

Vertical distribution, flight behaviour and evolution of wing morphology in *Morpho* butterflies

P. J. DeVries^{1*}, Carla M. Penz¹ and Ryan I. Hill²

¹Department of Biological Sciences, University of New Orleans, 2000 Lakeshore Drive, New Orleans, LA 70148, USA; and

²FAS Center for Systems Biology, Harvard University, 52 Oxford St, Cambridge, MA 02138, USA

Summary

1. Flight is a key innovation in the evolution of insects that is crucial to their dispersal, migration, territoriality, courtship and predator avoidance. Male butterflies have characteristic territoriality and courtship flight behaviours, and females use a characteristic flight behaviour when searching for host plants. This implies that selection acts on wing morphology to maximize flight performance for conducting important behaviours among sexes.

2. Butterflies in the genus *Morpho* are obvious components of neotropical forests, and many observations indicate that they show two broad categories of flight behaviour and flight height. Although species can be categorized as using gliding or flapping flight, and flying at either canopy or understorey height, the association of flight behaviour and flight height with wing shape evolution has never been explored.

3. Two clades within *Morpho* differ in flight behaviour and height. Males and females of one clade inhabit the forest understorey and use flapping flight, whereas in the other clade, males use gliding flight at canopy level and females use flapping flight in both canopy and understorey.

4. We used independent contrasts to answer whether wing shape is associated with flight behaviour and height. Given a single switch to canopy habitation and gliding flight, we compared contrasts for the node at which the switch to canopy flight occurred with the distribution of values in the two focal clades. We found significant changes in wing shape at the transition to canopy flight only in males, and no change in size for either sex. A second node within the canopy clade suggests that other factors may also be involved in wing shape evolution. Our results reinforce the hypothesis that natural selection acts differently on male and female butterfly wing shape and indicate that the transition to canopy flight cannot explain all wing shape diversity in *Morpho*.

5. This study provides a starting point for characterizing evolution of wing morphology in forest butterflies in the contexts of habitat selection and flight behaviour. Further, these observations suggest that exploring wing shape evolution for canopy and understorey species in other insects may help understand the effects of habitat destruction on biological diversity.

Key-words: ecomorphology, forest canopy, insect wing shape, vertical distribution

Introduction

Biological diversity in terrestrial ecosystems is dominated by insect species, and a key element of their success has been the evolution of flight (Grimaldi & Engel 2005). Flight has allowed insects to move within and among habitat types, seasons and elevations, and to expand across large geographical distributions (Johnson 1969; Johnson & Bowden 1973; Riley *et al.* 1995; Greenslade, Farrow & Smith 1999). Given the dominance of insects in ecological space and time and that they have evolved a diversity of flight mechanisms (Ellington

1991; Grimaldi & Engel 2005), it seems evident that selection to perform crucial behaviours has sculpted insect thoracic and wing morphology. Indeed, thoracic, and in particular, wing morphology have been associated with flight performance during dispersal, migration, territoriality, courtship and predator avoidance (e.g. Ellington 1984; Benson, Haddad & Zikán 1989; Srygley 1994; Dudley 2000; Breuker, Brakefield & Gibbs 2007; Dockx 2007; Johansson, Soderquist & Bokma 2009).

In general, longer narrower wings are considered aerodynamically more efficient for long gliding flights, while short wide wings are thought to be better suited for manoeuvrable flapping flight. Wing aspect ratio and non-dimensional

*Correspondence author. E-mail: phil.devries@gmail.com

moment parameters are commonly used to describe and quantify these characteristics of insect wing shape, particularly for butterflies (Ellington 1984; Betts & Wootton 1988; Dudley 1990; Srygley 1994). Here, wing aspect ratio indicates the proportion of wing length to the mean wing chord, and the first radial moment of wing area refers to the position of wing centroid (i.e. centre of wing area). Theoretically, a longer, narrower wing will be aerodynamically more efficient than its short, wide counterpart because it reduces induced drag and associated power expenditure (Vogel 1994). Wing centroid position is also highly correlated with the second and third moments of wing area (Ellington 1984; Betts & Wootton 1988), which are proportional to the mean lift force and profile power of the wing respectively (Ellington 1984). A distal positioning of wing centroid at constant wing area is therefore associated with increased force production and greater induced drag relative to a more basal position (Srygley 1994). Because both measures relate to the aerodynamics of flight, wing shape may be associated with flight behaviour. For example, butterfly species that glide extensively exhibit a relatively elongate wing shape, particularly the forewing, with high values of aspect ratio and low values of wing centroid that decreases the energetic costs of flight. Conversely, species with more rounded overall wing shape have lower values for wing aspect ratio and higher values for the position of the wing centroid and are predicted to be more manoeuvrable (Betts & Wootton 1988), and employ flapping flight to a greater degree (see Dudley 2000). In addition, such wing shape differences may also be exhibited in the context of sexual dimorphism within species (R.I.H., unpublished data).

All butterflies combine wing flapping and gliding, and the proportional allocation between the two behaviours probably affects the amount of energy used for flight. For example, flapping flight can be critical to powering take-off (Berwaerts, Matthysen & Van Dyke 2008), whereas maximizing flight time demands efficient flight and may be important for patrolling territories (Benson, Haddad & Zikán 1989) and during migration (Gibo & Pallett 1979; Dudley & DeVries 1990). Within the same species, migratory populations of insects often have significantly different wing shapes than non-migratory ones. Migratory individuals generally have a more elongate forewing apex that is energetically and aerodynamically more efficient for gliding flight (Williams 1958; Johnson 1969; Yao & Zhang 2001; Dockx 2007; Johansson, Soderquist & Bokma 2009). These observations suggest that, in general, natural selection has acted to influence insect flight behaviour and wing morphology relative to the costs of flight, albeit in the demanding context of migratory energetics.

The insect biota in tropical rain forests is distributed between the forest canopy and understorey. Although together they form a vertical continuum, the canopy and understorey differ in temperature, humidity, light levels, plant species and their life-forms, and these factors potentially influence the vertical distribution of the species inhabiting tropical forests (Pittendrigh 1950; Grubb *et al.* 1963; Allen,

Lemon & Muller 1972; Elton 1973; Richards 1976; Whitmore 1984; Fetcher, Oberbauer & Strain 1985; Basset *et al.* 2001). Various studies on tropical forest butterflies have shown vertical stratification among species (Jackson 1961; Burd 1994; Beccaloni 1997), and there is strong evidence that neotropical fruit-feeding Nymphalidae are partitioned between the understorey and canopy at all taxonomic levels (DeVries 1988; DeVries & Walla 2001; and references therein). Consideration of flight performance and wing shape, coupled with environmental differences between the canopy and understorey suggest that there is potential variance in wing shapes among butterfly species inhabiting these two strata.

