ADAPTIVE FLIGHT

Adaptive evolution of flight in *Morpho* butterflies

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The diversity of flying animals suggests that countless combinations of flight morphologies and behaviors have evolved with specific lifestyles, thereby exploiting diverse aerodynamic mechanisms. How morphology, flight behavior, and aerodynamic properties together diversify with contrasting ecology remains to be elucidated. We studied the adaptive codivergence in wing shape, flight behavior, and aerodynamic efficiency among *Morpho* butterflies living in different forest strata by combining high-speed videography in the field with morphometric analyses and aerodynamic modeling. By comparing canopy and understory species, we show that adaptation to an open canopy environment resulted in increased glide efficiency. Moreover, this enhanced glide efficiency was achieved by different canopy species through distinct combinations of flight behavior, wing shape, and aerodynamic mechanisms, highlighting the multiple pathways of adaptive evolution.

Insects display a diversity of flight patterns reflecting their different ecologies, ranging from the sustained, energy-efficient flight of long-range migrating species (*1*) to the highly maneuverable hovering of nectar-feeding species (*2*). This diversity of flight modes stems from morphological and behavioral adaptations that improve flight performance metrics such as speed, maneuverability, or energetic efficiency. Investigating insect flight aerodynamics is therefore crucial to understanding how natural selection shapes flight. Although insect flight has been studied in detail in several species, including *Drosophila*, mosquitoes, and hawkmoths (*3*), only the comparison of closely related species adapted to different habitats can unravel the impact of ecological constraints on the diversification of aerodynamic properties.

In this study, we addressed the ecological, behavioral, and morphological bases of the diversification of flap-gliding flight in closely related butterfly species. Butterflies are the only insects that regularly use flap-gliding flight, which combines periods of flapping interspersed with gliding. In contrast, many intermediate-sized birds use flap-gliding flight; this reduces energetic expenditure when the aerodynamic efficiency of gliding phases is high enough (*4*). We assessed the diversity of flap-gliding flight in the neotropical butterfly genus *Morpho*. Sympatric *Morpho* species display substantially different ecologies, with some species flying in the dense vegetation of the forest understory and others flying in the open canopy (*5*). The divergence between canopy and understory species occurred around 22 million years ago (*6*). This resulted in contrasting selective pressures acting on the evolution of flight behavior and morphology among species, whereby open canopy habitats may favor a more extensive gliding behavior (*5*).

