

Discrimination of flying mimetic, passion-vine butterflies *Heliconius*

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Wing-beat frequency and the degree of asymmetry in wing motion were more similar among mimics than among sister species of passion-vine butterflies in the genus *Heliconius*. Asymmetry in wing motion is not attributed to lift production, and serves as the first clear example of a mimetic behavioural signal for a flying organism. Because the similarities in wing motion are too subtle for humans to observe with the naked eye, they serve as a previously unexplored mimetic signal.

Keywords: locomotor mimicry; behaviour; mutualism; kinematics; tropical rainforest; signal

1. INTRODUCTION

Since bird retinas have a higher flicker-fusion rate than humans (Frost *et al.* 1994; McKee & Watamaniuk 1994; Delius *et al.* 1999), we presume that they are able to visually sense much more of the natural world. For insectivorous birds, vision is essential for detecting prey on background surfaces. Aerial insectivores must make rapid decisions of the profitability of a prey item before the insect is too distant for pursuit. Judging unpalatable prey from palatable ones profits both the predator and the prey. Hence, an increase in the predator's ability to process cues presented by the prey and an increase in the unpalatable prey's ability to reduce discrimination errors by the predator (MacDougall & Dawkins 1998) are both favoured by natural selection. Mimetic similarity of prey that are distasteful, i.e. Müllerian mimicry, evolves to reduce discrimination errors and increase foraging efficiency of the predator and survival of the prey.

One means of reducing discrimination errors by aerial insectivores is for unpalatable prey to have different flight behaviours from palatable ones (Chai & Srygley 1990). As a result, evolutionary convergence in flight behaviour should be selected to reduce discrimination errors. One likely place to look for convergence in flight behaviour is in butterflies that are associated with one another via colour mimicry. Butterflies in two clades within the genus *Heliconius*, the sylvaniform clade and the pupal-mating clade (figure 1a; Eltringham 1916; Brown 1981; Lee *et al.* 1992; Brower 1994), have served as a model system for coevolutionary studies of mimetic wing patterns in the neotropics (Eltringham 1916; Turner 1981; Gilbert 1983; Sheppard *et al.* 1985; Brower 1996). These coevolved, unpalatable, Müllerian mimics provide an opportunity to extend the investigation of mimicry from the classical convergence in coloration and shape to flight behaviour.

2. MATERIAL AND METHODS

We investigated the motion of the wings in a kinematic analysis of four species that are sympatric in Panamanian lowland rainforest (figure 1a,b). *Heliconius cydno chioneus* and *H. melpomene rosina* are members of the sylvaniform clade, whereas *H. sapho candidus* and *H. erato petiverana* are members of the pupal-mating clade (Eltringham 1916; Brown 1981; Lee *et al.* 1992; Brower 1994). *Heliconius cydno chioneus* and *H. sapho candidus* are one pair of mimics (co-mimics), whereas *H. melpomene rosina* and *H. erato petiverana* are another. All four species are highly distasteful to the rufous-tailed jacamar (Chai 1990).

Selection of these four subspecies permits the application of a powerful comparative technique to investigate character similarity as a result of two factors: (i) common ancestry, and (ii) the predatory selective force that the species have in common (Schluter 1986; Srygley 1999). For this study, we selected classes based on colour mimicry. The variance in the dependent variable is partitioned into that due to (i) membership in a mimicry group, (ii) membership in a phylogenetic lineage, (iii) interaction between mimicry and phylogeny, and (iv) error. As a result, the effects of mimicry and historical differences among the lineages were analysed simultaneously.

Butterflies were videotaped in insectaries or greenhouses flying within a flight tunnel that constrained the flight path to approximately perpendicular to the camera lens. The position of the head, wing base, tip of the abdomen, and near wing tip were digitized at 60 fields s⁻¹ for a flight sequence (for additional details, see Srygley (1999)). Flight sequences ranged from 0.25 to 0.5 s in duration. Three-dimensional motion of the near wing was reconstructed from successive fields (Ellington 1984; Srygley 1999).

