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Adaptive shifts underlie the divergence in wing morphology in bombycoid moths

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The evolution of flapping flight is linked to the prolific success of insects. Across Insecta, wing morphology diversified, strongly impacting aerodynamic performance. In the presence of ecological opportunity, discrete adaptive shifts and early bursts are two processes hypothesized to give rise to exceptional morphological diversification. Here, we use the sister-families Sphingidae and Saturniidae to answer how the evolution of aerodynamically important traits is linked to clade divergence and through what process(es) these traits evolve. Many agile Sphingidae evolved hover feeding behaviours, while adult Saturniidae lack functional mouth parts and rely on a fixed energy budget as adults. We find that Sphingidae underwent an adaptive shift in wing morphology coincident with life history and behaviour divergence, evolving small high aspect ratio wings advantageous for power reduction that can be moved at high frequencies, beneficial for flight control. By contrast, Saturniidae, which do not feed as adults, evolved large wings and morphology which surprisingly does not reduce aerodynamic power, but could contribute to their erratic flight behaviour, aiding in predator avoidance. We suggest that after the evolution of flapping flight, diversification of wing morphology can be potentiated by adaptative shifts, shaping the diversity of wing morphology across insects.

1. Introduction

The evolution of flight is thought to be a key innovation [1] foundational to the success of insects, one of the most speciose clades of animals on Earth. In flying insects, flight is critical for most aspects of life history including dispersal, migration, predator avoidance, feeding and courtship behaviours. The flight morphology of flying insects therefore probably faces strong selective forces to meet the functional demands of a species [2,3]. Selection can act on flight morphology to significantly impact flight performance [4]. Indeed, flying insects show an extraordinary diversity of wing and body sizes and shapes [2,5,6]. Revealing the phylogenetic patterns of insect flight morphology and the processes driving its evolution is a prime opportunity to examine how the evolution of aerodynamically important traits is linked to the divergence of diverse clades.

Clade divergence and the subsequent diversification of lineages and morphology can occur through different evolutionary processes. In the presence of an ecological opportunity, the tempo of trait evolution can accelerate and its mode can deviate from a random Brownian motion (BM) process, the null model of trait evolution. Early bursts [7–9] and discrete adaptative shifts [10–12] are two alternative processes hypothesized to give rise to exceptional morphological diversity. An early burst is associated with the adaptative radiation of a clade where morphological disparity is established early and followed by a subsequent slowdown in diversification rate [8,9]. Adaptive shifts are when discrete shifts occur along a single branch and are not followed by a slowdown in diversification rate [10–12]. Traits with known functional consequences (e.g. wing morphology) are more likely to reflect the ecology of a species [13], and therefore are more likely to be associated with non-BM processes when ecologically distinct clades evolve. Therefore, testing if insect wing and body morphology evolution deviates from BM and shifts in tandem with life history and behaviour will demonstrate the evolutionary processes driving morphological diversification as clades diverge to occupy different biological niches.

Wing size and shape, as well as body size, have known aerodynamic consequences for manoeuvrability, force production and power requirements. Nearly any aspect of shape can affect aerodynamics, but several metrics of wing morphology are common predictors of flight performance, notably wing loading (W_s), aspect ratio (AR) and radius of the second moment of area ($\hat{r}_2$). A lower W_s, the ratio between body mass (m_t) and wing area (S), typically enhances manoeuvrability, increasing the wing force production to body mass ratio, as seen in birds [14–16], bats [17–19] and moths and butterflies [20,21]. Larger AR wings (long, slender) can reduce the power requirements of flight [6,19,22], but can also reduce manoeuvrability [3,21,23]. High $\hat{r}_2$ wings will have more area concentrated distally, which increases force production because more of the wing is moving more quickly. But high $\hat{r}_2$ can increase power requirements and reduce manoeuvrability [24]. Finally, interspecific variation in wing and body morphology will have direct consequences for wing beat frequency (n) [6,25]. An increase in n increases active force generation [26], but at the cost of increasing inertial power (P_{acc}), the power required to oscillate the wing mass [27].

The moth superfamily Bombycoidea provides an opportunity to test hypotheses related to the evolution of flight morphology within closely related, but divergent clades. Bombycoidea is a globally distributed, diverse clade of more than 5000 species [28]. The most diverse families in the Bombycoidea are hawkmoths and wild silk moths (Sphingidae and Saturniidae, respectively); sister-families [29–31] of strikingly different life histories and flight behaviours. Hawkmoths are active, fast flyers [32] known for their manoeuvrability and hover feeding behaviour [33,34], where species can successfully track flower oscillations up to 14 Hz [33,34]. However, hovering requires a high power output [35]. Wild silk moths (hereafter 'silkmoths') display a flight behaviour that is often described as bobbing or erratic, but fast and agile when escaping from predators [32,36–38]. Silkmoths lack functional mouth parts and must rely on the strictly finite energy stores, gathered during the larval period, throughout their entire, albeit short, reproductive adult life stage [38]. The divergence in life history and flight behaviour between hawkmoths and silkmoths represent different niches, and would be expected to have correlated changes in flight morphology.

Here, we focus on the hawkmoths and silkmoths to test if each clade has evolved distinct flight morphology and determine what evolutionary processes led to extant morphological disparity. We hypothesize that hawkmoths evolved morphology favourable for manoeuvrability in order to rapidly track flower movements during hover feeding, while silkmoths evolved morphology favourable for power reduction in order to conserve limited energy as adult stage silkmoths do not feed. We next examine the morphological disparity through time (DTT) and compare different models of trait evolution to determine the processes that led to the diversity of extant flight morphology. We hypothesize that the distinct transitions in life history and flight behaviour between hawkmoths and silkmoths were accompanied by distinct adaptive shifts in flight morphology.

2. Material and methods

We created a time-calibrated Bombycoidea phylogeny, sampling representatives of all families, following published methods [31]. In total, the phylogenetic dataset of 606 loci included 57 species and one outgroup. The tree was inferred using a maximum likelihood approach and time calibrated based on the dates of corresponding nodes in a recently published Lepidoptera phylogeny that relied on 16 fossil calibrations with uniform priors and uncorrelated rates [30].

(a) Morphometrics

Body and wing morphology was digitized from museum images using StereoMorph (v. 1.6.2) [39]. Male specimens were analysed when available (53 of 57 species); males are known to exhibit higher flight activity in comparison to females [5,40]. Eight landmarks characterized the body; Bézier curves outlined the right forewing and hindwing (electronic supplementary material, figure S1).

