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Adaptive evolution of flight in *Morpho* butterflies

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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 (*I*), to the highly-maneuverable hovering

34 of nectar-feeding species (2). This diversity of flight modes stems from morphological and
35 behavioral adaptations that improve flight performance metrics such as speed, maneuverability or
36 energetic efficiency. Investigating insect flight aerodynamics is therefore crucial to understand
37 how natural selection shapes flight. Although insect flight has been studied in detail in several
38 species, including *Drosophila*, mosquitoes and hawkmoths (3), only the comparison of closely-
39 related species adapted to different habitats can unravel the impact of ecological constraints on the
40 diversification of aerodynamic properties.

41 Here, we addressed the ecological, behavioral and morphological bases of the diversification
42 of flap-gliding flight in closely-related butterfly species. Butterflies are the only insects that
43 regularly use flap-gliding flight, by combining periods of flapping interspersed with gliding. In
44 contrast, many intermediate-sized bird species use flap-gliding flight to reduce energetic
45 expenditure when the aerodynamic efficiency of gliding phases is high enough (4). In this study,
46 we assessed the diversity of flap-gliding flight in the neotropical butterfly genus *Morpho*.
47 Sympatric *Morpho* species display remarkably contrasted ecologies, some species flying in the
48 dense vegetation of the understory and others in the open canopy (5). The divergence between
49 canopy and understory species occurred around 22 million years ago (6). This resulted in
50 contrasted selective pressures acting on the evolution of flight behavior and morphology among
51 species, whereby open canopy habitats may favor a more extensive gliding behavior (5). We
52 examined the divergence of flight behavior among habitats and asked whether the evolution of
53 gliding flight in canopy species was enabled by increased aerodynamic efficiency through changes
54 in wing shape (7).

55 We performed a series of field and semi-field experiments in Amazonian Peru. Here, up-to-
56 twelve *Morpho* species co-occur, allowing us to investigate how habitat impacts the evolution of
57 flight in closely-related species in sympatry. We used high-speed videography to track and
58 characterize flight behavior of wild individuals in the field and in a large insectary (Movies S1-
59 S6). We then quantified the wing shape of these filmed butterflies using geometric morphometrics
60 and assessed shape covariation with flight. Finally, we used computational fluid dynamics (CFD)
61 modelling to assess the aerodynamic efficiency associated with the contrasted wing shapes of
62 species specialized in different habitats.

63 Using high-speed videography, we recorded 136 sequences of 80 wild *Morpho* butterflies freely
64 patrolling in nature, including four understory and three canopy species (Fig. 1, Movies S3-S6).

65 From the temporal positions of each wing stroke, we measured the flapping frequency, the gliding
66 phase duration, and the temporal flap-gliding ratio of each flight (Fig. 1). While little variation was
67 found in flapping frequency, two of the canopy species (*Morpho cisseis* and *Morpho telemachus*)
68 showed sharply longer gliding phases than all understory species, spending about half of their time
69 gliding. The third canopy species, *Morpho rhetenor*, differed from the other canopy species,
70 showing limited use of gliding, even less than all understory species (Fig. 1). These findings
71 corroborate field observations (5) and highlight that contrasted flight behaviors exist within the
72 canopy clade.

73 To finely characterize flight behaviors of canopy and understory species, we built a large
74 outdoor insectary equipped with a high-speed stereoscopic videography system. In this large cage,
75 we tracked the three-dimensional movements of 241 flights from 82 wild-caught *Morpho*
76 butterflies from eight understory and three canopy species (Fig. 2A,B, Movies S1-S2). We then
77 characterized all flights using eleven flight kinematics parameters, of which six characterized the
78 complete flap-gliding flight, three the flapping-phase, and two the gliding phase (supplementary
79 text, Table S1).

80 We first compared insectary flights to those recorded in the wild to assess the impact of captivity
81 on flight behavior (Fig. 1, Table S2). Captivity reduced the extent of flap-gliding flight in canopy
82 species. Understory species were little affected, suggesting that they may be accustomed to flying
83 in confined spaces. Overall, the interspecific variation in flight behavior in the insectary was
84 broadly consistent with that observed in the wild (Fig. 1).