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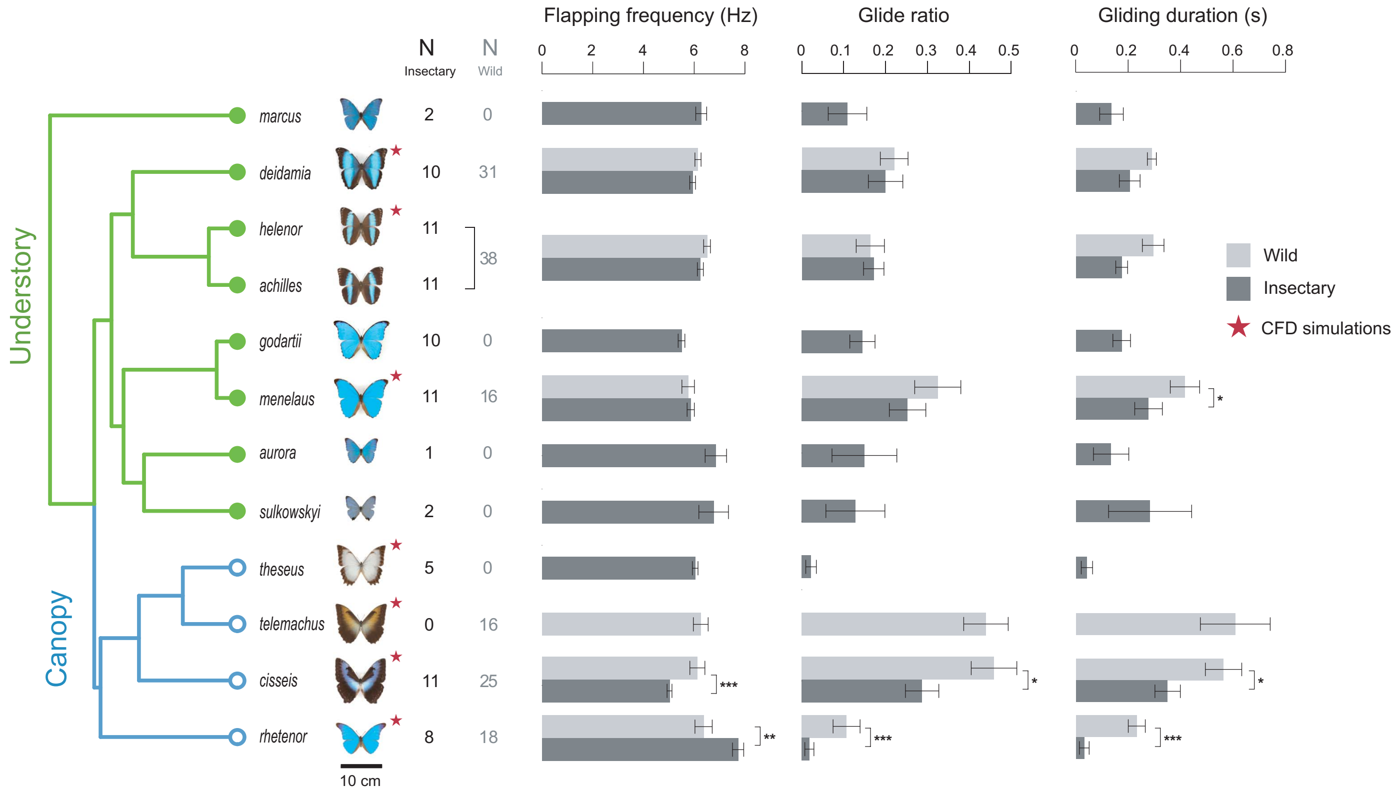

Fig. 1. Canopy butterflies use flap-gliding flight to a larger extent than understory butterflies. The 12 studied *Morpho* species are shown with their phylogenetic relationships. Differences in flap-gliding parameters between microhabitats were more notable in nature, as captivity reduced gliding in canopy species. Bars indicate the mean ± standard error, and stars indicate significant difference between nature and captivity. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Note that *M. helenor* and *M. achilles* cannot be distinguished during flight, and so corresponding data in nature were pooled for these two species.

We examined the divergence of flight behavior among habitats and sought to determine whether the evolution of gliding flight in canopy species was enabled by increased aerodynamic efficiency through changes in wing shape (*7*).

We performed a series of field and semifield experiments in Amazonian Peru. Here, up to 12 *Morpho* species co-occur, allowing us to investigate how habitat affects the evolution of flight in closely related species in sympatry. We used high-speed videography to track and characterize flight behavior of wild individuals in the field and in a large insectary (movies S1 to S6). We then quantified the wing shape of these filmed butterflies using geometric morphometrics and assessed shape covariation with flight. Finally, we used computational fluid dynamics (CFD) modeling to assess the aerodynamic efficiency associated with the contrasting wing shapes of species specialized in different habitats.

Using high-speed videography, we recorded 136 sequences of 80 wild *Morpho* butterflies freely patrolling in nature, including four understory species and three canopy species (Fig. 1 and movies S3 to S6). From the temporal positions of each wing stroke, we measured the flapping frequency, the gliding-phase duration, and the temporal flap-gliding ratio of each flight (Fig. 1). While little variation was found in flapping frequency, two of the canopy species (*M. cisseis* and *M. telemachus*) showed sharply longer gliding phases than all understory species, spending about half of their time gliding. The third canopy species, *M. rhetenor*, differed from the other canopy species, showing limited use of gliding, even less than all understory species (Fig. 1). These findings corroborate field observations (*5*) and highlight that different flight behaviors exist within the canopy clade.

