Locomotor mimicry in *Heliconius* butterflies: contrast analyses of flight morphology and kinematics

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Locomotor mimicry in *Heliconius* butterflies: contrast analyses of flight morphology and kinematics

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Müllerian mimicry is a mutualism involving the evolutionary convergence of colour patterns of prey on a warning signal to predators. Behavioural mimicry presumably adds complexity to the signal and makes it more difficult for Batesian mimics to parasitize it. To date, no one has quantified behavioural mimicry in Müllerian mimicry groups. However, morphological similarities among members of mimicry groups suggested that pitching oscillations of the body and wing-beat frequency (WBF) might converge with colour pattern. I compared the morphology and kinematics of four *Heliconius* species, which comprised two mimicry pairs. Because the mimics arose from two distinct lineages, the relative contributions of mimicry and phylogeny to variation in the species' morphologies and kinematics were examined. The positions of the centre of body mass and centre of wing mass and wing shape diverged among species within lineages, and converged among species within mimicry groups. WBF converged within mimicry groups, and it was coupled with body pitching frequency. However, body-pitching frequency was too variable to distinguish mimicry groups. Convergence in WBF may be due, at least in part, to biomechanical consequences of similarities in wing length, wing shape or the centre of wing mass among co-mimics. Nevertheless, convergence in WBF among passion-vine butterflies serves as the first evidence of behavioural mimicry in a mutualistic context.

Keywords: mimicry; *Heliconius*; comparative analysis; flight; mutualism

1. INTRODUCTION

An evolutionary approach to ecological morphology seeks to integrate morphological function with relevant environmental features in a historical context (Losos & Miles 1994). In comparisons among species, four questions must be addressed: (i) how is morphology related to function; (ii) what are the relevant environmental features; (iii) in what manner have morphology and function evolved with relation to the phylogeny of the organisms; and (iv) what is the relationship of history and ecology to phenotypic evolution.

Evolutionary convergence in morphology and function of species arising from historically dissimilar phenotypes has been used to infer evolutionary adaptation in form and function, and identify the selective agents that operate in common on the species. For example, mimetic resemblance of prey species is one of the most often cited examples of phenotypic evolution by natural selection (reviewed in Gilbert 1983), and visually acute, avian predators are the principal selective agents on similarities in colour pattern in insects (Brower & Brower 1964; Chai 1986). Two distantly related species that have similarities in signals are classically mimetic if a recipient of biological interest (such as a natural predator) associates the signal from one species with that of the other. As a result, mimetic similarity among two species suggests a common predatory environment.

More recently, mimicry has become an example of the elaborate coevolution that may result among prey species via common selective agents (Gilbert 1983). For example, mimetic resemblance in wing coloration of the distasteful passion-vine butterflies arises from two distinct lineages, sylvaniforms and pupal maters, within the genus *Heliconius*. Multiple, correlated changes in the wing patterns of species from these two groups occur across a broad geographic range from southern Brazil to Central America (Eltringham 1916). These changes in mimetic patterns are strong evidence for coevolution due to natural selection on signalling by predators (Turner 1981; Gilbert 1983; Sheppard *et al.* 1985; Brower 1996).

In Müllerian mimicry, species that are distasteful converge on a common colour pattern such that the association of the signals is beneficial for both prey species and predators (Müller 1879). Hence the environmental features that are relevant to the ecology and evolution of mimicry include a species' co-mimic, the predators or signal recipients and the butterfly species from which the signals are discriminated. In this paper, I will investigate the morphological and kinematic diversification among closely related *Heliconius* species that are members of different Müllerian mimicry groups to address: (i) the relationship of morphology to flight; (ii) the evolution of morphology and flight with respect to phylogenetic diversification; (iii) the evolution of morphology and flight

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with respect to mimetic convergence; and (iv) whether morphology and behaviour are used by predators as discriminatory signals.

(a) *Locomotor mimicry and relevant morphological and kinematic features*

Srygley (1994) analysed the flight-related morphology (refer to Ellington (1984*a*,*b*)) of the heliconiine butterflies and their mimics in order to evaluate those parameters that (i) were associated into character suites, (ii) converged within mimicry groups, and (iii) diverged among palatable and distasteful mimicry groups.

Centres of body and wing mass were positioned nearer to the wing base in mimicry groups composed of more palatable species, presumably decreasing radial moments of inertia and increasing flight speed and manoeuvrability (see Srygley (1994), fig. 3 and the appendix, in which the value for relative centre of body mass for female *Perrhybris pyrrha* should be changed to 0.318). Srygley & Dudley (1993) confirmed that positioning the centre of the body mass near to the wing base was associated with increases in flight speed and the ability to escape from the attack of birds.

Locomotor mimicry was a second hypothesis that emerged from this analysis: convergent selection on flight behaviour may reduce morphological differences of species within mimicry groups arising from distantly related lineages. Convergences in centres of mass within mimicry groups were predicted to result in convergences in oscillations of the body and wings (here referred to as body-pitching frequency (BPF) and wing-beat frequency (WBF)) during flight. In this paper to further our understanding of locomotor mimicry, I investigated the relative contributions of phylogeny and mimicry to the variance in flight-related morphological features and flight kinematics.

Using principal component analysis, three morphological features were identified that best represented three character suites (principal components are in capital letters). $\mathrm{CM}_{\mathrm{body}}$ was best associated with the position of the centre of body mass ($\mathrm{cm}_{\mathrm{body}}$) and the body's radius of gyration. $\mathrm{CM}_{\mathrm{body}}$ also served as a descriptor for body shape and mass allocation to the thorax and abdomen. Theoretically, this character suite influences flight speed, manoeuvrability (Srygley & Dudley 1993) and linear acceleration (Marden & Chai 1991). WING SHAPE was best associated with the centre of wing area [$r_1(S)$]. WING SHAPE was also associated with the aspect ratio and virtual mass of the wing. Theoretically, this character suite influences lift and drag generated by the wings (Ellington 1984*b*). $\mathrm{CM}_{\mathrm{wing}}$ was most heavily weighted by the position of the centre of wing mass ($\mathrm{cm}_{\mathrm{wing}}$), which is a non-dimensionalized predictor of the wing's rotational inertia. Theoretically, the wing's rotational inertia affects its acceleration and deceleration during the wing stroke and WBF (Sotovalta 1952, 1954; Greenewalt 1960; Ellington 1984*b*).

Wing loading (ρ), wing length, body mass and the area swept by the beating wings (sweep area, hereafter abbreviated to SA) were also tested for the effects of mimicry and phylogeny. Wing loading is a general predictor of airspeed across taxonomic groups (Srygley & Dudley 1993). During hovering and slow flight (defined by Ellington as an advance ratio, i.e. forward velocity relative to wing flapping velocity, less than 0.1), Ellington (1984*c*) demonstrated that the area swept by the wings was a more accurate predictor of the induced velocities generated by the wings than a previous theory based on the wing span alone. SA was investigated although the mean advance ratio mean advance ratio for the *Heliconius* presented here was 0.56 (range: 0.26 to 1.18). Body mass was included as a measure of body size and potential determinant of WBF (Norberg 1990).

Recently, Brower (1995) criticized a major assumption of Srygley (1994): mean values for mimicry groups served as independent observations in the association with palatability. Because of the potential for morphological similarities resulting from historical descent, Brower questioned whether similarities in the positions of centres of mass among colour-pattern mimics represent adaptive convergences. This paper presents a contrast analysis to evaluate the relative influence of phylogeny and mimicry on morphology and kinematics in two pairs of *Heliconius* co-mimics. An analysis of the morphology for this subset of the species analysed in Srygley (1994) was performed to accomplish three goals: (i) to evaluate my assumption of the relative role of mimicry and phylogeny in shaping butterfly morphology and address Brower's criticism; (ii) to investigate intraspecific variation which was not presented in Srygley (1994); and (iii) to determine whether the predicted differences in kinematics among mimicry groups follow from the member species' morphologies.