The genus *Morpho* (Nymphalidae) occurs from Mexico through Central and South America contains about 30 species (Lamas 2004), and includes some of the most visually obvious of all butterflies. Because they are so noticeable many observations have been made on *Morpho* butterflies, including characterizing two broad categories of flight behaviour and vertical distribution in forest habitats (Table 1). In some species, males predominantly use gliding flight to patrol at the canopy level whereas females of the same species use flapping flight to search for oviposition sites in both the canopy and understorey. Conversely, both sexes in other species fly in the mid to understorey, mainly employing flapping flight. In addition, long-term ecological studies also show vertical stratification among species of *Morpho* (DeVries, Murray & Lande 1997; DeVries & Walla 2001; P.J.D., unpublished data). Considered in concert, these observations indicate that spatial use of forest microhabitats differs among *Morpho* species.

The phylogenetic study of Penz & DeVries (2002) suggested that canopy-flying species constitute a monophyletic group within *Morpho*, and that understorey occupancy and predominantly flapping flight is an ancestral condition. Given existing linkages between butterfly flight performance and wing shape (Dudley 2000; Berwaerts, Matthysen & Van Dyke 2008), we expect differences in wing morphology to be related to flight kinematics in *Morpho*. Accordingly, this study tests whether evolution of wing morphology is associated with flight height and gliding. We do so by analysing forewing length, wing aspect ratio and centroid in species from two monophyletic groups within *Morpho*, and testing whether independent contrasts in these wing variables at the transition to gliding canopy flight are larger than contrasts above this node.

Materials and methods

SPECIES SAMPLED

Based on the phylogeny of Penz & DeVries (2002) we focused on the clade that includes most species of *Morpho*, and that comprised two sub-clades: the *achilles*- and *hecuba*-groups. Study specimens were the same as those used in Penz & DeVries (2002), where the specimen locality data and repositories are noted. For continuity, we employ the same taxonomic names as Penz & DeVries (2002). More recent changes in nomenclature and the authorities for species names may

Table 1. Observations on primary flight height and behaviours in members of the genus *Morpho*. Species marked with an asterisk were not included in the analysis, but listed here for completeness. Names in parentheses correspond to nomenclature in Lamas (2004), and show the differences in the status of some species or subspecies between Penz & DeVries (2002) and Lamas (2004). References: (1) Fruhstorfer (1913) includes his observations and those of H.W. Bates, F.D. Godman, P. Hahnel, A. Mabilde, O. Michael, O. Salvin and J.F. Zikan; (2) Otero (1986); (3) DeVries (1987) includes his observations and those of R. Canet and I. Chacón; (4) DeVries & Martínez (1993); (5) Neild (2008); (6) DeVries, unpublished data; (7) DeVries, pers. obs.; (8) Penz, pers. obs. Und, understorey; Can, canopy; M, male; F, female

Species	Height	Observations	Source
<i>adonis</i> * (<i>marcus</i>)	Und	Flies fast sometimes at ~3.5 m height	1
<i>eugenia</i> *	Und	Males show slow flapping flight in mid to understorey of forest	7
<i>aega</i> *	Und	One female observed flying in understorey searching for oviposition sites, flapping flight	2, 5, 7, 8
		Flap-sail, slow flight	
		understorey, sometimes ascending along canyon cliffs and waterfalls	
<i>portis</i> *	Und	Flies slowly, 1–2 m above the ground	1
<i>sulkowskyi</i> *	Und	Sails on grassy mountain slopes	1
<i>achilles</i>	Und	Fast flapping flight, low to the ground	1, 2, 5
		Trap data for Ecuador, Sucumbios, La Selva (10 years): 844 M and 547 F in understorey, 1 M and 2 F in canopy	6
		Trap data for Ecuador, Napo, Jatun Sacha (1 year): 98 individuals in understorey, none in canopy	6
<i>achillaena</i> (<i>helenor</i>)	Und	Both sexes fly ~1 m from the ground	1
<i>peleides</i> (<i>helenor</i>)	Und	Both sexes with floppy, zigzag flight in the understorey and along rivers and forest edges; flies with <i>granadensis</i>	1, 3, 5
		Trap data for Costa Rica, Tirimbina (5 years): 46 individuals in understorey, one in canopy	6
<i>granadensis</i> (<i>deidamia</i>)	Und	Both sexes with floppy, zigzag flight in the understorey and along rivers and forest edges; flies with <i>peleides</i>	3
		Trap data for Costa Rica, Tirimbina (5 years): 49 individuals in understorey, one in canopy	6
<i>catenarius</i> (<i>epistrophus</i>)	Und	Both sexes with slow floppy flight in the understorey, among trees or at forest edges	1, 7, 8
<i>polyphemus</i>	Und ^a	Flies from near the ground to the top of trees; males sail along the forest canopy or along river beds	1, 3
<i>laertes</i> (<i>epistrophus</i>)	Und	Both sexes with slow floppy flight in the understorey, among trees or at forest edges	
<i>deidamia</i>	Und	Fast flapping flight	1
		Trap data for Ecuador, Sucumbios, La Selva (10 years): four individuals in understorey, none in canopy	6
		Trap data for Ecuador, Napo, Jatun Sacha (1 year): five individuals in understorey, none in canopy	
<i>didius</i> (<i>menelaus</i>)	Und ^b		
<i>amathonte</i> (<i>menelaus</i>)	Und	Both sexes with floppy flight	3
		Trap data for Costa Rica, Tirimbina (5 years): 62 individuals trapped in understorey, none in canopy	6
<i>menelaus</i>	Und ^c	Flies high; females can be found near tree tops during oviposition times	1, 2
		Slow, floppy flight near the ground	
		Trap data for Ecuador Sucumbios La Selva (10 years): 173 M and 66 F in understorey, 1 M in canopy	6
		Trap data for Ecuador, Napo, Jatun Sacha (1 year): seven individuals in understorey, none in canopy	
<i>godartii</i> (<i>menelaus</i>)	Und ^b		
<i>rhetenor</i>	Can	Males fly 3–6 m height, flapping and gliding flight	1
		Females settle on wet river banks, fly slowly when disturbed	
		Trap data for Ecuador, Sucumbios, La Selva (10 years): 2 M in canopy; none in understorey	6
		Trap data for Ecuador, Napo, Jatun Sacha (1 year): 1 M in understorey at forest edge	
<i>cypris</i>	Can	Males patrol at or above canopy level with gliding flight	1, 3, 4
		Females weave in and out of the foliage with flapping flight	6
		Trap data for Costa Rica, Tirimbina (5 years): five individuals in canopy, none in understorey	
<i>anaxibia</i>	Can	Slow, sailing flight at canopy level	1, 2, 7, 8
		Females settle on wet river banks	
<i>cisseis</i>	Can	Sailing flight at canopy level	1, 5
<i>telemachus</i> *	Can	Gliding at canopy level in forest and along forest edges	5, 7
<i>hecuba</i>	Can	Flies at canopy level and for long distances (~30 km in 2–3 h); quiet, slow flight	1, 5, 7
<i>hercules</i>	Can	Gliding flight at or above canopy	1, 7
<i>amphitryon</i>	Can ^b		
<i>perseus</i> (<i>telemachus</i>)	Can	Gliding flight at or above canopy (8–10 m), scarcely flaps wings	1
<i>theseus</i>	Can	Soars at canopy level, males patrol breaks in the forest canopy	3, 5