From a prior analysis of morphology (Srygley 1994, 1999), we predicted a convergence in wing-beat frequency within mimicry groups. To estimate wing-beat frequency, we used values obtained by counting downstrokes of the wing as our starting value (Srygley 1999). We then assumed that the motion of the wing tip relative to the wing base was approximately sinusoidal over the measured time, and calculated the root mean square

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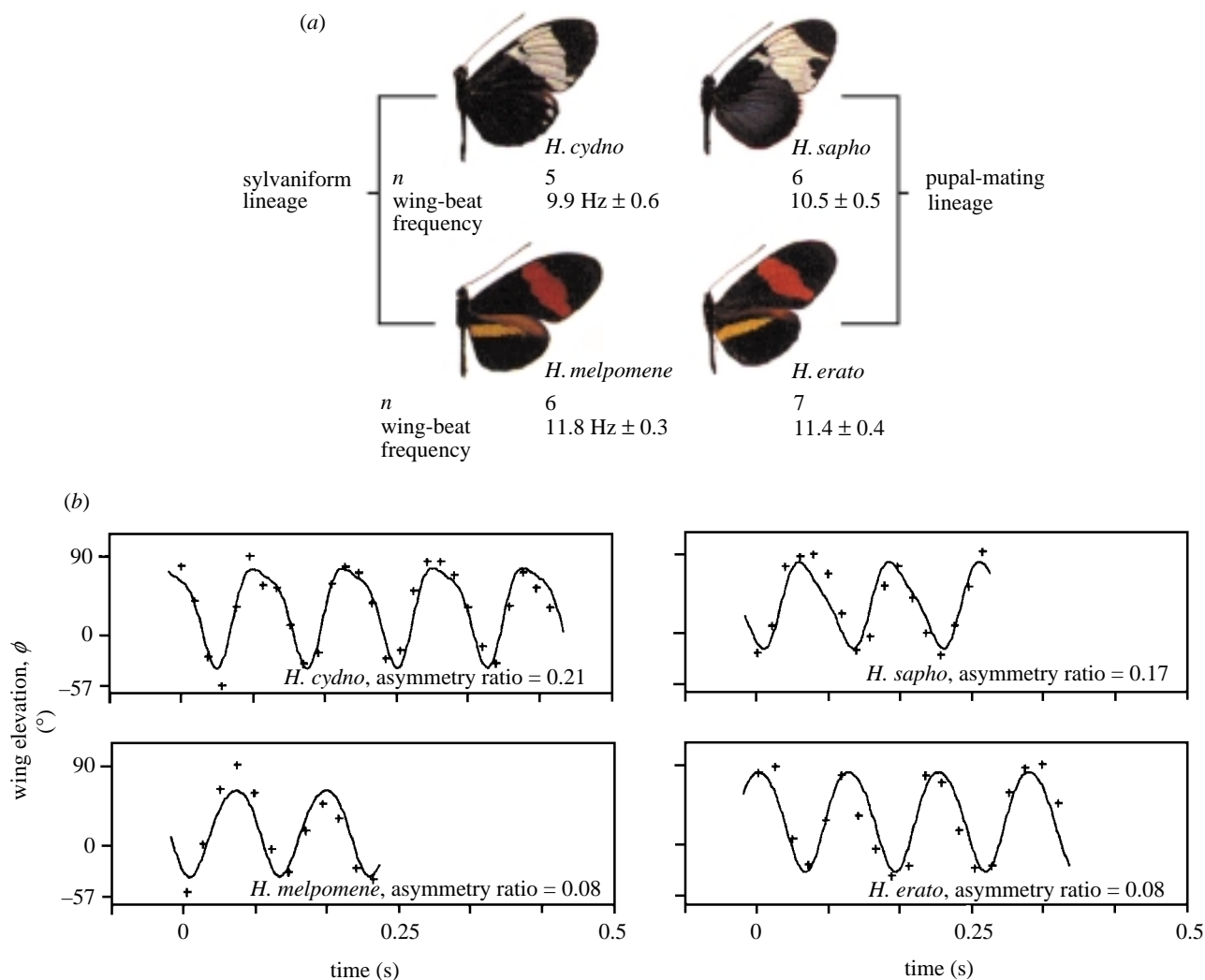


Figure 1. (a) Phylogeny and kinematics of *Heliconius* mimics. For each species, the sample size, mean, and standard deviation for wing-beat frequency are listed. *H. cydno* and *H. sapho* are black with a white forewing bar, and *H. erato* and *H. melpomene* are black with a red forewing bar and a yellow hindwing bar. (b) For one individual of each species, the elevation of the wing (ϕ) as smoothed by the Fourier series is shown as a waveform relative to time. The transition from upstroke to downstroke is at the peak of the wave, and downstroke to upstroke at the trough. When the wing is level with the wing base, $\phi = 0$. Raw data are shown as crosses. *H. cydno* and *H. sapho* with high asymmetry ratios have slowed or paused the wing motion at the beginning of the downstroke, whereas *H. melpomene* and *H. erato* with low asymmetry ratios have a more sinusoidal motion.

