Authors for correspondence:
Sebastián Mena
e-mail: dsmenag@gmail.com
María F. Checa
e-mail: mfcheca@puce.edu.ec

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Forest stratification shapes allometry and flight morphology of tropical butterflies

Sebastián Mena[1], Krzysztof M. Kozak[2], Rafael E. Cárdenas[1] and María F. Checa[1]

[1]Museo de Zoología QCAZ Invertebrados—Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito, Ecuador
[2]Smithsonian Tropical Research Institute, Panama City, Panama

iD SM, 0000-0001-7361-5127; KMK, 0000-0001-8980-3173; REC, 0000-0002-0375-9384;
MFC, 0000-0001-8825-3675

Studies of altitudinal and latitudinal gradients have identified links between the evolution of insect flight morphology, landscape structure and microclimate. Although lowland tropical rainforests offer steeper shifts in conditions between the canopy and the understorey, this vertical gradient has received far less attention. Butterflies, because of their great phenotypic plasticity, are excellent models to study selection pressures that mould flight morphology. We examined data collected over 5 years on 64 Nymphalidae butterflies in the Ecuadorian Chocó rainforest. We used phylogenetic methods to control for similarity resulting from common ancestry, and explore the relationships between species stratification and flight morphology. We hypothesized that species should show morphological adaptations related to differing micro-environments, associated with canopy and understorey. We found that butterfly species living in each stratum presented significantly different allometric slopes. Furthermore, a preference for the canopy was significantly associated with low wing area to thoracic volume ratios and high wing aspect ratios, but not with the relative distance to the wing centroid, consistent with extended use of fast flapping flight for canopy butterflies and slow gliding for the understorey. Our results suggest that microclimate differences in vertical gradients are a key factor in generating morphological diversity in flying insects.

1. Introduction

Flight has been fundamental to the evolution of insects [1–3]. As a result, morphological flight traits have been studied by ecologists in the context of spatial and temporal variation as potential adaptations to landscape structure, weather, dispersal and migration (e.g. [4–8]). Indeed, certain morphological traits predict flight performance [9–12]. For example, since more than 96% of insect thoracic mass consists of flight muscles [13,14], larger thoraxes have been associated with higher power outputs, leading to an increase in flight thrust and better manoeuvring during flapping flight [15,16]. Thoracic power can be characterized by measures that account for differences in body size, such as wing loading, flight muscle ratio, or the wing area (WA) to thoracic volume (TV) ratio [17–19], which are positively associated with wingbeat frequency [20,21] and metabolic activity [10,22].

Changes in wing shape also influence flight performance [22,23]. Long and narrow wings are aerodynamically more efficient than short and wide wings, because the former morphology reduces induced drag, economizing power at gliding [9,16]; these traits are represented by the aspect ratio, the proportion of wing length to the mean wing chord. A larger distance from the wing centroid (WC) to the wing base is associated with a better body response in the pitching plane and to increased manoeuvrability and force production [23–25].

Allometry, the relationship between size and shape, can also vary drastically between closely related species as a function of physiological and life-history traits [26–28]. It describes how different spatial niches can exert distinct selective pressures on flight behaviour, leading to changes in body proportions [29,30].

For example, the energetic cost of flight increases inversely with organism size [31]. Nevertheless, many insects and small birds successfully migrate over long distances despite their size, minimizing the energetic cost of locomotion through allometric shifts, allowing the use of alternating flapping–gliding flight modes [32,33].

Ecological factors driving morphological diversity and flight ability can be studied most effectively in diverse tropical habitats. As noted by McArthur [34]: 'increasing complexity of communities toward the equator may result in stronger species interaction, thus increasing species interdependence and intensifying selection for adaptive patterns'. In tropical rainforests, biotic and abiotic factors, such as temperature, light, humidity, wind and species, change significantly along a vertical gradient ranging from the ground level to the upper canopy [35–41]. Such variation results in a diversity of microhabitats, which support assemblages adapted to each range of conditions [42–44]. Niche partitioning along vertical scales has accordingly been suggested to contribute to the high biological diversity in these ecosystems [36,45–49].