85 A Principal Component Analysis (PCA) performed on the eleven kinematics parameters
86 showed that flight behaviors significantly differed between canopy and understory species (Fig.
87 2C). A phylogenetic MANOVA confirmed that this difference is higher than expected from a
88 Brownian model of character evolution (Table S3). This strong divergence in flight mode between
89 canopy and understory species therefore cannot be explained by their phylogenetic divergence
90 only, therefore pointing at an effect of the contrasted selection regimes acting on flight evolution
91 in the two microhabitats.

92 Principal component 1 (PC1) was driven by the relative use of gliding flight (variation in glide-
93 duration, glide-angle and glide-ratio), which was comparable in both canopy and understory
94 species when flying in captivity (Fig. 2C). PC2 reflected the aerodynamic force production during
95 flapping flight, and clearly opposed canopy and understory species: fast flight and high advance

96 ratio for understory species on the negative values and slow flight and curvy trajectories for canopy
97 species on the positive values. Understory species thus exhibit a more powerful wingbeat,
98 producing higher aerodynamic forces and leading to higher advance ratios and straighter high-
99 speed flights.

100 Glide angle and ascent angle diverged more between canopy and understory species than
101 predicted phylogenetically (Fig. 2D, Table S3), suggesting a strong effect of natural selection on
102 these two flight components. During their few flapping phases, canopy butterflies also climbed
103 more steeply, their mean ascent angle being 70% larger than in understory species (Fig. 2D;
104 $\gamma_{\text{ascend,canopy}}=22^{\circ}\pm 3^{\circ}$, mean \pm standard deviation, $n=70$ flapping phases; $\gamma_{\text{ascend,understory}}=13^{\circ}\pm 1^{\circ}$,
105 $n=171$ flapping phases). Thus, although understory species tended to fly at higher advance ratios
106 and flight speeds (Fig. 2C, PC2), the ascent angle of canopy species was higher (Fig. 2C, PC1;
107 Fig. 2D). This could stem from an increased behavioral tendency to fly up, and/or from a higher
108 climbing efficiency generated by their morphology.

109 During the gliding phases, canopy butterflies had a 36% lower glide angle than the understory
110 butterflies (Fig. 2D; $\gamma_{\text{glide,canopy}}=7^{\circ}\pm 2^{\circ}$, $n=61$ gliding phases; $\gamma_{\text{glide,understory}}=11^{\circ}\pm 1^{\circ}$, $n=135$ gliding
111 phases). Such shallow glides allow canopy butterflies to travel longer distances for a given height
112 loss, consistent with the longer gliding phases measured in the wild. Glide angle is directly related
113 to the aerodynamic efficiency parameter lift-to-drag ratio (8), and shallower angles detected in
114 canopy species might be promoted by their divergent wing shapes. This combination of field and
115 semi-field experiments shows that the evolutionary shift from understory to canopy resulted in an
116 increased use and efficiency of gliding flight (Fig. 2D), combined with a reduction in aerodynamic
117 force production during forward flapping flight (Fig. 1C, PC2).

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

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

133 Using Computational Fluid Dynamics (CFD), we then determine how glide performance differs
134 between canopy and understory species ($n_{\text{canopy}}=4$; $n_{\text{understory}}=3$; Figs. 1,4). For each species, we
135 produced *in-silico* wings based on our gliding flight experiments (Figs. 4E-J,S2). We then
136 performed gliding-flight CFD simulations to determine the lift-to-drag-ratio to angle-of-attack
137 curves ($L/D-\alpha$, Fig. 4A). Maximum lift-to-drag-ratio L/D_{max} was achieved at $\alpha=6^{\circ}-7^{\circ}$, and was
138 9% greater in canopy species ($L/D_{\text{max,canopy}}=5.62\pm 0.19$; $L/D_{\text{max,understory}}=5.18\pm 0.07$; phylogenetic-
139 generalized-least-squares: $F_{1,5}=13.48$, $P=0.01$; Fig. 4A,B), indicating that their wing shapes confer
140 higher glide efficiency.