To finely characterize flight behaviors of canopy and understory species, we built a large outdoor insectary equipped with a high-speed stereoscopic videography system. In this large cage, we tracked the three-dimensional movements of 241 flights of 82 wild-caught *Morpho* butterflies from eight understory species and three canopy species (Fig. 2A, fig. S1, and movies S1 and S2). We then characterized all flights using 11 flight kinematics parameters, of which six characterized the complete flap-gliding flight, three the flapping phase, and two the gliding phase (supplementary materials and methods and table S1).

We first compared insectary flights with those recorded in the wild to assess the impact of captivity on flight behavior (Fig. 1 and table S2). Captivity reduced the extent of flap-gliding flight in canopy species. Here, *M. theseus* displayed particularly short gliding phases in the enclosed environment, whereas it is known to glide extensively in natural canopy conditions (*5*). Understory butterflies were minimally affected, suggesting that they may be accustomed to flying in confined spaces. Overall, the interspecific variation in flight behavior in the insectary was broadly consistent with that observed in the wild (Fig. 1).

A principal components analysis performed on the 11 kinematics parameters showed that flight behaviors significantly differed between canopy and understory species (Fig. 2C). A phylogenetic multivariate analysis of variance confirmed that this difference is higher than expected from a Brownian model of character evolution (table S3). This strong divergence in flight mode between canopy and understory species therefore cannot be explained by their phylogenetic divergence alone,

