Estimating the relative fitness of local adaptive peaks: the aerodynamic costs of flight in mimetic passion-vine butterflies *Heliconius*

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In a related paper, we demonstrated that mimetic *Heliconius* butterflies have converged in wing-beat frequency and degree of asymmetry in the wing motion, whereas sister species are dissimilar in these same traits. Warning signals of sympatric, distasteful species converge in evolutionary models in order to educate their predators more efficiently that the signal is associated with unprofitable prey. Barring other constraints, the behaviours of the different co-mimetic pairs should ultimately converge on that behaviour which minimizes the energetic cost of flight. We estimated the energetic cost of each mimic's flight behaviour in order to predict the difference in height of each fitness peak and the direction of convergent selection qualitatively. Following adjustments for body mass, mimetic *Heliconius melpomene* and *Heliconius erato* required more aerodynamic power than *Heliconius cydno* and *Heliconius sapho*. This difference was attributed to the slower flight speeds and higher wing-beat frequencies of *H. melpomene* and *H. erato*. Consequently, *H. melpomene* and *H. erato* expended more energy per unit distance per unit body mass than *H. cydno* and *H. sapho*. However, differences in body mass may equalize energy budgets and stabilize the sympatric coexistence of the two pairs of co-mimics.

Keywords: locomotor mimicry; behaviour; biomechanics; coexistence; mutualism; energetics

1. INTRODUCTION

Distasteful butterflies in the genus *Heliconius* display a wide range of mimetic colour patterns across their range from south-eastern Brazil to the southern United States. Indeed, between localities within a single lineage, colour patterns have diverged such that a population from, for example, the pupal-mating lineage appears similar to that of a co-mimicking *Heliconius* from the sylvaniform lineage in the same locality. This radiation has been the subject of great interest in coevolutionary biology for decades (reviewed in Gilbert 1983). Even within a locality, more than one mimetic pair arising from these two lineages within *Heliconius* may occur in sympatry.

Why these Müllerian mimics do not converge when in sympatry so as to educate their predators more efficiently that the signal is associated with distastefulness remains a perplexing biological conundrum (Mallet 1993; Joron & Mallet 1998), as the benefits of increased survival are great. A number of hypotheses have been proposed (for a review, see Joron & Mallet 1998). Among these hypotheses is strong selection against novel mimetic phenotypes, which has been measured experimentally in the field (Benson 1972; Mallet & Barton 1989). As a result of strong selection against intermediate forms, mimetic species occupy local adaptive peaks.

Recently, Srygley (1994, 1999) found that, in addition to coloration, a suite of morphological traits relevant to flight (the positions of the centres of the body and wing mass and wing shape) also converge within mimicry groups, further defining these local adaptive peaks. Moreover, Srygley (1999) and Srygley & Ellington (1999) demonstrated that mimetic *Heliconius* butterflies have similar flight behaviours. *Heliconius cydno* and *Heliconius sapho* had lower wing-beat frequencies and a more asymmetrical wing motion due to a prolonged downstroke and shortened upstroke, whereas *Heliconius melpomene* and *Heliconius erato* had higher wing-beat frequencies and a near-sinusoidal wing motion with a more even downstroke and upstroke.

Lower energetic costs presumably translate into higher reproductive success. If one locomotion is energetically more costly than the other, then it may act as a constraint on the evolution of phenotypic convergence. At the very least, it should govern the direction of the evolution of convergence towards flight behaviour and morphology with lower energetic costs.

The purpose of this paper is to estimate the change in the energetic cost of flight associated with a change in wing motion among mimicry groups. By comparing two pairs of *Heliconius* mimics, within which one of each pair are sister species, we are able to address whether members of one mimicry group have incurred a cost relative to the flight energetics of their sister taxa.

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2. MATERIAL AND METHODS

(a) Kinematic analysis

The video recordings, selection of sequences and digitizing are detailed in Srygley (1999). The reconstruction of the wing motion by Fourier analysis is detailed in Srygley & Ellington (1999). To estimate the stroke plane of the wing, wing tip coordinates were plotted with the wing base fixed at the origin. A regression line calculated through the wing tip coordinates lies on the stroke plane and the slope of the line is the angle of the stroke plane relative to the horizontal (after Ellington 1984*b*).