141 Wing shape primarily affects induced drag of a wing, which inversely scales with the product
142 of wing aspect-ratio AR and span-efficiency e (9). Therefore, we tested how L/D_{max} scaled with
143 these parameters, and how this varied between species (Fig. 4B–D). The product $AR\cdot e$ was 24%
144 higher for canopy species ($AR\cdot e_{\text{canopy}}=1.83\pm 0.15$; $AR\cdot e_{\text{understory}}=1.48\pm 0.05$; phylogenetic-
145 generalized-least-squares: $F_{1,5}=15.38$, $P=0.01$; Fig. 4B), which was achieved by canopy species
146 in different ways (Figs. 4C,D): *M. rhetenor* has exceptionally high aspect-ratio wings (Figs. 4C),
147 whereas the other species have primarily an enhanced span-efficiency (Fig. 4D). Airflow
148 visualizations at L/D_{max} (Fig. 4E–J) show that all gliding butterflies produce a stable Leading-Edge-
149 Vortex and streamwise wingtip-vortices. Unlike canopy species, all understory species also
150 produce a highly-turbulent wingroot-vortex, which explains their reduced aerodynamic efficiency
151 (9). These results provide functional evidence that wing shape divergence among *Morpho* species
152 directly affects glide efficiency, and that different canopy species with contrasted wing shapes
153 achieve this using distinct aerodynamic properties.

154 Our combination of aerodynamic and ecological approaches revealed how natural selection
155 imposed by different microhabitats can drive the evolution of flap-gliding flight by jointly altering
156 wing shape and flight behavior. Butterflies from species evolving in the cluttered understory

157 habitat display powerful flapping phases, resulting in high flight speeds and advance ratios. In
158 contrast, evolution in the open canopy resulted in a more efficient gliding flight, illustrated by the
159 reduced descend angles during gliding phases observed in the canopy species.

160 Divergence in wing shape across forest strata has been documented in Amazonian butterflies
161 (5, 7, 10). Because any trait that reduces energetic cost-of-flight is likely under positive selection
162 (8), evolution in open habitats such as the canopy may favor traits enhancing glide efficiency. Most
163 animals flying in open environments indeed display this energy-saving gliding behavior (1, 11).
164 Intriguingly, our study also reveals an unexpected flight behavior in a canopy species that mostly
165 uses flapping flight (*M. rhetenor*; Fig.1 and Movies S4). This discrepancy between aerodynamic
166 performance and behavior suggests that conflicting selective pressures affect flap-gliding
167 behavior. The vigorous flight of *M. rhetenor* might have co-evolved with its blue iridescence, as
168 the blue flashes induced during wing flapping cause confusion in predators (12). Despite these
169 differences in flight behavior, all canopy species show increased glide efficiency compared to
170 understory species (Fig. 4), suggesting that the selection of aerodynamically-efficient wing shapes
171 prevails. Overall, our study illustrates how adaptive evolution is fueled by the flexible adjustment
172 of morphology, behavior and aerodynamic performance.

173 174 **References and Notes:**

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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)