There is abundant evidence of community niche partitioning among strata in Neotropical butterflies, usually occurring at the subfamily level [50–57], but also among species and populations [58]. Butterflies, due to their great phenotypic plasticity and strong sensitivity to environmental factors [59–63], are excellent models to study selective pressures moulding morphology to optimize flight performance across forest strata. However, few studies have examined the morphological differences across vertical strata in tropical butterflies. Among Amazonian Riodinidae, Hall & Willmott [64] found two groups of species with significantly different slopes for the allometric relation between WA and TV. They argued that if these groups corresponded to butterflies from different forest strata, then metabolic restrictions driven by differences in heat availability among used microhabitats might explain this pattern. Although field observations suggest differences in morphology among some Nymphalidae butterflies inhabiting canopy versus understorey [25,64–66], whether there is an evolutionary trend between stratification, allometry and flight morphology at a community level remains unknown.

The goal of this study was therefore to explore variation in allometry and flight morphology between the canopy and understorey, using a Nymphalidae butterfly community as a study model. The Nymphalidae is the most diverse butterfly family, one of the best studied, and shows the remarkable variation in size, morphology and ecology across the group, and is relatively easy to sample. Our data comes from a long-term butterfly monitoring program in the Ecuadorian Chocó biogeographic region, a locality broadly representative of Neotropical lowland rainforests [67]. We hypothesized that butterflies in the understorey should have a hypoallometric slope of WAs versus TVs compared with canopy butterflies, due to more restricted metabolic activity associated with lower temperatures in the understorey [64]. Consequently, we predicted an evolutionary trend between microhabitat specialization and morphology: as conditions in the canopy are favourable for higher wingbeat frequencies than in the understorey, an increasing preference for the canopy was hypothesized to be associated with butterflies with a lower ratio of WA to TV (i.e. extended use of fast flapping flight for canopy butterflies and slower gliding for understorey butterflies). Because these adaptations may affect speed and manoeuvrability in understorey butterflies, we also hypothesized that understorey butterflies may have shifted their wing shape as a means of compensation, towards higher aspect ratios and shorter distances to the WC (wings more elongated, wider towards the tip) compared with canopy butterflies.

2. Material and methods

(a) Sampling

We used ecological and morphological data from a long-term butterfly monitoring project (years 2006–2007, 2011–2013) at Reserva Río Canandé (00°28′ S, 79°12′ W), an Ecuadorian lowland rainforest within the Chocó biogeographic region (see [68,69]), one of the most diverse, threatened and least studied biomes on Earth [70–73]. The sampling design consisted of 32 Van Someren-Rydon traps [74] distributed in two transects, each about 280 m long. Along each transect, eight sampling sites were established, with each pair of sites spaced 40 m apart. At each site, one trap was positioned in each of the two strata: understorey (1–1.5 m) and canopy (20–30 m). All sites were similar in terms of altitude (approx. 500 masl) and topography (ridges with 10% inclination). The baits used were rotting banana (2 days of fermentation) and rotten shrimp (15 days of fermentation), based on Checa *et al.* [75]. Each sampling period consisted of 6 days and the traps were checked daily. Sampling was performed every two months. The collected butterflies were deposited at the Museum of Zoology, Invertebrates Section (QCAZ), of the Pontificia Universidad Católica del Ecuador, Quito, Ecuador, and identified using specialist literature (e.g. [30,76]) and consultation with taxonomists.

(b) Morphological traits

Because few females are captured with our sampling method [75], we only used males for analyses in this study. We selected 8–10 individuals of each species that were in the best physical conditions (i.e. undamaged wings and thoraxes) to be measured *ex situ*. Due to the limited number of individuals collected, we measured fewer individuals of the following species: *Adelpha naxia* ($n = 5$), *Memphis aulica* ($n = 6$), *Siproeta stelenes* ($n = 5$), *Heliconius hecale* ($n = 2$), *Hamadryas arinome* ($n = 2$), *Archaeoprepona demophon* ($n = 6$), *Catoblepia xanthicles* ($n = 7$) and *Magneuptychia mycalesis* ($n = 6$) (see [30] for a list of taxonomic authorities for all species). The morphological variables measured were WA, forewing length, thorax length, thorax height, thorax breadth and distance to centroid (see electronic supplementary material, appendix S3 for details of each measurement). Thorax breadth was measured with a manual calliper to the nearest 0.2 mm (Mitutoyo Corporation, Japan). To measure the remaining variables, digital images of individual specimens were used. Specimens were placed in a lightbox with the wings closed and the forewing and hindwing overlapped in a position similar to that seen during flight (using scale melanisation as proxies of wing overlapping; electronic supplementary material, appendix S3) [25,61]. Photographs were taken with a Canon EOS-1100 digital reflex camera (Canon Inc., Tokyo, Japan) with an 18–55 mm (at 55 mm) lens (Canon Inc., Tokyo, Japan) placed on a tripod and orientated in the same plane as the wings. Constant exposure parameters were used (shutter speed = 1/6; ISO = 100; diaphragm = f13). Measurements were made on the digital images using the measuring tool in ImageJ software [77]. WA (mm^2) was obtained using the Measure function, after the thoraxes were digitally removed.

