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Abstract: The diversity of flying animals suggests that countless combinations of flight morphologies and behaviors have evolved with specific lifestyles, thereby exploiting diverse aerodynamic mechanisms. Elucidating how morphology, flight behavior and aerodynamic properties together diversify with contrasted ecology remains rarely accomplished. Here, we studied the adaptive co-divergence 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 modelling. 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 strikingly distinct combinations of flight behavior, wing shape and aerodynamic mechanisms, highlighting the multiple pathways of adaptive evolution.

One Sentence Summary: Amazonian *Morpho* butterflies reveal a combined adaptive divergence of wing shape and flight behavior across forest strata.

Main Text:

Insects display a diversity of flight patterns reflecting their different ecologies, 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 understand 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.

Here, 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, by combining periods of flapping interspersed with gliding. In contrast, many intermediate-sized bird species use flap-gliding flight to reduce energetic expenditure when the aerodynamic efficiency of gliding phases is high enough (*4*). In this study, we assessed the diversity of flap-gliding flight in the neotropical butterfly genus *Morpho*. Sympatric *Morpho* species display remarkably contrasted ecologies, some species flying in the dense vegetation of the understory and others in the open canopy (*5*). The divergence between canopy and understory species occurred around 22 million years ago (*6*). This resulted in contrasted 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*). We examined the divergence of flight behavior among habitats and asked 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 semi-field experiments in Amazonian Peru. Here, up-to-twelve *Morpho* species co-occur, allowing us to investigate how habitat impacts 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-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) modelling to assess the aerodynamic efficiency associated with the contrasted 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 and three canopy species (Fig. 1, Movies S3-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 (*Morpho cisseis* and *Morpho telemachus*) showed sharply longer gliding phases than all understory species, spending about half of their time gliding. The third canopy species, *Morpho 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 contrasted 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 from 82 wild-caught *Morpho* butterflies from eight understory and three canopy species (Fig. 2A,B, Movies S1-S2). We then characterized all flights using eleven flight kinematics parameters, of which six characterized the complete flap-gliding flight, three the flapping-phase, and two the gliding phase (supplementary text, Table S1).

We first compared insectary flights to those recorded in the wild to assess the impact of captivity on flight behavior (Fig. 1, Table S2). Captivity reduced the extent of flap-gliding flight in canopy species. Understory species were little 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 Component Analysis (PCA) performed on the eleven kinematics parameters showed that flight behaviors significantly differed between canopy and understory species (Fig. 2C). A phylogenetic MANOVA 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 only, therefore pointing at an effect of the contrasted 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 in both canopy and understory species when flying in captivity (Fig. 2C). PC2 reflected the aerodynamic force production during flapping flight, and clearly opposed canopy and understory species: 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 species 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. 2D, 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 in understory species (Fig. 2D; $\gamma_{ascend,canopy}$=22°±3°, mean±standard deviation, n=70 flapping phases; $\gamma_{ascend,understory}$=13°±1°, n=171 flapping phases). Thus, although understory species tended to fly at higher advance ratios and flight speeds (Fig. 2C, PC2), the ascent angle of canopy species was higher (Fig. 2C, PC1; Fig. 2D). 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% lower glide angle than the understory butterflies (Fig. 2D; $\gamma_{glide,canopy}$=7°±2°, n=61 gliding phases; $\gamma_{glide,understory}$=11°±1°, n=135 gliding phases). Such shallow glides allow canopy butterflies to travel longer distances for a given height loss, consistent 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. 1C, 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 square analysis (Fig. 3A). Butterflies with more rounded wings and higher wing-loading flew at higher 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 co-evolves with wing shape.