(RMS) difference between the observed values for the wing elevation, ϕ , and those predicted by a Fourier series that fit the fundamental (first harmonic) to the data,

$$\phi = a/2 + c_1 \cos(2\pi ft + \theta_1),$$

where θ_1 is the phase angle of the fundamental, f is the wing-beat frequency and t is time. We varied the wing-beat frequency by approximately 0.1 Hz, and used that value which minimized the RMS.

We looked for convergence in the higher harmonics of wing motion. Using the wing-beat frequency which minimized RMS, we smoothed the motion of the wing tip by fitting the second, third, and fourth harmonic to the observed data. In all cases, irregularities in the waveform became obvious with addition of the fourth harmonic, indicating that it was fitting noise in the data. In order to ensure that the results were robust and reliable, we therefore stopped at the second harmonic.

However, irregularities were also evident with addition of the third harmonic for four butterflies. These four butterflies have been included only in the analyses of wing-beat frequency. From the Fourier analysis

$$\phi = a/2 + c_1 \cos(2\pi ft + \theta_1) + c_2 \cos(4\pi ft + \theta_2),$$

the following variables are sufficient to recreate the smoothed motion of the wing tip: the wing-beat frequency f , the mean elevation of the wing $a/2$, the first Fourier coefficient c_1 , the phase for the fundamental θ_1 , the second Fourier coefficient c_2 , and the phase for the second harmonic θ_2 (Willmott & Ellington 1997a). Deviations from sinusoidal wing motion were quantified with an asymmetry ratio equal to $c_2/(c_1 + c_2)$. Assuming that subsequent harmonics explain a negligible amount of the variation in the wing stroke cycle, the asymmetry ratio is equal to the amount of variation in ϕ that is explained by the second harmonic.

Table 1. Two-way MANOVA for the dependent variables wing-beat frequency and asymmetry ratio

source of variation	univariate <i>F</i> -tests									
	multivariate test statistics				asymmetry ratio (<i>n</i> = 19)			wing-beat frequency (<i>n</i> = 23)		
	d.f.	% explained	<i>F</i>	<i>p</i>	% explained	<i>F</i>	<i>p</i>	% explained	<i>F</i>	<i>p</i>
lineage	1,15	0.04	0.6548	0.4311	4.9	1.0577	0.3200	0.1	0.0280	0.8688
mimicry	1,15	47.0	13.3036	0.0024	21.2	4.5538	0.0498	33.0 ^a	9.5970 ^a	0.0059 ^a
interaction	1,15	0.04	0.6417	0.4356	0.9	0.1896	0.6694	3.6	1.0515	0.3180
overall model	3,15	48.7	4.7488	0.0160	—	—	—	—	—	—

^a For only those butterflies included in the MANOVA, the sample size was one less for each species than that presented in figure 1; and 46% of the variation in wing-beat frequency was explained by mimicry, $p = 0.0022$.

3. RESULTS

Wing motion was quantified by the asymmetry ratio and wing-beat frequency. The asymmetry ratio quantifies deviation from a sinusoidal wing motion in which the duration of the upstroke is even with that of the downstroke (figure 1*b*). As a null hypothesis, wing motion should be similar among sister species. In fact, a species' lineage explains an insignificant amount of the variation in two traits that describe the motion of the wings (table 1). However, a species' mimetic coloration explains a significant fraction of the variation. Hence, the asymmetry ratio and wing-beat frequency have diverged within lineages and converged on each species' respective co-mimic (figure 2).

On average, asymmetry ratios were higher within the *cydno-sapho* group than the *melpomene-erato* group (figure 2). A higher asymmetry ratio results from a larger contribution of the second harmonic to the wing motion. Examination of the waveform suggests that this asymmetry is typically due to the *cydno-sapho* members slowly accelerating the wing at the beginning of the downstroke and then rapidly executing the remainder of the downstroke (figure 1*b*). In some instances, it was the middle or bottom of the downstroke that was prolonged, but in all cases, the downstroke was prolonged relative to the upstroke. Kinematics for members of the *melpomene-erato* mimicry group were generally dominated by the fundamental, describing a wing motion that was much closer to sinusoidal and resulting in a low asymmetry ratio. For these species, the second harmonic also prolonged the downstroke relative to the upstroke, but the effect was much less pronounced than for the *cydno-sapho* group. Hence, wing motion within a wing cycle has diverged within lineages towards convergence to each species' respective co-mimic.