With these variables, we calculated the following flight-related parameters: aspect ratio, WA to TV ratio and relative distance to WC. Aspect ratio (AR) was calculated as $2 \times$ forewing length$^2 \times$ WA^{-1} [22]. The TV (mm^3) was estimated as thoracic length × thoracic breadth × thoracic height. The WA to TV ratio

(WA : TV) was estimated as: WA × TV$^{-2/3}$ [64]. The WC was obtained using the Centroid function in ImageJ (calculated as the average value of the central pixels of x and y coordinates of all pixels inside the WA) [77]. The distance of this vector from the wing base to the WC was measured digitally using ImageJ (DWC). This distance was subtracted from the square root of WA to correct for differences in wing size. The resulting value was named 'relative distance to wing centroid' (RDC). A total of 606 individuals were measured, from 67 species belonging to six subfamilies: Limenitidinae, Heliconinae, Nymphalinae, Satyrinae, Biblidinae and Charaxinae (electronic supplementary material, appendix S2).

(c) Stratification patterns

To test whether canopy and understorey butterfly assemblages were different in their structure and composition, a non-metric multidimensional scaling (NMDS) ordination was performed using data from canopy and understorey abundance for each species in each sampling period. A Bray–Curtis dissimilarity matrix and a Wisconsin double standardization were employed. We performed an analysis of similarities (ANOSIM) to test the significance of the difference. A SIMPER analysis was employed to evaluate how much each species contributes to the difference in assemblages between the strata. These analyses were performed using the Vegan package [78] in R [79]. Because traps were not placed in the same sites between 2006–2007 and 2011–2013, only data between 2011 and 2013 were used for these three analyses.

(d) Allometry and stratification

To test whether Nymphalidae species had a relationship between flight height and the ratio of WA to TV, as hypothesized by [64], we plotted both variables against each other (raising TV to the 2/3 power to control for geometric differences as area increases versus volume [64]). Since the plotted data appeared to show two distinct trends among species, we quantitatively assessed whether these trends were associated with preference for forest stratum by categorizing species as canopy (C) or understorey (U) according to a binomial test in SPSS v. 12 (IBM Corporation, Chicago, USA), using 0.5 as the theoretical value of the expected distribution of observations. We excluded the non-significant (NS) species and used an analysis of covariance (ANCOVA) in PAST v. 3.14 [80] to test if canopy and understorey butterflies differed in terms of their WA and TV regression slopes. To meet the assumptions of this test, data were log transformed, linearity of data was confirmed using least-square linear regressions, and normality of residuals was checked using a Shapiro–Wilk test ($p > 0.05$).

(e) Evolution of flight morphology and stratification

To test whether there were any significant associations between stratification and morphology, we used phylogenetic generalized least-squares models (PGLS) [81]. We fitted three PGLS models in Caper (after finding a significant phylogenetic signal for all morphological variables; see 'Accounting for phylogenetic bias' below) setting stratification values as our independent variable and WA : TV, AR and RDC as our respective dependent variables using maximum-likelihood estimation of the lambda escalation parameter.

To provide a continuous measure of the vertical distribution, we ran a hierarchical Bayesian model with 10 000 Markov chain Monte Carlo permutations using butterfly occurrence in either canopy or understorey for each sampling period [57], using the Bayespref package [57] in R [79]. We used the median of the canopy probability distribution as trait value: a value close to 0 indicated preference for understorey and a value close to 1 indicated preference for canopy. Only species with more than
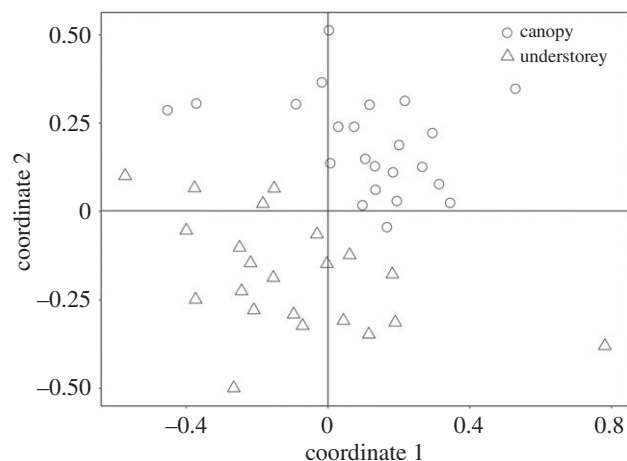
Figure 1. Non-metric multidimensional scaling plot. Each character represents a sampling period (one week) between 2011 and 2013. Canopy species assemblages are represented by circles, and triangles represent understorey assemblages. $p < 0.001$. Stress = 0.21 under a Bray–Curtis distance matrix.