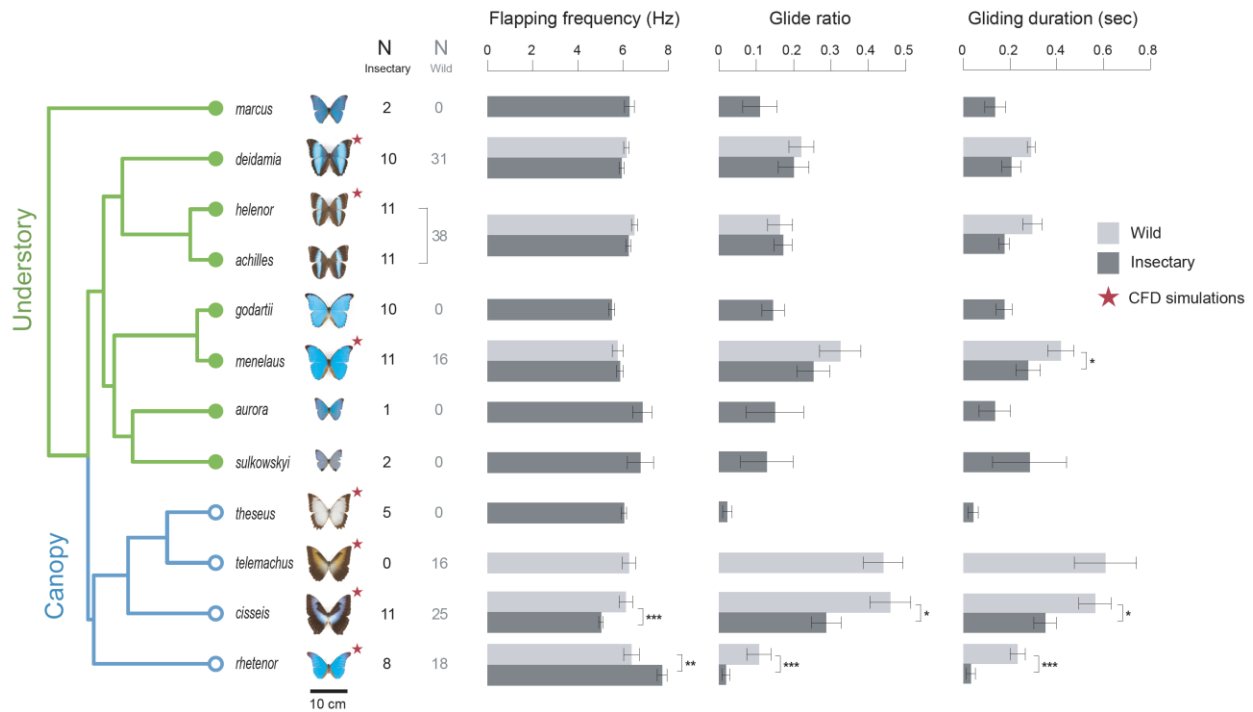


Fig. 1. Canopy butterflies use flap-gliding flight in a larger extent than understory butterflies. The twelve studied *Morpho* species are represented with their phylogenetic relationships. Differences in flap-gliding parameters between microhabitats were more striking in nature, as captivity reduced gliding in canopy species. Bars indicate the mean \pm 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.

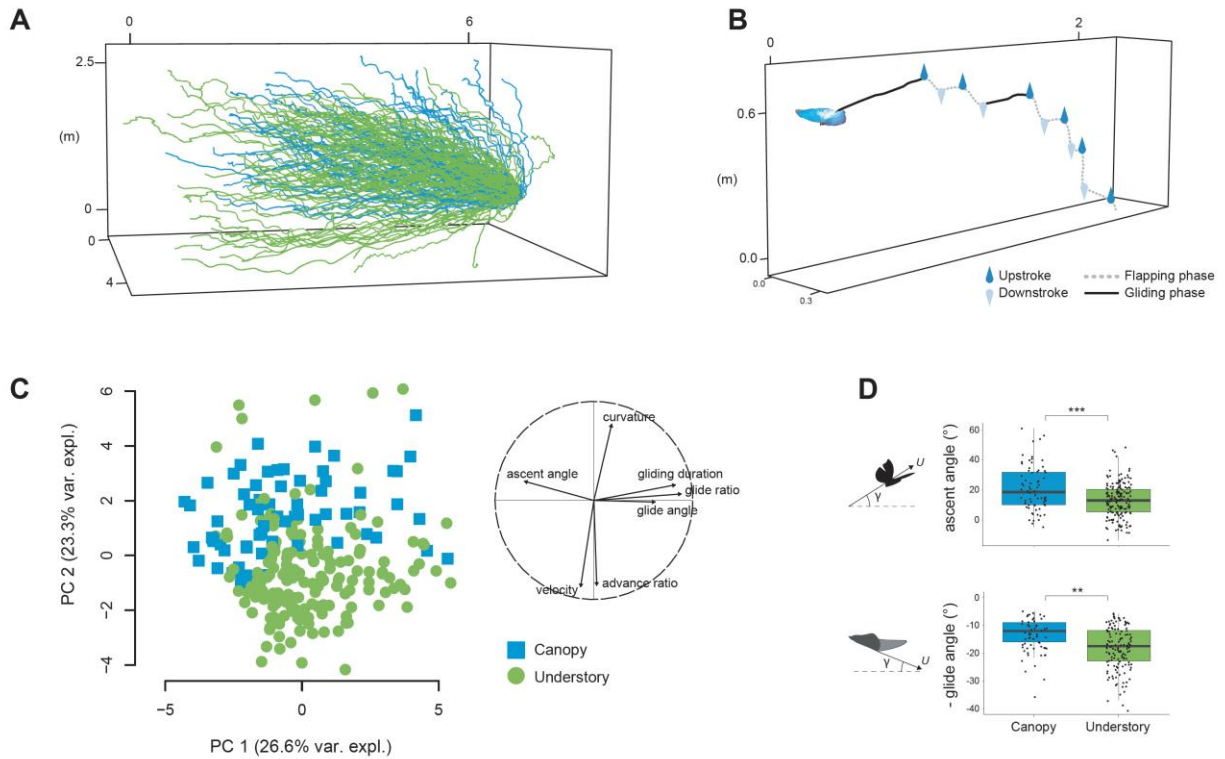


Fig. 2. Flight kinematics revealed differences in behavior and performance between canopy and understory species. (A) The 241 trajectories analyzed are shown together. (B) A single flight trajectory of an individual *M. cisseis* (duration=1.7 sec). Droplets indicate the uppermost and lowermost wing positions 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.

