1	0.16
		sex(male)		-6.76	11.64	-0.58	0.56	0.15
		dist.Eq.		-2.35	0.84	-2.80	0.005**	(0.15)
		altitude		-0.53	0.11	-4.88	0.00***	0.08
		aspect.ratio		-161	47.56	-3.40	0.00***	0.05
	H. melp.	(intercept)	704	1430	131.35	10.89	0.00	
		dist.Eq.		-5.51	0.86	-6.38	0.00***	0.33
		longitude		1.51	0.24	6.21	0.00***	0.18
		sex(female)		-39.5	12.13	-3.26	0.001**	0.18
		sex(male)		-13.8	11.39	-1.21	0.23	(0.18)
		aspect.ratio		-365	61.86	-5.91	0.00***	0.15
		AR*alt.		0.25	0.06	3.95	0.00***	0.10
		altitude		-0.51	0.13	-3.92	0.00***	0.05

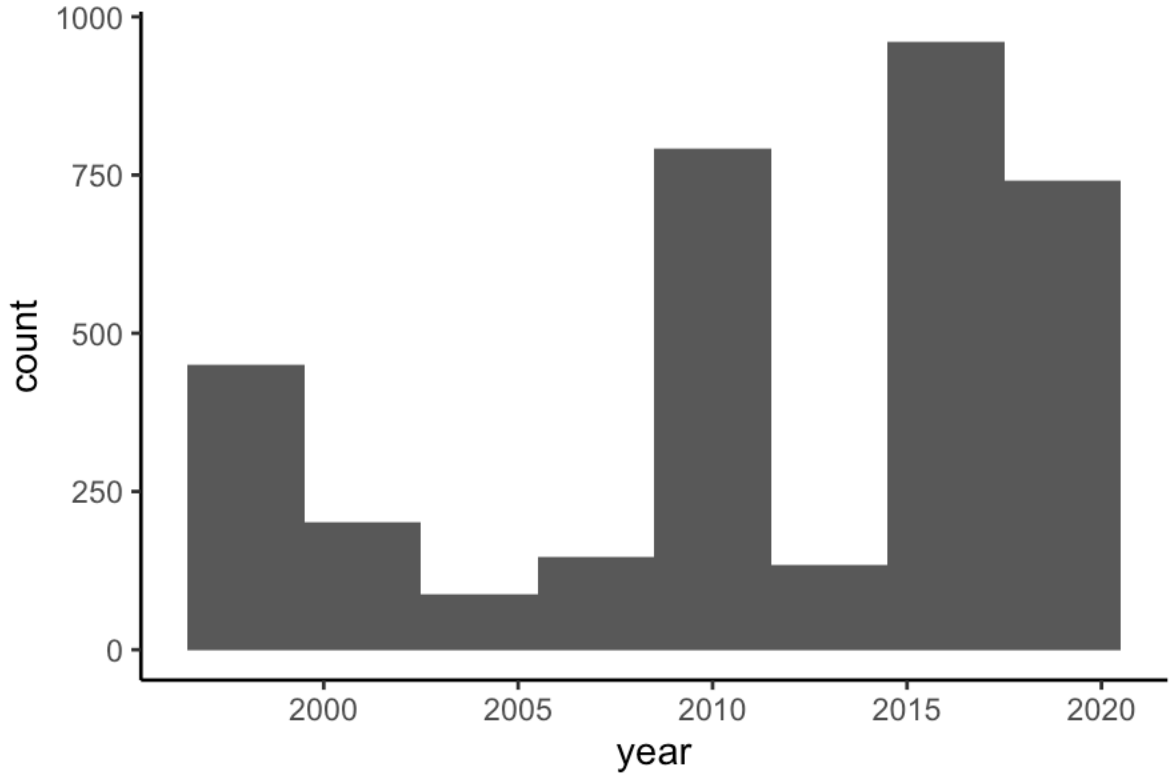

Figure S1. Number of *Heliconius* individuals in this study collected across 3-year intervals.