Other variables entered into the aerodynamic analysis included the stroke amplitude (Φ), estimated as $\phi_{\max} - \phi_{\min}$ and the mean position of the wing, estimated as $0.5(\phi_{\max} + \phi_{\min})$ where ϕ is the wing elevation. Finally, the advance ratio ($\mathcal{J}$) is the forward velocity relative to the flapping velocity of the wings:

$$\mathcal{J} = V/(2\Phi fR), \quad (1)$$

where V is the forward velocity, f is the wing-beat frequency and R is the wing length (see table 1 in Appendix A on the Royal Society Web site).

(b) Aerodynamic analysis

In order to compare flight in mimetic species, we modelled the lift coefficient and power requirements for flight. We used standard methods developed by Ellington for forward flight (see Wakeling & Ellington 1997; Willmott & Ellington 1997), but standard cautions also apply (Willmott & Ellington 1997).

The wing shape was reconstructed as a series of wing chords measured at 1 mm intervals from the wing base to the wing tip. At any moment, the instantaneous lift and profile drag forces acting on a wing element are given by

$$L = 0.5\rho c dr U_r^2 C_L, \quad (2)$$

and

$$D = 0.5\rho c dr U_r^2 C_{D,\text{pro}}, \quad (3)$$

where ρ is the air density, c is the chord length of the element, r is its width and U_r is its relative velocity. The vertical and horizontal components of the lift and drag forces were calculated for each wing element, summed along the wing span and averaged over the period of the downstroke and the upstroke (see Willmott & Ellington (1997) for formulae).

The mean lift coefficient (C_L) was estimated by assuming that the vertical force produced by the wings balanced the body weight (the vertical forces produced by the body were negligible). The mean lift coefficient can be solved using the formula

$$mg = C_L(A_{z,d} \pm A_{z,u}) + C_{D,\text{pro}}(B_{z,d} + B_{z,u}), \quad (4)$$

where m is the body mass, g is acceleration due to gravity, $C_L A_{z,d}$ equals the resolved lift force in the vertical direction over the downstroke, $C_L A_{z,u}$ equals the vertical lift force over the upstroke, $C_{D,\text{pro}} B_{z,d}$ equals the vertical component of the drag force over the downstroke and $C_{D,\text{pro}} B_{z,u}$ is the same over the upstroke. The coefficient of profile drag ($C_{D,\text{pro}}$) was assumed to take either of two values. During the production of lift forces, the wing angle of attack was assumed to be sufficiently high that the mean $C_{D,\text{pro}} = 7/(Re)^{0.5}$, which is Ellington's (1984*e*) approximation for insect wings operating at high attack angles and the Reynolds numbers (Re) less than a few thousand. When no lift was being produced, the wing angle of attack was assumed to be

at a minimum of $C_{D,\text{pro}} = 4.8/(Re)^{0.5}$. The Reynolds number for the wings was based on the mean chord $Re = U\bar{c}/\nu$, where U is the relative velocity of the mean chord $\bar{c}$ and ν is the kinematic viscosity of air ($1.55 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ at 23°C).

We assessed C_L in three scenarios.

- (i) The upstroke does not produce lift, i.e. C_L during the upstroke is equal to zero and $C_{D,\text{pro}}$ is at a minimum.
- (ii) Lift is produced during the upstroke but the wing flips over so that air is striking the dorsal surface of the wing, i.e. $A_{z,u}$ is subtracted from the vertical force balance and the mean $C_{D,\text{pro}}$ is at a maximum.
- (iii) Lift is produced during the upstroke but the wing does not flip so that air is striking the ventral surface, i.e. $A_{z,u}$ is added to the vertical force balance and the mean $C_{D,\text{pro}}$ is at a maximum.

