Large Hind Wings Enhance Gliding Performance in Ground Effect in a Neotropical Butterfly (Lepidoptera: Nymphalidae)

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Abstract

Neotropical butterflies in the nymphalid genera *Pierella* Herrich-Schäffer, 1865 (Lepidoptera, Nymphalidae) and *Cithaerias* Hübner, 1819 (Lepidoptera, Nymphalidae) are known to glide near ground level, and have a high forewing aspect ratio. The hind wings of *Pierella* are proportionately larger than those of *Cithaerias*, suggesting a potential role in gliding flight. We show that *Pierella helvina* (Hewitson, 1860) (Lepidoptera, Nymphalidae) is capable of gliding for longer periods of time than *Cithaerias pireta* (Stoll, 1780) (Lepidoptera, Nymphalidae) both in the field and a laboratory setting. Experimental reduction of hind wing area in *P. helvina* led to a decrease in male gliding performance, and an increase in female wing-beat frequency. We also found that escape velocity decreased in both sexes after hind wing area reduction, but the effect was strongest in females. Although the increased drag associated with large hind wings could be detrimental to gliding flight, the large hind wing area of *P. helvina* effectively enhances gliding performance in ground effect. This study is the first to investigate the role of hind wings on butterfly gliding performance.

Key words: Haeterini, *Pierella*, *Cithaerias*, patrolling flight

Flight is intimately linked to butterfly behavior and natural history, and selection on the performance of critical activities such as predator avoidance, mate-finding, courtship, and oviposition has led to a broad diversification of butterfly wing and body morphology. When employing flapping flight, thoracic muscle mass, wing loading, and forewing centroid position have all been shown to affect flight speed (Bartholomew and Casey 1978, Dudley 2000). During gliding flight, performance is enhanced by a high forewing aspect ratio that increases lift and reduces drag, thus promoting energy efficiency (Dudley 2000; see also Marden and Chai 1991, DeVries et al. 2010). The forewings are the first point of contact between the wings and incident air flow, and consequently, pressure gradients tend to be highest around their leading edges (Ancel et al. 2016). Thus, it is not surprising that forewing morphology is considered the prime determinant of butterfly flight performance (Dudley 2000, Berwaerts et al. 2006, Jantzen and Eisner 2008, Li et al. 2016, Le Roy et al. 2019). Male butterflies generally have a higher forewing aspect ratio than conspecific females, especially in species where males employ gliding flight to patrol mating territories (Betts and Wootton 1988, Berwaerts et al. 2002, DeVries et al. 2010, Cespedes et al. 2014). Selection is, therefore, expected to influence the shape and aerodynamic properties of the forewings to maximize performance during critical flight behaviors (DeVries et al. 2010, Shi et al. 2015, Chazot et al. 2016).

While the relationship between forewing morphology and flight is well established, comparatively little is known about the contribution of hind wings to butterfly flight (Le Roy et al. 2019). Using a moth and a butterfly species, Jantzen and Eisner (2008) showed experimentally that while complete hind wing removal caused a decrease in body acceleration and maneuverability, both species were capable of flight using the forewings only. The butterfly in that study, *Pieris rapae* (Linnaeus, 1758) (Pieridae), utilizes flapping flight almost exclusively, and its wide forewings presumably function to enhance flapping flight efficiency (Dudley 2000, Ha et al. 2013). To our knowledge, no study has evaluated the aerodynamic role of hind wings in butterfly species that regularly employ gliding flight. A suite of morphological parameters can play a role in butterfly gliding flight. Since the induced drag on a wing scales positively with mean chord length relative to wing length (Dudley 2000), selection for gliding efficiency should minimize chord by producing elongate forewings with high aspect ratio (see Le Roy et al. 2019 for a review). As forewings and hind wings operate in concert, the size and shape of the hind wings can influence induced drag as they contribute to the chord length of the wing pair (Dudley 2000, Ancel...
et al. 2016). Although the lift generated by the wings is proportional to their area (Dudley 2000), an enlarged hind wing would result in increased induced drag—thus constraining the contribution of the hind wing to efficient gliding flight. This constraint is, however, relaxed by the aerodynamic phenomenon of ground effect that occurs when a wing experiences a reduction in induced drag during gliding due to its close proximity to a fixed surface (Rozhdestvensky 2000, Cui and Zhang 2010, Rahimuddin et al. 2014). Since the strength of ground effect is inversely proportional to flight height above the ground, the wings of animals that regularly glide close to a surface should be optimized to take advantage of this phenomenon (Withers and Timko 1977, Hainsworth 1988, Rayner 1991). Consequently, butterflies that glide in ground effect present a unique opportunity to study hind wing aerodynamics.