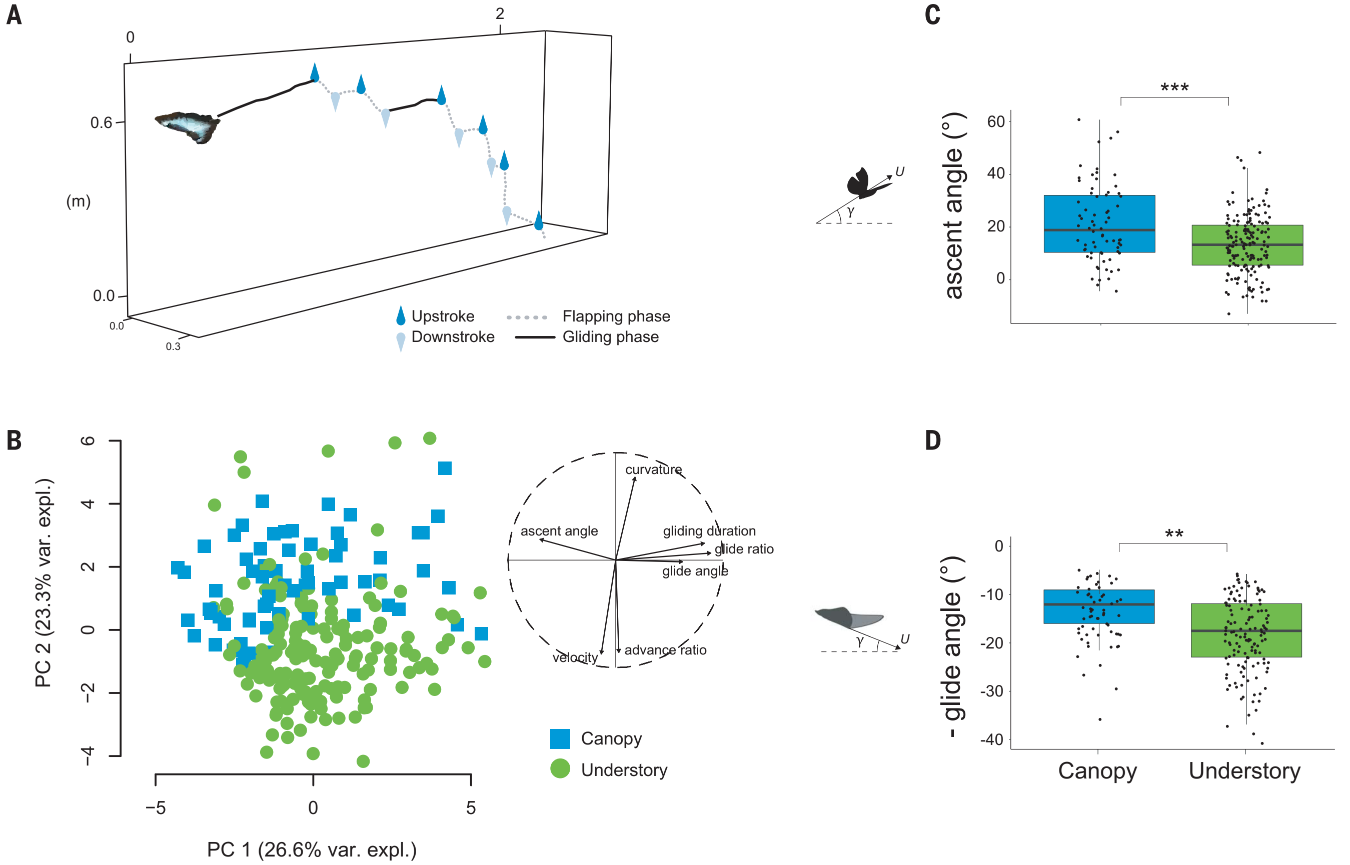

Fig. 2. Flight kinematics revealed differences in behavior and performance between canopy and understory species. (**A**) A single flight trajectory of an individual *M. cisseis* (duration = 1.7 s). Droplets indicate the uppermost and lowermost wing positions during upstroke and downstroke, respectively. (**B**) Principal components analysis showing the divergence of flight between canopy and understory butterflies. var. expl., variation explained. (**C** and **D**) Gliding and climbing efficiency was higher in canopy species and was found to diverge more strongly than expected from phylogenetic distance. $^{**}P < 0.01$; $^{***}P < 0.001$.

thus pointing at an effect of the contrasting selection regimes acting on flight evolution in the two microhabitats.

Principal component 1 (PC1) was driven by the relative use of gliding flight (variation in glide duration, glide angle, and glide ratio), which was comparable between canopy and understory species when flying in captivity (Fig. 2B). PC2 reflected the aerodynamic force production during flapping flight, for which canopy and understory species were clearly opposed: fast flight and high advance ratio for understory species on the negative values and slow flight and curvy trajectories for canopy species on the positive values. Understory butterflies thus exhibit a more powerful wingbeat, producing higher aerodynamic forces and leading to higher advance ratios and straighter high-speed flights.

Glide angle and ascent angle diverged more between canopy and understory species than predicted phylogenetically (Fig. 2, C and D, and table S3), suggesting a strong effect of natural selection on these two flight components. During their few flapping phases, canopy butterflies also climbed more steeply, their mean ascent angle being 70% larger than that of understory species (Fig. 2C; $\gamma_{\text{ascend,canopy}}$ = 22° ± 3°, mean ± standard deviation, n = 70 flapping phases; $\gamma_{\text{ascend,understory}}$ = 13° ± 1°, n = 171 flapping phases). Thus, although butterflies from understory species tended to fly at higher advance ratios and flight speeds (Fig. 2B, PC2), the ascent angle was higher in canopy species (Fig. 2B, PC1, and Fig. 2C). This could stem from an increased behavioral tendency to fly up and/or from a higher climbing efficiency generated by their morphology.