In all three instances, the lift production during the downstroke is such that air strikes the ventral wing surface, i.e. $A_{z,d}$ is positive and the mean $C_{D,\text{pro}}$ is at a maximum.

The induced power or power required to balance the body weight was estimated using the modified formula of Willmott & Ellington (1997): $P_{\text{ind}} = w_0(mg - B_{z,d}C_{D,\text{pro}} - B_{z,u}C_{D,\text{pro}})$. The induced velocity (w_0) generated by the flapping wings was estimated using momentum jet theory, such that

$$w_0 = [-V^2/2 + (k^4 w_{\text{RF}}^4 + V^4/4)^{0.5}]^{0.5}, \quad (5)$$

where V is the forward velocity, k is a constant and w_{RF} is the Rankin–Froude estimate of the induced velocity during hovering (see Ellington (1984*a,c,d*) for a discussion of the constant k and w_{RF}).

The power required to overcome the drag on the wings or profile power (P_{pro}) was estimated as the product of the profile drag and relative velocity for each element of the wing summed across the span and averaged over the course of the downstroke or upstroke.

The power required to overcome body drag or parasite power (P_{par}) was estimated by the product of the parasite drag and forward flight speed. We assumed that the parasite drag coefficient $C_{D,\text{par}} = 0.2$, a value within the realm of that of hawk-moths *Manduca sexta* (Willmott & Ellington 1997), *Urania fulgens* moths (Dudley & DeVries 1990) and dragonflies (Wakeling & Ellington 1997) at a body angle parallel to the oncoming air. The air flow around the body is likely to be complicated in flapping flight and this estimate of P_{par} is crude at best. However, at the flight speeds measured in this study, the parasite power is very small relative to P_{ind} and P_{pro} .

From these three estimates of power, we calculated the total aerodynamic power (P_{aero}) required to fly. Because of the high profile drag of the wings, it was assumed that P_{aero} was greater than the power required to accelerate the wings (P_{acc}). Thus, P_{aero} during the decelerating half of the downstroke and upstroke could not be supplied by the excess kinetic energy of the decelerating wing (P_{acc}) and the total power requirement for flight is simply equal to P_{aero} (see also Dudley 1991).

(c) Additional methodology for the outlier

For one *H. sapho*, the mass-specific P_{aero} appeared unusually high. With further evaluation of the wing kinematics, it became apparent that the stroke amplitude was unusually low (74°) compared to values of 110° that are more typical for *H. cydno* and *H. sapho*. Fourier analysis of the wing kinematics of this butterfly had underestimated the maximum wing elevation and, subsequently, stroke amplitude. Hence, for this butterfly an

additional method was employed to improve the fit of the Fourier to the data. We varied c_1 by 0.1 while conserving the ratio of c_1 to c_2 so as to preserve the form of the wave and found the value of c_1 which minimized the root mean square (RMS). At this new value for c_1 and the corresponding value for c_2 , we then altered the mean wing elevation $a/2$ by 0.05 and found that which minimized the RMS. The adjusted stroke amplitude for *H. sapho* was 90° , improving the fit to the data by 12%.

(d) Analysis of mimicry

Because body mass is a dominant feature determining the vertical force required to overcome the body weight, P_{aero} was covaried for body mass. We first tested for differences in P_{aero} versus body mass among species within mimicry groups. We used analysis of covariance (ANCOVA) to test for differences in the slopes of the regression lines (i.e. a significant interaction term) and differences between species in the mean mass-adjusted P_{aero} . If no differences between taxa were found, we then pooled species within mimicry groups and applied an ANCOVA to test for divergence in the mass-adjusted P_{aero} among mimicry groups.

In addition, body mass was correlated with wing length ($r = 0.87$ and $p = 0.0000$), which is a dominant feature determining the drag on the wings. To be consistent in the body size feature, P_{pro} and P_{aero} were also covaried with body mass.