Most species in the Neotropical butterfly tribe Haeterini (Nymphalidae, Satyrinae) use gliding flight while foraging, patrolling territories or searching for oviposition sites (Weymer 1910, Zikán 1942, Masters 1970, DeVries 1987). Observations and mark-recapture studies suggest that males use patrolling flight to move within their home ranges as part of mate-seeking behavior, whereas females move through the forest understory searching for larval host plants (DeVries and Alexander unpublished data, see also Murillo-Hiller 2009). Further, a comparative study in Costa Rica employing fruit-baited traps positioned at 15 cm and 1 m above the ground found that Pierella helvina (Hewitson, 1860) was sampled only in lower traps while Cithaerias pireta (Stoll, 1780) was captured in both (Alexander and DeVries 2012). These observations are consistent with the suggestion that there has been wing shape evolution in some Haeterini to enhance aerodynamic efficiency for gliding in ground effect (Cespedes et al. 2014).

Both P. helvina and C. pireta use gliding flight in the forest understory, but P. helvina appears to have larger hind wings relative to the forewings than does C. pireta (Fig. 1). Flight behavior and wing morphology thus led us to hypothesize that the hind wings of P. helvina could function to enhance gliding flight in ground effect. To test this hypothesis, we compared wing morphology and flight performance of P. helvina and C. pireta. To assess potential differences between sexes and species, we measured forewing and hind wing areas and forewing aspect ratio, and used high-speed videography to address the following questions relevant to flight performance: 1) Does gliding flight in P. helvina and C. pireta conform to predictions based on forewing aspect ratio alone? 2) Does hind wing area affect gliding flight performance in P. helvina? 3) Does hind wing area affect flight speed in P. helvina? We then discuss similarities and differences in flight performance of P. helvina and C. pireta in the field and laboratory. Finally, we conclude that hind wing area in P. helvina enhances gliding in ground effect.

Methods

Study Site and Sampling

This investigation was conducted at the Tirimbina Biological Reserve, Heredia Province, Costa Rica (10°29′50.3″S; 76°22′28.9″W) in December 2016. The Tirimbina 345 hectare reserve is composed of approximately 85% primary lowland rain forest, and has an elevation range of 180–220 m. See DeVries et al. (2012) for a more detailed description.

Individuals of P. helvina and C. pireta were captured in the field with hand nets between 0700 and 1100 hours, placed in glassine envelopes, and subsequently transferred to 500 ml transparent plastic containers kept in an ambient temperature laboratory. All individuals were allowed to feed ad libitum on overripe fruit. Butterflies captured on a given morning were stored for no more than 3 h before experimental flights in the lab. The ambient temperature indoor flight arena consisted of an open room (ca. 4 × 5 m in area) with ambient light from a screened door.

Wing Measurements and Manipulations

Captured individuals were photographed next to a metric scale, and the images were processed using Adobe Photoshop to yield a cutout of each wing to measure length and area (Combes and Daniel 2003). We standardized wing length measurements by fitting the smallest possible circle around each cutout in Adobe Photoshop, and the diameter of this circle corresponded to wing length. The left wing pair was used to measure length and area. To calculate aspect ratio we used the equation AR = (span^2)/(total FW area) where span refers to twice the length of one forewing. The combined areas of one forewing and one hind wing were doubled to estimate total wing area. All measurements were performed with the NIH software ImageJ (https://imagej.nih.gov/ij/, last accessed January 2017).