During the gliding phases, canopy butterflies had a 36% smaller glide angle than the understory butterflies (Fig. 2D; $\gamma_{\text{glide,canopy}}$ = 7° ± 2°, n = 61 gliding phases; $\gamma_{\text{glide,understory}}$ = 11° ± 1°, n = 135 gliding phases). Such shallow glides allow canopy butterflies to travel longer distances for a given height loss, consistent

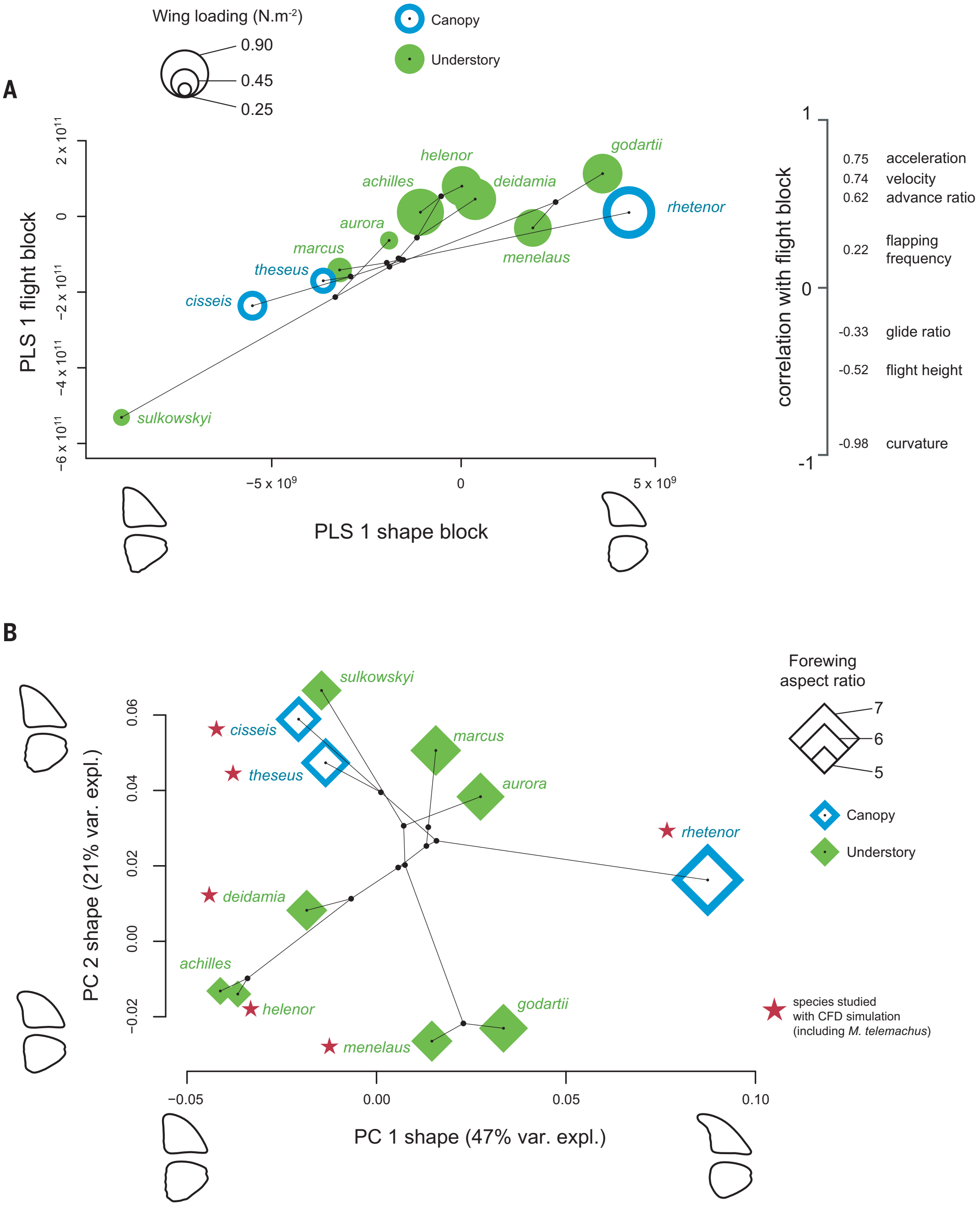

Fig. 3. Wing shape and wing loading jointly covary with flight behavior. (**A**) Phylogenetic partial least squares (PLS) analysis shows the covariation between wing shape and flight behavior (coefficient of covariation r-PLS = 0.89; P = 0.02; 74% of covariation explained). This covariation opposes triangular to rounded wings, respectively associated with slow, curvy flight and straighter, more powerful flight. Wing loading (depicted by circle size) also covaries with flight, suggesting that the evolution of flight is linked to both wing shape and body morphology. The contributions of flight parameters to the PLS 1 flight block are indicated on the right. (**B**) Phylogenetic morphospace depicting variation in wing shape among species. Diamond size indicates wing aspect ratio.

with the longer gliding phases measured in the wild. Glide angle is directly related to the aerodynamic efficiency parameter lift-to-drag ratio (*8*), and shallower angles detected in canopy species might be promoted by their divergent wing shapes. This combination of field and semi-field experiments shows that the evolutionary shift from understory to canopy resulted in an increased use and efficiency of gliding flight (Fig. 2D), combined with a reduction in aerodynamic force production during forward flapping flight (Fig. 2B, PC2).