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

Using Computational Fluid Dynamics (CFD), we then determine how glide performance differs between canopy and understory species (n_{canopy}=4; $n_{understory}$=3; Figs. 1,4). For each species, we produced *in-silico* wings based on our gliding flight experiments (Figs. 4E-J,S2). We then performed gliding-flight CFD simulations to determine the lift-to-drag-ratio to angle-of-attack curves (L/D-α, Fig. 4A). Maximum lift-to-drag-ratio L/D_{max} was achieved at α=6°–7°, and was 9% greater in canopy species ($L/D_{max,canopy}$=5.62±0.19; $L/D_{max,understory}$=5.18±0.07; phylogenetic-generalized-least-squares: $F_{1,5}$=13.48, P=0.01; Fig. 4A,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 aspect-ratio AR and span-efficiency *e* (*9*). Therefore, we tested how L/D_{max} scaled with these parameters, and how this varied between species (Fig. 4B–D). The product AR·*e* was 24% higher for canopy species (AR·e_{canopy}=1.83±0.15; AR·$e_{understory}$=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 (Figs. 4C,D): *M. rhetenor* has exceptionally high aspect-ratio wings *(*Figs. 4C), whereas the other species have primarily an enhanced span-efficiency (Fig. 4D). Airflow visualizations at L/D_{max} (Fig. 4E-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 contrasted 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 descend 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 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 flight behavior in a canopy species that mostly uses flapping flight (*M. rhetenor*; Fig.1 and Movies S4). This discrepancy between aerodynamic performance and behavior suggests that conflicting selective pressures affect flap-gliding behavior. The vigorous flight of *M. rhetenor* might have co-evolved with its blue iridescence, as the blue flashes induced during wing flapping cause confusion in predators (*12*). Despite these differences in flight behavior, all canopy species show increased glide efficiency compared to 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:

Materials and Methods

Figs. S1 to S13

Tables S1 to S6

Captions for Movies S1 to S6

Source data (excel file)

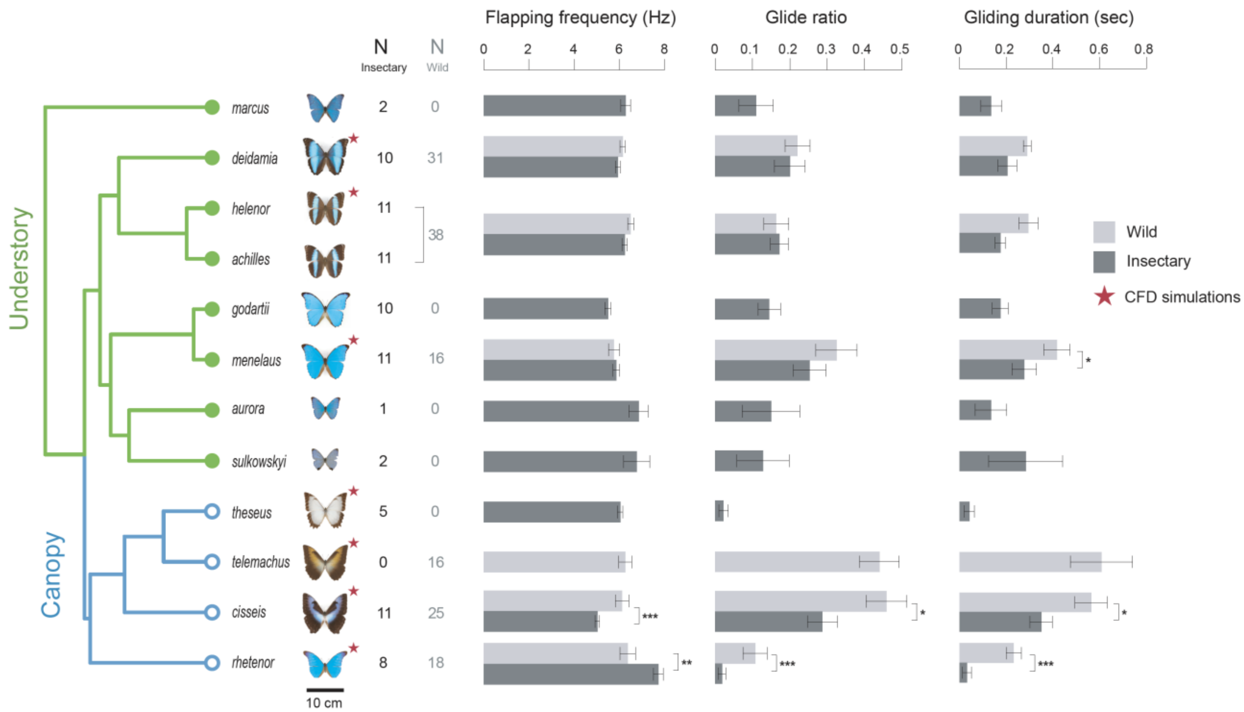

Fig. 1. Canopy butterflies use flap-gliding flight in a larger extent than understory butterflies. The twelve studied *Morpho* species are represented with their phylogenetic relationships. Differences in flap-gliding parameters between microhabitats were more striking in nature, as captivity reduced gliding in canopy species. Bars indicate the mean ± standard error, and stars indicate significant difference between nature and captivity. Note that *M. helenor* and *M. achilles* cannot be distinguished during flight: corresponding data in nature were thus pooled.