To explore the functional role of the enlarged hind wings of P. helvina (Fig. 1a), we experimentally trimmed hind wings of male and female specimens (Fig. 1b) to approximate the relative hind wing area of C. pireta (Fig. 1c). Two straight cuts were made across the images were processed using Adobe Photoshop to yield a cutout of each wing to measure length and area (Combes and Daniel 2003). We standardized wing length measurements by fitting the smallest possible circle around each cutout in Adobe Photoshop, and the diameter of this circle corresponded to wing length. The left wing pair was used to measure length and area. To calculate aspect ratio we used the equation AR = (span^2)/(total FW area) where span refers to twice the length of one forewing. The combined areas of one forewing and one hind wing were doubled to estimate total wing area. All measurements were performed with the NIH software ImageJ (https://imagej.nih.gov/ij/, last accessed January 2017).

To explore the functional role of the enlarged hind wings of P. helvina (Fig. 1a), we experimentally trimmed hind wings of male and female specimens (Fig. 1b) to approximate the relative hind wing area of C. pireta (Fig. 1c). Two straight cuts were made across male specimens (Fig. 1b) to approximate the relative hind wing area of C. pireta (Fig. 1c). Two straight cuts were made across...
the paired hind wings of each individual to approximate a 10% reduction in hind wing contribution to total wing area. To measure the actual percent reduction in area we compared photographs of individuals before and after the wings had been cut. Only butterflies with undamaged hind wings were used for hind wing size reduction, and after cutting, they showed no obvious signs of physical or behavioral stress.

Videoigraphy
We used iPhones SE, 240 fps (Apple Inc.) to record high-speed videos of butterfly flight in the field and in the lab. Due to low light levels in the rainforest understory, methods for filming in the field differed from those used in the lab.

To record natural flight behavior of *P. helvina* and *C. pireta*, butterflies were video-taped in forest light gaps. As they were not captured and sexed, flight data were pooled by species. While flapping and gliding flight was discernable in these videos, limited light precluded accurate kinematic analysis, estimates of flight path, or flight speeds. A recorded flight was deemed suitable for frame-by-frame analysis if a single camera captured at least five consecutive wing-beat cycles.

To assess flight performance in the lab, two high-speed video cameras were set up in stereo to create an overlapping field of view. Video-capture in stereo allowed us to use the angle of a flying butterfly from each of the cameras to triangulate its three-dimensional position within the flight arena (Almbro and Kullberg 2007, Chakravarthy et al. 2009, de Margerie et al. 2015; Supp. Fig. 1 [online only]). These kinematic data, plus temporal data derived from the precise frame rate of each camera were used to estimate individual flight path, speed, glide duration, wing-beat frequency, wing-beats per total flight time, and the ratio of gliding to total flight time.

Experimental Flight Protocol
At the start of each experimental flight, we used a light pulse from a flashlight to synchronize both cameras, and then an individual butterfly was gently placed on the floor approximately 1 m from the cameras in the center of the flight arena. Once placed, individuals either took flight immediately or were gently prodded on a hind leg to induce flight. We assume that these flights constituted reasonable approximations of the maximum flight capabilities when startled (escape-flight; see Almbro and Kullberg 2007), and that conditions affecting flight behavior were approximately constant for all experimental individuals.

After an individual with intact wings had been video-taped for two consecutive flights, the hind wings were experimentally reduced, and it was immediately flown and video-taped twice more. These butterflies were sacrificed and stored in glassine envelopes as voucher specimens.

Video Analysis and Data Extraction
When synced, the two videos of each flight remained consistent on a frame-by-frame basis. Consistency at a frame rate of 240 fps yields a high degree of precision in the measurement of flight time by frame-number (Dudley 1990, Brodsky 1991, Jantzen and Eisner 2008). Since behavioral and kinematic analysis of flight performance depend directly on the measurement of time, such temporal precision was critical to our analyses.