Wing measurements for all species began by re-orienting each wing to a comparable orientation consistent with known flight position. The forewing was rotated so its long axis was perpendicular to the long axis of the body. In Sphingidae, the hindwing long axis was also rotated perpendicular to the long axis of the body; the approximate orientation during flight. The hindwing of Saturniidae and the 'other bombycoid families' were kept in the same orientation of dried museum specimens, which is the approximate orientation during flight and provides a consistent and comparable orientation across species. A combined wing outline was created from the non-overlapping portions of the rotated forewing and hindwing, resampled to generate 75 evenly spaced points.

Analysis of wing shape traits was conducted in Matlab (R2018b–9.5.0.944444). Wing parameters (R, $\bar{c}$, S, AR, $\hat{r}_2$ and W_s) were calculated following Ellington [24]. n was estimated from morphology [25].

(b) Phylogenetic comparisons

A phylogenetic principal components analysis (pPCA) [41] was conducted on forewing, hindwing and combined shapes. The dominant pPC axes for wing shape were determined using the broken stick method implemented in the bsDimension function of the PCDimension R package v. 1.1.11 [42].

For each trait, we performed a DTT analysis [8] (1000 simulations); a maximum likelihood estimation of the presence of shifts and their positions using PhylogeneticEM [43]; and compared the fit of 10 different models of trait evolution using mvMORPH [44]. These analyses were conducted in RStudio (v. 1.1.383) using R (v. 4.0.2). Unabridged methods are available in the electronic supplementary material. See electronic supplementary material, table S1 for list of all variables and derivation. Data are available on Dryad [45].

3. Results

(a) Phylogeny

Phylogenetic relationships of the 57 species in this study show a monophyletic, well-supported clade of the Sphingidae and Saturniidae as sister-lineages, with the Bombycidae as the sister to those two (figure 1*a*; electronic supplementary material, figure S2). Relationships are congruent with previous studies [29,31,46,47].

(b) Hawkmoths and silkmoths each have diverse, but clustered wing shapes in morphospace

We first used a pPCA to assess the variation in extant wing shape in a data-driven, evolutionary framework. For all three wing shapes (forewing, hindwing and combined), most of the variation is explained by the first two pPC axes (figure 1*b–e*; electronic supplementary material, table S2); pPC three or four explained no more than 14% of the variation (electronic supplementary material, figure S3*a–d* and table S2). Hindwing and combined wing morphospaces capture the evolution of hindwing tails in some silkmoth species, but hawkmoths and silkmoths remain clustered (figure 1*c,d*). When tailed species (nos. 1, 2, 3, 4, 20, 28) are removed (figure 1*b*), families remain clustered in combined wing shape space; variation along pPC1 generally corresponds to AR.

The wing shapes of hawkmoths and silkmoths are well separated in morphospace. We conducted a MANOVA on each wing shape; pPC1–4 scores were the response variables and clade (hawkmoth; silkmoth; other bombycoid families, abbreviated OB) was the factor. Each wing shape is significantly separated between clades (forewing: $F = 14.91$, $p < 10^{-13}$; hindwing: $F = 10.84$, $p < 10^{-10}$; combined wing: $F = 14.96$, $p < 10^{-13}$). Separation persists when considering only hawkmoths and silkmoths (forewing: $F = 44.42$, $p < 10^{-14}$; hindwing: $F = 10.84$, $p < 10^{-10}$; combined wing: $F = 101.17$, $p < 10^{-15}$), and for the combined wing when tailed silkmoths are removed from the analysis (all families: $F = 16.19$, $p < 10^{-13}$; hawkmoths versus silkmoths: $F = 144.06$, $p < 10^{-15}$).

(c) Wing area is greater in silkmoths than hawkmoths

In addition to shape, we determined if wing size is larger for a given body size between the two clades. We conducted a linear regression between S and m_t (figure 1*f*), constraining the *y*-intercept for each family to zero (hawkmoths: $r^2 = 0.90$, $F = 234.4$, $p < 10^{-13}$; silkmoths: $r^2 = 0.75$, $F = 66.8$, $p < 10^{-7}$). An ANCOVA with family as a factor reveals significant differences in regression slope ($F = 8.732$, $p = 0.0005$), indicating wing area is larger for a given body size in silkmoths than hawkmoths. Next, before accounting for phylogeny, the relative wing area of each species (S/m_t) is significantly different between hawkmoths and silkmoths (two-tailed *t*-test, $p < 10^{-9}$). A comparison of absolute wing area between the clades reinforces these differences (figure 1*f,g*; electronic supplementary material, figure S4*a,b*).

(d) Aerodynamic features of the wing and body also separate between clades

To complement the data-driven pPCA and relate variation in wing and body shape and size to aerodynamic metrics, we next quantified several specific morphological variables: nondimensional radius of second moment of area ($\hat{r}_2$), aspect ratio (AR), wing loading (W_s) and the fraction of body length occupied by the abdomen ($\hat{l}_{\text{abd}}$) and thorax ($\hat{l}_{\text{tho}}$). Before accounting for phylogeny, combined wing AR, W_s and $\hat{r}_2$ are all significantly greater in hawkmoths than in silkmoths (figure 1*g*; electronic supplementary material, table S3). Finally, while variation in total body length (l_b) spans a similar range within each family, clade average $\hat{l}_{\text{abd}}$ is significantly longer in hawkmoths than silkmoths and $\hat{l}_{\text{tho}}$ is generally greater in silkmoths than in hawkmoths (electronic supplementary material, table S3). To further ensure these multiple comparisons did not bias our statistics, we conduct a separate MANOVA of the wing ($\hat{r}_2$, AR, W_s) and body ($\hat{l}_{\text{abd}}$, $\hat{l}_{\text{tho}}$) traits between hawkmoths and silkmoths and, in both cases, find significant separation between the clades (wing: $F = 107.15$, $p < 10^{-15}$; body: $F = 11.432$, $p < 10^{-5}$).

(e) Wing beat frequency diverges between hawkmoths and silkmoths

Wing beat frequency (n) is also an important feature of flight that depends on wing and body size. n, estimated from scaling relationships (electronic supplementary material, table S1; [25]), is distinct from wing shape, but not independent of wing and body size (total body mass, m_t, and the mass of the wing pair, m_w, were estimated from museum specimens; see selectronic supplementary material, figure S6 and table S5). Based on morphological differences, n is significantly greater in hawkmoths (n: mean ± s.d.: 29.37 ± 9.89 Hz) compared to silkmoths (n: mean ± s.d.: 14.34 ± 5.21 Hz, $p < 0.0001$; electronic supplementary material, table S3).