2. STUDY ORGANISMS

To evaluate the relative influence of mimicry and phylogeny on morphological and kinematic features, four *Heliconius* species were selected so that two pairs of close relatives had one member in each of two mimicry groups. This design permitted use of two-way analysis of variance (ANOVA) and related parametric statistical analyses to determine the proportion of the variance in the features that was due to each of the two independent factors: mimicry group and phylogenetic lineage.

Heliconius cydno chioneus (figure 1; subspecies range: Costa Rica to Colombia) and *H. melpomene rosina* (Mexico to Panama) are members of the sylvaniform clade of *Heliconius* (Brown 1981; Brower 1994), whereas *H. sapho candidus* and *H. erato petiverana* (Mexico to Panama) are members of the pupal-mating clade (Brown 1981; Lee *et al.* 1992; Brower 1994). The subspecies are sympatric in south-eastern Costa Rica and Panama.

Heliconius cydno chioneus and *H. sapho candidus* are co-mimics, whereas *H. melpomene rosina* and *H. erato petiverana* are another pair of co-mimics. All four species were highly distasteful to the rufous-tailed jacamar (Chai & Srygley 1990). These pairs and related subspecies were subjects for coevolutionary studies of mimetic wing patterns (Eltringham 1916; Turner 1981; Gilbert 1983; Sheppard *et al.* 1985; Brower 1996).

Prior to measuring flight-related morphological features in the heliconiine butterflies and their co-mimics (detailed in Srygley (1994)), the butterflies were videotaped for kinematic analysis. Of this larger array of species, the four *Heliconius* species analysed here represent

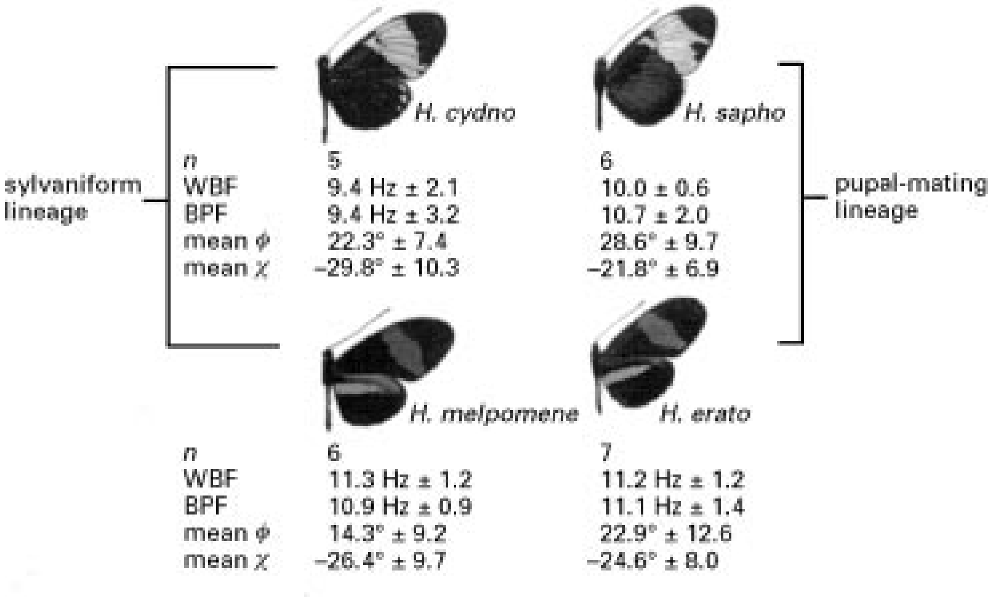

Figure 1. Phylogeny and kinematics of *Heliconius* co-mimics. The sample size, means and standard deviations for kinematics are listed for each species. Wing-beat frequency (WBF) was the only feature that converged significantly within mimicry groups, whereas none of the features were significantly associated with phylogenetic lineage. *H. cydno* and *H. sapho* are black with a white forewing bar, and *H. erato* and *H. melpomene* are black with a red forewing bar and a yellow hindwing bar.

a subset for which the phylogeny is sufficiently resolved to evaluate the relative influence of mimicry and phylogeny using the contrast method developed in this paper.

3. MATERIALS AND METHODS

During December 1987 to January 1988, June and September 1988, January to February 1989 and August to September 1989, butterflies were captured in the field on Barro Colorado Island or Pipeline Road in lowland Panamanian wet forest, and held overnight in an insectary on Barro Colorado Island. Butterflies were fed 25% sugar-water or 10% honey-water in the early morning. The original set of taped sequences comprised the following number of individuals: *Heliconius cydno*: four females, nine males; *H. erato*: six females, twelve males; *H. melpomene*: seven females, ten males; *H. sapho*: one female, six males. For *H. melpomene*, technical difficulties reduced the sample size to only two males. Hence, an additional two females and two males were reared and videotaped in the greenhouse in Austin during May 1989. Body mass and wing loading of males and females from the greenhouse did not differ significantly from that of male and female *H. melpomene* captured in the field (Student's *t*-tests: $p > 0.50$ for all four cases).

Butterflies were flown through a 1.5 m tube of 0.5 half-inch mesh hardware cloth (0.25 inch in Austin) that directed their flightpath horizontally and perpendicular to the video camera lens. A Panasonic video camera (model AG160 in Panama, WV3250 in Austin) was levelled in three dimensions on a tripod so that the lens was perpendicular to the length of the tunnel, parallel to the ground and approximately 450–800 mm from the midline of the flight tunnel. The zoom lens was set to wide angle (focal lengths: 9 mm in Panama, 10.5 mm in Austin), and the distance from the flight cage was measured to adjust for perspective (see below).

Each butterfly was removed from the holding bag (*ca.* 0.5 × 0.2 m, height × diameter), and its surface thoracic temperature and ambient temperature were measured with an Omega alumel-chromel surface thermocouple and an Omega thermometer. The butterfly was released into the tunnel and chased along the first 1–1.5 m of the tunnel with rapid hand motions to direct it forward at a steady speed. An unchased portion of flight in the final 0.5 m of the tunnel was captured on tape.

All butterflies were flown for at least five sequences and as many as ten sequences, while ambient temperature and body temperature were varied to determine the optimum temperatures for flight (R. B. Srygley, unpublished data). In Panama, ambient conditions were varied without artificial means with the use of two insectaries (one in shaded and one in sunlit habitats). In Austin, ambient temperature was varied independently of the sun by adjusting the greenhouse thermostat. Precautions were taken to minimize ambient wind. In Panama, butterflies were taped in the morning and only when there was no detectable wind. In Austin, heating or cooling units were turned off after the desired ambient temperature was reached. Mean body temperature was not significantly different among the species (one-way ANOVA, $F = 1.0$, $p = 0.455$).

Following videotaping, butterflies were frozen and flight-related morphological features were measured following guidelines in Srygley (1994). The positions of $\mathrm{cm}_{\mathrm{body}}$, $\mathrm{cm}_{\mathrm{wing}}$ and wing centroid were selected to represent the three character suites ($\mathrm{CM}_{\mathrm{body}}$, $\mathrm{CM}_{\mathrm{wing}}$ and WING SHAPE, respectively), identified in Srygley (1994). The body's rotational inertia ($\mathrm{I}_{\mathrm{body}}$) was also included because it serves as a direct measure of the pendular oscillating frequency of the body. In addition, I calculated two general predictors of insect flight kinematics and aerodynamics: wing loading ($\rho =$ body mass/wing area) and sweep area (SA = the arc swept by the wings in radians multiplied by wing length squared).