Video-taping each flight in stereo enabled the triangulation of positional (x,y,z) data when butterflies were simultaneously visible to both cameras. Analysis of positional data was limited to the first five wing-beats of each flight. To extrapolate positional data from raw digital videos, it was first necessary to construct a standard curve relating the distance of a butterfly from left-of-frame to the angle of that butterfly from each camera. This was measured in pixels using ONDER RULERS for Mac (Supp. Fig. S1a [online only]). These two angles were then used to triangulate the (x,y) position of a butterfly at the beginning (pronation), middle (supination), and end (pronation) of each wing-beat cycle, as well as at the beginning and end of each glide. Height (z) was measured in a similar fashion. See Supp. Table S1 and Fig. S1b (online only) for detailed descriptions of these positional calculations. To test the accuracy of our stereo camera rig, a tape measure was set in the flight arena parallel to, and 1.5 m from, the camera plane, and the (x,y,z) position of each marking on the tape was measured using ONDER RULERS as described above. Triangulation of over 50 points of known position produced an average error of 3.56 mm and a maximum error of 8.32 mm, which was considered sufficiently accurate for kinematic measurements (Jantzen and Eisner 2008, Chakravarthy et al. 2009).

Flight Data
To compare the flight behaviors and performance of *P. helvina* and *C. pireta*, we used temporal data derived from the precise frame rate of each camera. To quantify gliding flight, we measured glide durations and the proportion of gliding to total flight time for each individual (Dudley 1990). To quantify flapping flight, we measured both wing-beat frequency and the number of wing-beats per total flight time for each individual.

To compare the kinematic flight performance of *P. helvina* before and after experimental hind wing reduction, we used (x,y,z) positional data to measure flight path and flight speed. Flight path was calculated as the sum of linear displacements between each successive point in the wing-beat cycle. Flight speed was calculated as flight path divided by flight time. This was done for each flight of each individual.

Statistical Analyses
We used one-tailed *t*-tests to assess differences in wing morphology between species and sexes, as these data fit a normal distribution. Mann–Whitney *U* tests were used to compare proportions of gliding per total flight time and wing-beats per total flight time because these proportions were homoscedastic, but not normally distributed. We used Kruskal–Wallis tests to compare samples of wing-beat frequencies and glide durations because these variables were neither homoscedastic nor normally distributed. All analyses were performed in the statistical suite JASP (Version 0.9.1) for Mac.

Results
Relative Hind Wing Area, Forewing Aspect Ratio, and Range of Ground Effect
The hind wings of *P. helvina* comprised a greater portion of the total wing area than those of *C. pireta* (Table 1, Fig. 2). Male *P. helvina* had larger hind wings than conspecific females, but in *C. pireta* the sexes were the same (Table 1). Experimental hind wing reduction in *P. helvina* (Fig. 1b) resulted in a similar relative hind wing area as possessed by *C. pireta* (Figs. 1c and 2).

Male *P. helvina* had a significantly higher forewing aspect ratio than male *C. pireta*, but females of these species did not differ (Table 1). We also found that male *P. helvina* had a significantly higher forewing aspect ratio than conspecific females, but *C. pireta* showed no difference between sexes (Table 1).

The strength of ground effect is greatest when flying within one wingspan from the ground (Rayner 1991, Cui and Zhang 2010).
Table 1. Relative area of the hind wings (HW), forewing aspect ratio, and wingspans