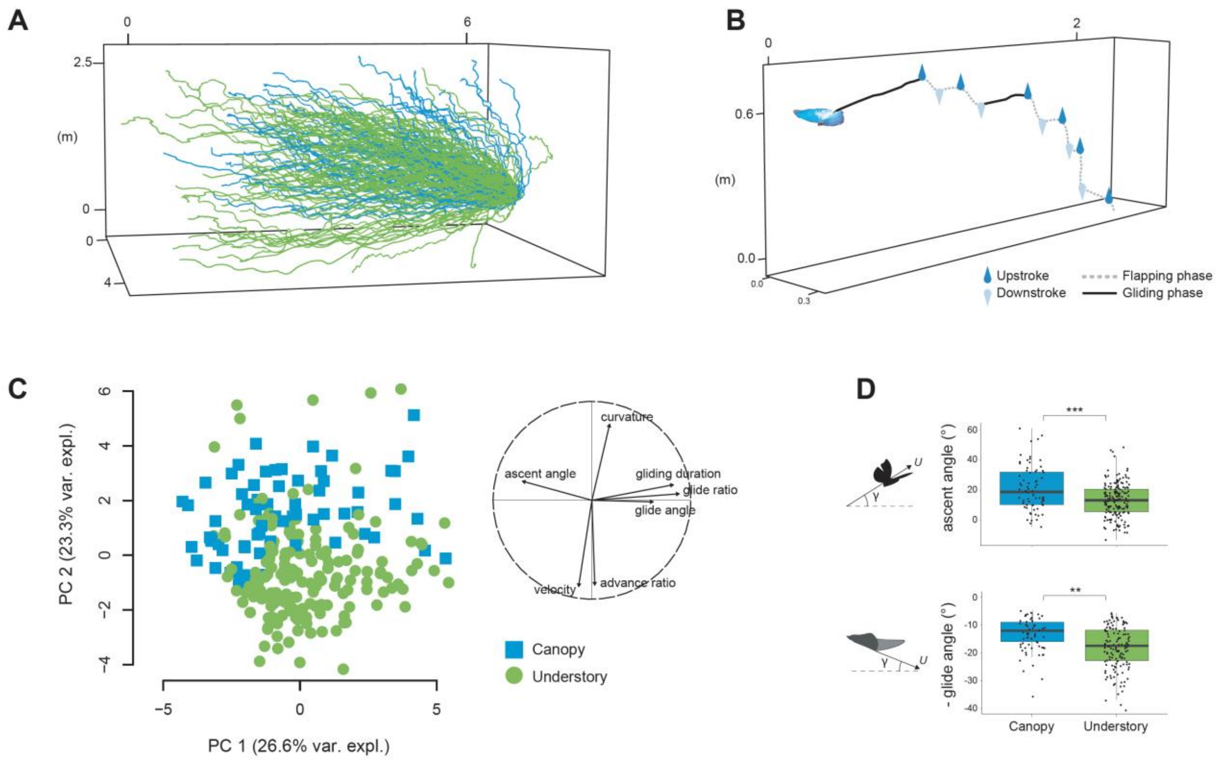

Fig. 2. Flight kinematics revealed differences in behavior and performance between canopy and understory species. (**A**) The 241 trajectories analyzed are shown together. (**B**) A single flight trajectory of an individual *M. cisseis* (duration=1.7 sec). Droplets indicate the uppermost and lowermost wing positions during up-stroke and downstroke, respectively. (**C**) Principal Component Analysis showing the divergence of flight between canopy and understory butterflies. (**D**) Gliding and climbing efficiency was higher in canopy species, and found to diverge more strongly than expected from phylogenetic distance.