(a) *Digitizing and reconstructing flight sequences*

Each 1/60 s image was digitized using a PEAK motion analysis system. Coordinates (x, y) for four points were extracted from each image (figure 2): anterior end of the head, wing base, posterior end of the abdomen and the wing-tip nearest to the camera. For those frames in which both the near and far wing-tips were visible, motion was apparently symmetrical. The butterflies' two-dimensional (2D) coordinates were adjusted for perspective following the methods of Ellington (1984*b*) with the assumption that the butterfly was flying down the vertical midplane of the chamber. If the flight path deviated from the midplane to the front or back of the chamber, then the

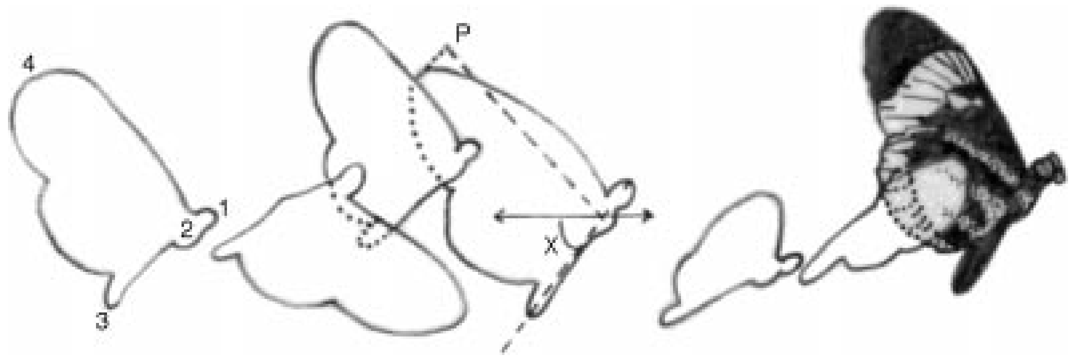

Figure 2. The first 13 frames of a *Heliconius cydno* flight sequence, of which only the odd set (1/30 s between images) is shown for clarity. Numbers in frame one are near to the four digitized points: (1) head, (2) wing base, (3) abdomen tip, (4) near wing-tip. The derived kinematic features are presented in frame four. The body angle (χ) is the angle formed by the body axis (connecting points 1 and 3) and the horizontal axis (solid axis through the wing base). The stroke plane (P) is that plane nearest to the path of the wing tips, lying perpendicular to the page, and passing through both wing bases. The elevation of the wing (ϕ) is the angle formed by the nearest projection of the wing-tip onto the stroke plane (dotted line to P) and the horizontal axis that lies perpendicular to the page running through both wing bases (not drawn).

maximum perspective error in the coordinates was 12–23%. These errors were most significant for measurements of flight speed at either end of the tunnel and negligible for the analyses of kinematics presented here.

(b) *Selection of sequences*

Only sequences in which the flight path was visibly smooth and uninterrupted by sudden bursts of acceleration or deceleration were selected. In addition, I excluded sequences of butterflies in slow, deliberate flight, as they apparently searched the cage for an escape route. In some instances, portions of a sequence were deemed acceptable and the remainder excluded.

Furthermore, the following *a priori* assumptions were tested empirically, leading to the exclusion of some sequences or parts of a sequence. First, to use wing length to calculate flight speed, butterflies were assumed to be flying without moving from one side of the flight tunnel to the other. The criterion was that the forewing length, when positioned fully upwards, varied negligibly as the butterfly moved through the tunnel. Negligible variance in body length was used as an independent confirmation of a relatively straight flight path. However, if the wings were never positioned fully upwards and body length varied minimally during the flight sequence, body length was used to calculate flight speed and adjust the wings for perspective to calculate wing elevation (see below). Second, to combine a series of frames to achieve higher resolution of body and wing motion, only those sequences in which the butterflies flew evenly with negligible change in velocity and altitude (sloping less than 10° between the beginning and end of a sequence; i.e. a 5% change in vertical lift production for a 130 mg butterfly with a forward velocity of 0.8 m s^{-1}) were selected.

For those sequences that met these criteria, one acceptable sequence was selected randomly for each individual. Any turning of the butterfly was assumed to be negligible and motion of the near wing was assumed to reflect that of the far wing, thus minimizing the sensitivity of the kinematic results to error due to perspective.

(c) *Kinematic analysis*

To calculate butterfly kinematics for each sequence, perspective-adjusted x,y coordinates for the digitized points were run through a Pascal program written by the author.

Mean flight speed was calculated as the mean displacement in the position of the wing base between two frames (wing lengths per frame), multiplied by 60 (frames s^{-1}), and multiplied by the wing length.

WBF (in Hz) was estimated by examining sequential frames to determine whether the y-coordinate of the near wing-tip crossed that of the wing base. Half of the total number of sweeps across the wing base per sequence was divided by the number of video frames minus one, and then multiplied by 60 frames s^{-1}. For two out of 24 sequences, WBF was adjusted because the wing approached but did not cross the wing base. Error in WBF was equal to WBF divided by the number of frames sampled. Errors were typically ± 0.25 cycles (range: ± 0.15 to ± 0.4 cycles).

The near wing-tip was projected from the wing base to yield three-dimensional (x,y,z) coordinates following the methods of Ellington (1984*b*). For each frame relative to a horizontal plane that bisected the wing base (see figure 2), I estimated the wing elevation (ϕ) as the projection of the wing above or below the wing base along the stroke plane angle. Wing elevation was positive when dorsal to the wing base. The body angle (χ) was estimated as the projection of the line segment connecting the head and the tip of the abdomen. Body angle was positive when above horizontal. For each sequence, mean values of ϕ and χ were calculated as the sum of their respective values divided by the number of frames (mean ϕ differed from that of Ellington (1984*a*) for which mean ϕ is the midpoint between the dorsal and ventral ends of the wing stroke cycle). BPF (in Hz) was estimated as half the number of pitches of χ across mean χ divided by the number of frames per sequence minus one and multiplied by 60 frames s^{-1} (errors were typically the same as that for WBF). I added to this two pitches of χ when an oscillation in the body angle that did not cross mean χ was clearly evident (10 out of 24 sequences). Stroke amplitude (in radians) was estimated as the maximum ϕ in the sequence less the minimum ϕ, and SA as wing length^2 × stroke amplitude.