<table>
<thead>
<tr>
<th></th>
<th>P. helvina</th>
<th>C. pireta</th>
<th>P</th>
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<tbody>
<tr>
<td>HW/total wing area (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male (7)</td>
<td>56.2 ± 1.2</td>
<td>Male (6)</td>
<td>46.4 ± 0.3</td>
</tr>
<tr>
<td>Female (7)</td>
<td>54.9 ± 1.2</td>
<td>Female (4)</td>
<td>46.8 ± 0.4</td>
</tr>
<tr>
<td>P</td>
<td>0.026</td>
<td></td>
<td>0.056</td>
</tr>
<tr>
<td>Forewing aspect ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (7)</td>
<td>5.77 ± 0.17</td>
<td>Male (6)</td>
<td>5.34 ± 0.13</td>
</tr>
<tr>
<td>Female (7)</td>
<td>5.45 ± 0.18</td>
<td>Female (4)</td>
<td>5.35 ± 0.09</td>
</tr>
<tr>
<td>P</td>
<td>0.002</td>
<td></td>
<td>0.484</td>
</tr>
<tr>
<td>Wingspan (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (7)</td>
<td>6.80 ± 0.25</td>
<td>Male (6)</td>
<td>5.63 ± 0.20</td>
</tr>
<tr>
<td>Female (7)</td>
<td>7.42 ± 0.16</td>
<td>Female (4)</td>
<td>6.10 ± 0.13</td>
</tr>
</tbody>
</table>

Specimens measured are the same as those used in the flight experiment, and sample sizes are in parentheses. Values are sample means and standard deviations. P-values correspond to one-tailed t-tests, and significant differences are in bold type.

Fig. 2. Allometric intercepts for individual Pierella helvina with intact and reduced hind wing area, and Cithaerias pireta with intact wings.

Based on average wingspans (Table 1), we estimate the range of ground effect for P. helvina to be 6.8–7.4 cm, and 5.6–6.1 cm for C. pireta. We presume that individuals flying above these ranges would lose the advantage of ground effect.

Flight Behavior in the Field

In the field, P. helvina exhibited greater glide durations and proportions of gliding relative to total flight time than C. pireta, and also had a narrower range of variation in gliding time (Table 2).

Although P. helvina showed higher wing-beat frequencies than C. pireta, they employed fewer wing-beats per total flight time—likely because they spent more time gliding (Table 2). In both species the proportion of gliding flight was positively correlated with wing-beat frequency (Fig. 3) and negatively correlated with wing-beats per total flight time (Fig. 4a). The field camera was positioned 20.2 cm from the ground. We found that P. helvina, but not C. pireta, flew at or below mid-frame. This observation suggests that P. helvina flew within the estimated range of ground effect.

Comparisons Between P. helvina and C. pireta

Flight comparisons from different environments revealed potential laboratory effects on gliding. Although field and lab flights did not use the same individuals, Fig. 4 suggests that butterflies of both species generally spent more time gliding over total flight time in the field than in the lab. As all butterflies were handled in the same way, this can be interpreted as an effect of being flown in an enclosure and responding to a startle stimulus (see Methods).

All butterflies flown in the lab took off from the ground and ascended towards a source of natural light. As dictated by the wingspan of each individual, we assumed they did not utilize ground effect because they flew above the critical height from the ground. Nonetheless, we detected differences in gliding flight performance between species and sexes. Male P. helvina with intact wings showed significantly longer glide durations than male C. pireta, but the range of individual variation was broader in P. helvina (Table 2). Although the proportion of gliding over the total flight time was higher for male P. helvina than C. pireta, the difference was not significant (Table 2). Finally, male P. helvina and C. pireta differed in wing-beat frequencies but not in wing-beats over total flight time. We did not detect differences between species in male glide durations, proportions of gliding over total flight time, wing-beat frequencies or wing-beats over total flight time (Table 2).

Effects of Hind Wing Reduction on Flight Performance

After hind wing area reduction, both sexes of P. helvina showed an overall decline in gliding ability (Fig. 4b), but only males had a significant decrease in the ratio of gliding to total flight time (Table 3). Females exhibited a significant increase in wing-beat frequency, and while both sexes had a greater number of wing-beats per total flight time, this difference was not significant (Table 3). Kinematic analysis of lab-flown P. helvina indicated that all butterflies reached a stable forward velocity within two wing-beats from takeoff (Fig. 5). This led us to partition forward velocity into two components: the distance traveled after the first wing-beat divided by the time interval of that wing-beat (escape velocity; x,y,z positional data), and the stable rate of horizontal, forward motion during the third to fifth wing-beats (cruising velocity; x,y positional data). We found that escape velocity decreased in both sexes after hind wing area reduction, but the effect was strongest in females (Table 3). In contrast, cruising velocity was not affected by hind wing reduction in either sex. In sum, hind wing reduction in male P. helvina significantly diminished gliding flight performance, but in females, it produced both an increase in wing-beat frequency and a decrease in escape velocity (Table 3, Figs. 4b and 5).