Table 1. Number of species in canopy, understorey and non-significant stratification (NS), categorized according to the binomial test.

subfamily	canopy	understorey	non-significant	total
Biblidinae	11	2	2	15
Charaxinae	9	2	4	15
Heliconiinae	1	3	4	8
Limenitidinae	5	1	0	6
Nymphalinae	1	1	5	7
Satyrinae	2	13	3	18
Total	29	22	18	

Significant levels of two-tailed probabilities for the binomial tests are **$p < 0.01$, *$p < 0.05$, n.s. = not significant, at $\alpha = 0.05$.

Table 2. Results of the phylogenetic signal tests for stratification and flight morphology traits. P for Pagel's λ is the calculated p-value when λ is compared with $\lambda = 0$.

trait	Pagel's λ value	P	Bloomberg's K value	P
stratification	0.660	<0.001	0.434	0.011
WA : TV	0.968	<0.001	0.829	<0.001
AR	0.858	<0.001	1.020	<0.001
RDC	0.984	<0.001	1.380	<0.001

10 captured specimens were considered. Sixty-four species had morphological, stratification and phylogenetic data, and thus were included in this analysis.

(f) Accounting for phylogenetic bias

We analysed our data in a phylogenetic context to account for the influence of phylogeny on traits and resulting potential loss of

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	stratification	WA:TV	AR	RDC
Colobura annulata	0.50	18.5	3.02	13.1
Colobura dirce	0.59	19.3	2.93	11.8
Tigridia acesta	0.38	25.3	2.86	9.5
Smyrna blomfildia	0.95	17.8	2.56	16.6
Eresia alsina	0.26	34.0	3.89	6.9
Siproeta stelenes	0.43	35.3	2.87	16.9
Catonephele orites	0.39	19.6	2.76	14.1
Catonephele numilia	0.77	22.2	2.53	15.4
Nessaea aglaura	0.35	23.3	2.54	14.2
Hamadryas laodamia	0.83	19.8	2.61	14.2
Hamadryas arinome	0.56	18.6	2.80	14.1
Hamadryas amphinome	0.97	20.8	2.89	13.4
Pyrrhogyra otolais	0.23	42.2	2.67	11.9
Pyrrhogyra amphiro	0.84	26.0	2.54	14.9
Pyrrhogyra crameri	0.73	26.7	2.71	10.8
Temenis laothoe	0.79	18.3	2.91	9.5
Temenis pulchra	0.71	18.3	2.93	9.1
Callicore atacama	0.96	19.0	2.87	9.8
Callicore guatemalena	0.86	21.4	2.88	8.2
Diaethria marchalii	0.82	22.6	2.78	8.3
Heliconius hecale	0.69	32.0	4.33	9.1
Heliconius atthis	0.18	36.6	3.71	10.0
Heliconius cydno	0.16	34.3	3.71	11.1
Heliconius doris	0.61	32.0	3.88	10.5
Heliconius erato	0.04	35.5	3.77	9.7
Heliconius hecalesia	0.94	27.1	4.52	9.0
Heliconius sara	0.59	33.1	4.33	8.2
Heliconius eleuchia	0.09	36.4	3.54	10.6
Dryas iulia	0.49	35.6	4.29	10.5
Adelpha phylaca	0.88	19.1	2.70	13.1
Adelpha erotia	0.93	15.2	2.61	14.8
Adelpha heraclea	0.76	20.0	2.68	12.7
Adelpha naxia	0.84	18.7	3.01	11.8
Adelpha barnesia	0.86	21.1	2.67	13.6
Adelpha cytherea	0.68	33.4	2.68	10.7
Cithaerias pireta	0.04	45.3	2.84	11.2
Haetera piera	0.04	41.4	2.86	15.8
Dulcedo polita	0.07	48.7	2.79	12.8
Pierella helvina	0.05	40.7	2.38	17.4
Manataria maculata	0.49	33.6	2.73	13.9
Magneuptychia mycalesis	0.85	47.5	2.61	12.2
Hermeuptychia sp.	0.17	33.5	2.51	7.0
Cissia confusa	0.57	48.0	2.59	10.5
Megeuptychia antonoe	0.79	37.4	2.49	16.0
Opsiphanes invirae	0.33	16.6	2.43	16.9
Opsiphanes cassiae	0.52	16.4	2.63	21.4
Catoblepia xanthicles	0.25	41.0	2.14	27.1
Catoblepia orgetorix	0.08	40.9	2.20	25.2
Caligo eurilochus	0.04	37.9	2.29	44.0
Caligo zeuxippus	0.05	38.6	2.40	32.7
Caligo atreus	0.01	38.3	2.24	43.5
Anitirrhea pterocopha	0.05	39.2	2.59	21.0
Caerois gerdrudtus	0.29	62.0	2.60	21.2
Memphis chaeronea	0.83	17.6	2.32	15.0
Memphis cleomestra	0.75	16.5	2.51	14.2
Fountainea ryphea	0.78	21.4	2.40	15.1
Memphis aulica	0.47	19.0	2.36	14.6
Consul panariste	0.29	36.0	2.59	16.5
Prepona laertes	0.82	13.7	3.32	17.2
Prepona philipponi	0.88	14.4	3.17	19.5
Archaeoprepona demophoon	0.66	17.6	2.97	23.8
Archaeoprepona demophon.	0.55	11.6	3.16	18.7
Archaeoprepona camilla	0.22	19.0	3.07	24.5
Zaretis isidora	0.61	19.9	2.50	16.1