(d) *Statistical analyses and pooling of sexes*

When the observations met the standard assumptions of parametric analyses, two-way ANOVAs, two-way MANOVAs (multivariate analysis of variance) or two-way ANCOVAs (analysis of covariance) were applied to partition the variance in the dependent variable(s) into: (i) if applicable, that due to the

Table 1. *Morphology of* Heliconius *co-mimics with kinematic data: (sample size) mean ± s.d.*

	H. cydno	*H. sapho*	*H. melpomene*	*H. erato*
body mass (mg)	(5) 160 ± 30	(6) 130 ± 40	(6) 120 ± 20	(7) 100 ± 20
wing length (mm)	(5) 41 ± 1	(6) 35 ± 3	(6) 35 ± 2	(7) 34 ± 2
$\mathrm{cm}_{\mathrm{wing}}{}^{*}$	(5) 0.33 ± 0.03	(6) 0.34 ± 0.01	(2) 0.37 ± 0.02	(7) 0.35 ± 0.02
$\mathrm{I}_{\mathrm{wing}}$ (mg mm^2)	(5) 5.53 ± 0.85	(6) 3.34 ± 1.22	(2) 3.18 ± 1.20	(7) 2.35 ± 0.97
wing centroid*	(5) 0.48 ± 0.01	(6) 0.47 ± 0.01	(6) 0.44 ± 0.01	(6) 0.44 ± 0.02
$\mathrm{cm}_{\mathrm{body}}{}^{*}$ males	(3) 0.21 ± 0.03	(5) 0.23 ± 0.04	(4) 0.20 ± 0.05	(2) 0.16 ± 0.01
$\mathrm{cm}_{\mathrm{body}}{}^{*}$ females	(1) 0.25	(1) 0.26	(2) 0.22 ± 0	(1) 0.21
$\mathrm{I}_{\mathrm{body}}$ (g mm^2)	(4) 16 ± 4.5	(6) 8.7 ± 5.1	(6) 8.0 ± 3.1	(3) 6.3 ± 0.4
ρ (mg cm^{-2})	(5) 9.2 ± 2.7	(6) 8.6 ± 1.7	(6) 8.5 ± 0.7	(7) 7.5 ± 1.0

* Non-dimensionalized.

covariate; (ii) that due to membership in a mimicry group; (iii) that due to membership in a phylogenetic lineage; (iv) that due to interaction between mimicry and phylogeny; and (v) error. As a result, the effects of mimicry and historical differences among the lineages were analysed simultaneously.

This analysis contrasts quantitative and continuous, independent features of close relatives that are members of discrete ecological classes. It is quantitatively identical to the analysis of ecological similarity developed by Schluter (1986). For this study, I selected classes based on colour mimicry. This contrast analysis is advantageous over those of Felsenstein (1985) or Purvis & Rambaut (1995) because it not only adjusts the feature of interest for variance resulting from historical descent, but it explicitly measures that variance so that historical constraints are tested statistically. Furthermore, it is advantageous because the two-way ANOVAs, MANOVAs and ANCOVAs are part of standard statistical packages. However, the disadvantage is that the analysis is restricted to questions that arise from two pairs of closely related species within which the species potentially differ as a result of an independent, dichotomous factor. Hence, the hypotheses can be analysed with statistical analyses of a two-by-two species matrix, with phylogenetic lineage comprising one dimension and a biological factor the other.

Intraspecific variation in the morphological features identified in Srygley (1994: wing centroid, $\mathrm{cm}_{\mathrm{wing}}$, $\mathrm{cm}_{\mathrm{body}}$, $\mathrm{I}_{\mathrm{body}}$) were analysed among individuals for which flight sequences had met the criteria outlined and additional individuals for which flight sequences either were not videotaped or excluded. Within each species, males and females were not significantly different in the following morphological traits: $\mathrm{I}_{\mathrm{body}}$, $r_1(S)$, and $\mathrm{cm}_{\mathrm{wing}}$ ($\alpha = 0.05$), whereas males and females differed significantly in $\mathrm{cm}_{\mathrm{body}}$ for *H. cydno* ($p = 0.010$) and *H. melpomene* ($p = 0.015$). Morphological data for males and females were pooled except for $\mathrm{cm}_{\mathrm{body}}$, for which sexes were analysed separately. All variables met the assumptions for parametric statistics (Bartlett's test for homoscedasticity, $\alpha = 0.05$, Systat for Windows v. 5; Shapiro–Wilk test for normality, $\alpha = 0.05$, JMP v. 3.0), except $\mathrm{I}_{\mathrm{body}}$. The effects of mimicry group and phylogenetic lineage on wing centroid and $\mathrm{cm}_{\mathrm{wing}}$ were modelled with MANOVA. The effects of mimicry and phylogeny on $\mathrm{I}_{\mathrm{body}}$ were analysed with Kruskal–Wallis one-way ANOVAs (Systat v. 5), and for each sex, their effects on $\mathrm{cm}_{\mathrm{body}}$ were analysed with a two-way ANOVA (Systat v. 5).

From the one-to-four sequences that were digitized for each individual, a single sequence was selected randomly to meet the assumption of independent observations for statistical analyses. The selection of sequences reduced the kinematic data set to: *Heliconius cydno*: one female, four males; *H. erato*: two females, four males; *H. melpomene*: two females, four males; *H. sapho*: one female, five males. WBF and mean elevation of the wings (ϕ) were dependent variables and mimicry group and phylogenetic lineage were independent variables modelled with MANOVA (Systat). Mean body angle did not meet the test for normality, and so the effects of mimicry and phylogeny on mean body angle were analysed with Kruskal–Wallis one-way ANOVAs.

Because sampling may have caused the subset of individuals to be different from the larger morphological data set, results of a second MANOVA that analysed the effects of mimicry group and phylogenetic lineage on wing centroid $[r_1(S)]$ and the centre of wing mass ($\mathrm{cm}_{\mathrm{wing}}$) were compared to those of a larger data set which included individuals that lacked kinematic data. The corresponding morphological data set $[r_1(S)$, and $\mathrm{cm}_{\mathrm{wing}}]$ for which kinematic data existed included: *Heliconius cydno*: one female, four males; *H. melpomene*: zero females, two males; *H. sapho*: one female, five males; *H. erato*: two female, four males. Males and females were pooled for all of the morphological features except $\mathrm{cm}_{\mathrm{body}}$, for which sexes differed in the larger data set. For $\mathrm{cm}_{\mathrm{body}}$, only males were analysed with a two-way ANOVA (sample sizes in table 1). The effects of mimicry and phylogeny on $\mathrm{I}_{\mathrm{body}}$ were analysed with Kruskal–Wallis one-way ANOVAs.

To assess the effects of mimicry and phylogeny on four gross predictors of flight kinematics, SA (a composite morphological and kinematic feature), ρ, wing length and body mass were the dependent variables for a MANOVA. Wing loading was log transformed ($\log \rho$) to meet assumptions for MANOVA. This data set was composed of the same individuals as that for the kinematics listed above.

Using regression analyses (Systat v. 5), I tested for morphological determinants of WBF and morphological and kinematic determinants of ϕ and BPF.

4. RESULTS

(a) *Flight-related morphology*

Except for $\mathrm{I}_{\mathrm{body}}$, the proportion of variance in a species' morphological features that was explained by its membership in a mimicry group greatly outweighed the proportion of variance that was explained by its lineage of origin. For both the larger data set (table 2*a*) and its subset (table 2*b*), variance in $r_1(S)$ and $\mathrm{cm}_{\mathrm{wing}}$ were significantly greater among mimicry groups than within (see multivariate test statistics). However, mimicry explained a much smaller proportion of the variance in $r_1(S)$ in the

Table 2. *Statistical summary for two-way MANOVAs for the effects of mimicry group and phylogenetic lineage on wing centroid and the centre of wing mass for all individuals*

	multivariate test statistics			source of variation in wing centroid			source of variation in centre of wing mass		
	Wilks's lambda	d.f.	p	% explained	F	p	% explained	F	p
(*a*) all individuals (for univariate test, d.f. = 1,28)									
mimicry group	0.542	2,42	0.00001	23.67	30.077	0.001	32.47	21.486	0.00003
phylogenetic lineage	0.996	2,42	0.916	0.05	2.191	0.150	2.47	0.161	0.690
interaction	0.961	2,42	0.430	3.29	12.590	0.001	2.30	1.519	0.224
(*b*) subset of individuals for which kinematic data measured (for univariate tests, d.f. = 1,15)									
mimicry group	0.334	2,14	0.0005	62.36	25.607	0.0001	30.64	4.976	0.019
phylogenetic lineage	0.970	2,14	0.806	0.92	0.376	0.549	0.60	0.140	0.714
interaction	0.955	2,14	0.724	0.20	0.099	0.757	2.89	0.659	0.430

Table 3. *Statistical summary for a two-way MANOVA for the effects of mimicry group and phylogenetic lineage on WBF and BPF*

	multivariate test statistics			source of variation in WBF			source of variation in BPF		
	Wilks's lambda	d.f.	p	% explained	F (d.f. = 1,20)	p	% explained	F (d.f. = 1,20)	p
mimicry group	0.725	2,19	0.047	22.5	6.16	0.022	2.5	0.54	0.470
phylogenetic lineage	0.852	2,19	0.219	1.3	0.36	0.556	4.0	0.87	0.362
interaction	0.933	2,19	0.515	2.9	0.80	0.382	0.0	0.00	0.987

larger data set than in its subset. Similarities among the data sets in the overwhelming effects of mimicry relative to phylogeny on these traits pertaining to wing kinematics suggest that the wing kinematic data were not an anomaly due to sampling.