Discussion

Effects of Forewing Aspect Ratio and Wing Allometry on Gliding Flight

High forewing aspect ratio promotes energy conservation during gliding flight by reducing induced and thus overall drag (Dudley 2000, Ancel et al. 2016). The study of Haeterini by Cespedes et al.
Table 2. Comparison of flight performance between *P. helvina* (intact wings) and *C. pireta* in the field and laboratory

<table>
<thead>
<tr>
<th>Field</th>
<th><em>P. helvina</em> (16)</th>
<th><em>C. pireta</em> (16)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glide duration (ms)(^a)</td>
<td>96 (17–396)</td>
<td>63 (21–179)</td>
<td>1.90E-09</td>
</tr>
<tr>
<td>Gliding/total flight time</td>
<td>0.56 (0.36–0.70)</td>
<td>0.28 (0.17–0.48)</td>
<td>3.20E-07</td>
</tr>
<tr>
<td>Wing-beat frequency(^b)</td>
<td>15.9 (12.7–24.3)</td>
<td>12.9 (11.8–15.7)</td>
<td>2.87E-05</td>
</tr>
<tr>
<td>Wing-beats/total flight time(^c)</td>
<td>7.5 (5.45–9.02)</td>
<td>9.0 (7.58–10.37)</td>
<td>1.50E-05</td>
</tr>
</tbody>
</table>

Laboratory

| | Males | | Females | | |
| | *P. helvina* (7) | *C. pireta* (6) | P-values | *P. helvina* (7) | *C. pireta* (3) | P-values |
| Glide duration (ms)\(^a\) | 67 (8–166) | 50 (21–121) | 0.034 | 29 (4–171) | 42 (17–88) | 0.332 |
| Gliding/total flight time | 0.35 (0.11–0.38) | 0.18 (0.11–0.25) | 0.073 | 0.16 (0.04–0.25) | 0.14 (0.11–0.19) | 0.833 |
| Wing-beat frequency\(^b\) | 13.0 (12.3–14.6) | 11.1 (10.1–13.1) | 0.022 | 11.0 (10.1–13.1) | 11.5 (11.0–14.2) | 0.305 |
| Wing-beats/total flight time\(^c\) | 8.37 (7.93–11.94) | 8.74 (8.27–11.71) | 0.731 | 10.45 (8.06–11.00) | 9.53 (9.36–12.61) | 0.667 |

Sample sizes are in parentheses following species names. Values are sample medians followed by ranges in parentheses. Kruskal–Wallis tests were used for comparisons of glide duration and wing-beat frequency between groups, as these data failed both tests of normality (Shapiro-Wilks) and homoscedasticity (Levene’s Test). Mann–Whitney U tests between sample distributions were used for all other comparisons. Significant differences are in bold type.

\(^a\)Glide duration (milliseconds) reflects all gliding events across all flights of all individuals pooled for a particular sample.

\(^b\)Measured in wing-beats/second.

\(^c\)Wing-beats/total flight time is the amount of flapping per second of a recorded flight time for an individual, and reflects its flight energy expenditure.