4. DISCUSSION

Differences in asymmetrical motion of the wing have little effect on aerodynamic force production, at least for hawkmoths (Willmott & Ellington 1997*b*). Hence, these differences are more likely to arise as a result of direct selection on signalling behaviours by predators. Variation from nearly sinusoidal to more asymmetrical wing motion adds a previously unexplored complexity to the

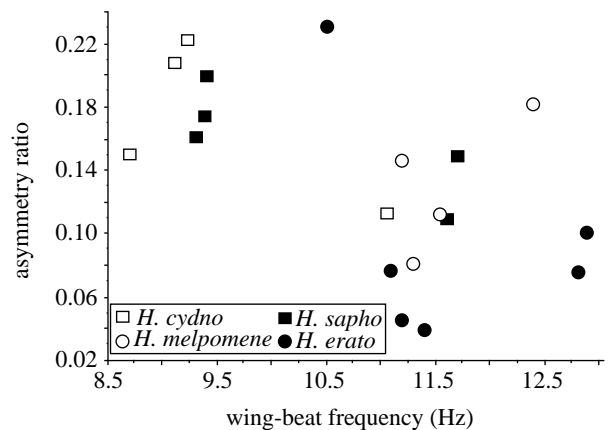


Figure 2. Asymmetry ratio versus wing-beat frequency for the two mimicry groups. Open symbols, sylvaniform clade; closed symbols, pupal-mating clade.

mimetic signal. Because the species are unpalatable, the behaviours may serve as warning signals.

This analysis serves as direct evidence that behavioural mimicry has occurred in a mutualistic or Müllerian context. Aerial predators that choose to attack or ignore butterflies in flight, such as rufous-tailed jacamars (Chai 1986, 1990; Chai & Srygley 1990; Srygley & Chai 1990), are the most likely selective agents for convergence in flight behaviour.

Wing-beat frequency averaged 10.2 Hz (± 0.3 , s.e.m.) for the *cydno-sapho* group and 11.6 Hz (± 0.3) for the *melpomene-erato* group, a difference of *ca.* 10%. Convergence in wing-beat frequency may be the direct result of predators using wing-beat frequency as a cue to distinguish among Müllerian mimicry groups. However, morphological convergence in mimics may also result in wing-beat frequency convergence for reasons of aerodynamic force production (Srygley 1999). The higher wing-beat frequencies of the *melpomene-erato* group require greater aerodynamic power and thus they are energetically more costly than the relatively low wing-beat frequencies in the *cydno-sapho* group (Srygley & Ellington 1999).

Evolutionary models of mimicry predict that the aposematic signals of sympatric, distasteful butterflies should converge when exposed to a common predatory environment (Joron & Mallett 1998). Asymmetries in wing motion probably reflect asymmetries in muscle

contractions, as evident in the asymmetrical muscle contractions and wing kinematics of bumble-bees (Gilmour & Ellington 1993). We suggest that because of its complexity, locomotor mimicry makes convergence of aposematic morphs more unlikely than colour mimicry alone. In addition, parasitization of the signal by palatable, Batesian mimics is more difficult to evolve.

The ability of birds to perceive differences in wing-beat frequency or wing motion has not previously been investigated. However, lepidopterists use the pattern of pause and stroke during the wing-beat to distinguish among taxonomic groups (e.g. the pause at the top of the upstroke characteristic of satyrids). Motion discrimination in humans requires integration of velocity for 100–200 ms to obtain a precise speed signal. Higher-order signals, such as acceleration, require additional time and greater differences to distinguish (Frost *et al.* 1994; McKee & Watamaniuk 1994). The downstroke for a butterfly in the *cydno-sapho* group endured, on average, 59 ms (± 2 , s.e.m.), whereas butterflies in the *melpomene-erato* group have a briefer half-stroke (47 ± 2 ms). Hence, differences among the mimicry groups presented here are probably not detectable by humans. Comparable data are not available for birds. However, using flicker-fusion rates as a gauge, the temporal resolution in bird retinas are 1.5–3 times faster than that in humans (pigeons: 74–145 Hz, Frost *et al.* 1994; humans: 50–100 Hz, McKee & Watamaniuk 1994). Discrimination of wing strokes that differ in velocity and acceleration over a time-span of 50 ms suggests that birds perceive motion two to four times faster than humans.

Locomotor mimicry may be a general phenomenon that is likely to arise even in the absence of colour mimicry. For example, bats are not likely to use colour to distinguish moths in flight; but, motion of the wings and body may allow bats to distinguish edible from distasteful moth species, and hence locomotor mimicry might be selected. The passion-vine butterflies, and other examples of convergent evolution in flight behaviour among mimetic species, provide test subjects for theoretical analyses of biomechanical design and physiology relevant to flight.

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