(f) Relative subclade disparity through time shows both an early and recent accumulation of morphological diversity

A DTT analysis determines how morphological disparity accumulated over time. The relative subclade disparity of each shape is similar through time. Early in evolutionary history, relative subclade disparity is less than expected by BM for all three wings; the lowest values fall just inside the 95% confidence interval of BM trait simulation at the point when hawkmoths and silkmoths split (approx. 66 Ma; figure 2*a–c*). From that time, subclade disparity remained relatively static until sharply and significantly rising above BM expectations approximately 38 Ma (figure 2*a–c*), indicating younger subclades evolved a greater proportion of modern disparity than expected under BM. Removing tailed species from the analysis produces a similar result, but the rise in relative subclade disparity above the BM expectation now occurs more recently (electronic supplementary material, figure S4*c*). The DTT of combined wing metrics (W_s, n, S/m_t) follow similar patterns (figure 2*d–h*), with the exception of $\hat{r}_2$ (figure 2*e*). Notably, relative subclade disparity of AR significantly deviates below the BM expectation coincident with the divergence of the two sister-clades (figure 2*d*). Again, at approximately 38 Ma, the disparity of these wing traits begins to rise above the BM expectation, but only S/m_t and W_s significantly rise above the expectation under a BM process (figure 2*f,g*). A multivariate DTT of normalized functional wing metrics reveals a similar overall trend (electronic supplementary material, figure S5).

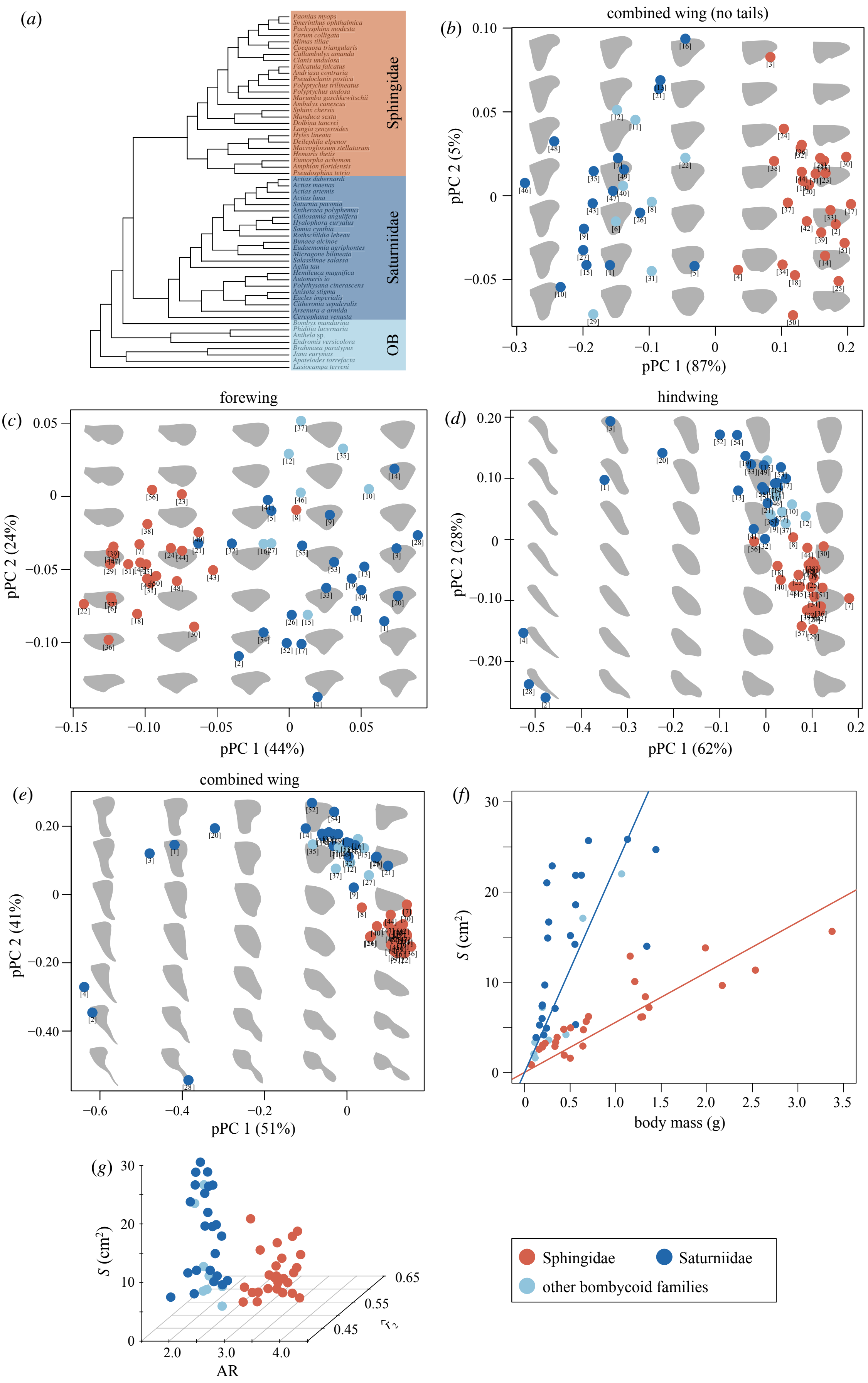

Figure 1. The evolution and trajectory of wing shape diversity. (*a*) The phylogenetic relationships of bombycoids and outgroups (node labels in electronic supplementary material, figure S2*b*). OB refers to other bombycoid families (the name we give to all long-branched species that do not belong to either the Saturniidae or Sphingidae clades). Clade colour is consistent across figures. Projections of shapes from (*b*) combined wing without tails, (*c*) forewing, (*d*) hindwing and (*e*) combined wing onto the first two pPCs demonstrates the separation between extant hawkmoths and silkmoths (pPC 3 and 4 and species number key in electronic supplementary material, figure S3). (*f*) Wing size and (*g*) combined wing functional shape metrics also diverge between hawkmoths and silkmoths. (Online version in colour.)