Within the larger data set, mimicry explained a significant proportion of the variance in cm_{body} for both males and females (10.5%, $p = 0.041$, and 24.2%, $p = 0.020$, respectively), whereas phylogenetic lineage did not have a significant effect (1.9%, $p = 0.378$, and 12.6%, $p = 0.082$, respectively). Females were too few to analyse for the subset of individuals. For males, mimicry did not explain a significant proportion of the variance in cm_{body} (19.8%, $p = 0.115$), and phylogenetic lineage explained less than 2% of the variance.

For the larger data set, I_{body} did not differ among mimicry groups (Mann–Whitney $U = 547$, $p = 0.117$), and differed significantly among lineages ($U = 652$, $p = 0.001$). For the subset, I_{body} tended to differ among mimicry groups (Mann–Whitney $U = 67$, $p = 0.07$), and did not differ among lineages ($U = 62$, $p = 0.16$). Hence, a strong effect of lineage on I_{body} was evident in the larger data set, but not the data subset.

(b) *Flight kinematics*

In general, the proportion of variance in a species' flight kinematics that was explained by its membership in a mimicry group greatly outweighed the proportion of variance that was explained by its lineage of origin (table 3). Variance in WBF and BPF were significantly greater among mimicry groups than within (see multivariate test statistics). WBF was significantly affected by mimicry, whereas BPF was not affected (see univariate tests).

WBF was a significant determinant for BPF during a flight sequence (figure 3). In general, the downstroke forces the wing base up relative to the cm_{body}, causing χ to decrease; and the upstroke forces the wing base down relative to cm_{body}, causing χ to increase. During hovering, three pitching moments resulting from the wing's motion might influence the body's pendular motion: (i) that resulting from the lift force acting on the flapping wings; (ii) that resulting from the drag force acting on the moment arm between wing base and cm_{wing}; and (iii) that resulting from the inertial wing forces and virtual mass of the wing acting on the same moment arm (Ellington 1984*b*). Forward flight results in induced drag acting on the wings and that acting on the body which further influence the body's pitching.

To determine whether the phase relationship between the oscillating wings and body might serve as an additional signal to the predators, I investigated the lag between the peak in the wing-beat cycle (ϕ_{max}) and that in the body-pitching cycle (χ_{max}, note this is opposite to

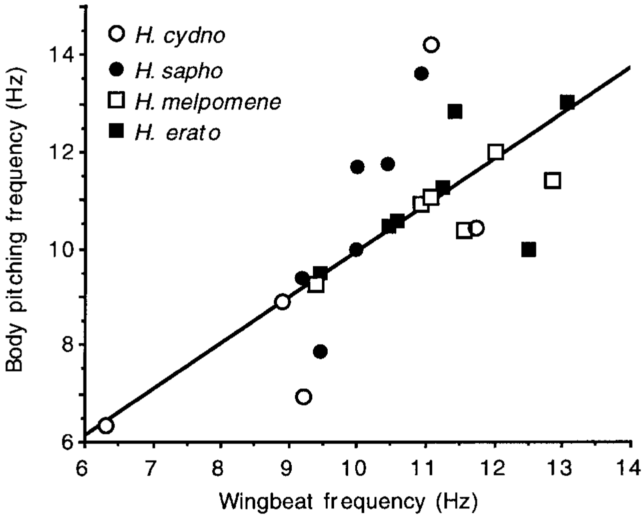

Figure 3. Correlation of wing oscillations with body oscillations among the four *Heliconius* species. Confidence intervals for the slope did not differ significantly from one, indicating a close correspondence between their oscillating frequencies. A few points were shifted slightly to avoid overlap. $y = 0.95x + 0.44$; $r = 0.727$; $p = 0.0001$.

$\chi_{\max}$ of Ellington (1984*b*) where χ is positive below horizontal). In addition I investigated the relative lag in $\phi_{\min}$ and $\chi_{\min}$. Errors in lag increased linearly with oscillation frequency due to videoframe sampling at 60 Hz (s.e. = 0.017 frequency). Errors were typically 0.16 of a wing stroke cycle for *H. cydno*–*H. sapho* and 0.19 of a wing stroke cycle for *H. erato*–*H. melpomene*. Among individuals, lag in $\phi_{\max}$–$\chi_{\max}$ was not associated with WBF ($r = -0.33$, $p = 0.113$), whereas lag in $\phi_{\min}$–$\chi_{\min}$ was significantly associated with WBF ($r = -0.49$, $p = 0.014$). As a result at low WBFs, the oscillation of the body and wings are approximately one-quarter cycle out of phase. At higher WBFs, the ventral end of the wing stroke and pitching of the body remained one-quarter cycle out of phase, whereas the dorsal ends of the wing stroke and pitching of the body were one-half cycle out of phase. This asymmetry is most likely due to non-sinusoidal motion of the wings (also evident from the analysis of the mean elevation of the wings presented below). Unfortunately, videoframe analysis gave insufficient resolution to determine which of the above forces predominated in determining this trend.

I asked whether this phase relationship might vary among mimicry groups and lineages. The relative lag between the top of the upstroke of the wing ($\phi_{\max}$) and pitching forward of the body ($\chi_{\max}$) was not significantly different among mimicry groups nor phylogenetic lineages (two-way ANOVA, d.f. = 1,20: variance explained by mimicry group = 0.18%, $p = 0.92$; by phylogenetic lineage = 0.03%, $p = 0.92$; by interaction = 7.39%, $p = 0.17$). The relative lag between $\phi_{\min}$ and $\chi_{\min}$ tended to differ among mimicry groups and was less affected by phylogeny (two-way ANOVA, d.f. = 1,20: variance explained by mimicry group = 12.40%, $p = 0.084$; by phylogenetic lineage = 8.26%, $p = 0.132$; by interaction = 0.83%, $p = 0.62$).

The mean elevation of the wings (mean ϕ) was strongly correlated with the proportion of time during the sequence that the butterflies held their wings aloft, arbitrarily assigned as greater than one radian (i.e. > 57°; $n = 24$, $r = 0.90$, $p = 0.001$). In the field, many lepidopterists use the pattern of pause and stroke during the wingbeat as a characteristic to distinguish among taxonomic groups (e.g. the characteristic pause at the top of the upstroke for satyrids). For this reason, I tested whether mean ϕ converged within mimicry groups. Mean ϕ tended to be affected to a similar degree by phylogenetic descent and mimicry (two-way ANOVA, d.f. = 1,20: by mimicry group = 11.0%, $F = 2.89$, $p = 0.105$; variance explained by phylogenetic lineage = 12.6%, $F = 3.31$, $p = 0.084$; by interaction = 0.5%, $F = 0.15$, $p = 0.707$).