We also assessed the relationship between mass-specific P_{aero} (W kg^{-1}) and velocity between species both within and among mimicry groups. We first tested for differences in mass-specific P_{aero} versus velocity among species within mimicry groups. As for the relationship between P_{aero} and body mass, an ANCOVA was applied to test for differences in the slopes of the regression of P_{aero} and velocity and then for differences between species in the mean mass-adjusted P_{aero} . If no differences between taxa were found, we pooled species within mimicry groups and applied an ANCOVA to test for divergence in the mass-adjusted P_{aero} among mimicry groups.

3. RESULTS

(a) Lift coefficient

The lift coefficient decreased with flight velocity (figure 1a). However, it was always greater than 1.0 and, thus, well above the maximum C_L achieved by Lepidoptera wings under steady flow (e.g. 0.7 for *M. sexta*; Willmott & Ellington 1997; also see Dudley 1991). Hence, mechanisms for generating lift via unsteady aerodynamics must be operating in *Heliconius* butterflies.

Of the three scenarios, an active, lift-generating upstroke during which the wing flips over so that the air strikes the dorsal side resulted in the lowest C_L at velocities below 0.7 m s^{-1} (figure 1b). At velocities greater than 0.8 m s^{-1} , an active upstroke without the wing flipping consistently yielded the lowest C_L . In general the difference between the C_L when the wing was not flipped and that when the wing flipped over was 25–100%. For all cases of up to 1.2 m s^{-1} , the inactive upstroke resulted in less than a 27% increase in C_L relative to the scenario producing the lowest C_L . However, in no instance did an inactive upstroke result in the lowest C_L .

For the remainder of this paper, we assume that an inactive upstroke operates for the range of velocities investigated ($0.4\text{--}1.4 \text{ m s}^{-1}$) because the increase in C_L is slight, but the drag on the wings is reduced (resulting in a

Figure 1. (a) Decline of the lift coefficient with increasing flight velocity under the assumption of an inactive upstroke. Open symbols are the sylvaniform clade and closed symbols are the pupal-mating clade. (b) Variation in the lift coefficient under the assumption of an inactive upstroke versus a lift-producing upstroke (i) where the wing flips over (open symbols) or (ii) without wing flipping (closed symbols). An inactive upstroke always yielded a C_L intermediate between the two active assumptions. Note the near mirror image of the difference between the C_L resulting from the inactive and both active upstrokes. These differences were significantly related to velocity ($p = 0.0000$ in both cases). Above 0.75 m s^{-1} , the active upstroke without the wing flipping yielded the lowest C_L , whereas below 0.75 m s^{-1} , the active upstroke with wing flipping yielded the lowest C_L . Squares are the *H. cydno*–*H. sapho* co-mimics and circles are the *H. melpomene*–*H. erato* co-mimics.

5% saving in total aerodynamic power; see §3(c)). Without further evaluation of the air flow around the wings, it is not possible to determine whether or when the activity of the upstroke changes.

(b) Differences in the lift and drag coefficients between mimics

The mean lift coefficients for the wing-beat with an inactive upstroke did not differ between mimicry groups (Student's *t*-test d.f.=17 and $p=0.103$). However, the drag coefficients for the active and inactive portions of the stroke were significantly different (d.f.=17 and $p=0.0017$), because the Reynolds number was significantly different between mimicry groups ($p=0.0014$).

(c) Power requirements

The profile power required to overcome the drag on the wings was 13% greater for an active upstroke than for an inactive upstroke ($P_{\text{pro(active)}} = 1.13P_{\text{pro(inactive)}} + 0.01$, $R^2=98.5$, $n=19$ and $p=0.0000$). The increase in P_{pro} resulted in a 5% increase in total aerodynamic power ($P_{\text{aero(active)}} = 1.05P_{\text{aero(inactive)}} + 0.00$, $R^2=99.8$, $n=19$ and $p=0.0000$). Because $P_{\text{aero(active)}}$ can be predicted from $P_{\text{aero(inactive)}}$, we only present the power requirements for an inactive upstroke (see table 2 in Appendix A on the Royal Society Web site). However, the associations of $P_{\text{aero(inactive)}}$ with body size and mimicry are qualitatively identical to those that result from using $P_{\text{aero(active)}}$.