We then investigated the contribution of morphological divergence in the adaptive evolution of flight between canopy and understory species. We precisely quantified wing shape of the filmed butterflies using geometric morphometrics and detected a strong covariation between wing shape and flight behavior using a phylogenetic partial least squares analysis (Fig. 3A). Butterflies with more-rounded wings and higher wing loading flew at greater flight speed and advance ratio and accelerated more rapidly. These results suggest that the evolution of smaller (high wing loading), more-rounded wings indeed increased force production during flapping flight. Our analyses demonstrate that flight power therefore tightly coevolves with wing shape.

In contrast to flapping-flight parameters, gliding parameters were weakly correlated with wing shape and aspect ratio (AR) (Fig. 3B and table S4). Canopy species are efficient gliders yet exhibit notably diverse wing loadings, aspect ratios, and wing shapes (Fig. 3): Two of the studied canopy species are slow fliers with low-AR, low–wing loading triangular wings (*M. cisseis* and *M. theseus*), whereas the fast-flying *M. rhetenor* has high-AR elongated wings with high wing loading. This begs the question of how this divergence in wing shape among species altered gliding efficiency.

Using CFD, we then determined how glide performance differs between canopy and understory species (n_{canopy} = 4; $n_{\text{understory}}$ = 3; Figs. 1 and 4). For each species, we produced in silico wings based on our gliding-flight experiments (Fig. 4, E to J, and fig. S2). We then performed gliding-flight CFD simulations to determine the lift-to-drag ratio (L/D) to angle-of-attack (α) curves (Fig. 4A). Maximum lift-to-drag ratio ($L/D_{\max}$) was achieved at α = 6° to 7° and was 9% greater in canopy species ($L/D_{\max,\text{canopy}} = 5.62 \pm 0.19$; $L/D_{\max,\text{understory}} = 5.18 \pm 0.07$; phylogenetic generalized least squares: $F_{1,5} = 13.48$, $P = 0.01$; Fig. 4, A and B), indicating that their wing shapes confer higher glide efficiency.