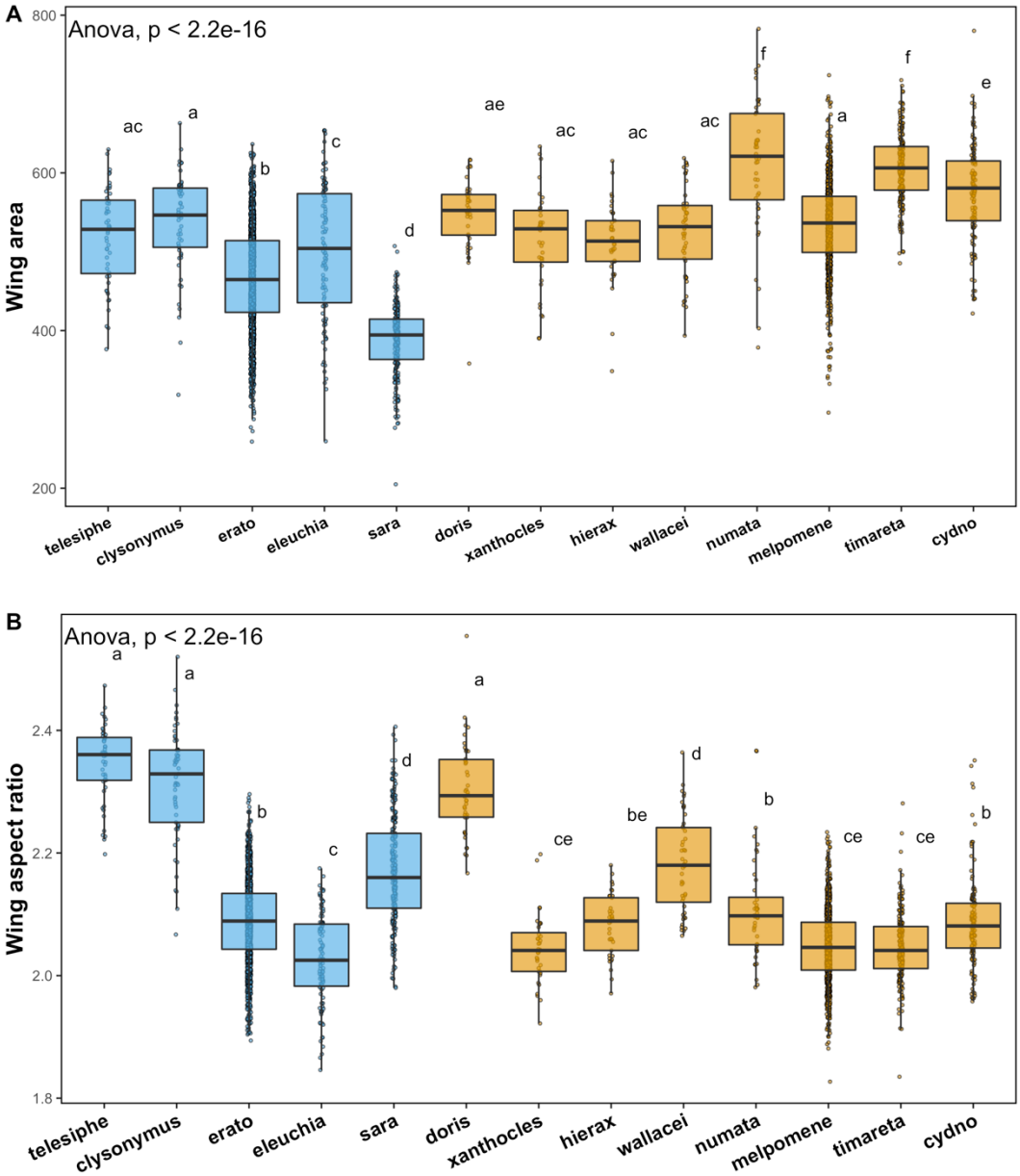

Figure S2. Wing area (mm^2, A) and wing aspect ratio (wing roundness, B) variation across species. Species sharing a letter are not significantly different (Tukey-adjusted comparisons). Species are ordered phylogenetically (for phylogeny see Fig. 3) and coloured by the two major clades.

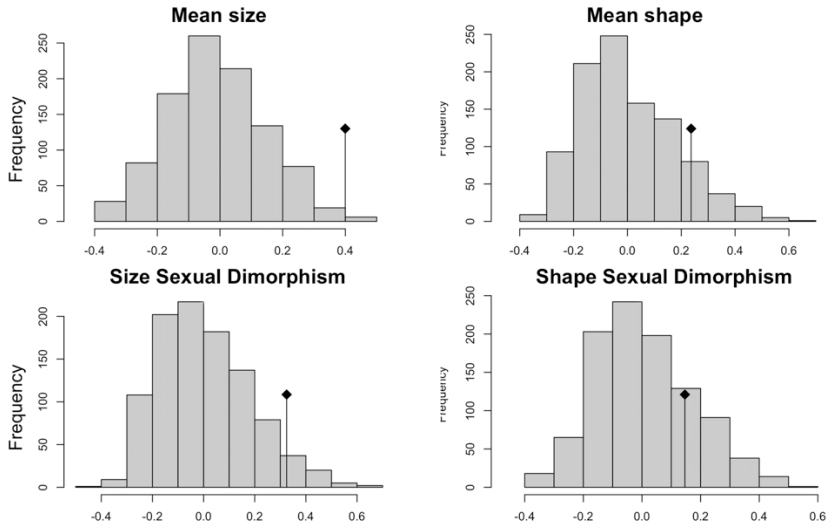

Figure S3. Abouheif C-mean distribution plots for six variables. Black dots depicts the observed C-mean statistic relative to the null hypothesis of randomisations along the tips of the phylogeny.