^aInterpreted as 'within the forest', or understorey microhabitat. ^bBased on character optimization (canopy/ understorey flight) using MACCLADE (Maddison & Maddison 2000). ^cConflicting reports, but Fruhstorfer's description seems to infer inner forest.

be found in Lamas (2004) checklist. Unless indicated otherwise, measurements were taken from two males and one female from 12 species in the *Morpho achilles*-group (*achilles*, *achillaena* (1 M, 1 F), *peleides*, *granadensis*, *deidamia*, *laertes*, *polyphemus*, *catenarius*, *amathonte* (1 M, 1 F), *didius*, *menelaus* and *godartii*) and nine species in the *Morpho hecuba*-group (*hecuba*, *cisseis*, *hercules*, *perseus*, *theseus*, *anaxibia*, *amphitryon* (1 M), *cypriis* and *rhetenor*). Although we measured few individuals per species, our sampling design probably captured the spectrum of interspecific variation within the two focal sub-clades inasmuch as our analyses focused on comparing morphological variation between two ecologically distinct species-groups rather than within species variation. Because the taxa *achillaena*, *peleides* and *achilles* formed an unresolved polytomy in Penz & DeVries (2002), analyses here were performed with each of the three possible topological resolutions.

FLIGHT HEIGHT

We consider flight height (canopy or understorey) to be correlated with, and to provide a good indication of microhabitat use among *Morpho* species. Information on primary flight heights was distilled from three sources: quantitative canopy and understorey fruit-trap samples, published and unpublished natural history records, and personal observations (Table 1). In some cases, we were able to use all three sources, but in others we could not. For example, all three sources were in agreement with respect to flight height for *Morpho achilles*. On the other hand, Fruhstorfer's (1913) and our observations indicate that *Morpho anaxibia* flies in the canopy, but no trap data were available. In three cases (*Morpho didius*, *Morpho godartii*, *Morpho amphitryon*), we used character optimizations performed with MACCLADE (Maddison & Maddison 2000) to predict species flight height. Because some observations indicate differences between sexes (Table 1), our analyses treated males and females separately.

MORPHOLOGICAL VARIABLES

Wing variables were measured from images obtained by placing individual specimens against a 5-mm paper grid. Images were taken with a Nikon Coolpix 900 camera (Nikon Corporation, Japan) mounted on a tripod and oriented in the same plane as the wings. Forewing length was calculated with NIH IMAGE software (<http://rsb.info.nih.gov/nih-image>). Separate photographs of the forewing and hindwing were joined into a single composite image using Adobe Photoshop (Adobe systems, Incorporated, San Jose, California, USA) by overlapping the hindwing costal margin with forewing vein Cu2 to approximate the overlap seen in natural flight (Betts & Wootton 1988; S. Horisawa, pers. comm.).

Composite images representing the aerodynamically active wing surface were used to calculate values of forewing length, aspect ratio and wing centroid (Fig. 1; *Morpho* wings exhibit amplexiform coupling flight). These images were processed in Adobe Illustrator (Adobe systems, Incorporated, San Jose, California, USA) by overlaying 14 concentric lines radially equidistant from one another to obtain 15 concentric areas. This circular plot was centred on the forewing base, and the outermost circle was adjusted so that it touched the forewing apex. This image was laser-printed on paper, cut out, and the concentric strips then cut along each line to yield 15 wing sectors. Each sector was weighed, and these weights coupled with known areal mass density of paper were used to calculate aspect ratio and the wing centroid (first radial moment of wing area) using the equations in Ellington (1984). Mean values of forewing length, aspect ratio and wing centroid were calculated separately for males and females (Table 2).

INDEPENDENT CONTRASTS

In the phylogeny of Penz & DeVries (2002), the transition to canopy flight within *Morpho* occurred a single time (see *hecuba*-group in

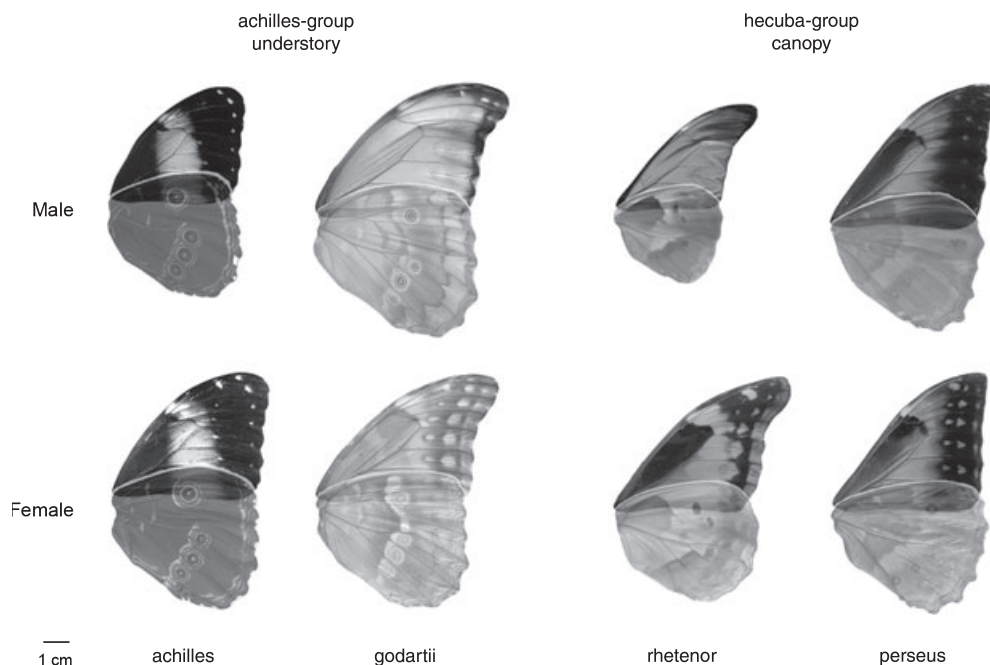


Fig. 1. Photographic images showing how a dorsal view of forewings and a ventral view of hindwings were aligned for analysis. Two representatives of the *Morpho achilles*- and *hecuba*-groups were selected to demonstrate the range in wing shape variation within each group.