Subfamilies: Nymphalinae, Biblidinae, Heliconiinae, Limenitidinae, Satyrinae, Charaxinae. Scale: −60 −50 −40 −30 −20 −10

Figure 2. Phylogeny of the 64 Nymphalidae species collected at Reserva Río Canandé. Stratification values and means of the WA to TV ratio (WA : TV), aspect ratio (AR) and relative distance to wing centroid (RDC) are shown. Scale bar indicates time in MY.

statistical independence among our species data points [82,83]. We built a time-calibrated phylogeny using eight published [84] and 56 newly obtained partial sequences of the mitochondrial gene *cytochrome oxidase subunit I* (COI) from the species in our butterfly community (see [69] for details on DNA extraction,

marker amplification and sequencing). COI sequences were aligned in CLUSTALW (0.9% missing data and 304 distinct alignment patterns). A maximum-likelihood phylogeny was estimated in RAxML v.8.2 using a GTR + gamma model of DNA sequence evolution; a separate partition for third codon positions

to account for the variation in rates of substitution; and 800 bootstrap replicates. The novel COI data enabled us to investigate species without existing sequence data, but the use of a single, fast-evolving marker and the inclusion of a limited fraction of all Nymphalidae may result in poor estimates of the deeper topology due to saturation and long-branch attraction. To account for these problems, we constrained the ML tree search-space with a backbone topology (TreeBase 26598) representing 21 previously resolved splits above the genus level [85,86]. The resulting tree was ultrametricized using the RelTime procedure in MEGA7 [87,88] using 18 secondary calibration points derived from previous studies as reported in Timetree [89] (electronic supplementary material, appendix S5).

We tested the phylogenetic signal (the tendency for related species to resemble each other more than they resemble species drawn at random from a phylogeny, [90]) for stratification using Pagel's λ [91] and Blomberg's K [92] using, respectively, the Geiger [93] and Caper [94] packages in R [79].