(d) Relationships of aerodynamic power to body mass and mimicry

Body mass was a significant covariate for the total aerodynamic power required to fly for both mimicry groups (for both mimicry groups, $p<0.0005$). Within the *H. melpomene*–*H. erato* mimicry group, the slopes of P_{aero} on body mass were not significantly different ($p=0.235$) and there was no difference between the species in the mass-adjusted P_{aero} ($p=0.375$). These pooled species will hereafter be referred to as the *m–e* co-mimics. Within the *H. cydno*–*H. sapho* mimicry group, the slopes were also not significantly different ($p=0.250$) and there was no difference between the species ($p=0.919$). This result did not change when the unadjusted P_{aero} value for the outlying *H. sapho* was used ($p=0.641$ and species $p=0.372$). When pooled, these species will hereafter be referred to as the *c–s* co-mimics.

The slopes relating P_{aero} to body mass were not significantly different between the mimicry groups ($p=0.347$). Following adjustment for body mass, the total aerodynamic cost of flight was significantly different between mimicry groups ($p=0.047$). The mass-adjusted aerodynamic power was greater for the *m–e* co-mimics relative to that of the *c–s* co-mimics (figure 2, *m–e* co-mimics $P_{\text{aero}} = -0.0462 + 0.0065$ mass and *c–s* co-mimics $P_{\text{aero}} = -0.1157 + 0.0065$ mass). Using the unadjusted P_{aero} value for the outlying *H. sapho*, the result of the ANCOVA changed such that the *m–e* co-mimics only tended to have a higher mass-adjusted aerodynamic power relative to the *c–s* co-mimics ($p=0.067$).

The relationship of P_{aero} to body mass results from the fact that all of the components of P_{aero} are proportional to body size. For all butterflies, the power to offset the drag on the wings (P_{pro}) and the induced power to offset body weight (P_{ind}) were both proportional to body mass ($R^2=0.627$ and $p<0.0001$ and $R^2=0.728$ and $p<0.0001$, respectively). The power to offset the drag on the body also increased with body mass, but the relationship was not linear and, relative to P_{pro} and P_{ind} , the magnitude was small.

Figure 2. Regression of the total aerodynamic power (P_{aero}) assuming an inactive upstroke versus body mass. The mimicry groups did not differ in slope but mass-adjusted P_{aero} was significantly higher in the *H. melpomene*–*H. erato* co-mimics (upper regression line) relative to the *H. cydno*–*H. sapho* co-mimics (lower regression line $P=0.047$). Open symbols are the sylvaniform clade and closed symbols are the pupal-mating clade.

(e) Relationships of aerodynamic power to velocity and mimicry

Aerodynamic power is a composite of $P_{\text{pro}} + P_{\text{ind}} + P_{\text{par}}$. Contributing 0.12 W kg^{-1} or less, P_{par} is a minor component of the total P_{aero} . The slopes of the mass-specific P_{pro} , P_{ind} and P_{aero} on velocity for *H. melpomene* were not significantly different from those for *H. erato* ($p=0.639$, $p=0.469$ and $p=0.107$, respectively) and there was no difference between the species in the velocity-adjusted, mass-specific P_{pro} , P_{ind} and P_{aero} ($p=0.491$, $p=0.358$ and $p=0.662$, respectively). Hence, we pooled the data for these individuals within the *m–e* co-mimics. Without the exclusion of the *H. sapho* flying at a velocity of 1.3 m s^{-1} , *H. cydno* and *H. sapho* were significantly different in the slopes of the mass-specific P_{pro} , P_{ind} and P_{aero} on velocity. From an examination of figure 3, it is obvious that this outlying *H. sapho* is making the relations nonlinear, as expected should the U-shaped relation of power to velocity apply (for a discussion of power curves, see Ellington (1991)). With its exclusion, the slopes of the mass-specific P_{pro} , P_{ind} and P_{aero} on velocity for *H. cydno* were not significantly different from those for *H. sapho* ($p=0.798$, $p=0.133$ and $p=0.184$, respectively) and there was no difference between the species in the velocity-adjusted, mass-specific P_{pro} , P_{ind} and P_{aero} ($p=0.938$, $p=0.118$ and $p=0.250$, respectively). For the analyses that follow, we have excluded the *H. sapho* flying at a velocity of 1.3 m s^{-1} and analysed the linear relations between the mass-specific P_{pro} , P_{ind} and P_{aero} for the *m–e* and *c–s* co-mimics between 0.4 and 1.2 m s^{-1} .