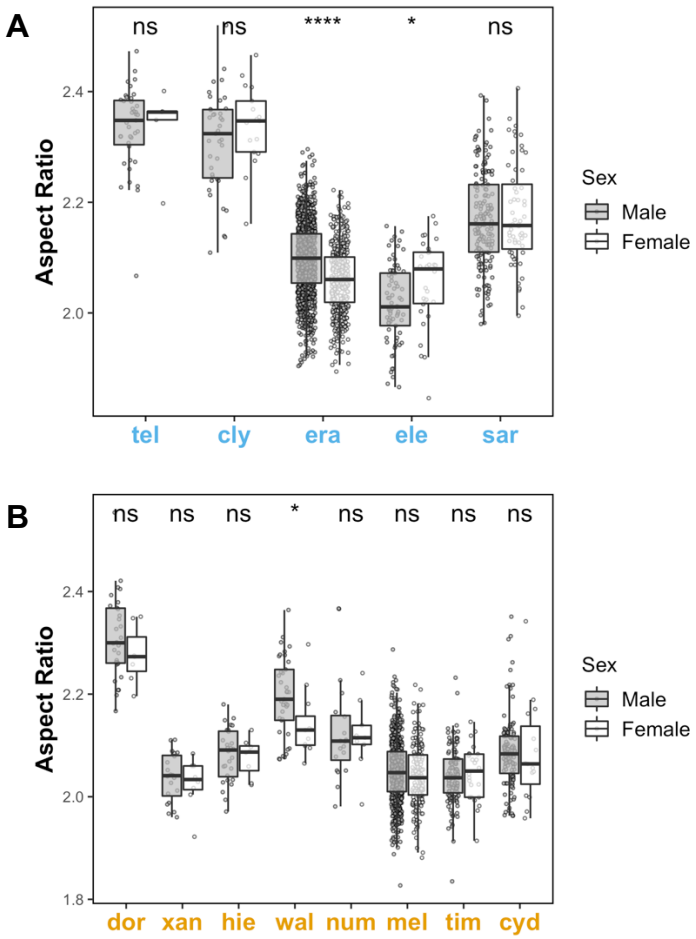

Figure S4. Sexual wing aspect ratio dimorphism across species of the erato cade (A) and the melpomene clade (B). Wing aspect ratio differences between males (grey) and females (white). Error bars represent 95% confidence intervals of the means. Stars represent significance levels of two sample t-tests between female and male wing areas for each species (•<0.08, *< 0.05, **<0.01, ***<0.001), for full t-tests output see Table S2.