Wing shape primarily affects induced drag of a wing, which inversely scales with the product of wing AR and span efficiency e (*9*). Therefore, we tested how $L/D_{\max}$ scaled with these parameters and how this varied between

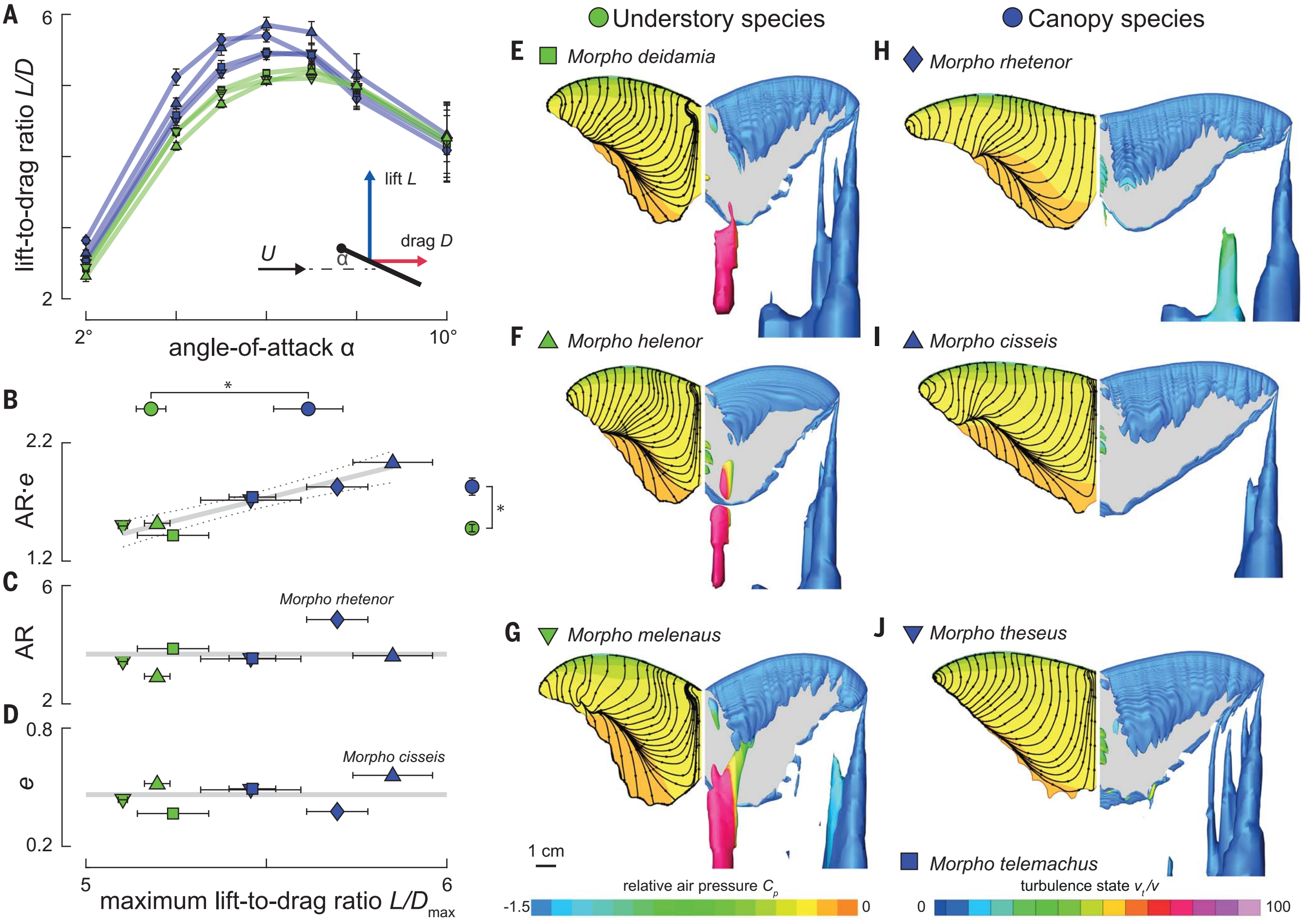

Fig. 4. The different wing shapes of canopy species similarly increase glide efficiency. Canopy butterflies have increased (**A**) maximum lift-to-drag ratio ($L/D_{\max}$) and (**B**) aerodynamic efficiency (AR·e), as shown by phylogenetic generalized least squares analyses [top and right of (B), respectively]. *$P < 0.05$. Canopy species *M. rhetenor* and *M. cisseis* achieve increased aerodynamic efficiency via increased wing aspect ratio AR (**C**) and span efficiency e (**D**), respectively. (**E** to **J**) *Morpho* wings at $L/D_{\max}$, with relative air pressure and streamlines (left wing) and vorticity fields color coded with turbulence state (right wing). Understory butterflies produce highly turbulent wingroot vortices [(E) to (G)], canopy butterflies do not [(H) to (J)]. Species-specific error bars show numerical uncertainties, and for canopy and understory groups, these are standard errors.

species (Fig. 4, B to D). The product AR · e was 24% higher for canopy species (AR · e_{canopy} = 1.83 ± 0.15; AR · $e_{\text{understory}}$ = 1.48 ± 0.05; phylogenetic generalized least squares: $F_{1,5} = 15.38$, $P = 0.01$; Fig. 4B), which was achieved by canopy species in different ways (Fig. 4, C and D): *M. rhetenor* has exceptionally high AR wings (Fig. 4C), whereas the other species have primarily an enhanced span efficiency (Fig. 4D). Airflow visualizations at $L/D_{\max}$ (Fig. 4, E to J) show that all gliding butterflies produce a stable leading-edge vortex and streamwise wingtip vortices. Unlike canopy species, all understory species also produce a highly turbulent wingroot vortex, which explains their reduced aerodynamic efficiency (*9*). These results provide functional evidence that wing shape divergence among *Morpho* species directly affects glide efficiency and that different canopy species with contrasting wing shapes achieve this using distinct aerodynamic properties.

Our combination of aerodynamic and ecological approaches revealed how natural selection imposed by different microhabitats can drive the evolution of flap-gliding flight by jointly altering wing shape and flight behavior. Butterflies from species evolving in the cluttered understory habitat display powerful flapping phases, resulting in high flight speeds and advance ratios. In contrast, evolution in the open canopy resulted in a more efficient gliding flight, illustrated by the reduced descent angles during gliding phases observed in the canopy species.