3. Results

(a) Stratification patterns

The composition and abundance of butterfly assemblages were different between canopy and understorey (NMDS, figure 1). These differences between the strata were highly significant (ANOSIM, $p < 0.001$), and 30 species explained greater than 75% of the variation (SIMPER; electronic supplementary material, appendix S4). Twenty-nine species were significantly associated with canopy (C) (electronic supplementary material, appendix S1, binomial test). Of these, 11 belong to the Biblidinae and 9 to the Charaxinae. Five of 6 Limenitidinae were categorized as canopy species. Six species (*Hamadryas amphinome*, *Callicore atacama*, *Smyrna blomfildia*, *Eunica pomona*, *Heliconius hecalesia* and *Adelpha erotia*) had the highest preference for the canopy as indicated by the Bayespref analysis (stratification > 0.9). Twenty-two species were significantly more likely to be found in the understorey (U). Thirteen of those belong to Satyrinae (table 1, binomial test). The species showing the highest preference for the understorey (stratification < 0.1) were *Antirrhea philaretes*, *Caligo atreus*, *C. eurilochus*, *C. zeuxippus*, *Catoblepia orgetorix*, *Cithaerias pireta*, *Dulcedo polita*, *Eresia clara*, *Haetera piera*, *Heliconius eleuchia*, *H. erato* and *Pierella helvina*. Five out of 7 Nymphalinae showed no stratification (NS) (binomial test; table 1). Phylogenetic signal for stratification was highly significant ($\lambda = 0.660$, $p < 0.001$; K = 0.434, $p = 0.011$; table 2), meaning that closely related butterflies in our community tend to fly at similar heights above the ground.

(b) Allometry and stratification

Caerois gerdrudtus (stratification = 0.29 U), *Cissia confusa* (stratification = 0.57 NS) and *Magneuptychia mycalesis* (stratification = 0.85 C) had the largest WA : TV ratio (62.0, 48.0 and 47.5 respectively). *Memphis cleomestra* (stratification = 0.75 C), *Archaeoprepona demophon* (stratification = 0.55 NS) and *Prepona philipponi* (stratification = 0.88 C) had the smallest ratios (11.5, 11.6 and 14.4, respectively) (figure 2). Canopy and understorey butterflies differed significantly in their WA and TV allometric slopes (ANCOVA, $p < 0.001$; figure 3).

(c) Flight morphology and stratification

Strong phylogenetic signal was found for WA : TV, AR and RDC (table 2). PGLS analyses showed highly significant
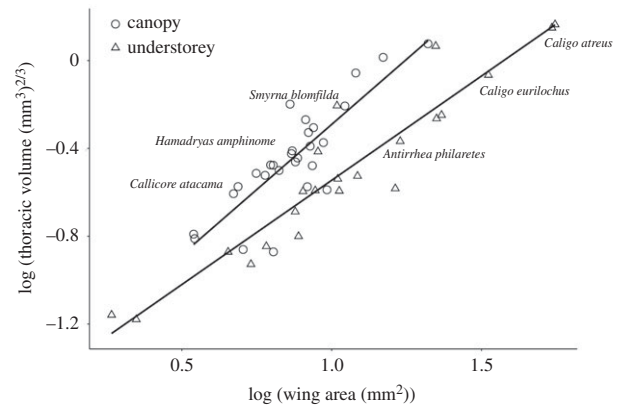
Figure 3. Allometric relationships between thoracic volume and wing area of canopy and understorey Nymphalidae species. Circles represent canopy and triangles represent understorey butterflies, categorized according to the binomial test. Canopy and understorey regression slopes were significantly different (ANCOVA, $p < 0.001$). The three butterflies with the strongest preference for each stratum (according to the Bayesian analysis) are noted.
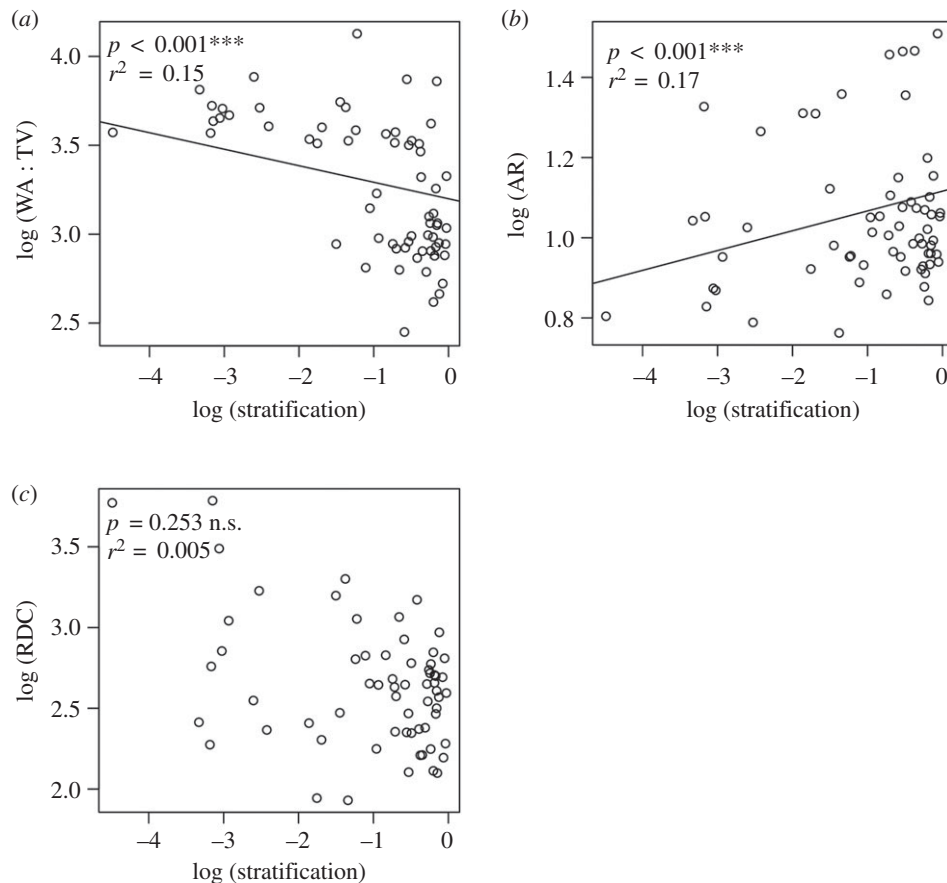
associations between stratification and WA : TV ($p < 0.001$, $r^2 = 0.15$) and AR ($p < 0.001$, $r^2 = 0.17$). For WA : TV the association was negative, whereas for AR the association was positive. In other words, butterflies in the canopy have relatively larger thoraxes and smaller, more elongated wings than understorey butterflies. No association was found between stratification and RDC ($p = 0.253$, $r^2 = 0.005$).

