

Asymmetry costs: Effects of wing damage on hovering flight performance in the hawkmoth
Manduca sexta

María José Fernández^{1*}, M. Ellis Driver¹ and Tyson L. Hedrick¹

¹Department of Biology, University of North Carolina, Chapel Hill, North Carolina
thedrick@bio.unc.edu

*Current address: Department of Life Sciences, University of Roehampton, London, SW15 4JD

Abstract

Flight performance is fundamental to the fitness of flying organisms. Whilst airborne, flying organisms face unavoidable wing wear and wing area loss. Many studies have tried to quantify consequences of wing area loss to flight performance with varied results; suggesting that not all types of damage are equal and different species may have different means compensating for some forms of wing damage with little to no cost. Here, we investigate the cost of control during hovering flight with damaged wings, specifically wings with asymmetric and symmetric reductions in area, by measuring maximum load lifting capacity and the metabolic power of hovering flight in hawkmoths (*Manduca sexta*).

We found that while asymmetric and symmetric reductions are both costly in terms of maximum load lifting and hovering efficiency, asymmetric reductions are approximately twice as costly in terms of wing area lost. The moths also did not modulate flapping frequency and amplitude as predicted by a hovering flight model, suggesting that the ability to do so, possibly tied to asynchronous versus synchronous flight muscles, underlies the varied responses found in different wing clipping experiments.

Key words:

asymmetry, metabolism, stability, wing wear, respirometry

Introduction

Flying organisms face unavoidable wing wear and wing area loss due to collisions with the environment, competition and even predation. Losing wing area may have negative consequences in organisms' flight performance; these negative consequences may be exacerbated by bilateral asymmetries in the area loss. Here we investigate the costs to maximum load lifting capacity and flight efficiency (here overall lift to power ratio) brought about by asymmetric and symmetric wing damage.

Asymmetric wing damage results in a stability challenge as well as a reduction in aerodynamic efficiency and maximum lift force because if the wings are asymmetric, symmetric wing kinematics no longer produce symmetric forces and torques. Therefore, the animal must use asymmetric wing kinematics to produce symmetric forces and torques, potentially increasing the difficulty and cost of compensating for the wing damage. Nevertheless, animals with asymmetric wing damage are able to maintain flight stability and perform complex manoeuvres (Haas and Cartar, 2008), suggesting that insects may possess capabilities for reducing the costs of wing asymmetry.

Animals are able to maintain stability when perturbed using active neural mechanisms, passive non-neural mechanisms or a combination of both (Dickinson et al., 2000; Nishikawa et al., 2007). For example, studies of flapping flight show that organisms are able to overcome some types of perturbation with minimal neural input through the intrinsic stabilizing effects of flapping (Hedrick et al., 2009; Ristroph et al., 2010). On the other hand, organisms also use active neural modulation to perform voluntary manoeuvres, like yaw turns in aerial locomotion (Springthorpe et al., 2012). Moreover, Fernández et al. (Fernández et al., 2012) showed that hawkmoths with asymmetric wings control and maintain stability during hovering through neural modulation of muscle activity once the amount of wing area loss exceeds ~10%. In this case, the moths have to constantly steer to counteract the torque produced by the wing asymmetry. The constant manoeuvring required of the neuromuscular system with asymmetric wings may be costly to other aspects of flight performance such as maximum lift production, where some aerodynamic force will be assigned to flight control rather than weight support. Furthermore it

will affect the animal's metabolic power requirement, since some additional flight force beyond that required to support the body must be generated and devoted to steering.

Many studies have tried to quantify consequences of wing area loss to flight performance. However they have produced disparate results; suggesting that not all types of damage are equal and different species may have different means compensating for some forms of wing damage with little to no cost. For example, a study of metabolic power expenditure in hovering hummingbirds found no costs associated with natural reductions in wing area during moulting (Chai, 1997). Similarly, Hedenström, et al. (Hedenström et al., 2001) showed no increase in metabolic power when artificially reducing wing area in bumblebees. The same outcome has also been found in bats with naturally asymmetric wings (Voigt, 2013) and birds were found to suffer more from symmetric than asymmetric damage of similar total magnitude (Hambly et al., 2004). Furthermore, studies of insects have found that individuals with reduced wing area had fitness similar to that of individuals without damaged wings (e.g. butterflies (Kingsolver, 1999) and bumblebees (Haas and Cartar, 2008)). However, other studies have shown decreases in various measures of flight performance following natural and artificial wing damage in birds (Swaddle, 1997) and insects (Combes et al., 2010; Vance and Roberts, 2014; Mountcastle et al, 2016).

Here we study the cost of hovering flight with damaged wings, specifically wings with asymmetric and symmetric reductions in area. We investigate the cost of control during hovering flight with asymmetric wings by measuring maximum load lifting capabilities and the metabolic power of flight through respirometry (oxygen consumption and carbon dioxide production) in hawkmoths (*Manduca sexta*).