Below 1.2 m s^{-1} , the slopes of the mass-specific P_{pro} , P_{ind} and P_{aero} on velocity for the *m–e* co-mimics were not significantly different from those for the *c–s* co-mimics ($p=0.177$, $p=0.170$ and $p=0.807$, respectively) and there was no difference between the species in the velocity-adjusted, mass-specific P_{pro} , P_{ind} and P_{aero} ($p=0.299$, $p=0.214$ and $p=0.405$, respectively). Between 0.4 and 1.2 m s^{-1} , the mass-specific P_{pro} (W kg^{-1}) increased

Figure 3. Regressions of mass-specific (a) profile power, (b) induced power and (c) total aerodynamic power versus velocity. Between velocities of 0.4 and 1.2 m s^{-1} , the regressions for the $m-e$ and $c-s$ morphs were not significantly different and so a single line was drawn. Assuming that this line represents one side of the U-shaped power curve typical for many flying organisms, we excluded the *H. sapho* with a velocity greater than 1.3 m s^{-1} as an outlier. Both power components for that *H. sapho* are plotted; that derived from a stroke amplitude of 72° is the asterisk and that from a stroke amplitude of 90° is a closed square at the corresponding velocity. Open symbols are the sylvaniform clade and closed symbols are the pupal-mating clade.

linearly with velocity (figure 3a), the mass-specific P_{ind} decreased linearly with velocity in the $m-e$ co-mimics (figure 3b) and the mass-specific P_{aero} also decreased linearly with velocity (figure 3c).

Following standard methodology, we analysed the ratio of power to body mass (W kg^{-1}) with velocity. Because P_{aero} increases linearly with body mass, the results using ratios typically do not differ from those using the residuals from the regression of P_{aero} on body mass. However, the intercepts of the regression lines relating P_{aero} to body mass differed significantly for the two pairs of co-mimics and this might result in a difference between the two pairs of co-mimics in the intercepts of the mass-adjusted P_{aero} regressed on velocity. When we regressed the residual mass-adjusted P_{aero} on velocity, the results did not change qualitatively. The slopes did not differ between the pairs of co-mimics ($p = 0.778$) and, when covaried with velocity, the residual mass-adjusted P_{aero} did not differ significantly between pairs of co-mimics ($p = 0.577$).

Hence, the data for 0.4 – 1.2 m s^{-1} may describe one side of the classic U-shaped power curve. However, from a single observation we cannot conclude whether power will increase with velocity above 1.2 m s^{-1} or remain level.

(f) Velocities and advance ratios of mimics

Velocity was not associated with body mass ($p = 0.37$), yet the $c-s$ co-mimics flew significantly faster than the $m-e$ co-mimics (Student's t -test d.f. = 17 and $p = 0.033$). Covarying velocity with body mass removed some of the variation in velocity between the mimicry groups so that velocity only tended to differ ($p = 0.056$).

The advance ratios also tended to be greater in the $c-s$ co-mimics compared with that of the $m-e$ co-mimics (Student's t -test d.f. = 17 and $p = 0.051$). Although the relationship between body mass and the advance ratio was not significant ($p = 0.385$), the differences in the advance ratio between the mimicry groups were removed ($p = 0.086$) when the advance ratio was covaried with body mass.