(2014) suggested that species in which males glide near the ground to patrol territories have a higher forewing aspect ratio than those that use mainly flapping flight (e.g., *Dulcedo d’Almeida*, 1951). That study used a polygon derived from landmarks as a proxy for aspect ratio, but here we used the forewing outline to measure aspect ratio directly. This allowed us to demonstrate that the forewings of our focal species have a high aspect ratio in both sexes, but most importantly, we showed that the higher aspect ratio of male *P. helvina* is associated with increased gliding performance (Tables 1–3). Intact male *P. helvina* showed longer glide durations than male *C. pireta* in the field and lab, and significantly greater proportions of gliding over total flight time in the field (Table 2). In contrast, females of these species had similar forewing aspect ratios and did not differ with regard to either of these parameters. Although female *Pierella Herrich-Schaffer*, 1865 and *Cithaerias Hübner*, 1819 also utilize gliding flight, it is likely that this behavior is employed more often by patrolling males than by females. Together, our analyses of flights in the field and lab appear to support the hypothesis that the proportions of gliding versus flapping flight in Haeterini can be predicted by forewing aspect ratio alone.

Although male *P. helvina* have a higher forewing aspect ratio than male *C. pireta*, they also possess proportionately larger hind wings (Table 1). As such, the aerodynamic gain from a high forewing aspect ratio could potentially be offset by the increased drag and additional mass of an enlarged hind wing in *Pierella*. By video-taping the same *P. helvina* individuals with intact and experimentally reduced wings, we were able to assess the effect of hind wing area on gliding performance. We showed that reduced hind wing area resulted in both shorter glide durations and decreased proportions of gliding over total flight time, particularly in males (Fig. 4b, Table 3). Thus, our experimental results strongly suggest that in *P. helvina* gliding performance is enhanced by increased lift production due to their large hind wing area. Wing allometry is genetically determined (Frankino et al. 2007), and independent evolution of butterfly forewings and hind wings can lead to sexual dimorphism in shape (Chazot et al. 2016, Hegedus...
Table 3. Comparisons of *P. helvina* flight performance with intact (Full-HW) and reduced hind wings (Cut-HW)

<table>
<thead>
<tr>
<th></th>
<th>Males (7)</th>
<th>Females (7)</th>
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<tbody>
<tr>
<td></td>
<td>Full-HW (Cut-HW)</td>
<td>P-value</td>
</tr>
<tr>
<td>Glide duration (ms)</td>
<td>67 (8–166) 48 (4–100)</td>
<td>3.85E-04</td>
</tr>
<tr>
<td>Gliding / total flight time</td>
<td>0.35 (0.11–0.38) 0.13 (0.04–0.24)</td>
<td>0.026</td>
</tr>
<tr>
<td>Wing-beats / total flight time</td>
<td>8.37 (7.93–11.94) 11.58 (8.91–13.85)</td>
<td>0.073</td>
</tr>
<tr>
<td>Wing-beat frequency (hz)</td>
<td>13.0 (12.3–14.6) 12.2 (10.3–14.6)</td>
<td>0.406</td>
</tr>
<tr>
<td>Escape Velocity (m/s)</td>
<td>0.40 (0.28–0.59) 0.39 (0.08–0.66)</td>
<td>0.437</td>
</tr>
<tr>
<td>Cruising Velocity (m/s)</td>
<td>1.1 (0.68–1.91) 1.08 (0.69–1.76)</td>
<td>0.503</td>
</tr>
</tbody>
</table>

Sample sizes of sexes are in parentheses. Values are sample medians followed by ranges in parentheses. Kruskal–Wallis tests were used for comparisons of glide duration and wing-beat frequency between groups, as these data failed both tests of normality (Shapiro–Wilks) and homoscedasticity (Levene’s Test). Mann–Whitney U tests between sample distributions were used for all other comparisons.

Glide duration (milliseconds) reflects all gliding events across all flights of all individuals pooled for a particular sample. Individuals that did not glide were recorded as a single glide duration of 0 ms.

Wing-beats/total flight time is the amount of flapping per second of a recorded flight time for an individual, and reflects its flight energy expenditure.

Our analyses suggest that male *P. helvina* evolved proportionately larger hind wings relative to conspecific females as a response to the gliding flight demands of their patrolling behavior (Table 1). We acknowledge that the area reduction employed here also changed hind wing shape, but we did not assess how this could have affected gliding flight.