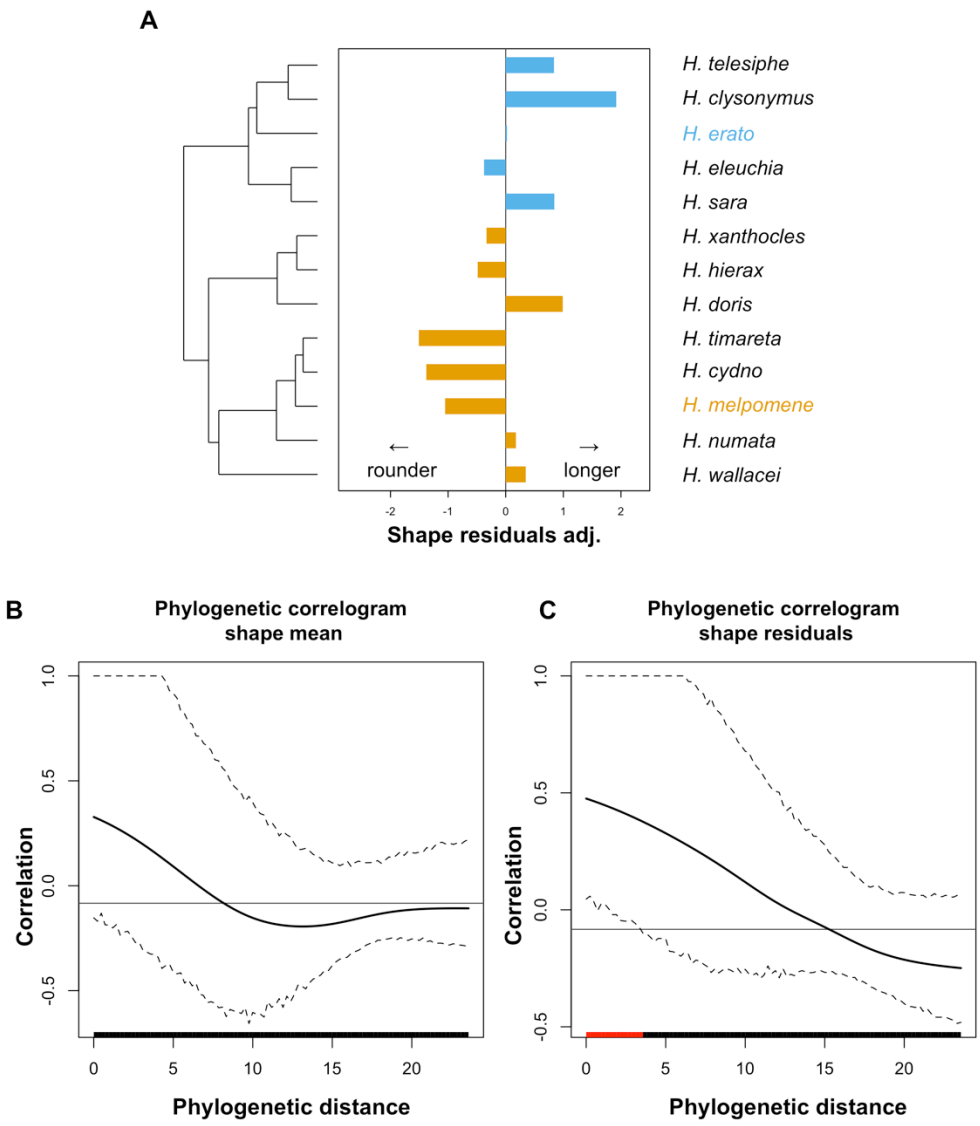

Figure S5. Phylogenetic signal in wing shape. A) Z-transformed wing shape residuals across the *Heliconius* phylogeny. B) phylogenetic correlogram of species mean wing shape. C) phylogenetic correlogram of species wing shape model residuals. The solid black line represents Moran's I index of autocorrelation and the dashed black lines represent the lower and upper bounds of the confidence 95% confidence interval. The horizontal black line represents the expected value of Moran's I under the null hypothesis of no phylogenetic autocorrelation. The coloured bars in the x-axes show whether the autocorrelation is significant (based on the confidence interval): red for significant positive autocorrelation and black for nonsignificant autocorrelation. All figures were obtained with the package phylosignal (Keck et al. 2016).

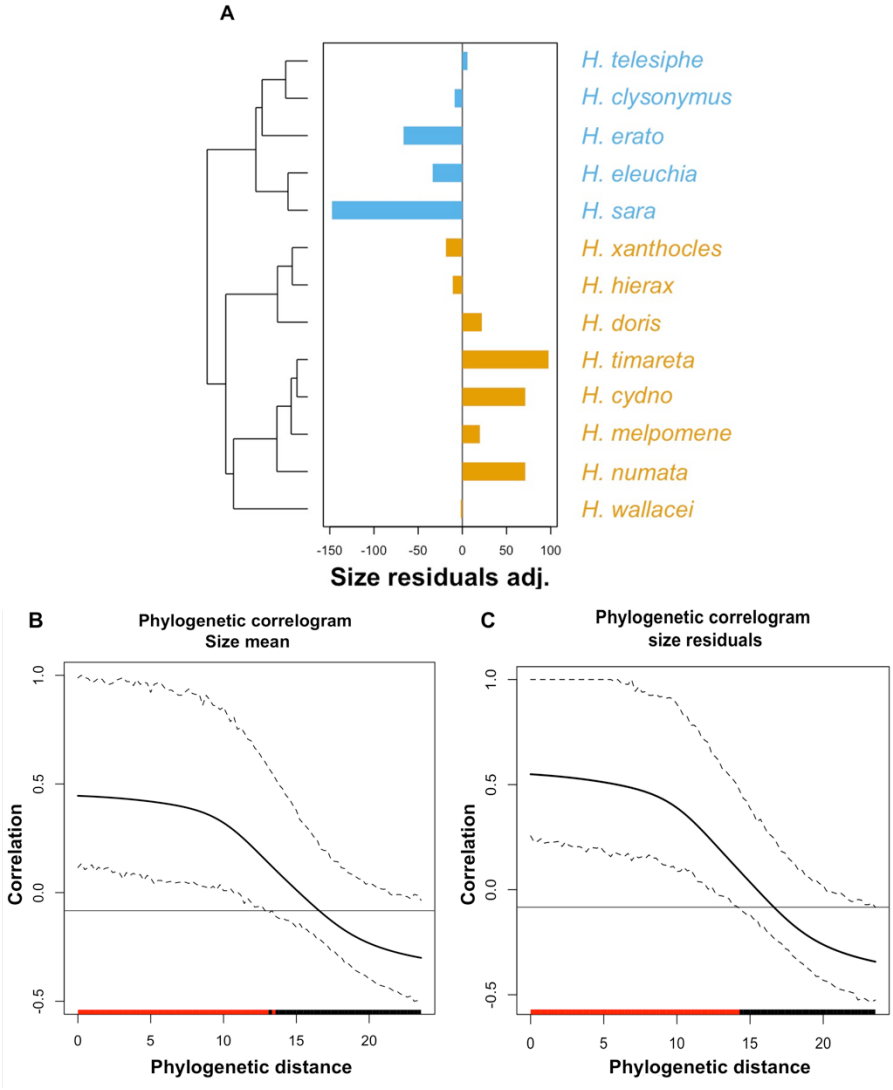

Figure S6. Phylogenetic signal in wing size. A) Centered wing size residuals across the *Heliconius* phylogeny. B) phylogenetic correlogram of species mean wing size. C) phylogenetic correlogram of species wing size model residuals. The solid black line represents Moran's I index of autocorrelation and the dashed black lines represent the lower and upper bounds of the confidence 95% confidence interval. The horizontal black line represents the expected value of Moran's I under the null hypothesis of no phylogenetic autocorrelation. The coloured bars in the x-axes show whether the autocorrelation is significant (based on the confidence interval): red for significant positive autocorrelation and black for nonsignificant autocorrelation.