Table 2. Forewing length (FW), aspect ratio (AR) and wing centroid (WC) for males and females (M and F) in the understorey *achilles*-group and canopy dwelling *hecuba*-group. Values here were rounded from those used in calculating contrasts based on the tree in Fig. 2

Species	Sex	FW (mm)	AR	WC
<i>achilles</i> -group				
<i>achillaena</i>	M	62.0	2.79	0.54
	F	70.0	2.81	0.53
<i>achilles</i>	M	65.5	2.67	0.54
	F	75.5	2.58	0.54
<i>amathonte</i>	M	89.5	3.23	0.51
	F	91.0	3.04	0.51
<i>catenarius</i>	M	67.8	2.97	0.53
	F	73.2	2.86	0.53
<i>deidamia</i>	M	75.3	2.96	0.52
	F	78.1	2.95	0.53
<i>didius</i>	M	84.8	2.92	0.51
	F	87.0	2.98	0.51
<i>godarti</i>	M	80.2	3.33	0.51
	F	80.7	3.35	0.51
<i>granadensis</i>	M	66.3	2.77	0.53
	F	67.8	2.58	0.54
<i>laertes</i>	M	68.9	3.05	0.53
	F	74.6	2.96	0.53
<i>menelaus</i>	M	77.2	3.05	0.51
	F	73.8	2.88	0.53
<i>peleides</i>	M	65.8	2.90	0.52
	F	74.4	2.61	0.54
<i>polyphemus</i>	M	82.0	2.75	0.52
	F	78.2	3.31	0.53
<i>hecuba</i> -group				
<i>amphitryon</i>	M	87.2	3.50	0.49
	F	No data	No data	No data
<i>anaxibia</i>	M	76.4	3.43	0.51
	F	84.2	3.27	0.50
<i>cisseis</i>	M	89.1	3.24	0.51
	F	90.5	3.20	0.52
<i>cypris</i>	M	62.7	3.88	0.48
	F	75.3	3.32	0.50
<i>hecuba</i>	M	90.4	3.34	0.51
	F	107.6	3.60	0.51
<i>hercules</i>	M	75.9	3.20	0.51
	F	74.1	2.88	0.53
<i>perseus</i>	M	72.0	3.19	0.51
	F	72.9	3.03	0.53
<i>rhetenor</i>	M	70.1	4.20	0.48
	F	84.7	3.85	0.48
<i>theseus</i>	M	83.5	3.24	0.50
	F	78.2	3.57	0.51

Fig. 2). Furthermore, because all canopy species descended from a common ancestor, their flight behaviour and wing characteristics are confounded by phylogeny. This scenario precludes a standard correlated evolution approach using independent contrasts to identify significant relationships between ecological and morphological traits. Instead, we used an approach developed by Garland *et al.* (1993) to investigate home range size differences between carnivores and ungulates, a scenario similar to that described here for *Morpho*. This approach focuses on the particular node where the shift in ecology occurs to ask if there is significantly more change at this node than elsewhere in the tree. The logic is that if a trait differs between two clades it will show the largest change at the split between the two

clades. Accordingly, we tested whether independent contrasts in forewing length, wing aspect ratio and wing centroid were larger at the node between the two *Morpho* sub-clades compared to contrasts above this node. Independent contrasts (Felsenstein 1985) of the three morphological variables were calculated with the PDAP package (Midford, Garland & Maddison 2003) in Mesquite (Maddison & Maddison 2009). Because the phylogeny did not have an estimate of branch lengths, these were arbitrarily set equal to one, and we standardized the contrasts by dividing by their standard deviations (the square root of their summed branch lengths). The adequacy of contrast standardization was assessed with correlations between the absolute value of standardized contrasts, and the standard deviation of contrasts for each trait (Garland, Harvey & Ives 1992).

To test whether body size and wing shape changed with the shift to canopy flight, we compared the basal node between the two sub-clades (node 'S' in Fig. 2) with the distribution of changes within the entire clade above that node (Garland *et al.* 1993). Forewing length was used as a proxy for body size since it is correlated with body mass across butterflies (Dudley 1990) and within butterfly groups (R.I.H., unpublished data). Mean forewing length appeared to be greater in the *hecuba*-group for both sexes. Thus, we used a one-tailed single observation *t*-test adapted for this situation (Garland *et al.* 1993, p. 278) to test whether size increased with the shift to flying in the canopy. To test whether changes in aspect ratio and wing centroid were related to changes in size (= forewing length), we used least-square linear regressions through the origin (Garland, Harvey & Ives 1992). Regressions excluded the contrast at node 'S' since this was the contrast of interest for sub-clade differences (see below). Contrasts in forewing length were converted to positive values, and the sign of corresponding contrasts in aspect ratio and wing centroid were changed accordingly (Garland, Harvey & Ives 1992).

To test whether wing shape changed with flight height, the value of the node 'S' contrasts for aspect ratio and wing centroid were compared to the 95% prediction interval of the regressions against forewing length. Since regressions were computed through the origin, we followed Garland *et al.* (1993) by calculating the prediction intervals using the equations in Neter, Wasserman & Kutner (1989). If the contrast at node 'S' fell outside the prediction interval, then more change in wing shape occurred at the transition to canopy flight relative to changes within the two groups. As the *hecuba*-group was known *a priori* to have higher average aspect ratio and lower average wing centroid than the *achilles*-group, we used one-tailed *t*-tests for the comparisons.

Because males and females can differ in their flight behaviours, and *Morpho cypris* and *Morpho rhetenor* are strongly sexually dimorphic (Fruhstorfer 1913; DeVries 1987), we partitioned our analyses by sex and tested for differences in wing morphology. Inasmuch as it was known *a priori* in both the *achilles*- and *hecuba*-groups that females had higher mean forewing lengths, lower mean aspect ratios, and higher mean wing centroids than males, we used one-tailed *t*-tests to compare the standardized contrasts between sexes. Statistics were done using R (R Development Core Team 2008).