Flapping Flight Performance in *P. helvina* and *C. pireta*

Flapping flight was evaluated by estimating wing-beat frequencies and the number of wing-beats over recorded flight time. Intact male *P. helvina* had higher wing-beat frequencies than male *C. pireta* in the field and lab (Table 2, Fig. 4). The relatively larger thoracic mass
of *P. belvina* likely allows higher wing-beat frequencies (e.g., Betts and Woottton 1988), suggesting that male *P. belvina* might be capable of generating the momentum and power required to sustain longer periods of gliding than *C. pireta*. Although this hypothesis will require future investigation, to our knowledge the effect of thoracic mass on the proportion between flapping and gliding during continuous flight has not been critically examined for any butterfly species. Finally, females of both species showed similar wing-beat frequencies and glide durations in the lab (Table 2), implying that the proportion of flapping versus gliding during flight has a sex-specific component. This is consistent with abundant field observations indicating that sex-related differences in flight behavior (e.g., male patrolling, female oviposition) can readily be determined by eye (P.J.D. and C.M.P., personal observation).

Kinematic analysis showed that hind wing reduction in *P. belvina* decreased escape velocity, and that this effect was strongest in females (Table 3). Insect flight requires the production of both vertical lift and of nose-down torque to avoid stalling at low speeds (Ellington 1999, Dudley 2000). Since increased abdominal mass due to egg load can affect flight in female butterflies (Karlsson and Wickman 1990, Berwaerts et al. 2002, Almbro and Kullberg 2007), it is not surprising that reduction of hind wing area diminished female escape velocity (Table 3). This implies that hind wings play a role in lift production during takeoff and that the increased wing-beat frequency after hind wing reduction could represent a behavioral compensation for diminished lift. In female bumblebees, hind wing removal decreased wing-beat frequency and capacity for maximum total force production during takeoff (Buchwald and Dudley 2010). We found that hind wing reduction in *P. helvina* increased wing-beat frequency during takeoff, but once airborne, relative hind wing area had little effect on their cruising velocity (Table 3). This suggests that the role of butterfly hind wings in flapping flight might be more important for takeoff than for sustained flight. We did not, however, assess the flight effects of hind wing removal in *P. belvina*.

### Gliding in Ground Effect

High-speed videography of *P. belvina* and *C. pireta* in the field supported the hypothesis of Cespedes et al. (2014) that these butterflies utilize the drag-reducing properties of ground effect to glide above the rainforest floor. Our study also assessed natural variation in the range of flight altitudes between species. In the field, *P. belvina* rarely ascended 20 cm above the forest floor, while the flight height of *C. pireta* ranged up to 1 m above the ground (personal observation, see also Alexander and DeVries 2012). These observations suggest that *P. belvina* glides more often within the range of ground effect than *C. pireta*, and could contribute to the observed differences in gliding time between species (Table 2). Finally, mark-release-recapture data suggest that *P. belvina* moves substantially greater distances on a daily basis than other sympatric Haeterini (Alexander and DeVries, unpublished data), a behavioral outcome that could be facilitated by gliding in ground effect.

### Concluding Remarks

This is the first study to evaluate the aerodynamic role of hind wings in butterfly species that regularly employ gliding flight. We demonstrated that although forewing aspect ratio was a reliable predictor of flight behavior in our focal species, experimental hind wing reduction had a significant effect on gliding flight performance. Within Haeterini, a tighter association with the forest floor and greater use of ground effect likely explains the large hind wing area of *Pierella* as compared to *Cithaerias*. We also provide evidence that enlarged hind wings aid in the takeoff flight of *P. belvina*, and hypothesize that the sex-specific effect of hind wing area reduction is due to larger abdominal mass of females (egg loading). Future work on flight-associated morphology in butterflies should include hind wing area as an additional factor that influences flight performance.

### Supplementary Data

Supplementary data are available at Annals of the Entomological Society of America online.

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