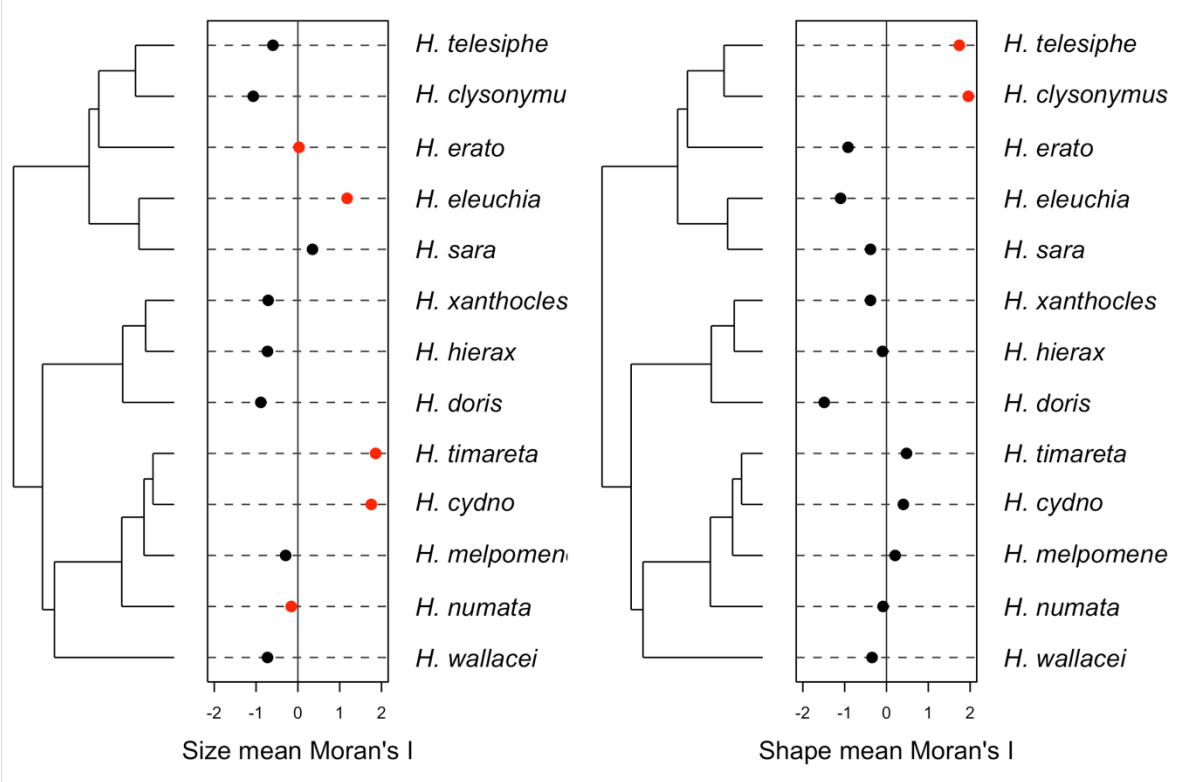

Figure S7. Local Moran's I index values for each species for wing area mean (left) and wing aspect ratio mean (right). Red points indicate significant positive autocorrelation in mean traits among neighbours in the phylogeny. Estimated and plotted with the package *phylosignal* (Keck et al. 2016).