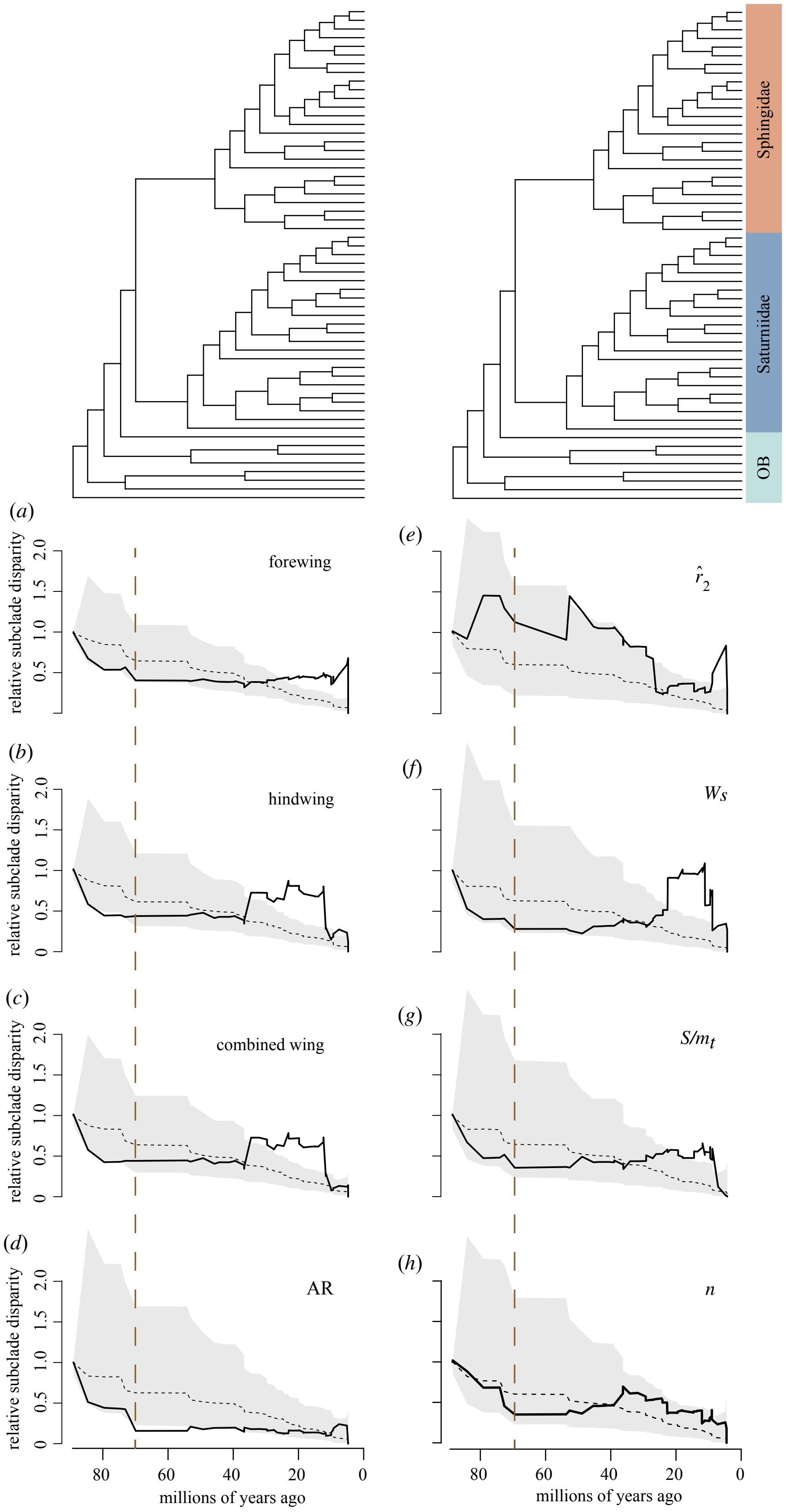

Figure 2. Disparity through time reveals that wing morphology diverged early between the clades and additional variation accumulated within each clade in more recent time. In each panel, the dashed line represents the median simulated subclade disparity under a single-rate BM process and includes the 95% confidence interval in grey. The observed relative subclade disparity is presented as a solid black line. All traits other than $\hat{r}_2$ show a similar trend in relative subclade disparity with low values deviating below the BM expectation in the early evolutionary history of the clade and high values in recent time. The low values in the early history of the clade indicate the disparity between clades was established early and the high values in recent history indicate disparity within each clade was established in more recent time. The brown vertical dashed line represents the time at which hawkmoths and silkmoths split. (Online version in colour.)

As relative subclade disparity shifts from consistently low values below the BM expectation to high values above the BM expectation in recent evolutionary history, morphological disparity index (MDI) values for each trait are near zero and not statistically significant (other than AR: −0.221 ± 0.202; $p = 0.022$; electronic supplementary material, table S6). While MDI values of approximately zero typically indicate a BM process, here, wing morphology deviates from the BM simulation in both deep and recent time. Instead, our findings suggest that morphological disparity was established *between* subclades early in the evolutionary history of the group (indicated by values below the BM expectation) and the additional disparity was established within each subclade in recent time (indicated by values above the BM expectation). While this pattern deviates from the BM expectation, the two deviations are in opposite directions, which is why we find an MDI near zero.

(g) Adaptive shifts account for differences in the evolution of several traits of wing morphology

Next, we tested whether an adaptive shift is responsible for the divergence in wing shape and its associated traits between hawkmoths and silkmoths without *a priori* hypotheses of shift location(s). We found support for an adaptive shift at the ancestral node of the hawkmoth clade for combined wing shape, AR and W_s (figure 3*a–c*). More recent adaptive shifts also occurred for combined wing shape, W_s, $\hat{r}_2$, S/m_t and n (figure 3*a–f*). The recent adaptive shifts in silkmoth combined wing shape are associated with the independent tail evolutions (figure 3*a*). The recent adaptive shift for n occurs in the hawkmoth subfamily, Macroglossinae, known for its particularly high n (figure 3*f*). Adaptive shifts did not occur at the ancestral node for either sister family for combined wing $\hat{r}_2$, S/m_t or n. In the absence of an adaptive shift, a trait can still have diverged between the sister-clades through other evolutionary processes. However, a single adaptive shift is inferred at the ancestral hawkmoth node when all functional (normalized) wing metrics are analysed together, supporting the findings that hawkmoth wing morphology undergoes an adaptive shift (electronic supplementary material, figure S5).

(h) Wing morphology does not evolve under a single-rate Brownian motion process

Next, we determined which model best fit the evolution of combined wing shape and its associated morphological features. For all traits, the model representing the adaptive shifts detected in the PhylogeneticEM analysis always fit best (electronic supplementary material, table S7). However, an adaptive shift was not detected in the previous analysis at the node for either sister family for $\hat{r}_2$, S/m_t or n, and this study is focused on the sister-clade divergence; the absence of an adaptive shift is likely due to the complex selective pressures on these traits that depend on both body and wing morphology.

4. Discussion

Flight morphology can have a strong influence on the aerodynamic performance of flying animals. We find that early in the evolutionary history of the moth superfamily Bombycoidea, wing shape and size were generally conserved until the ancestors of the hawkmoth and silkmoth sister-clades rapidly diverged (figure 3*a–c*), which is consistent with the early establishment of morphological disparity *between* clades (figure 2).

The evolutionary split between these two families has been dated to have occurred approximately 66 (confidence interval: 56.9–75.4) Ma [30], suggesting that these wing morphology trajectories may have been evolving since then. The initial divergence in wing morphology between hawkmoths and silkmoths was followed by subsequent diversification within each group, indicated by the rise in relative subclade disparity above a BM expectation coinciding with the more recent speciation events occurring within each family (figure 2). However, despite recent diversification, wing morphology did not converge between the two sister-families, indicated by the strong separation between the families in phylogenetic morphospace (figure 1).