At slow flight speeds, the cost of flight per unit body mass per unit distance ($\text{J kg}^{-1} \text{ m}^{-1}$) is greater. Following adjustment for variance due to lineages, the mimics were significantly different in their cost of transport (two-way ANOVA: lineage $p = 0.251$, mimicry group $p = 0.032$ and interaction $p = 0.685$). As a result of flying more slowly than the $c-s$ co-mimics, the $m-e$ co-mimics had a higher cost of transport. The cost of transport was on average $9.3 \pm 0.9 \text{ J kg}^{-1} \text{ m}^{-1}$ (mean $\pm$ s.e.) for the $m-e$ co-mimics and $6.3 \pm 0.9 \text{ J kg}^{-1} \text{ m}^{-1}$ for the $c-s$ co-mimics.

4. DISCUSSION

(a) Aerodynamic power requirements and mimicry

We conclude that the aerodynamic cost of flight for butterflies in the *H. melpomene*–*H. erato* mimicry group was significantly greater than that for the *H. cydno*–*H. sapho* co-mimics. However, because the lift coefficients were > 1 in all cases, the quasi-steady assumption can only partly explain the lift generation in *Heliconius*. As a result, the power requirements derived from quasi-steady aerodynamic theory are a first but, at present, the only estimation available.

The mass-adjusted cost of flight was greater for the $m-e$ co-mimics relative to the $c-s$ co-mimics. This may be due to the slower flight speeds and the higher wing-beat frequencies of the $m-e$ co-mimics. At slow flight speeds, relatively more of the vertical lift must be generated by the flapping motion of the wings as opposed to the forward

velocity. The induced power decreases with air speed because the mass of air acted upon per unit time increases. Hence, for the same weight support, a smaller downwash needs to be imparted to that larger air mass. Theoretically, the faster wing-beat frequency of the $m-e$ co-mimics has a second order effect on P_{ind} relative to the effect of velocity. However, in a stepwise regression analysis of the dependent variable P_{aero} , the independent variables body mass ($p=0.0000$), wing-beat frequency ($p=0.014$) and velocity ($p=0.022$) were selected by the analysis in that order. The departure from theoretical expectation is due to the fact that, including the outlier, P_{aero} was curvilinearly related to velocity. The resulting equation is

$$P_{\text{aero}} = -0.311 + 0.0067m + 0.0284f - 0.1176V, \quad (6)$$

and the cumulative variance explained by the model is 97%. With the outlier excluded, body mass ($p=0.0000$), velocity ($p=0.0008$) and wing-beat frequency ($p=0.021$) were selected by the analysis in that order. All of the differences in P_{aero} between the mimicry groups were explained by these models.

As a result of the reduction in the power requirements for flight, it would be economical for the $m-e$ co-mimics to converge on the faster flight speeds and lower wing-beat frequency of the $c-s$ co-mimics. There are three main reasons that convergence may not occur in nature. First, wing-beat frequency may serve as a signal to predators and, like coloration, it is under stabilizing selection. Second, the differences in velocity and wing-beat frequency observed in this study may result from differences in the effect of the artificial environment on members of each mimicry group. Third, although the cost of transport was different between the mimicry groups, the daily energy budget may nevertheless be similar resulting in fitness peaks of the same height.

If we assume that predators select against novel behavioural phenotypes like they select against novel coloration (Benson 1972; Mallet & Barton 1989), flight behaviour may be under stabilizing selection. If predators recognize the combination of traits as a mimetic signal, then they may select against the evolution of a single trait away from the local adaptive phenotype more strongly than concordant changes in all of the traits. Hence, wing coloration, wing-beat frequency and asymmetries in the wing motion (Srygley & Ellington 1999) may all have to change in concordance. If a higher wing-beat frequency is necessary to balance the body weight given the smaller wing length of the $m-e$ co-mimics (Srygley 1999), then body mass may also have to change in concordance with the other traits.