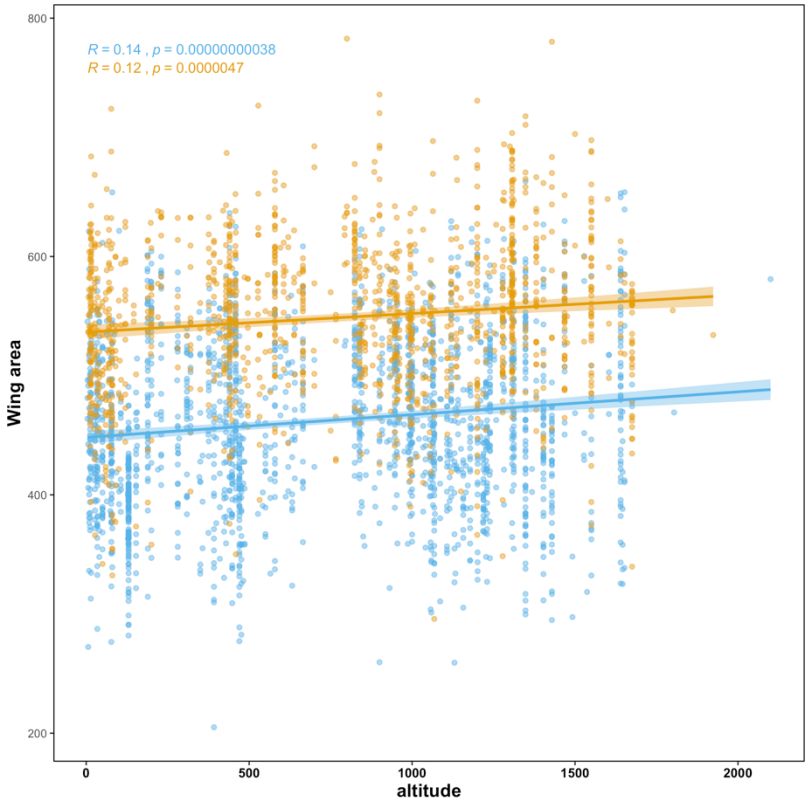

Figure S8. Wing area variation with altitude across individuals from all species of the erato clade (blue) and the melpomene clade (orange). Each point represents an individual. Lines show best linear fit and are colored by clade. Shaded areas show confidence bands at 1 standard error. Pearson correlation coefficients and p-values are shown for each regression plotted.

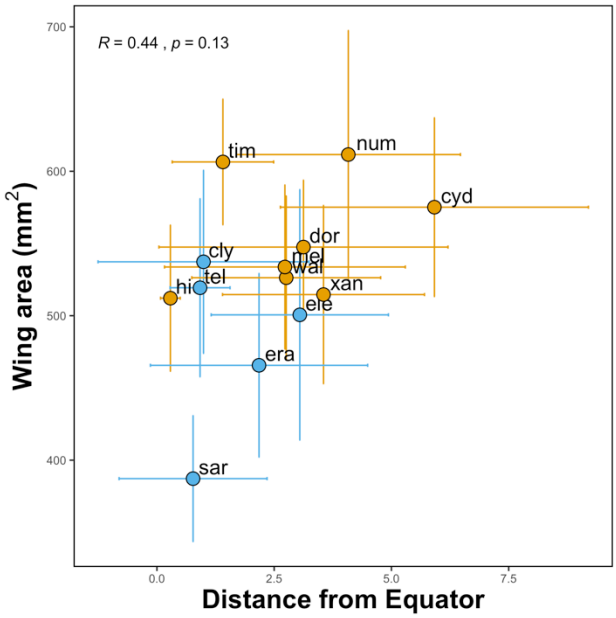

Figure S9. Species variation in wing area. Plot shows the correlation between distance from the Equator (degrees) and species mean wing area (mm2). Points represent species mean raw values per species. Horizontal and vertical lines show standard error for species mean distance from Equator and mean wing area, respectively. The point labels correspond to the first three characters of the following *Heliconius* species: *H. telesiphe*, *H. clysonymus*, *H. erato*, *H. eleuchia*, *H. sara*, *H. doris*, *H. xanthocles*, *H. hierax*, am*H. wallacei, H. numata*, *H. melpomene*, *H. timareta*, *H. cydno.*

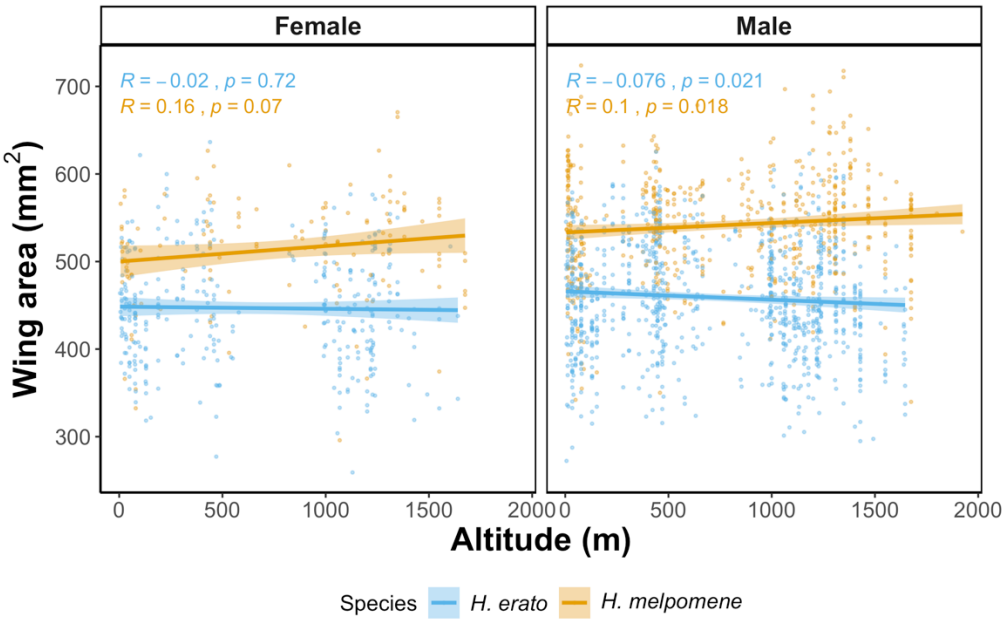

Figure S10. Within-species variation in wing area (mm^2) across alt.s in *H. erato* (blue) and *H. melpomene* (orange), females (left) and males (right). Lines show best linear fit and are colored by species. Shaded areas show confidence bands at 1 standard error. Pearson correlation coefficients and p-values are shown for each regression plotted.