Even specific species that converged in life history did not fully converge to employ overlapping wing shapes. For example, while the majority of hawkmoths are known for their hovering nectaring behaviour as adults, members of the hawkmoth subfamily, Smerinthinae (Node 67; figure 1*a* and electronic supplementary material, figure S2*b*), have lost the ability to feed as adults [38], convergent with silkmoths. However, the combined wing morphology (shape, size and most associated traits) of Smerinthinae species (Node 67 in electronic supplementary material, figure S2*b*) remains divergent from silkmoths, implying that Smerinthinae wing morphology is constrained by its evolutionary history. Finally, while we chose species to broadly cover the groups within bombycoids, sampling is far from complete. Therefore, we remain conservative in our interpretation, focusing on the split between hawkmoths and silkmoths for which we were able to accumulate broad sampling for our analysis. In sum, these data provide phylogenetic evidence supporting our hypothesis that distinct flight morphology evolved in each sister-clade.

(a) The evolutionary divergence of wing morphology has implications for flight performance

Given that the hawkmoth and silkmoth clades diverged in wing morphology, we can explore the consequences of these two morphologies for flight performance. While flight performance depends on many other factors, most notably wing movement, shape and size do have implications for aerodynamics. Contrary to our expectations, we did not observe morphological changes that were consistent with extreme manoeuvrability in hawkmoths and extreme power reduction in silkmoths. Hawkmoths, known to be manoeuvrable hover feeders, have evolved small wings of high AR, W_s and $\hat{r}_2$; all metrics typically associated with power reduction, efficient force production and lower degrees of manoeuvrability. By contrast, silkmoths, a group that does not feed as adults and is known for its bobbing (erratic) flight behaviour, have evolved large wings of low AR, W_s and $\hat{r}_2$.

(b) Hawkmoth wing morphology likely reduces power without sacrificing manoeuvrability

The high AR and $\widehat{r_2}$ wings of hawkmoths might act to reduce power and increase force production efficiency while *not* sacrificing manoeuvrability in comparison to silkmoths that are employing wings of lower AR and $\widehat{r_2}$. All else being equal, high AR and $\widehat{r_2}$ wings will reduce the induced power (P_{ind}) requirements of flight [6,19,22] and increase force production

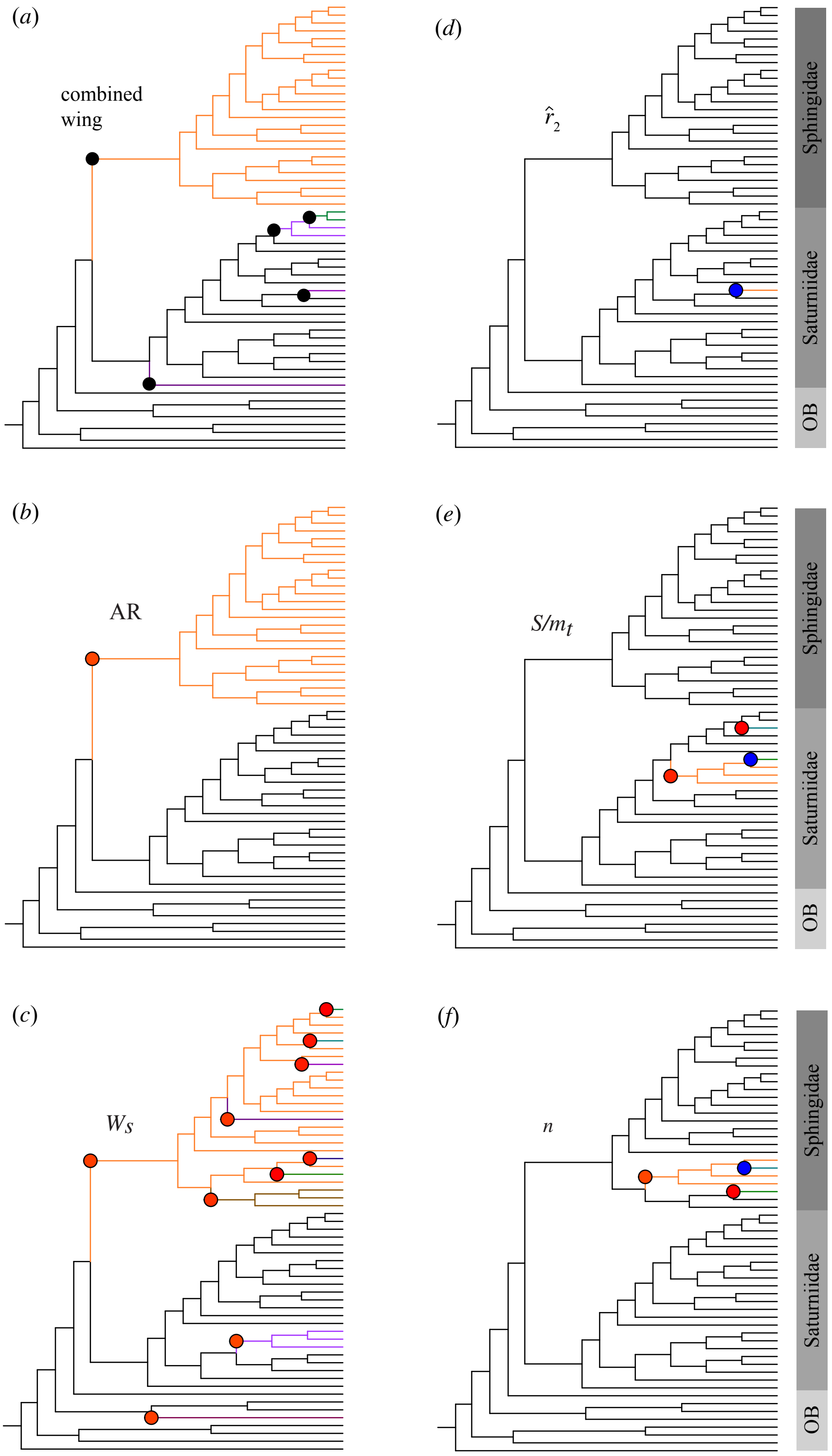

Figure 3. An adaptive shift is responsible for divergence in (*a*) wing shape, (*b*) aspect ratio and (*c*) wing loading between hawkmoths and silkmoths. Each branch colour indicates a separate regime (a set of branches evolving under a different set of model parameters). All branches sharing the same colour also share the same evolutionary mode. Shifts to new regimes are indicated by dots. For univariate traits, red dots indicate shifts to a larger trait value optima and blue dots indicate shifts to a smaller trait value. Black dots are used for shifts in multivariate traits, but do not indicate a direction. (Online version in colour.)