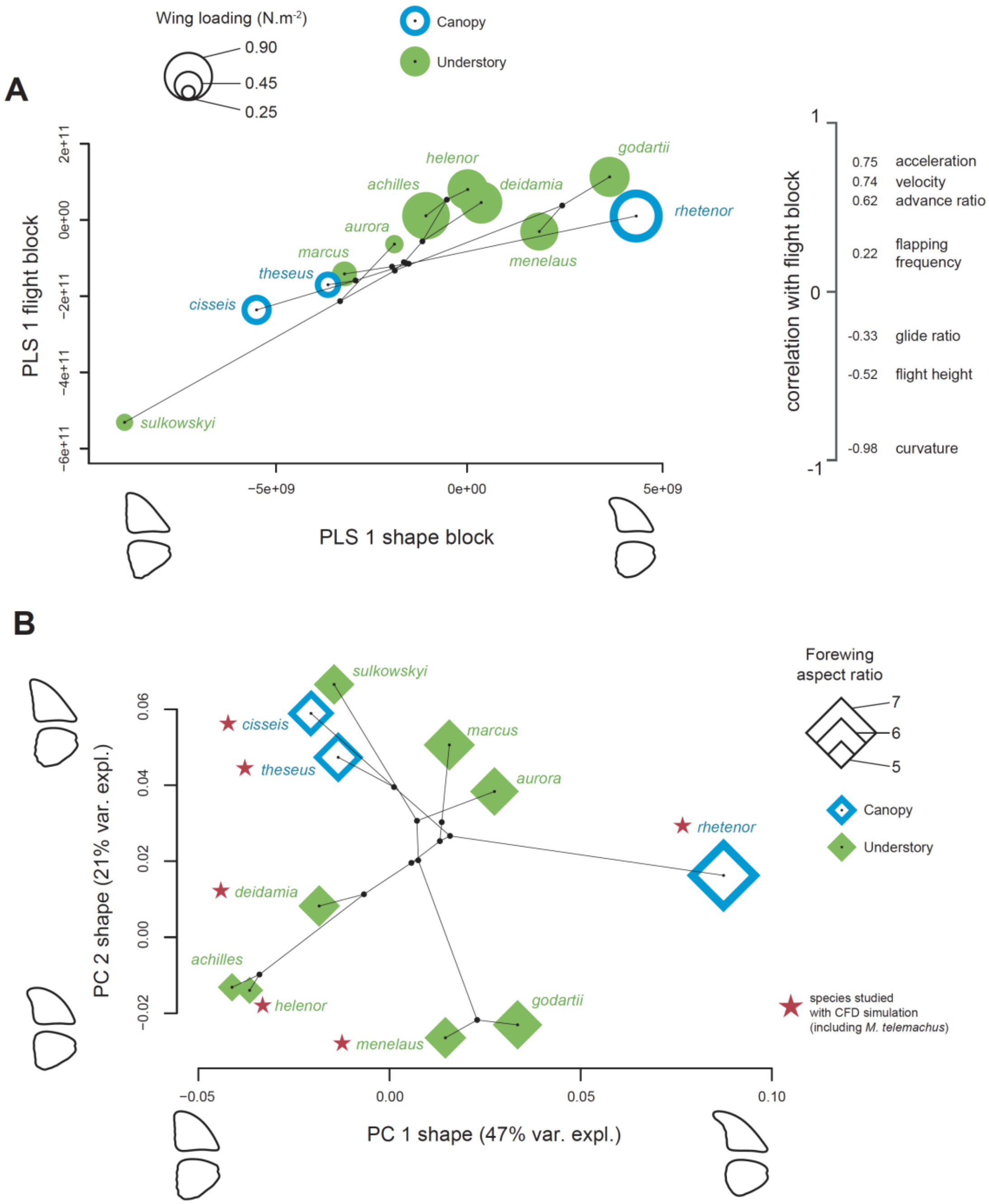

Fig. 3. Wing shape and wing-loading jointly covary with flight behavior. (**A**) Phylogenetic partial-least square analysis shows the covariation between wing shape and flight behavior (r-PLS = 0.89; P = 0.02; 74% of covariation explained). This covariation opposes triangular to rounded wings, respectively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