4. Discussion

The use of trait-based approaches to explore species responses to their biotic and abiotic environment are central to our understanding of the structure of natural assemblages [95], proving effective in tropical rainforests (e.g. [96,97]). Here, we report a consistent pattern of habitat specialization linked to the evolution of flight traits.

We found strong vertical stratification, consistent with previous studies of Neotropical butterflies [50,53–55,57,98]. Most species typically occupy a specific stratum, although the phylogenetic signal of stratification was lower than reported by Fordyce & DeVries [99] in Costa Rican Nymphalidae, in a study done at the genus level. Given the similarities in composition and structure, this difference probably reflects a divergence in flight height preferences within a genus, which highlights the importance of vertical dimensions as a niche partitioning mechanism for closely related taxa. Indeed, *Colobura dirce* and *C. annulata* were considered a single species until a closer examination revealed different larval morphology and ecology, with some evidence for divergence in flight height [100]. Here, we found several notable examples of sister species that differ in flight height as well as flight morphology, in *Pyrrhogyra*, *Catonephele*, *Hamadryas* and *Heliconius*. Furthermore, Nice *et al.* [58] showed that genetic divergence in sympatric canopy and understorey populations of *Archaeoprepona demophon* is comparable with that among isolated localities, separated by 1500 km. These examples suggest that a shift in flight height is difficult, but those lineages that manage to move into a new vertical stratum may find opportunities for adaptive radiation.

A number of factors, such as forest physiognomy and structure, resource availability and behaviour, have been associated with arthropod stratification [101], but these

Figure 4. Plotted PGLS models for the relations between stratification and morphological traits: (*a*) wing area to thoracic volume (WA : TV), (*b*) aspect ratio (AR) and (*c*) relative distance to wing centroid (RDC). Significant levels are ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, n.s. = not significant at $\alpha = 0.05$.

relationships may be highly specific. For example, vertical stratification in ants has been linked to species' preferred food source [102], whereas canopy fig wasps apparently use wind to passively carry them to their hosts [103]. Flight height was found to be correlated with the height of their larval host plants in clearwing butterflies and arctiid and geometrid moths [52,104], although it remains unclear if larval resource availability actually influences adult stratification. Within Morphini and Haeterini butterflies, a relationship between sex-specific behaviours, flight height and wing morphology has been reported [25,66]. Although each taxon has its own ecological niche, which may exert differential selection pressure, our results showed that a relationship between flight height and morphology can arise at a community level. This suggests that microhabitat preference may be an important factor contributing to the evolution of flight morphology [25,66,105].