efficiency [5,48,49], respectively. However, both traits could come at the cost of reduced manoeuvrability due to an increase in the moments of inertia of the wing pair [3,5,6,21,23]. For a wing of constant area, uniform thickness and density, a larger AR and $\hat{r}_2$ will necessarily make the wing longer (increasing AR) while also concentrating more area distally along the span of the wing (increasing $\hat{r}_2$). Both scenarios correspond to an increase in wing moments of inertia, suggesting silkmoths should be more manoeuvrable than hawkmoths [5,24]. However, wing size will also have a strong impact on wing moment of inertia, and silkmoths have evolved larger wings (per body size) than hawkmoths (figure 1*f*,*g*; electronic supplementary material, table S3). Hawkmoths evolved high AR by reducing mean chord length, $\bar{c}$, rather than through an increase in wing span, *R* (figure 1*b*; electronic supplementary material, table S4). Therefore, while selection for economical flight (increased AR) might often reduce manoeuvrability, the evolution of small, high AR wings in the hawkmoth clade (achieved through a reduction in $\bar{c}$) could act to increase economy while not necessarily sacrificing manoeuvrability.

The potential cost of small wing size is that proportionally smaller wings could reduce wing stroke-averaged aerodynamic force production, if wing movement remains constant. However, in flapping or revolving wings, when all other things are equal, the greater $\hat{r}_2$ and n (inferred through scaling relationships) of hawkmoths would increase their magnitude of torque production relative to silkmoths. The velocity of a wing section increases with its distance from the axis of rotation, and aerodynamic force production is proportional to velocity squared. Therefore, shifting more area distally (increasing $\hat{r}_2$) and moving the wing at higher speeds (increasing n) will increase aerodynamic force production (e.g. [26,48,49]). Additionally, increasing n allows for more frequent modification of force vectors, which could enhance flight control and manoeuvrability. Natural selection could thus act on wing shape, size and frequency (tradeoffs through scaling relationships) to modify the means of force production, power and flight control across species.

(c) Lower wing loading (W_s) in silkmoths could contribute to manoeuvrability and erratic flight

It is possible that inter-clade differences in W_s contribute to inter-clade differences in flight behaviour between families. A lower W_s increases both manoeuvrability [14–21] and flight path unpredictability [50]. Silkmoths, which evolved significantly lower W_s in comparison to hawkmoths (figure 1*g*; electronic supplementary material, table S3), are well known for their erratic flight patterns [32,38] where the vertical position is regularly changing throughout their flight bout. An erratic, or unpredictable flight path, can enhance predator avoidance [15,51], and therefore survival and fitness. In hummingbird flight, positional predictability and W_s are positively correlated where hummingbirds with lower wing loading are less predictable [50]. If the relationship between W_s and predictability is true in other systems, then the divergence in W_s between hawkmoths and silkmoths is precisely the expectation based on the divergence in flight behaviour between the two clades. Therefore, it is likely that the evolution of silkmoth wing morphology, particularly low W_s, is directly tied to the production of erratic flight patterns and the ability to avoid predation.

(d) Body shape evolution might aid predator avoidance in silkmoths

Next, we examined the implications of body size evolution for flight performance. In comparison to hawkmoths, silkmoths have a shorter l_b and a longer thorax compared to the abdomen, thereby decreasing I_{yy} and I_{zz} of the body and likely increasing manoeuvrability. These patterns could allow silkmoths greater angular accelerations during pitch and yaw manoeuvres and might be complemented by a reduction in the distance between the centre of mass and wing hinge [52]. Indeed, species of neotropical butterflies equipped with a shorter abdomen and larger thorax were more successful at evading predators than species with shorter thoraces and longer abdomens [52]. Therefore, in addition to wing elaborations [32,38,46] and bobbing flight behaviour [32,36–38], our data suggest that the evolution of a large thorax and short abdomen is an additional mechanism contributing to predator avoidance in silkmoths.

(e) Adaptive shifts are responsible for the divergence in wing morphology between hawkmoths and silkmoths

An adaptive shift is found at the stem of hawkmoths for wing shape, AR and W_s (figure 3*a–c*), indicating that the shape and relative size of hawkmoth wings are evolving around an adaptive peak. Although disparity was established early in the evolutionary history of the clade (figure 2), rather than slow down in diversification rate, which would occur in an early burst [8,9], the initial divergence in life history and flight morphology gives rise to the accumulation of additional disparity within each clade in recent time (figure 2). Indeed, the recent accumulation of disparity within a subclade is associated with evolution around an adaptive peak [53], and the absence of evidence for an early burst in the diversification of wing morphology is consistent with major inter-continental radiations in other systems [7,10,11].

The discrete adaptive shift in hawkmoth wing morphology parallels the evolution of the hover feeding behaviour in hawkmoths and the loss of adult-stage feeding in silkmoths. The adaptive shift in hawkmoth wing morphology to small, slender wings of high AR that can be moved at high frequencies might be directly related to the evolution of hover feeding, which requires enhanced flight control and high power output [35], as high AR wings are known to reduce flight power requirements [6].

An adaptive shift at the stem of hawkmoths was not found for all wing morphology traits, suggesting a potential decoupling of the processes, and, therefore, selective pressures, driving the evolution of overall wing shape, size and specific features. It should not be expected that all features of wing morphology evolve under the same process. Wing metrics, like $\hat{r}_2$, which is related to force production efficiency [24], appear to be more conserved, and those related to both wing and body size, like n and S/m_t, might be under particularly complex selective pressures.

Differently, an adaptive shift was never found for any trait at the stem of the silkmoth clade, which could be expected given the less drastic separation in wing morphology traits between silkmoths and the other bombycoid families (figure 2). By contrast, more recent adaptive shifts were detected and associated with the evolution of hindwing tails in silkmoths (figure 3*a*) and high n in diurnal hawkmoths (figure 3*f*). While these recent shifts need to be supported through further sampling within these specific groups, it is exciting that they might be indicative of recent shifts in flight morphology within these clades, providing a potential opportunity to identify specialized species or subclades for future functional studies in live animals.

The overall combined wing morphology is derived from two functionally linked and overlapping wing structures (forewing and hindwing) that can each potentially evolve independently in size and shape, unlocking additional complexities unachievable by a single wing alone. While forewing and hindwing morphology also diverge between groups, the absolute values of these traits are different between the fore- and hindwing (electronic supplementary material, figure S4). Different components of the same functional system often evolve at different tempos and modes [54], raising questions of whether or not certain aspects of wing morphology constitute evolutionary modules. The integration of techniques from developmental and evolutionary biology will be particularly fruitful when investigating the modularity of insect wing units.