Results

Contrasts in wing shape variables indicated that whereas some wing measures differed significantly between males and females, others did not. We found no difference in the *achilles*-group contrasts of forewing length, aspect ratio and wing centroid between males and females, pointing to morphological homogeneity among sexes and species in this group

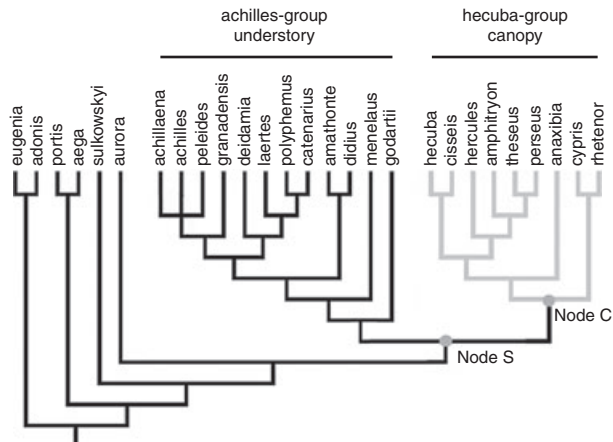


Fig. 2. Phylogeny of *Morpho* (after Penz & DeVries 2002) indicating taxa in the *achilles*- and *hecuba*-groups and their flight heights. Node 'C' is the basal-most contrast within the canopy inhabiting *hecuba*-group. Node 'S' marks the shift to canopy flight based on the optimization of 'flight height' onto the cladogram.

(Table 3). Although male and female forewing length did not differ in the *hecuba*-group, the contrasts in wing centroid and aspect ratio differed significantly between sexes (Table 3). Here, the smaller contrasts for males indicated less variation in male wing shape among species (Fig. 3). We note that analyses using the three different topologies to account for the polytomy among *Morpho peleides*, *Morpho achillaena* and *M. achilles* made no qualitative difference to any of our results.

The associations between forewing length and aspect ratio or wing centroid varied with sex. Standardized contrasts in forewing length showed no significant correlation with aspect ratio or wing centroid contrasts in males (Table 4). There were, however, significant correlations between contrasts of forewing length and aspect ratio and wing centroid in females (Table 4). Thus, changes in wing shape are size independent for males, but not females.

Comparing the pooled contrasts (*achilles*- and *hecuba*-groups combined), above node 'S' with the contrast at this basal node allowed us to compare changes in wing shape between clades. The contrasts showed that forewing length at node 'S' did not differ from the distribution of pooled contrasts above node 'S' for either sex (Table 3). Further, there was no detectable association between body size, flight height and flight behaviour, even in the *hecuba*-group where large variation in wing length was evident (Fig. 3). Nevertheless, the contrast at node 'S' for aspect ratio and wing centroid differed significantly in males, but not females (Fig. 3). This indicates that the switch to flight at canopy height is associated with marked changes in male wing morphology in the *hecuba*-group.

As a result of their notably elongate forewing apex the sister species *M. cypris* and *M. rhetenor* exhibited the highest aspect ratio within the *hecuba*-group (Table 2). This outcome strongly affected the calculated contrast between the clade containing *cypris* and *rhetenor* and all other species in the group (contrast at node 'C' in Fig. 2). These sister taxa also

Table 3. Comparisons of contrasts in forewing length (FW), aspect ratio (AR) and wing centroid (WC). Values for *t* and *P* here are for the polytomy resolution with *achillaena* and *achilles* as sister taxa. However, topology differences did not qualitatively alter the results. Note that for 'group', all contrasts within a clade were used, whereas 'node' implies only a single contrast. See Fig. 2 to locate specific groups and nodes. M, male; F, female

Comparison			<i>t</i>	<i>P</i> -value
<i>t</i> -Tests				
Sex, <i>achilles</i> -group	FW	M vs. F	0.28	0.61 NS
	AR	M vs. F	-0.009	0.50 NS
	WC	M vs. F	0.16	0.56 NS
Sex, <i>hecuba</i> -group	FW	M vs. F	1.14	0.14 NS
	AR	M vs. F	2.06	0.030 *
	WC	M vs. F	-2.12	0.029 *
Node 'S' vs. All contrasts above node 'S'	FW	M	0.66	0.26 NS
		F	0.30	0.38 NS
Regression (95% prediction interval)				
Node 'S' vs. All contrasts above node 'S'	AR	M	-	< 0.05 *
		F	-	> 0.05 NS
Node 'S' vs. All contrasts above node 'S'	WC	M	-	< 0.05 *
		F	-	> 0.05 NS

affected the overall variance within the *hecuba*-group to the extent that, for males, the contrast at node 'C' fell outside the 95% prediction interval (Fig. 2). This result suggests that the elongate forewing apex in *M. cypris* and *M. rhetenor* reflects further morphological evolution beyond the general changes shown by other members of the *hecuba*-group.

Discussion

Here, we used field observations on flight height, flight behaviour and quantitative measures of wing morphology to test for associations among species in two sister groups of *Morpho* butterflies. Our results support the hypothesis that natural selection has acted to influence flight behaviour and wing morphology relative to the costs of flight. Specifically, we found that smaller contrasts and variances in forewing length, aspect ratio and wing centroid among species in the *achilles*-group (Table 3) were consistent with field observations indicating that both sexes fly in the understory and use a similar flapping flight (Table 1). Given observations that only members of the *hecuba*-group utilize gliding flight extensively, we expected that parameters of wing shape related to gliding would change at the node 'S'; where the switch to canopy flight height occurred (Fig. 2). Although there was no significant change in forewing length for either sex (Table 3), we found significantly greater change in aspect ratio and wing centroid at node 'S' in males, but not females (Fig. 3).

Our study of *Morpho* butterflies revealed significant differences in aspect ratio and wing centroid among sexes, including that wing shape changes were independent of body size in males, but not females (Fig. 3, Table 3). These findings are consonant with work showing sex-related differences in flight