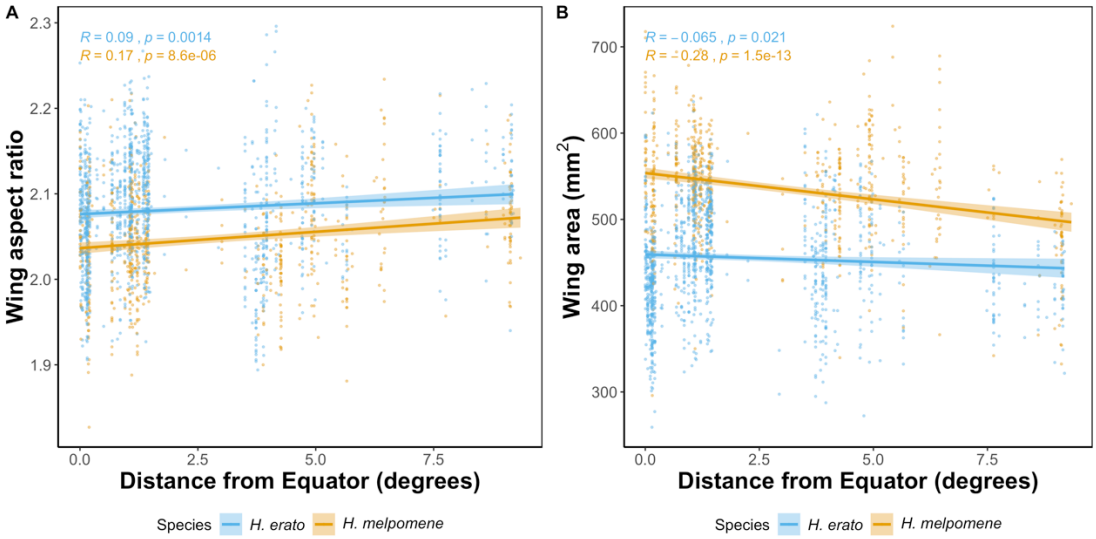

Figure S11. Species variation in raw wing aspect ratio (A) and wing area (B) in *H. erato* (blue) and *H. melpomene* (orange). Points represent individual values. Lines show best linear fit for significant effects. Shaded areas show confidence bands at 1 standard error. Pearson correlation coefficients and p-values are shown for each regression plotted.

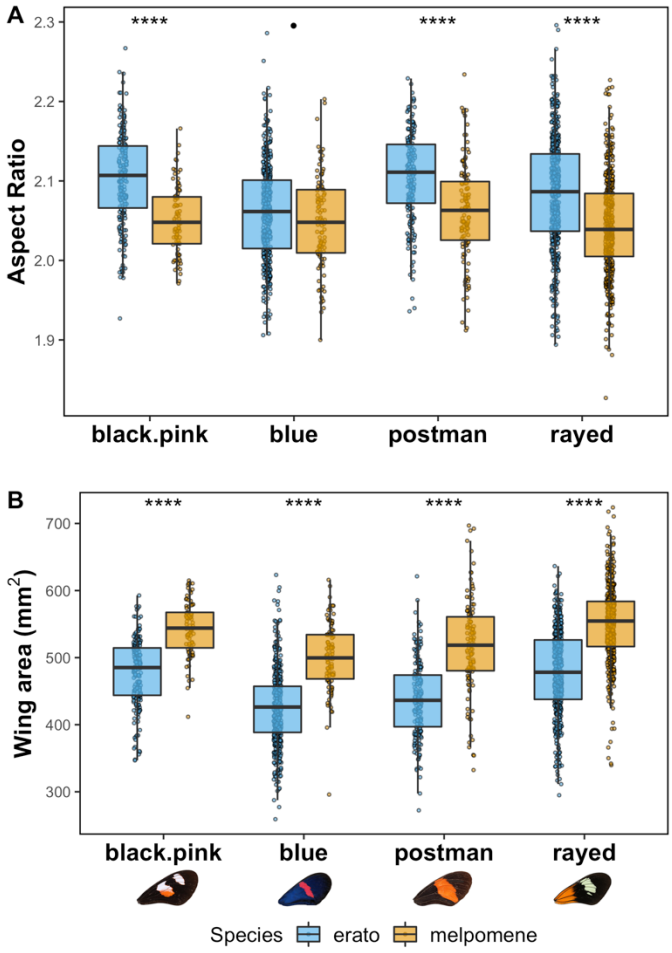

Figure S12. Wing aspect ratio (A) and area (B) variation across mimicry ring wing patterns of the two most abundant species, *H. erato* (blue) and *H. melpomene* (orange). Error bars represent 95% confidence intervals of the means. Stars represent significance levels of two sample t-tests between *H. erato* and *H. melpomene* wings for each mimicry ring (•<0.08, *< 0.05, **<0.01, ***<0.001)