Classic flapping flight aerodynamic models provide a predictive framework for examining the effects of wing area loss on flight performance using the 2nd and 3rd moments of wing area, which weigh the aerodynamic importance of wing area by its distance from the wing root or base (Ellington, 1984b; Weis-Fogh, 1973). From these models, we hypothesize that the maximum load lifted by the moth decreases proportional to the cube root of the fractional change in 2nd moment of wing area, while the metabolic cost of hovering flight will increase in proportion to the decrease in the lift to power ratio; the hypothesized increase in cost is in proportion to the reduction in the 3rd moment of wing area raised to the -0.5 power. Alternatively, if the moths are not able to adjust their flapping frequency or amplitude, maximum

load lifted is predicted to decline in linear proportion to the decrease in the 2nd moment of wing area. In all cases, we predict that asymmetric wing damage will lead to reduced performance compared to symmetric damage of similar magnitude, as has been found previously in flying birds (Balmford et al., 1993; Swaddle, 1997). Please see the aerodynamic model section below for the equations and more details about the models.

Materials and Methods

Animals and experimental design

We obtained male *Manduca sexta* (Linnaeus 1763) pupae from the Department of Biology at Duke University. After eclosion adult moths were housed in mesh fabric cages (30 x 30 x 30 cm) at $25 \pm 3^\circ\text{C}$ under a 22:2 hours light:dark photoperiod to minimise activity and avoid unintended wing damage. We trained moths, after their third day of eclosion, by presenting them with a natural and artificial flower to stimulate their feeding behaviour and therefore elicit stable hovering flight. Moths became experimental candidates after demonstrating prolonged and stable hovering flight in front of the flower. We measured asymptotical load-lifting and respirometry (oxygen consumption and carbon dioxide production; see below for details) in moths exposed to three experimental conditions (Fig. 1): (i) fully intact wings (unclipped), (ii) asymmetric wing clipping (one forewing clipped; side randomly chosen), and (iii) approximately symmetric wing clipping (both forewings similarly clipped). To perform all treatments in each individual, we applied the treatments in order, randomly varying which wing received the damage in the asymmetric treatment. We clipped each individual forewing tip, from the trailing edge to the leading edge, with scissors (Fig. 1), resulting in a total wing area reduction between 7 to 24%. These area reductions are similar in magnitude to what is found in nature in wild bumblebees (Cartar, 1992). Wing clipping typically caused the moths to attempt to seek shelter and not respond to the food and flower stimulus used to elicit flight behaviour. Thus, moths were given 12 – 24 hours to recover from clipping before they were used in the next assay. Moths were housed in the aforementioned mesh cages and given access to water *ad libitum* during this time.

Asymptotical load-lifting

We performed asymptotical load-lifting to quantify maximum vertical aerodynamic force production (Buchwald and Dudley, 2010; Chai et al., 1997; Dillon and Dudley, 2004). Briefly, we attached a rubber band with a 2 cm loop of string near the moth's centre of body mass (between the thorax and abdomen). A beaded string with 7 to 9 beads at 2 cm intervals (0.2 ± 0.0001 grams each; Adventurer Pro, OHAUS Corporation, USA) was then attached 3 cm below the loop. We placed the trained moth in the bottom of a flight chamber ($0.7 \times 0.7 \times 0.7 \text{ m}^3$) illuminated with eight infrared lights (760 nm, Roithner LaserTechnik GmbH, Austria). After placing the individuals in the bottom of the chamber they began to fly upwards, progressively lifting more beads off the ground until reaching their load-carrying capacity. To encourage the moths to fly vertically and hover stably with a maximum load we used a flower stimulus. Load lifting trials were recorded with high-speed video and typically lasted less than 10 seconds. We discarded recordings where the individual did not fly vertically or its proboscis contacted the flower.

Each individual was exposed to three experimental treatments (as mentioned above), unclipped, asymmetric wing clipping, and symmetric wing clipping. During the load-lifting trials we removed between 7 – 23% of the area of a single wing, which corresponded to a 17 - 49% decrease in second moment of area, when clipping the wings for the asymmetric clipping treatment (see the electronic supplementary material, Table S1).

We calculated maximum load by adding the mass of all beads lifted and the moth mass. After each treatment we measured the individuals' body mass. Maximum vertical force (F_{Vmax}) was then calculated as the product of the maximum load and gravitational acceleration. During load-lifting, flight kinematics were recorded at 1,000 Hz using three orthogonally positioned high-speed cameras (two Phantom v7.1 and one Phantom v5.1; Vision Research, Wayne, NJ). From the videos we extracted wing stroke amplitude (θ) and wingbeat frequency (η) for each treatment through a 3-D kinematic reconstruction (DLTdv5 (Hedrick, 2008), see the electronic supplementary material, Table S2).