Divergence in wing shape across forest strata has been documented in Amazonian butterflies (*5*, *7*, *10*). Because any trait that reduces the energetic cost of flight is likely under positive selection (*8*), evolution in open habitats such as the canopy may favor traits enhancing glide efficiency. Most animals flying in open environments indeed display this energy-saving gliding behavior (*1*, *11*). Intriguingly, our study also reveals an unexpected flapping-flight behavior in one canopy species (*M. rhetenor*; Fig. 1 and movie S4). This discrepancy between aerodynamic performance and behavior suggests that conflicting selective pressures affect flap-gliding behavior. Contrasting trade-offs might influence flight evolution in the different sympatric canopy species, as they likely occupy different ecological niches within the canopy habitat. The vigorous flight of *M. rhetenor* might have coevolved with its blue iridescence, as the blue flashes induced during wing flapping may cause confusion in predators (*12*). Despite these differences in flight behavior, all canopy species show increased glide efficiency compared with understory species (Fig. 4), suggesting that the selection of aerodynamically efficient wing shapes prevails. Overall, our study illustrates how adaptive evolution is fueled by the flexible adjustment of morphology, behavior, and aerodynamic performance.

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SUPPLEMENTARY MATERIALS

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Science

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Strategic fliers

Forests are often crowded and complex, presenting numerous and varied challenges for species flying through them. Le Roy *et al*. looked at the Amazonian *Morpho* butterfly group and found differences in both morphological and behavioral perspectives across species that occupy the canopy relative the understory. Species that evolved to occupy the canopy have improved gliding abilities because of a combination of wing shape and flight behavior. The combination of these traits varied across species even within this single genus, which suggests that there was not one route that led to colonization of this part of the forest. —SNV

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