5. Conclusion

Silkmoths and hawkmoths evolved distinct flight morphology through an adaptive shift in hawkmoth wing morphology, which occurred in parallel to the evolution of the hover feeding behaviour in hawkmoths. The sister-clade divergence of wing morphology metrics, which are historically derived for fixed-winged aircrafts, is not totally consistent with initial expectations of flight performance based on the life history of species in each clade. However, aerodynamic performance emerges from the interaction of wing shape, size and movement [6,55], and it is likely that hawkmoths achieve high levels of flight control through high n and other kinematic adjustments. Our findings indicate that aerodynamically important morphological traits can experience drastic shifts in parallel to the divergence in life history and flight behaviour. While the evolution of flapping flight in insects is thought to be a key innovation [1], diversification can be further potentiated by more recent adaptive shifts, helping to shape the diversity of wing morphology seen across extant aerial animals.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.fj6q573v7 [45].

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References

1. Nicholson DB, Ross AJ, Mayhew PJ. 2014 Fossil evidence for key innovations in the evolution of insect diversity. *Proc. R. Soc. B* **281**, 20141823. (doi:10.1098/rspb.2014.1823)
2. Wootton RJ. 1992 Functional-morphology of insect wings. *Annu. Rev. Entomol.* **37**, 113–140. (doi:10.1146/Annurev.En.37.010192.000553)
3. DeVries PJ, Penz CM, Hill RI. 2010 Vertical distribution, flight behaviour and evolution of wing morphology in Morpho butterflies. *J. Anim. Ecol.* **79**, 1077–1085. (doi:10.1111/j.1365-2656.2010.01710.x)
4. Ray RP, Nakata T, Henningsson P, Bomphrey RJ. 2016 Enhanced flight performance by genetic manipulation of wing shape in *Drosophila*. *Nat. Commun.* **7**, 10851. (doi:10.1038/ncomms10851)
5. Le Roy C, Debat V, Llaurens V. 2019 Adaptive evolution of butterfly wing shape: from morphology to behaviour. *Biol. Rev.* **94**, 1261–1281. (doi:10.1111/brv.12500)
6. Dudley R. 2000 *The biomechanics of insect flight: form, function, evolution*. Princeton, NJ: Princeton University Press.
7. Harmon LJ *et al.* 2010 Early bursts of body size and shape evolution are rare in comparative data. *Evol. Int. J. Org. Evol.* **64**, 2385–2396. (doi:10.1111/j.1558-5646.2010.01025.x)
8. Harmon LJ, Schulte JA, Larson A, Losos JB. 2003 Tempo and mode of evolutionary radiation in iguanian lizards. *Science* **301**, 961–964. (doi:10.1126/science.1084786)
9. Gavrilets S, Losos JB. 2009 Adaptive radiation: contrasting theory with data. *Science* **323**, 732–737. (doi:10.1126/science.1157966)
10. Arbour JH, Curtis AA, Santana SE. 2019 Signatures of echolocation and dietary ecology in the adaptive evolution of skull shape in bats. *Nat. Commun.* **10**, 2036. (doi:10.1038/s41467-019-09951-y)
11. Cooney CR, Bright JA, Capp EJR, Chira AM, Hughes EC, Moody CJA, Nouri LO, Varley ZK, Thomas GH. 2017 Mega-evolutionary dynamics of the adaptive radiation of birds. *Nature* **542**, 344–347. (doi:10.1038/nature21074)
12. Simpson GG. 1944 *Tempo and mode in evolution*. New York, NY: Columbia University Press.
13. Wainwright PC. 2007 Functional versus morphological diversity in macroevolution. *Annu. Rev. Ecol. Evol. Syst.* **38**, 381–401. (doi:10.1146/annurev.ecolsys.38.091206.095706)
14. Burns JG, Ydenberg RC. 2002 The effects of wing loading and gender on the escape flights of least sandpipers (*Calidris minutilla*) and western sandpipers (*Calidris mauri*). *Behav. Ecol. Sociobiol.* **52**, 128–136. (doi:10.1007/s00265-002-0494-y)
15. Hedenstrom A, Rosen M. 2001 Predator versus prey: on aerial hunting and escape strategies in birds. *Behav. Ecol.* **12**, 150–156. (doi:10.1093/beheco/12.2.150)
16. Dakin R, Segre PS, Straw AD, Altshuler DL. 2018 Morphology, muscle capacity, skill, and maneuvering ability in hummingbirds. *Science* **359**, 653–657. (doi:10.1126/science.aao7104)
17. Aldridge HDJN. 1987 Turning flight of bats. *J. Exp. Biol.* **128**, 419–425. (doi:10.1242/jeb.128.1.419)
18. Norberg U. 1990 *Vertebrate flight: mechanics, physiology, morphology, ecology and evolution*. Berlin, Germany: Springer-Verlag.
19. Norberg UM, Rayner JMV. 1987 Ecological morphology and flight in bats (Mammalia, Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Phil. Trans. R. Soc. Lond. B* **316**, 337–419. (doi:10.1098/rstb.1987.0030)
20. Berwaerts K, Van Dyck H, Aerts P. 2002 Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. *Funct. Ecol.* **16**, 484–491. (doi:10.1046/j.1365-2435.2002.00650.x)
21. Betts CR, Wootton RJ. 1988 Wing shape and flight behavior in butterflies (Lepidoptera, Papilionoidea and Hesperioidea): a preliminary analysis. *J. Exp. Biol.* **138**, 271–288. (doi:10.1242/jeb.138.1.271)
22. Pennycuick CJ. 1968 Power requirements for horizontal flight in pigeon columba livia. *J. Exp. Biol.* **49**, 527. (doi:10.1242/jeb.49.3.527)
23. Cespedes A, Penz CM, DeVries PJ. 2015 Cruising the rain forest floor: butterfly wing shape evolution and gliding in ground effect. *J. Anim. Ecol.* **84**, 808–816. (doi:10.1111/1365-2656.12325)
24. Ellington CP. 1984 The aerodynamics of hovering insect flight. 2. Morphological parameters. *Phil.*

Trans. R. Soc. Lond. B **305**, 17–40. (doi:10.1098/Rstb.1984.0050)