The angular position of the body relative to horizontal (χ) may also convey a characteristic profile during flight. The long, heavy abdomens of many distasteful species hang well below their hind wings during flight (for an example, see profiles of ithomiines in fig. 1 of Chai & Srygley (1990)). However, mean χ was not significantly different among mimicry groups (Mann–Whitney $U = 75.5$, $p = 0.817$) nor among phylogenetic lineages ($U = 46.0$, $p = 0.139$).

Velocity tended to be greater in the cydno-sapho mimicry group (mean velocities: *H. cydno*: 886 mm s^{-1}; *H. sapho*: 1017 mm s^{-1}) than the melpomene-erato group (*H. melpomene*: 773 mm s^{-1}; *H. erato*: 781 mm s^{-1}). Mimicry explained 14.8% of the variance in velocity ($p = 0.071$), whereas phylogenetic lineage explained 2.2% of the variance ($p = 0.539$). Among all individuals ($n = 24$), velocity was not correlated with WBF ($r = -0.34$, $p = 0.108$). However, mean ϕ and mean χ were both positively associated with velocity ($r = 0.42$, $p = 0.038$; $r = 0.47$, $p = 0.022$, respectively).

Because wing length and SA are theoretically relevant to induced power, or that power required to accelerate air downwards in support of body weight (during hovering), one might expect that wing length and SA would be proportional to the body mass that must be lifted. Mimicry and phylogeny both explained significant proportions of the variance in SA, wing loading, wing length and body mass (table 4, see multivariate test statistics). In univariate tests, wing loading was the only variable that was not significantly associated with mimicry or phylogeny. SA and wing length were more strongly influenced by phylogeny than mimicry, whereas body mass was much more strongly influenced by mimicry than phylogeny. Although, the interaction term was not significant overall, both SA and wing length were significantly affected by an interaction between mimicry and phylogeny in univariate tests.

(c) *Functional relationships*

Wing centroid [$r_1(S)$], cm$_{\text{wing}}$, and cm$_{\text{body}}$ were found to converge within mimicry groups in this analysis of the *Heliconius* butterflies. Srygley (1994) predicted that convergences in cm$_{\text{body}}$ and cm$_{\text{wing}}$ within mimicry groups might result in convergences in WBF and BPF, respectively. Among the species means, cm$_{\text{wing}}$ explained 92.3% of the variance in WBF ($n = 4$ species, $r^2 = 0.92$, $p = 0.039$). However, $r_1(S)$ explained slightly greater proportion of the variance ($n = 4$, $r^2 = 0.98$, $p = 0.009$). When regressed separately, wing length ($r^2 = 0.65$,

Table 4. *Statistical summary for a two-way* MANOVA *for the effects of mimicry group and phylogenetic lineage on sweep area, log wing loading, wing length and body mass for that subset of individuals for which kinematic data were measured*

multivariate test statistics	Wilks's lambda	d.f.	p
mimicry group	0.529	4,17	0.022
phylogenetic lineage	0.453	4,17	0.007
interaction	0.676	4,17	0.135
univariate F tests:			
source of variation in sweep area	% explained	F (d.f. = 1,20)	p
mimicry group	12.2	5.978	0.024
phylogenetic lineage	37.6	18.407	0.0004
interaction	9.4	4.611	0.044
source of variation in log wing loading	% explained	F (d.f. = 1,20)	p
mimicry group	7.3	1.70	0.208
phylogenetic lineage	6.3	1.49	0.237
interaction	1.3	0.29	0.599
source of variation in wing length	% explained	F (d.f. = 1,20)	p
mimicry group	25.3	14.23	0.001
phylogenetic lineage	27.0	15.19	0.0009
interaction	12.1	6.80	0.017
source of variation in body mass	% explained	F (d.f. = 1,20)	p
mimicry group	32.9	12.05	0.002
phylogenetic lineage	11.3	4.14	0.055
interaction	1.1	0.40	0.534

$p = 0.20$), SA ($r^2 = 0.43$, $p = 0.35$), and log ρ ($r^2 = 0.24$, $p = 0.51$) explained less of the variance than $\mathrm{cm_{wing}}$ and were not significant determinants of WBF.

Because phylogeny was responsible for insignificant fractions of the variance in $\mathrm{cm_{wing}}$, $r_1(S)$ and WBF (less than 3%), I pooled data for individuals from the four species and regressed WBF on $\mathrm{cm_{wing}}$ and $r_1(S)$. The position of centre of wing mass explained a significant proportion of the variance in WBF (figure 4*a*), and there was a trend for $r_1(S)$ to explain a significant proportion of the variance in WBF when regressed separately ($n = 22$, $r^2 = 0.15$, $p = 0.077$). Contrary to the predicted result (Srygley 1994), WBF increased as $\mathrm{cm_{wing}}$ was positioned further from the wing base, whereas WBF decreased as $r_1(S)$ was positioned further from the wing base.

Wing length was a significant determinant of the radial moment of wing inertia ($\mathrm{I_{wing}}$ in $\mathrm{mg\,mm^2}$) both among species means ($\mathrm{I_{wing}} = -11.9 + 0.43$ wing length, $r = 0.99$, $p = 0.014$) and among all individuals ($\mathrm{I_{wing}} = -11.2 + 0.41$ wing length, $r = 0.92$, $p = 0.0001$). Similar to wing length, $\mathrm{I_{wing}}$ had a mimetic and phylogenetic component (two-way ANOVA, d.f. $= 1{,}16$: by mimicry group $= 28.2\%$, $F = 10.23$, $p = 0.006$; variance explained by phylogenetic lineage $= 23.0\%$, $F = 8.35$, $p = 0.011$; by interaction $= 4.6\%$, $F = 1.69$, $p = 0.212$). Among all individuals, WBF was proportional to the inverse of $\mathrm{I_{wing}}$ (figure 4*b*), and WBF declined with wing length.

The role of wing length in determining $\mathrm{I_{wing}}$ and WBF suggested an alternative mechanism by which predation might result in convergence in WBF: selection for similarity in body size might lead to the convergence in WBF rather than selection for similarity in WBF, *per se*. To investigate whether WBF converged independently of body size, the independent variable WBF was covaried with wing length and body mass while analysing for the effects of mimicry and phylogeny (table 5). In a two-way ANCOVA, neither wing length nor body mass explained significant fractions of the variance. However, mimicry group was no longer a significant factor with inclusion of the covariates. A model with only wing length as a covariate yielded a similar result, suggesting that changes in wing length among mimicry groups may be sufficient to explain changes in WBF.

The best model for WBF was a two-way ANCOVA with log ρ as a covariate. In this model, log ρ as a composite size variable (mass per unit wing area), explained some of the variance ascribed to error in the ANOVA (table 3). As a result, mimicry remained a highly significant factor in this model.

To test whether the convergence in WBF within mimicry groups might be due to selection by predators on wing shape, I added $r_1(S)$ as a covariate to the best model (log ρ). Although this model explained little more of the variance than inclusion of log ρ alone, mimicry group was no longer a significant factor, suggesting that changes in wing shape among mimicry groups may, in part, explain differences in WBF. Unfortunately, $r_1(S)$ was not available for one individual, such that the lack of association between mimicry and WBF may also be due to loss of one degree of freedom.