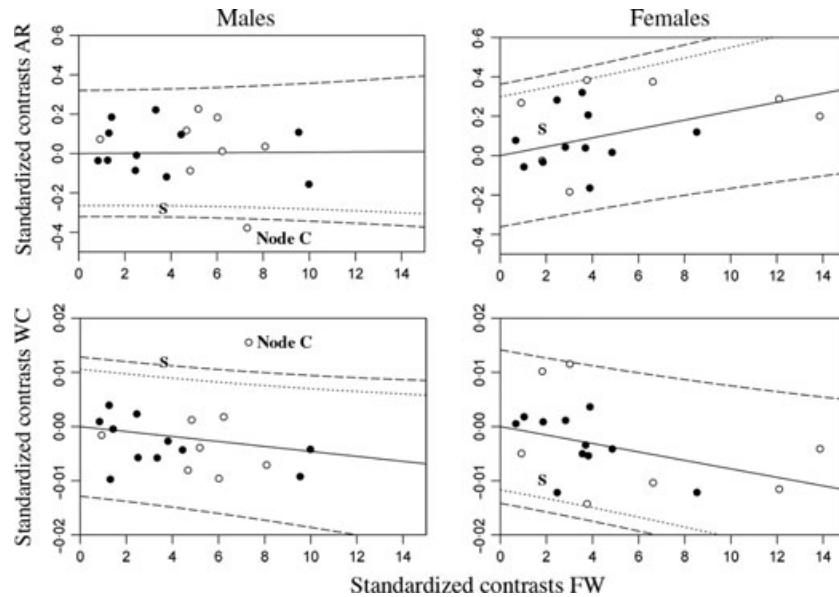


Fig. 3. Scatterplots of standardized contrasts in forewing length (FW) vs. aspect ratio (AR, top row) and wing centroid (WC, bottom row) for males (left) and females (right). Contrasts within the *achilles*-group (solid circle) and *hecuba*-group (open circle) are plotted, with node 'S' indicated with a letter. Solid lines represent the least-square linear regression through the origin for the contrasts above node 'S'. Wide dashed lines represent the two-tailed prediction interval, dotted lines the one-tailed prediction interval. Node 'S' falls outside of the one-tailed prediction interval for both variables in males, but not females. The basal *hecuba*-group contrast (labelled node 'C') falls outside the two-tailed prediction interval for males.

Table 4. Least-square linear regression for contrasts for males (M) and females (F) within the *achilles* and *hecuba* clades above node 'S'. Regressions were through the origin using positivized standardized contrasts in forewing length (FW) with aspect ratio (AR) and wing centroid (WC) adjusted accordingly. Females showed a correlated evolution between forewing length and wing shape, but males did not. The statistics presented here refer to the topology in which *achillaena* and *achilles* are sister taxa, but topology did not affect results (see text)

<i>X</i> vs. <i>Y</i>	Sex	Slope	r^2	<i>F</i>	<i>P</i> -value
FW vs. AR	M	0.00067	0.0006	0.010	0.92 NS
	F	0.0225	0.371	10.02	0.006 **
FW vs. WC	M	-0.00046	0.140	2.92	0.10 NS
	F	-0.00078	0.317	7.87	0.012 *

performance in other butterflies. For example, in European *Pararge aegeria* (Linnaeus), males show better flight performance at increased ambient temperatures than females, and flight performance can differ between populations in forested and open areas (Merckx, Karlsson & Van Dyke 2006). Secondly, male territorial and flight behaviour in *P. aegeria* is genetically associated with aspect ratio, body mass and relative thoracic mass (Berwaerts, Matthysen & Van Dyke 2008). Finally, the study by Breuker, Brakefield & Gibbs (2007) on wing morphology and sex-specific dispersal in European *Melitaea cinxia* (Linnaeus) showed that changes in forewing shape were associated with dispersal only in females.

In nature, changes in flight behaviour from flapping to gliding represent subtle adjustments directly linked to an

individual butterfly's energy budget and physiology (Srygley & Thomas 2002; Dudley & Srygley 2008). Our study found that wing shape within the genus *Morpho* is associated with flight height. Observations from canopy towers and hilltops indicate that males in the *hecuba*-group (e.g. *Morpho hecuba*, *Morpho telemachus*, *Morpho theseus*, *Morpho hercules*) often glide at canopy height for distances of over 1 km (P.J.D., pers. obs.). Given that gliding is less costly energetically than flapping (Wakely & Ellington 1997), we reason that the shift to gliding flight at canopy height (Fig. 2) is associated with wing shape changes that reflect long distance patrolling behaviours of the male sex. This observation is consistent with the hypothesis that wing shape evolved to improve aerodynamic efficiency (Dudley 1990). Since female wing shape did not change significantly at the shift to canopy (Figs 2 and 3, Table 3), aerodynamic efficiency only partially explains wing shape patterns within *Morpho*. Female wing morphology appears more constrained and correlated with size, perhaps as result of increased predation pressure on females (Ohsaki 1995), and/or increased mass allocation to the abdomen related to reproductive tissues (Marden & Chai 1991). Here, sex differences combined with correlated evolution of size and shape in females (Fig. 3) imply that there is a conflict between optimal wing shape for male and female flight characteristics according to the predominant activities performed by each sex (e.g. see Johansson, Soderquist & Bokma (2009) for mate-guarding and migrating in dragonflies). In sum, we conclude that the shift to gliding flight at canopy height in *hecuba*-group males is coincident with the evolution of wing shape that reduces the energetic costs of patrolling in the can-

opy, and in contrast, wing morphology of *hecuba*-group females has remained more conserved and similar to males and females of understory species.

In addition to detecting a shift in wing shape at the switch to canopy flight (see Fig. 2, node 'S'), the contrast at node 'C' showed a large change in male wing shape within the *hecuba*-group itself. Node 'C' corresponds to the contrast between the branch leading to *M. cypris* and *M. rhetenor* and the rest of the *hecuba*-group. It is thus affected by the two species with wing shape at the far end of the continuum in *Morpho* (Fig. 1) that show extreme values of aspect ratio and wing centroid (Table 2). The large difference at node 'C' indicates unique wing shape evolution within the *hecuba*-group that cannot be explained by the shift to canopy flight alone.

Although pronounced colour pattern dimorphism in *Morpho* may have evolved (or was lost) multiple times (Penz & DeVries 2002), *M. cypris* and *M. rhetenor* are the only strongly sexually dimorphic species in the *hecuba*-group with intensely iridescent blue males and dull, yellow-brown females. In contrast to other species of *Morpho*, the forewings of *M. cypris* and *M. rhetenor* are markedly elongate (i.e. high aspect ratio). As noted in Penz & DeVries (2002), the evolution of sex-linked dimorphism may reflect either the interplay between female choice for iridescent males (Darwin 1874; Fruhstorfer 1913), and/or selection for crypsis in females (Wallace 1889). It has also been suggested that female iridescence is driven by male–male interactions (Silberglied 1988) that represents a male-biased sensory exploitation system (Vane-Wright 1985; Ryan *et al.* 1990). Whatever the factors leading to sexual dimorphism, it would be of interest to investigate its potential contribution to the evolution of wing shape and colour in *M. cypris* and *M. rhetenor* within the context of the entire *hecuba*-group.

Some studies have suggested that tropical canopy insect species are smaller relative to those that occur in the understory (Wolda 1979; Erwin 1983). Indeed, among fruit-feeding nymphalid butterflies in Costa Rica, DeVries (1988) found that species trapped in the canopy had smaller mean wing-lengths than those trapped in the understory. That study also showed that wing length and height of capture could not, however, be separated from phylogenetic relatedness. In this investigation, we accounted for phylogenetic relatedness within *Morpho*, yet found no evidence that forewing length changed with the shift to canopy flight in either sex (Table 3), and that wing shape was independent of body size for males but not females (Fig. 3). This suggests that body size differentials among canopy and understory insect species require further investigation.