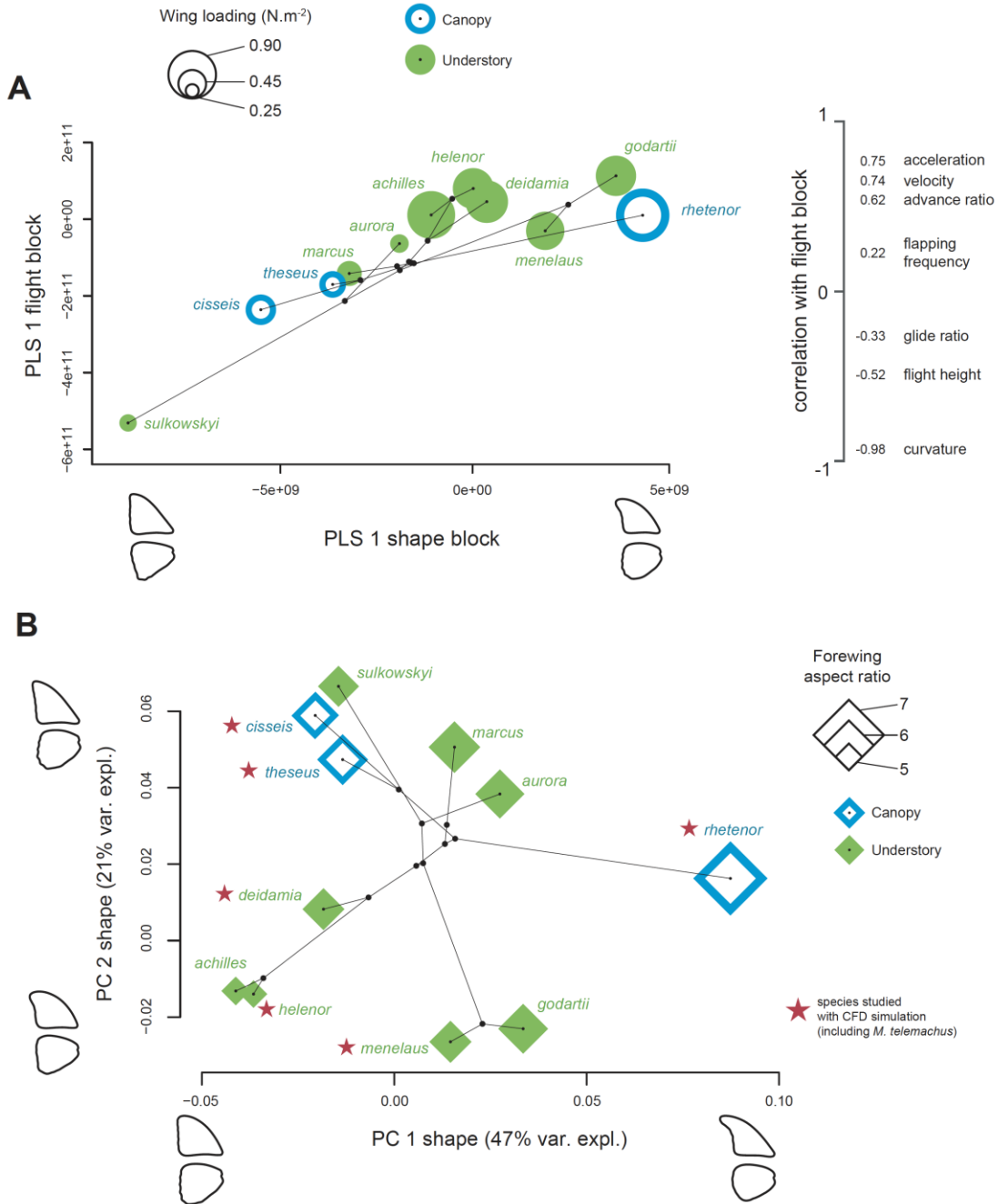


Fig. 3. Wing shape and wing-loading jointly covary with flight behavior. (A) Phylogenetic partial-least square analysis shows the covariation between wing shape and flight behavior (r-PLS = 0.89; $P = 0.02$; 74% of covariation explained). This covariation opposes triangular to rounded wings, respectively 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.