For small ectotherms that operate at the scale of microhabitats, microscale climate systems are especially important [106,107]. It has been suggested that differences in available light and temperature influence butterflies inhabiting different microhabitats [50,57], as the microhabitat conditions select for distinct morphologies, which in turn determine flight modes [25,65]. In tropical lowland rainforests, vertical strata at the same location may have even steeper climatic gradients than those found across the dimensions of latitude and elevation [41]. Whereas there is a global theoretical drop in temperature of 1°C for every 100 m increase in altitude [108], or a drop of 1°C for every 154 km shift from northwards the equator [109], a lowland tropical forest can experience canopy-understorey daytime differences of 4–10°C in Gamboa,

Panama [110], or 5.8°C at midday in Durango, Ecuador (100 km NW from our study area; P. A. Salazar & S. Mena 2014–2016, unpublished data); with light availability in the understorey being 1% that of the canopy [111].

Because butterflies are poikilotherms, their metabolic rates are limited by available environmental heat [112]. Insect wingbeat frequencies increase with environmental temperature and hence higher heat availability [113,114]. A reduction in wing area is typically compensated for by an increase in wingbeat frequency, which increases energetic requirements [115]. Experimental reductions in WA significantly increased wingbeat frequencies in *Pontia occidentalis* and *Pierella helvina* [116,117]. Canopy butterflies might tend to have low WA : TV ratio because these morphologies are more suited for high performances in environments with high heat availability. Likewise, understorey butterflies may make more use of gliding flight, which is less energetically expensive than flapping flight [118,119] and would not require such high amounts of heat energy. Because acceleration is dependent on wing size and wing beat frequency [120], an increase in wing size in understorey butterflies may help to compensate for a decrease in thorax size. An elevated wing : thorax size ratio has been proposed to be adaptive for flight at cold temperatures in *Drosophila* [115,121], and large hindwings have been shown to enhance gliding performance in Haeterini (Nymphalidae) butterflies [118]. Moreover, because wings participate in thermoregulation by circulating haemolymph through their veins and thus gaining heat from the sun when they are opened [112], it is likely that a larger wing area is also advantageous for increasing body temperature in understorey butterflies. Consistent with these

arguments, Xing *et al.* [122] found butterflies with larger wing-spans and darker colours in cool, closed-canopy rainforests than in adjacent and hotter open woodlands in Australian tropical–rainforest ecosystems (after accounting for phylogenetic relationships). Furthermore, they found such butterflies to be more active in the shade and during crepuscular hours, while brighter and small-winged butterflies were more active in the sun and midday hours (temperature differences of 2.3°C at crepuscular hours and 3.4°C at midday; figure 4).

We hypothesized that shifts between morphological traits might help compensate for performance constraints imposed by habitat in understorey butterflies. Studies have shown a negative relation between butterfly WA : TV ratio (or analogous parameters) and flight speed [9,10,64,123]. First, because having a high aspect ratio (AR) when using gliding flight reduces metabolic costs compared with those of powered flight [118], understorey butterflies were expected to have high ARs as a possible compensation for a more limited metabolic energy availability. Second, because a centroid located distally to the body improves flapping flight performance, the RDC was expected to be positively associated with strata preference [16,61,123]. However, AR was higher for canopy butterflies and no consistent relation was found for RDC. Because high values of AR and low WA : TV ratios were found to be associated with high performance in terms of speed and manoeuvrability both in temperate [9,59,60] and tropical butterflies [10], it is tempting to think that the evolution of butterfly morphology in the canopy might, in general, favour a faster and more agile flight compared with the understorey. If this is the case, aspects of ecology related to flight may shift consistently between the strata. For example, in a Bornean butterfly community, predator abundance was higher in the canopy, creating a selective pressure towards fast and agile flight [44]. Nevertheless, other morphological traits, such as having a dark, hairy and large absolute thorax (to dissipate heat more slowly), combined with behavioural strategies, such as a short flight duration, shifts in wing coupling, or basking in sunlit gaps, may permit high flight performances in understorey butterflies [124,125]. These and other aspects remain to be explored to fully understand the links between flight performance and ecology.

In conclusion, our results support the hypothesis that butterfly habitat preference for canopy and understorey is associated with a set of morphological flight traits. These results highlight the importance of vertical dimensions in the generation of morphological and ecological diversity.

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