In fact, the flight speeds in the artificial environment of this study are likely to be lower than flight speeds in nature. *Heliconius* intercepted flying naturally over Lake Gatun had flight speeds in the order of $2-3 \text{ m s}^{-1}$, considerably faster than those recorded in this study (Srygley & Dudley 1993, also see the discussion in Srygley (1999)). However, little work on the context dependence of flight speeds has been performed. Additional measures of flight speed in different natural contexts are needed to evaluate the role of flight speed as a mimetic signal. The changes in the kinematics and aerodynamics of *Heliconius* over a broad range of flight speeds also remain unexplored.

If the two pairs of co-mimics fly in different habitats, then the co-mimics with faster flight speeds might be expected to inhabit more open areas. In nature, the opposite occurs: the slower $m-e$ co-mimics tend to be encountered more frequently in open areas (see the discussion in Srygley & Chai (1990)), whereas the faster $c-s$ co-mimics are more frequently found in forest understory (R. B. Srygley, unpublished data). However, because they frequent more open habitats (albeit with a great degree of overlap with the $c-s$ co-mimics), the $m-e$ co-mimics may have reacted to the enclosures by slowing its flight speed disproportionately more than the $c-s$ co-mimics.

Finally, despite the differences in the power requirements and cost of transport, the energy budget of the two pairs of co-mimics may not differ. We found that, as a result of differences in velocity and associated power requirements, the cost of transport was much greater for the $m-e$ co-mimics relative to that for the $c-s$ co-mimics. However, when we account for the differences in body mass between the two groups, the energetic costs per metre flown are very similar. *Heliconius* feed on nectar and pollen, as well as having stored fats. Assuming muscle efficiency is 10% (the remaining 90% being lost as heat), the corresponding cost of flight per metre for the $m-e$ co-mimics is $0.26 \mu\text{g}$ fat or $0.57 \mu\text{g}$ sugar for a mean body mass of 108 mg. Because body mass was greater for the $c-s$ co-mimics than the $m-e$ co-mimics, the cost of flight ($0.25 \mu\text{g}$ fat or $0.56 \mu\text{g}$ sugar per metre for a mean body mass of 156 mg) was not significantly different from that of the $m-e$ co-mimics ($p=0.860$).

In order to relate our estimate for the energetic cost per metre flown to the butterfly's relative fitness, we would need a daily budget of flight distances for each *Heliconius*. The differences in feeding habits are not great (R. B. Srygley, personal observation), but the species do differ in mating habits. Pupal-mating *Heliconius* males (*H. erato* and *H. sapho* in this study) seek pupal females and await their emergence (Deinert *et al.* 1994), whereas *H. melpomene* and *H. cydno* apply more typical mate-seeking behaviours. Each of the mimicry groups has one pupal mater and, thus, there may be no differences in the distances flown between mimicry groups.

Hence, the differences in body mass between mimicry groups (with the additional assumption that the mean daily activity budgets are similar between mimicry groups) result in similar estimates of daily energy expended for flight. If the fitnesses of the butterflies within each mimicry group are related to their daily energy expenditures, then fitness may also be similar. As a result, differences in body mass may serve to stabilize the sympatric coexistence of the two pairs of co-mimics. Without a difference in energetics, it is unlikely that a selective force exists for the convergence of one mimetic behaviour to another as long as the difference in body mass between the mimicry groups remains.

(b) *Aerodynamic power requirements and body mass*

Body mass was a predictive factor for flight energetics with P_{aero} increasing linearly. Although little explored, this simple relationship between body mass and P_{aero} is evident in other data sets as well. Dudley (1991) calculated P_{aero} for 15 butterfly species. We have excluded two

of these because they were very large (greater than 1000 mg) relative to the other species (range 24–454 mg) and their inclusion had a significant effect on the relationship of P_{aero} to body mass. For the other 13 species, P_{aero} was significantly dependent on body mass ($R^2=0.94$ and $p=0.0000$) and the intercept was not significantly different from zero. Hence, within this range of body mass, P_{aero} was uniformly 7.5 W kg^{-1} body mass (95% confidence limits $6.3\text{--}8.8 \text{ W kg}^{-1}$ body mass). This result is not significantly different from our estimate of 6.5 W kg^{-1} for both the $m-e$ and $c-s$ co-mimics.

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