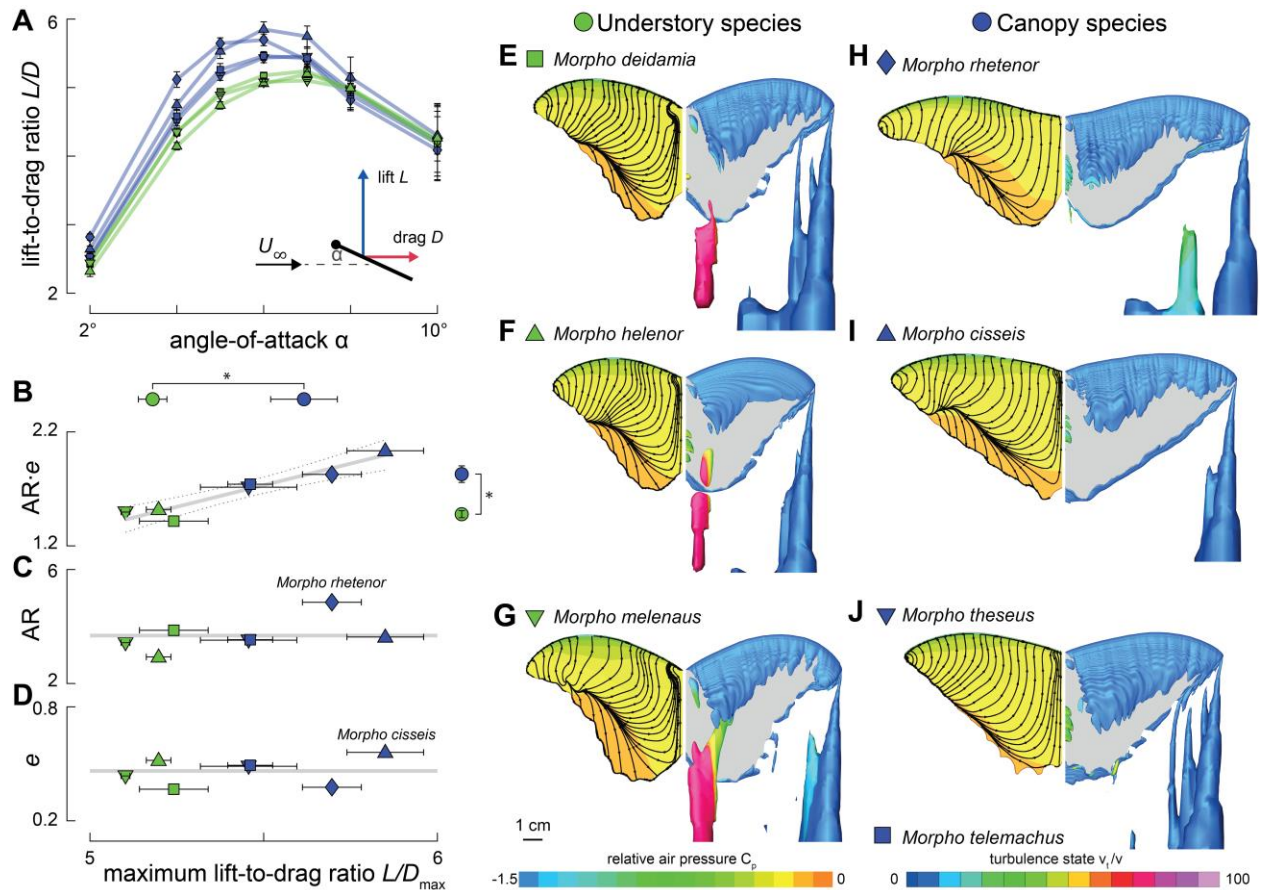


Fig. 4. Canopy species achieve greater glide efficiency through different wing shapes. (A,B) Canopy species have increased maximum lift-to-drag ratio (L/D_{\max}) and aerodynamic efficiency ($AR \cdot e$), as shown by phylogenetic-generalized-least-squares analyses (top and right of B, respectively). (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.