25. Deakin MAB. 2010 Formulae for insect wingbeat frequency. *J. Insect. Sci.* **10**, 96. (doi:10.1673/031.010.9601)
26. Hedrick TL, Cheng B, Deng X. 2009 Wingbeat time and the scaling of passive rotational damping in flapping flight. *Science* **324**, 252–255. (doi:10.1126/science.1168431)
27. Ellington CP. 1984 The aerodynamics of hovering insect flight. VI. Lift and power requirements. *Phil. Trans. R. Soc. Lond. B* **305**, 145–181. (doi:10.1098/Rstb.1984.0054)
28. Kitching IJ, Rougerie R, Zwick A, Hamilton CA, St Laurent RA, Naumann S, Mejia LB, Kawahara AY. 2018 A global checklist of the Bombycoidea (Insecta: Lepidoptera). *Biodivers. Data J.* **6**, e22236. (doi:10.3897/BDJ.6.e22236)
29. Breinholt JW, Earl C, Lemmon AR, Lemmon EM, Xiao L, Kawahara AY. 2018 Resolving relationships among the megadiverse butterflies and moths with a novel pipeline for anchored phylogenomics. *Syst. Biol.* **67**, 78–93. (doi:10.1093/sysbio/syx048)
30. Kawahara AY *et al.* 2019 Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *Proc. Natl Acad. Sci. USA* **116**, 22 657–22 663. (doi:10.1073/pnas.1907847116)
31. Hamilton CA, St Laurent RA, Dexter K, Kitching IJ, Breinholt JW, Zwick A, Timmermans M, Barber JR, Kawahara AY. 2019 Phylogenomics resolves major relationships and reveals significant diversification rate shifts in the evolution of silk moths and relatives. *BMC Evol. Biol.* **19**, 182. (doi:10.1186/s12862-019-1505-1)
32. Janzen D. 1984 Two ways to be a tropical big moth: Santa Rosa saturniids and sphingids. In *Oxford surveys in evolutionary biology* (eds R Dawkins, M Ridley), pp. 85–140. Oxford, UK: Oxford University Press.
33. Sponberg S, Dyhr JP, Hall RW, Daniel TL. 2015 Luminance-dependent visual processing enables moth flight in low light. *Science* **348**, 1245–1248. (doi:10.1126/science.aaa3042)
34. Stöckl AL, Kihlstrom K, Chandler S, Sponberg S. 2017 Comparative system identification of flower tracking performance in three hawkmoth species reveals adaptations for dim light vision. *Phil. Trans. R. Soc. B* **372**, 20160078. (doi:10.1098/rstb.2016.0078)
35. Willmott AP, Ellington CP. 1997 The mechanics of flight in the hawkmoth *Manduca sexta*. II. Aerodynamic consequences of kinematic and morphological variation. *J. Exp. Biol.* **200**, 2723–2745. (doi:10.1242/jeb.200.21.2723)
36. Jacobs D, Bastian A. 2016 *Predator–prey interactions: co-evolution between bats and their prey*. Cham, Switzerland: Springer.
37. Lewis FP, Fullard JH, Morrill SB. 1993 Auditory influences on the flight behavior of moths in a nearctic aite. 2. Flight times, heights, and erraticism. *Can. J. Zool.* **71**, 1562–1568. (doi:10.1139/z93-221)
38. Tuskes P, Tuttle J, Collins M. 1996 *The wild silk moths of North America: a natural history of the saturniidae of the United States and Canada*. Ithaca, NY: Cornell University Press.
39. Olsen AM, Westneat MW. 2015 StereoMorph: an R package for the collection of 3D landmarks and curves using a stereo camera set-up. *Methods Ecol. Evol.* **6**, 351–356. (doi:10.1111/2041-210X.12326)
40. Gilchrist GW. 1990 The consequences of sexual dimorphism in body size for butterfly flight and thermoregulation. *Funct. Ecol.* **4**, 475–487. (doi:10.2307/2389315)
41. Revell LJ. 2009 Size-correction and principal components for interspecific comparative studies. *Evol. Int. J. Org. Evol.* **63**, 3258–3268. (doi:10.1111/j.1558-5646.2009.00804.x)
42. Wang M, Kornblau SM, Coombes KR. 2018 Decomposing the apoptosis pathway into biologically interpretable principal components. *Cancer Inform.* **17**, Artn 1176935118771082. (doi:10.1177/1176935118771082)
43. Bastide P, Ane C, Robin S, Mariadassou M. 2018 Inference of adaptive shifts for multivariate correlated traits. *Syst. Biol.* **67**, 662–680. (doi:10.1093/sysbio/syy005)
44. Clavel J, Escarguel G, Merceron G. 2015 mvMORPH: an R package for fitting multivariate evolutionary models to morphometric data. *Methods Ecol. Evol.* **6**, 1311–1319. (doi:10.1111/2041-210x.12420)
45. Aiello B *et al.* 2021 Data from: Adaptive shifts underlie the divergence in wing morphology in bombycoid moths. Dryad Digital Repository. (doi:10.5061/dryad.fj6q573v7)
46. Barber JR, Leavell BC, Keener AL, Breinholt JW, Chadwell BA, McClure CJW, Hill GM, Kawahara AY. 2015 Moth tails divert bat attack: evolution of acoustic deflection. *Proc. Natl Acad. Sci. USA* **112**, 2812–2816. (doi:10.1073/pnas.1421926112)
47. Kawahara AY, Barber JR. 2015 Tempo and mode of antibat ultrasound production and sonar jamming in the diverse hawkmoth radiation. *Proc. Natl Acad. Sci. USA* **112**, 6407–6412. (doi:10.1073/pnas.1416679112)
48. Fernandez MJ, Driver ME, Hedrick TL. 2017 Asymmetry costs: effects of wing damage on hovering flight performance in the hawkmoth *Manduca sexta*. *J. Exp. Biol.* **220**, 3649–3656. (doi:10.1242/jeb.153494)
49. Muijres FT, Iwasaki NA, Elzinga MJ, Melis JM, Dickinson MH. 2017 Flies compensate for unilateral wing damage through modular adjustments of wing and body kinematics. *Interface Focus* **7**, 20160103. (doi:10.1098/rsfs.2016.0103)
50. Berberi I, Segre PS, Altshuler DL, Dakin R. 2020 Unpredictable hummingbirds: flight path entropy is constrained by speed and wing loading. *bioRxiv* 2020.08.11.246926. (doi:10.1101/2020.08.11.246926)
51. Humphries DA, Driver PM. 1970 Protean defence by prey animals. *Oecologia* **5**, 285–302. (doi:10.1007/Bf00815496)
52. Srygley RB, Dudley R. 1993 Correlations of the position of center of body-mass with butterfly escape tactics. *J. Exp. Biol.* **174**, 155–166. (doi:10.1242/jeb.174.1.155)
53. Lopez-Fernandez H, Arbour JH, Winemiller KO, Honeycutt RL. 2013 Testing for ancient adaptive radiations in neotropical cichlid fishes. *Evol. Int. J. Org. Evol.* **67**, 1321–1337. (doi:10.1111/evo.12038)
54. Evans KM, Taylor S, Fenolio DB. 2019 Bony patchwork: mosaic patterns of evolution in the teleost skull. *Integr. Comp. Biol.* **59**, E64.
55. Warrick DR, Dial KP. 1998 Kinematic, aerodynamic and anatomical mechanisms in the slow, maneuvering flight of pigeons. *J. Exp. Biol.* **201**, 655–672. (doi:10.1242/jeb.201.5.655)