Species assemblages of fruit-feeding nymphalid butterflies in neotropical forests are known to be partitioned between the understory and canopy (DeVries 1988; DeVries, Murray & Lande 1997; Shahabuddin & Terborgh 1999; DeVries & Walla 2001; P.J.D., unpublished data), and there is increasing evidence for vertical stratification among species of African forest butterflies (Fermon, Walt-ert & Mulenberg 2003; Molleman *et al.* 2006). By focusing on the diversity and variation within *Morpho* butterflies,

our study provides a starting point for characterizing the evolution of wing morphology of fruit-feeding nymphalid communities within the contexts of flight behaviour and habitat selection. It seems apparent that forest destruction by activities such as logging creates ecological situations where the integrity of the forest understory, when left to regenerate, can recover more quickly than the canopy. This will have a measurable effect on resident canopy species. Given that flight has contributed considerably to the success of insects, in a general context, elucidating the evolution of wing shape for canopy and understory species in many insect groups may be important to how we interpret, and ultimately understand, ecological and evolutionary effects of habitat destruction on biological diversity.

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References

- Allen, L., Jr, Lemon, E. & Muller, L. (1972) Environment of a Costa Rican forest. *Ecology*, **53**, 102–111.
- Basset, Y., Aberlenc, H.P., Barrios, H., Curlett, G., Berenger, J.M., Vesco, J.P., Causse, P., Haug, A., Henniom, A.S., Lesobre, L., Marques, F. & O'Meara, R. (2001) Stratification and diel activity in a lowland rainforest in Gabon. *Biological Journal of the Linnean Society*, **72**, 585–607.
- Beccaloni, G.W. (1997) Vertical stratification of ithomiine butterfly (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host-plant height. *Biological Journal of the Linnean Society*, **62**, 313–341.
- Benson, W.W., Haddad, C.F.B. & Zikán, M. (1989) Territorial behavior and dominance in some heliconiine butterflies (Nymphalidae). *Journal of the Lepidopterists' Society*, **43**, 33–49.
- Berwaerts, K., Matthysen, E. & Van Dyke, H. (2008) Take-off flight performance in the butterfly *Pararge aegeria* relative to sex and morphology: a quantitative genetic assessment. *Evolution*, **62**, 2525–2533.
- Betts, C.R. & Wootton, R.J. (1988) Wing shape and flight behavior in butterflies (Lepidoptera: Papilionoidea and Hesperioidea): a preliminary analysis. *Journal of Experimental Biology*, **138**, 271–288.
- Breuker, C., Brakefield, P.M. & Gibbs, M. (2007) The associations between wing morphology and dispersal is sex-limited in the Glanville fritillary butterfly *Melitaea conxia* (Lepidoptera: Nymphalidae). *European Journal of Entomology*, **104**, 445–452.
- Burd, M. (1994) Butterfly wing colour patterns and flying heights in the seasonally wet forest of Barro Colorado Island, Panama. *Journal of Tropical Ecology*, **10**, 601–610.
- Darwin, C.R. (1874) *The Descent of Man and Selection in Relation to Sex*. John Murray, London.
- DeVries, P.J. (1987) *The Butterflies of Costa Rica and Their Natural History. Vol. 1: Papilionidae, Pieridae, Nymphalidae*. Princeton University Press, Princeton, NJ.
- DeVries, P.J. (1988) Stratification of fruit-feeding nymphalid butterflies in a Costa Rican rainforest. *Journal of Research on the Lepidoptera*, **26**, 98–108.
- DeVries, P.J. & Martinez, G.E. (1993) The morphology, natural history, and behavior of the early stages of *Morpho cypris* (Nymphalidae: Morphinae) –