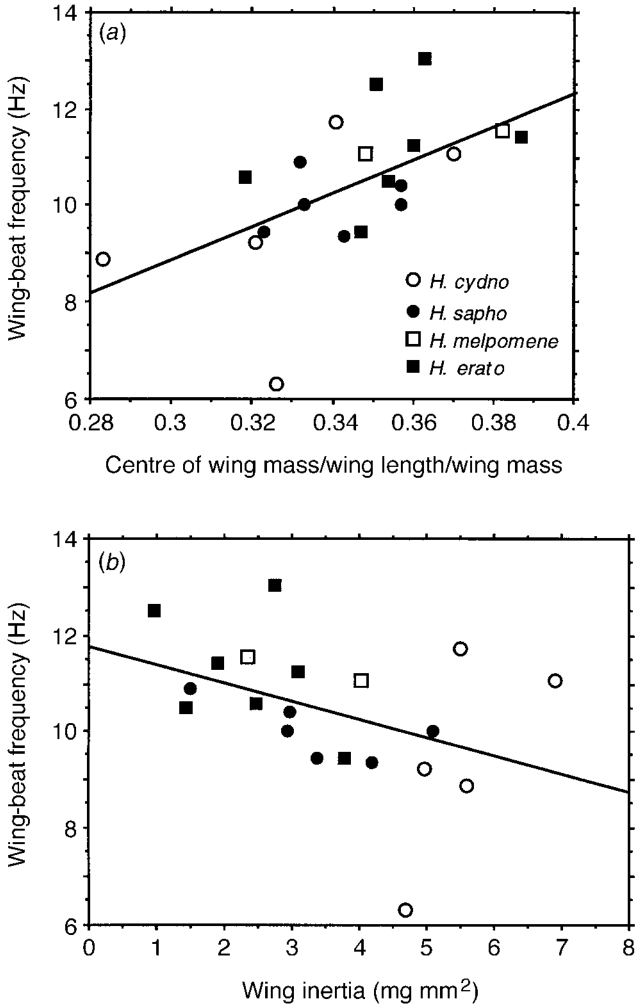

Figure 4. (*a*) Regression of WBF on the position of centre of wing mass (non-dimensionalized) for all individuals. Among the mean values for the species, WBF was also positively and linearly related to the position of the centre of wing mass. $y = 34.4x - 1.4$; $r = 0.57$; $p = 0.009$. (*b*) Regression of WBF on the wings' rotational inertia for all individuals. $y = 11.76 - 0.37x$; $r = -0.4$; $p = 0.076$.

5. DISCUSSION

(a) *The evolution of morphology and flight with respect to mimetic convergence*

In addition to the classic morphological features associated with mimicry (e.g. wing pattern and coloration), convergence in the position of the centre of wing mass, wing centroid and the position of centre of body mass distinguished the *Heliconius* Müllerian mimicry pairs. Moreover, WBF converged within the Heliconius co-mimics. This serves as the first direct evidence that aerial predators that choose to attack or ignore butterflies in flight, such as rufous-tailed jacamars (Chai 1986, 1990; Chai & Srygley 1990), may result in convergence in kinematic characters such as WBF. This also serves as the first direct evidence that behavioural mimicry has occurred in a mutualistic or Müllerian, rather than a parasitic or Batesian, context. Convergence in WBF may be the direct result of predators using WBF as a cue to distinguish among Müllerian mimicry groups. Alternatively, WBF may converge because of selection by predators on similarities in wing length or wing shape within mimicry groups.

Body size features, such as body mass and wing length, differed depending on the species' phylogenetic history. The sylvaniforms, *Heliconius cydno* and *H. melpomene*, were more massive and had longer wings than their pupal-mating co-mimics. An analysis of a larger data set (that used for table 1; $n = 77$) confirmed this influence of phylogeny on body mass and wing length. Moreover, wing loading (body mass per wing area) was strongly associated with phylogeny but not with mimicry in this larger data set. Because wing loading was relatively conserved among phylogenetic lineages, convergent selection on wing length was apparently independent from that on other body size attributes, such as body mass and wing area. Convergent selection has worked on these phylogenetic backgrounds to result in more similar body masses and wing lengths among co-mimics.

For flying organisms in general, WBF decreases with body mass and increases with wing loading (Norberg 1990). However, body mass did not explain any of the variance in WBF among *Heliconius* (table 5). In contrast, the association of WBF with mimicry was stronger when WBF was covaried with wing loading. This model suggests that wing loading explained variance in WBF above and beyond that due to the effects of mimicry. Hence, WBF converged among co-mimics despite differences in wing loading that were attributable to historical differences. This serves as strong support for the hypothesis that WBF converged as a result of predation.

Srygley (1994) predicted that evolutionary convergence in $\mathrm{cm_{wing}}$ within mimicry groups might arise as a result of convergent selection on WBF. The association of $\mathrm{cm_{wing}}$ and $r_1(S)$ with WBF is strong evidence for a functional relationship (figure 3). In agreement with the theoretical and empirical constructs of Sotavalta (1952) and Greenewalt (1960), WBF was inversely proportional to the wing's rotational inertia, and the wing's rotational inertia was directly proportional to wing length.

The position of the centre of wing mass, wing centroid and centre of body mass diverged significantly within the *Heliconius* lineages. Wing length and the wing's rotational inertia were poorer predictors of WBF than $r_1(S)$ and $\mathrm{cm_{wing}}$. Hence, the convergence of $r_1(S)$ and $\mathrm{cm_{wing}}$ within mimicry groups is a useful predictor for the convergence of WBF (Ellington 1984*b*; Srygley 1994).

This study emphasizes that a suite of characters relating directly to inertial, lift and drag forces generated by the wings, including wing length, body mass, $r_1(S)$ and $\mathrm{cm_{wing}}$, have converged within mimicry groups. An examination of kinematics in mimicry groups that include species of widely different wing lengths (e.g. *Eresia mechanitis* and *Mechanitis polymnia* of the tiger mimicry group), and an examination of kinematics in groups that include species of different wing shapes (e.g. *Consul fabius* and *Lycorea cleobaea* in the tiger mimicry group) would be useful for separating the size and shape features that influence WBF. Furthermore, experimental manipulation of the position of centre of wing mass (e.g. Sotavalta 1952) and wing shape are essential to understanding their direct determination of WBF.

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Table 5. Analyses of covariance for the dependent variable WBF

	% variance explained				
covariates entered	covariates	mimicry group		phylogeny	total*
none		27.6	$p = 0.011$	0.9	28.9
body mass	0.0	18.6	0.049	0.7	29.0
wing length	3.0	11.4	0.128	0.1	31.4
wing length, body mass	4.9	12.1	0.122	0.1	32.2
log wing loading	6.2	31.1	0.005	2.3	35.6
log wing loading, $r_1(S)$	6.8	18.2	0.052**	3.4	36.5

*Interaction of mimicry × phylogeny was never significant.
**One individual excluded due to missing datum.