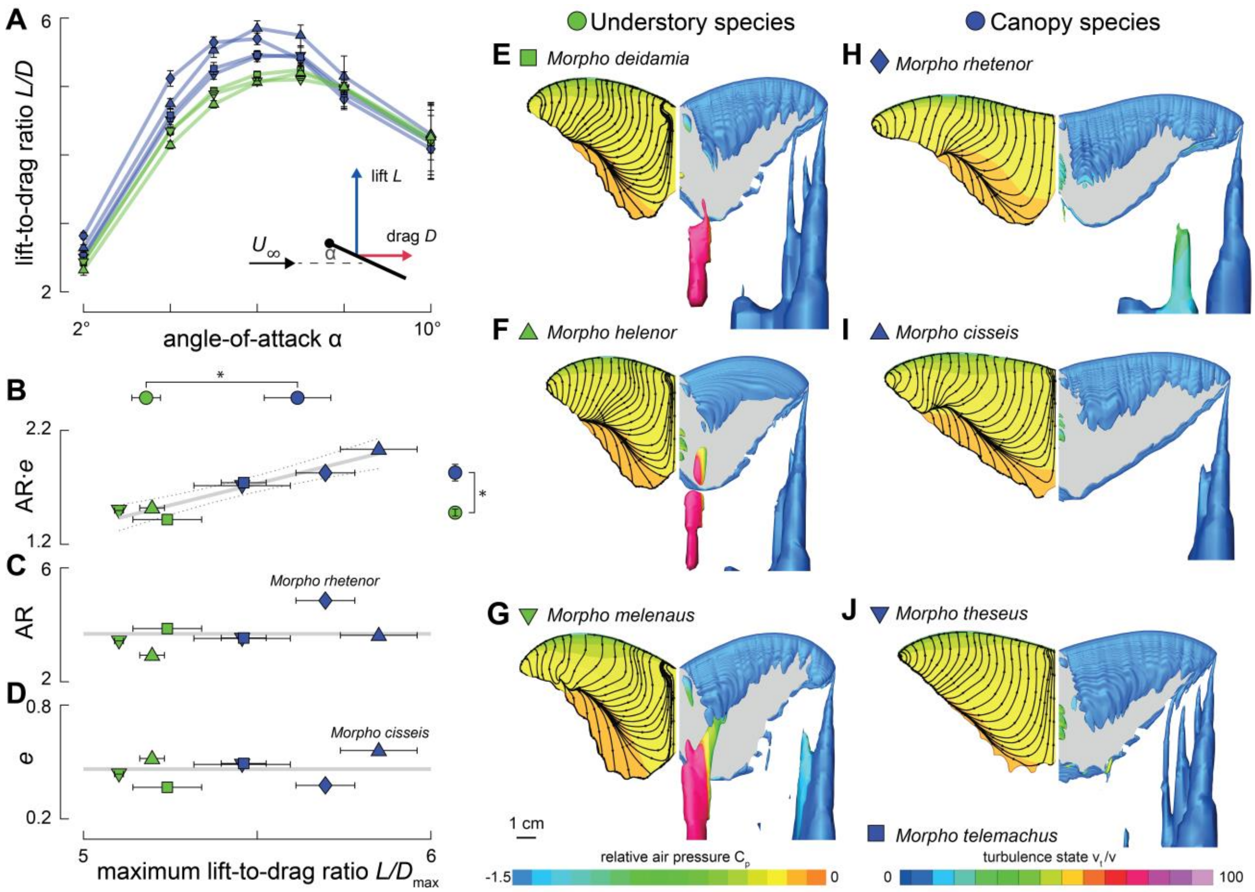

Fig. 4. Canopy species achieve greater glide efficiency through different wing shapes. (**A,B**) Canopy species have increased maximum lift-to-drag ratio (L/D_{max}) and aerodynamic efficiency (AR·*e*), as shown by phylogenetic-generalized-least-squares analyses (top and right of **B**, respectively). (**B**-**D**) Canopy-species *M. rhetenor* and *M. cisseis* achieve increased aerodynamic efficiency via increased aspect ratio (**C**) and span efficiency (**D**), respectively. (**E**-**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 species produce highly-turbulent wingroot vortices (**E**-**G**), canopy species do not (**H**-**J**). Species-specific error bars show numerical uncertainties, and for canopy/understory groups these are standard errors.