- 140 years after formal recognition of the butterfly. *Journal of the New York Entomological Society*, **101**, 515–530.
- DeVries, P.J., Murray, D. & Lande, R. (1997) Species diversity in vertical, horizontal, and temporal dimensions of a fruit-feeding butterfly community in an Ecuadorian rainforest. *Biological Journal of the Linnean Society*, **62**, 343–364.
- DeVries, P.J. & Walla, T.R. (2001) Species diversity and community structure in neotropical fruit-feeding butterflies. *Biological Journal of the Linnean Society*, **74**, 1–15.
- Dockx, C. (2007) Directional and stabilizing selection on wing size and shape in migrant and resident monarch butterflies, *Danaus plexippus* (L.), in Cuba. *Biological Journal of the Linnean Society*, **92**, 605–616.
- Dudley, R. (1990) Biomechanics of flight in neotropical butterflies: morphometrics and kinematics. *Journal of Experimental Biology*, **150**, 37–53.
- Dudley, R. (2000) *The Biomechanics of Insect Flight: Form, Function, Evolution*. Princeton University Press, Princeton, NJ.
- Dudley, R. & DeVries, P.J. (1990) Flight physiology of migrating *Urania fulgens* (Uranidae) moths: kinematics and aerodynamics of natural free flight. *Journal of Comparative Physiology*, **63**, 235–251.
- Dudley, R. & Srygley, R.B. (2008) Airspeed adjustment and lipid reserves in migratory Neotropical butterflies. *Functional Ecology*, **22**, 264–270.
- Ellington, C.P. (1984) The aerodynamics of hovering insect flight. II. Morphological parameters. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, **305**, 17–40.
- Ellington, C.P. (1991) Aerodynamics and the origin of insect flight. *Advances in Insect Physiology*, **23**, 171–210.
- Elton, C.S. (1973) The structure of invertebrate populations inside neotropical rainforest. *Journal of Animal Ecology*, **42**, 55–104.
- Erwin, T.L. (1983) Beetles and other insects of tropical forest canopies at Manaus, Brazil, sampled by insecticidal fogging. *Tropical Rainforests: Ecology and Management* (eds S.L. Sutton, T.C. Whitmore & A.C. Chadwick), pp. 59–75. British Ecological Society. Special Publication No. 2. Blackwell Scientific publications, Oxford, UK.
- Felsenstein, J. (1985) Phylogenies and the comparative method. *The American Naturalist*, **125**, 1–15.
- Fermon, H., Waltert, M. & Mulenberg, M. (2003) Movement and vertical stratification of fruit-feeding butterflies in a managed West African rainforest. *Journal of Insect Conservation*, **7**, 7–19.
- Fetcher, N., Oberbauer, S.F. & Strain, B.R. (1985) Vegetation effects on microclimate in lowland tropical forest in Costa Rica. *International Journal of Biometeorology*, **29**, 145–155.
- Fruhstorfer, H. (1913) Family: Morphidae. *Macrolepidoptera of the World*, Vol. 5 (ed. A. Seitz), pp. 333–356. Kernan, Stuttgart.
- Garland, T., Jr, Harvey, P.H. & Ives, A.R. (1992) Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology*, **41**, 18–32.
- Garland, T., Jr, Dickerman, A.W., Janis, C.M. & Jones, J.A. (1993) Phylogenetic analysis of covariance by computer simulation. *Systematic Biology*, **42**, 265–292.
- Gibo, D.L. & Pallett, M.J. (1979) Soaring flight of monarch butterflies, *Danaus plexippus* (Lepidoptera: Danaidae), during the late summer migration in southern Ontario. *Canadian Journal of Zoology*, **57**, 1393–1401.
- Greenslade, P., Farrow, R.A. & Smith, J.M.B. (1999) Long distance migration of insects to a subantarctic island. *Journal of Biogeography*, **26**, 1161–1167.
- Grimaldi, D. & Engel, M.S. (2005) *Evolution of the Insects*. Cambridge University Press, Cambridge.
- Grubb, P.J., Lloyd, J.R., Pennington, T.D. & Whitmore, T.C. (1963) A comparison of montane and lowland rainforest in Ecuador. I. The forest structure, physiogamy, and floristics. *Journal of Ecology*, **51**, 576–601.
- Jackson, T.H.E. (1961) Entomological observations from a high tower in Mpanga forest, Uganda. IX. Observations on Lepidoptera (Rhopalocera). *Transactions of the Royal Entomological Society, London*, **113**, 346–350.
- Johansson, F., Soderquist, M. & Bokma, F. (2009) Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings. *Biological Journal of Linnean Society*, **97**, 362–372.
- Johnson, C.G. (1969) *Migration and Dispersal of Insects by Flight*, 763 pp. Meuthen, London.
- Johnson, C.G. & Bowden, J. (1973) Problems related to the transoceanic transport of insects, especially between the Amazon and Congo areas. *Tropical Forest Ecosystems in Africa and South America: A Comparative Review* (eds B.J. Meggars, E.S. Ayensu & W.W. Duckworth), pp. 207–222. Smithsonian Institution Press, Washington, DC.
- Lamas, G. (2004) *Atlas of Neotropical Lepidoptera – Checklist: Part 4A Hesperioidea–Papilionoidea*. Scientific Publishers, Gainesville, FL.
- Maddison, D.R. & Maddison, W.P. (2000) *MacClade 4*. Sinauer, Sunderland.
- Maddison, W.P. & Maddison, D.R. 2009. *Mesquite: a modular system for evolutionary analysis*. Version 2.71. Available at: <http://mesquiteproject.org>.
- Marden, J.H. & Chai, P. (1991) Aerial predation and butterfly design how palatability, mimicry and the need for evasive flight constrain mass allocation. *The American Naturalist*, **138**, 15–36.
- Merckx, T., Karlsson, B. & Van Dyke, H. (2006) Sex- and landscape-related differences in flight ability under suboptimal temperatures in a woodland butterfly. *Functional Ecology*, **20**, 436–441.
- Midford, P.E., Garland, T., Jr & Maddison, W.P. (2003) *PDAP Package*. <http://mesquiteproject.org>.
- Molleman, F., Kop, A., Brakefield, P., DeVries, P.J. & Zwan Bas, J. (2006) Vertical and temporal patterns of fruit-feeding butterflies in a tropical forest in Uganda. *Biodiversity and Conservation*, **15**, 107–121.
- Neild, A. (2008) *Butterflies of Venezuela*, Vol. II. Meridian Publications, London.
- Neter, J., Wasserman, W. & Kutner, M.H. (1989) *Applied Linear Regression Models*. Richard D. Irwin, Inc., Boston, MA.
- Ohsaki, N. (1995) Preferential predation of female butterflies and the evolution of Batesian mimicry. *Nature (London)*, **378**, 173–175.
- Otero, L. (1986) *Borboletas. Livro do Naturalista*. Fundacao de Assistencia ao Estudante, Lafayette.
- Penz, C.M. & DeVries, P.J. (2002) Phylogenetic analysis of *Morpho* butterflies (Nymphalidae, Morphinae): implications for classification and natural history. *American Museum Novitates*, **3374**, 1–33.
- Pittendrigh, C.S. (1950) The ecoclimatic divergence of *Anopheles bellator* and *homunculus*. *Ecology*, **4**, 43–63.
- R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Richards, P.W. (1976) *The Tropical Rain Forest*. Cambridge University Press, Cambridge.
- Riley, J.R., Reynolds, D.R., Mukhopadhyay, S., Ghosh, M.R. & Sarkar, T.K. (1995) Long-distance migration of aphids and other small insects in north-east India. *European Journal of Entomology*, **92**, 639–653.
- Ryan, M.J., Fox, J.H., Wilczynski, W. & Rand, A.S. (1990) Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature*, **343**, 66–67.
- Shahabuddin, G. & Terborgh, J.W. (1999) Frugivorous butterflies in Venezuelan forest fragments: abundance, diversity and the effects of isolation. *Journal of Tropical Ecology*, **15**, 703–722.
- Silberglied, R. (1988) Visual communication and sexual selection in butterflies. *The Biology of Butterflies* (eds R.I. Vane-Wright & P.R. Ackery), pp. 207–223. Princeton University Press, Princeton, NJ.
- 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 of London, Series B: Biological Sciences*, **343**, 145–155.
- Srygley, R.B. & Thomas, A.L.R. (2002) Unconventional lift-generating mechanisms in free-flying butterflies. *Nature*, **420**, 660–664.
- Vane-Wright, R.I. (1985) The role of pseudosexual selection in the evolution of butterfly colour patterns. *The Biology of Butterflies* (eds R.I. Vane-Wright & P.R. Ackery), pp. 251–253. Princeton University Press, Princeton, NJ.
- Vogel, S. (1994) *Life in Moving Fluids: The Physical Biology of Flow*. Princeton University Press, Princeton, NJ.
- Wakely, J.M. & Ellington, C.P. (1997) Dragonfly flight I. Gliding flight and steady-state aerodynamic forces. *Journal of Experimental Biology*, **200**, 543–556.
- Wallace, A.R. (1889) *Darwinism*. Macmillan, London.
- Whitmore, T.C. (1984) *Tropical Rain Forests of the Far East*, 2nd edn. Oxford University Press, Oxford.
- Williams, C.B. (1958) *Insect Migration*, 235 pp. Collins, London.
- Wolda, H. (1979) Abundance and diversity of Homoptera in the canopy of a tropical forest. *Ecological Entomology*, **4**, 181–190.
- Yao, Q. & Zhang, Z. (2001) Analysis of wing-shape characteristics of migrating lepidopterous insects. *Entomologica Sinica*, **8**, 183–192.

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