Among these closely related lineages, the lack of association of $\mathrm{cm_{body}}$, $r_1(S)$ and $\mathrm{cm_{wing}}$ with phylogeny supports Srygley's (1994) assumption that these locomotory features have converged within *Heliconius* mimicry groups and are phylogenetically independent. Moreover, this result is contrary to Brower's (1995) criticism that four mimicry groups within *Heliconius* (*H. cydno*–*H. sapho*, *H. melpomene*–*H. erato*, *H. pachinus*–*H. hewitsoni* and *H. sara*–*H. doris*) constituted a single observation for statistical analyses because the uniformity of these taxa in morphological characters other than wing coloration was due to common ancestry. Srygley (1994) argued that $\mathrm{cm_{body}}$, wing shape and $\mathrm{cm_{wing}}$ of the co-mimicry pairs (based on wing coloration) were independent because these features diverged among members of the pupal-mating lineage (*H. sapho*, *H. erato*, *H. hewitsoni* and *H. sara*) and converged with that of each species' non-pupal-mating co-mimic. Similarly, these features also diverged among members of the sylvaniform lineage (*H. cydno*, *H. melpomene* and *H. pachinus*) and converged on the features of their co-mimics. Here, I only included two of these species pairs. The third pair *H. pachinus*–*H. hewitsoni* was excluded because kinematic data were not collected for *H. pachinus*. The fourth pair was excluded because Srygley (1994) recognized *H. doris* as a member of the sister genus *Laparus* (*sensu* Brown 1981). Concerning this fourth pair, Brower's criticism (1995) was founded on his more recent analysis of mitochondrial sequence data (Brower 1994). His data weakly supported a regrouping of *Laparus* and *Eueides* within *Heliconius* (CI = 0.32). However, Penz (1999) separates *Heliconius* (bootstrapped chance of monophyly = 84) from a clade containing *Laparus* and *Eueides* (bootstrapped value = 90) in a more recent analysis of morphological traits. Problems with phylogeny aside, the analysis of *H. cydno*–*H. sapho* and *H. melpomene*–*H. erato* indicated that flight-related morphology and kinematics were subject to convergent selection due to locomotor mimicry and were not strongly influenced by historical descent.

Srygley (1994) also predicted that evolutionary convergence in $\mathrm{cm_{body}}$ within mimicry groups might arise as a result of convergent selection on BPF. Mimicry significantly affected $\mathrm{cm_{body}}$ and phylogeny had a significant effect on $\mathrm{I_{body}}$. However, the rotation of the body about the centre of body mass was due primarily to the drag force acting on the moment arm between wing base and $\mathrm{cm_{wing}}$; and secondarily to the lift force acting on the flapping wings. Consequently, the BPF was tightly coupled to the WBF. Variance in the BPF that was not due to WBF resulted in a lack of association with mimicry.

(b) *Evidence that predators use behaviours as discriminatory signals*

No direct evidence that birds are capable of distinguishing differences in WBF among prey exists. In birds, neural analysis of motion (reviewed in Frost *et al.* 1994), retention of motion patterns and discrimination from other motion patterns involves much more than perception of flickering light, yet flicker-fusion rates probably serve as an upper bound on visual processes. Measures of the flicker-fusion rates of avian eyes suggest that resolving differences of 1 Hz when the WBF is near to 10 Hz may be physically possible. The flicker-fusion rate was experimentally measured for pigeon eyes with electroretinograms at 74–145 Hz and estimated with behavioural tests for chickens at 100 Hz (reviewed in Emerton 1983). The frame-to-frame analyses of video sequences performed here at 60 Hz was within the lower bound of the temporal resolution for avian eyes. Even at this lower frequency, detection of the periodicity of, for example, maximum positions of the wings would be feasible.

Wing length and wing shape are also probably detectable by a prudent predator, including aerial insectivores hawking butterflies in flight and gleaners selecting butterflies at rest. Either of these features explained at least some of the variation in WBF among mimicry groups, and both features theoretically play a role in determining the generation of lift, thrust and drag by the flapping wings.

However, WBF is not likely to be a consequence of only wing shape and wing length. Neural stimulation of the flight muscle is synchronous with elevation and depression of the wings in butterflies (Kammer & Rheuben 1981). Hence, WBF is a behaviour that is subject to modification by the individual and it is not solely affected by the resonant properties of flight tissues and appendages. For this reason, the convergence in WBF within mimicry groups suggests that predators use WBF as a cue in distinguishing prey items.

Behavioural mimicry has been investigated in detail for two cases that involve deception of the signal recipient for offensive purposes (aggressive mimicry, Wickler (1968)). Sabre-toothed blennies mimic the colour, shape and locomotor behaviour of the cleaner wrasse in order to

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deceive potential prey that expect to be cleaned (Wickler 1968; Springer & Smith-Vaniz 1972), and the young of the nest-parasitic African widowbird mimic the begging behaviour of their waxbill-host nestlings (Nickolai 1964, 1974; Wickler 1968, p. 197). In both these cases, advergent evolution (i.e. the evolution of the mimic toward the model and the model away from the mimic, Brower & Brower (1972)) was evident.

In Müllerian mimicry, the association of the signals is beneficial for both prey species and predators (Müller 1879). As a result, Müllerian mimicry may involve signals that reinforce the mimetic association. For example, recent studies suggest that Müllerian mimics are partitioned among spatial niches (Papageorgis 1975; Srygley & Chai 1990; Mallet 1993) that may improve the resemblance between species in Müllerian mimicry systems and more reliably signal their aposematism to potential predators.

Natural selection may favour convergence in WBF among Müllerian mimics because it lends complexity to the mimetic signal and makes it more difficult for Batesian mimics to parasitize. In contrast to Batesian and aggressive mimics, Müllerian mimics should converge on common signals. The fact that they have often diverged (presumably under different selective regimes in allopatry) and do not subsequently converge when sympatric remains a perplexing biological conundrum (Mallet 1993).

Qualitative differences in flight behaviours suggest that mimicry is likely to be context dependent. For this paper, we constrained the flight sequences to 'cruising flight', whereas a single butterfly may display a diversity of flight styles in nature. For example, during the late afternoon when they aggegate at roost sites, *Heliconius* have a fluttering flight that is 'display-like' in quality and observed when they approach the dormitory and when they are disturbed. Flight behaviours among mimetic species at the roost sites appear remarkably similar (personal observation), although divergence within lineages has not been documented. If true, natural selection may have resulted in mimetic similarities in a number of flight contexts. For another example, the forward airspeed of *H. erato* in natural free flight over Lake Gatún (Srygley & Dudley 1993) was 2–3 m s^{-1}, whereas those *H. erato* released over Lake Gatún flew more slowly 1.6–1.8 m s^{-1}, and those in the flight tunnel flew more slowly still (e.g. the average 2D flight speed was 0.8 m s^{-1}). Qualitative differences in velocity suggest that kinematics may also vary with methodology, but this aspect of the kinematics remains unexplored.

Variance in WBF both within and among individuals is indicative of the contributions of environmental and motivational differences, as well. For example, WBF for one *Heliconius cydno* male varied from 6–8 Hz between three flight sequences. Rather than constraining WBF, the position of cm$_{\text{wing}}$ may affect the resonant frequency of the tissues (Sotavalta 1952) and the energetic efficiency that is achieved during flight.

Locomotor mimicry provides an opportunity to investigate adaptation of form with relation to the slow-flapping flight characteristic of butterflies in a historical setting. Members of distinct lineages within *Heliconius* demonstrated convergence in flight-related morphological features and WBF within mimicry groups. Behavioural similarity among co-mimics may, in part, be due to convergences in wing lengths and moments of wing inertia from historically dissimilar body sizes. Hence, the evolutionary convergence of WBFs might be achieved through convergence in body size. Similarities in wing shape and the position of centre of wing mass suggest that lift, drag and inertial forces operating on the wings should also converge within mimicry groups. The convergence in aerodynamic power with these morphological and kinematic features is under investigation.

The co-evolution of the sylvaniform and pupal-mating *Heliconius* has resulted in a diversity of colour patterns, and potentially a diversity of flight behaviours, across a wide geographic range (Eltringham 1916; Turner 1981; Gilbert 1983; Sheppard *et al.* 1985). Additional investigations of morphological and kinematic diversity in these co-evolving lineages will further our understanding of the role of flight behaviour in signalling to predators.

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