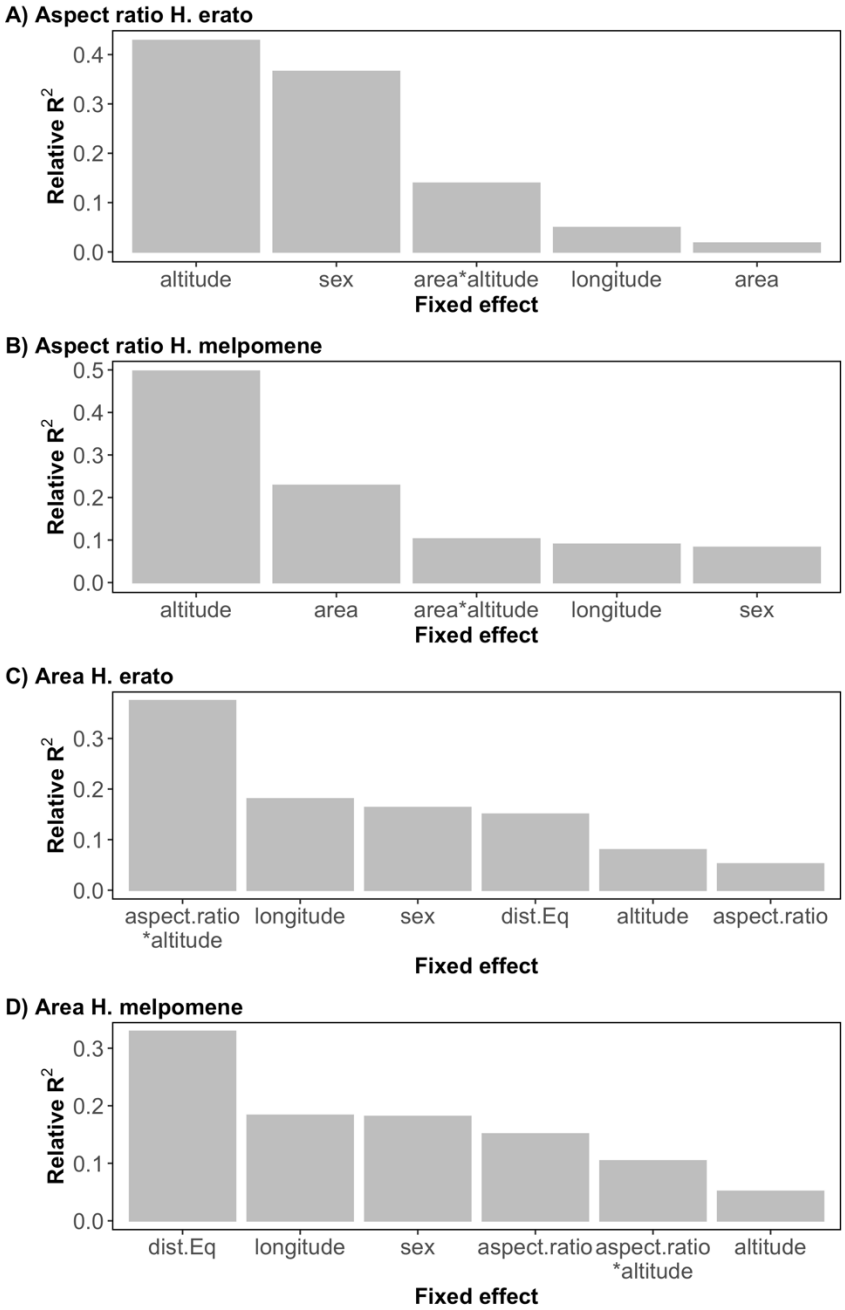

Figure S13. Relative importance of model predictors of within species variation wing aspect ratio (A, B) and wing area (C, B) in *H. erato* (A, C) and *H. melpomene* (B, D). Total model adjusted R^2 values are A) 0.13, B) 0.14, C) 0.19, D) 0.19.

References

Grömping, U. 2006. Relative Importance for Linear Regression in *R*: The Package **relaimpo**. Journal of Statistical Software, doi: 10.18637/jss.v017.i01.

Keck, F., F. Rimet, A. Bouchez, and A. Franc. 2016. Phylosignal: An R package to measure, test, and explore the phylogenetic signal. Ecology and Evolution, doi: 10.1002/ece3.2051.

Kozak, K. M., N. Wahlberg, A. F. E. Neild, K. K. Dasmahapatra, J. Mallet, and C. D. Jiggins. 2015. Multilocus species trees show the recent adaptive radiation of the mimetic heliconius butterflies. Systematic Biology 64:505–524.