After the last treatment, individuals were frozen for wing preservation. Clipped forewings and their fragments were later placed together and scanned for morphological measurements (see Table S1). We investigated the effect of symmetric and asymmetric wing damage (as fixed effects) on maximum vertical aerodynamic force production (F_{Vmax}) and wing

kinematics (η and θ) using a linear mixed-effects model [nlme package of R 3.3.1 (Pinheiro and Bates, 2000; Pinheiro et al., 2014; R Development Core Team, 2016)] with random intercepts for each individual (Pinheiro and Bates, 2000).

We also categorised the magnitude of the wing clipping to further examine the effect of wing area loss on maximum vertical force. A small area clipped was $< 12\%$ of wing area which is similar to what is found in nature in wild bumblebees (Cartar, 1992), while a larger area clipped was $> 12\%$ (maximum of 23%); around an 18% decrease in wing area is the upper extreme of wear normally found in nature (Cartar, 1992). We chose those magnitudes as it has been shown that individuals with 10% or less of wing area loss are more likely to use a passive mechanism to maintain flight stability while individuals with more than 10% wing damage are more likely to use an active mechanism suggesting a more costly flight performance (Fernández et al., 2012); we used the 12% dividing line to split our dataset in half near this previously supported point. To investigate the magnitude of the clipping we used a subset of the data (asymmetric and symmetric clipping only) in a linear mixed-effects model [nlme package of R 3.3.1 (Pinheiro and Bates, 2000; Pinheiro et al., 2014; R Development Core Team, 2016)] of the relationship between maximum vertical aerodynamic force production and clipping treatments.

Respirometry

We determined moth metabolic rate during the above mentioned three experimental treatments to quantify the cost of hovering with asymmetric and symmetric wings. The measured volume of O_2 consumption and CO_2 production was divided by the time the moth spent hovering in a sealed Plexiglas chamber (9.4 litres) to obtain the individuals metabolic rate (see the electronic supplementary material, Table S3). Measurements were done at room temperature $23 \pm 2^\circ C$.

Immediately following a warm-up flight moths were placed in a cylindrical enclosure of 20 cm in diameter and 30 cm in height (Fig. 2). Moths flew between 3 and 12 min, measured with a chronometer. Once the moth stopped flying we took a 50 ml sample of air from the flight chamber with a large syringe (60 ml). We injected the gas sample through a three-way valve to the FOXBOX oxygen and carbon dioxide analyser (Sable Systems International, Las Vegas, NV, USA) passing through a column of desiccant (Drierite, Xenia, OH, USA) for the removal of water vapour. This method allowed us to obtain CO_2 production and measured O_2 consumption in moths, therefore enabling us to calculate the respiratory quotient, RQ (i.e. the ratio of $\dot{V}CO_2$ to

$\dot{V}O_2$) and allowing us to know the fuel that moths were using. As such our methodology was preferable to the more conventional flow-through respirometry approach which, with the equipment available here, would not have permitted O_2 measurement in the large flight volume.

During the analysis we only considered trials that lasted more than 2 minutes with the moth flying continuously in the centre of the chamber. For calculation of the flight efficiency among treatments, we used the ratio between the predicted mechanical power, using Eqn. 1, 2 and Eqn. 5, and the metabolic power calculated from the raw O_2 consumption data (see aerodynamic model section for equations details).

After the last treatment, as we did with individuals used during the asymptotical load-lifting, individuals were frozen for wing preservation and clipped forewings were later reconstructed and scanned for morphological measurements (see Table S1). Note that the set of individuals used for the respirometry experiments ($n = 6$) differs from the set used for load lifting ($n = 8$) and that the respirometry rig and recording duration precluded use of high speed video to measure wing kinematics.

We investigated the effect of treatment (symmetric and asymmetric wing clipping), as fixed effects, on metabolic power, $\dot{V}O_2$ consumption, $\dot{V}CO_2$ production, and the respiratory quotient RQ using a linear mixed-effects model [nlme package of R 3.3.1 (Pinheiro and Bates, 2000; Pinheiro et al., 2014; R Development Core Team, 2016)] with random intercepts for each individual (Pinheiro and Bates, 2000).

Aerodynamic model

Following Ellington (Ellington, 1984b), the lift from a flapping wing with zero forward velocity, i.e. when hovering, may be modelled as:

$$L = \frac{1}{8} \rho S_2 n^2 \phi^2 \overline{C_l} \overline{(d\hat{\phi}/d\hat{t})^2} \quad \text{Eqn. 1}$$

where ρ is air density, S_2 is the second moment of wing area, n is the flapping frequency, ϕ is the flapping amplitude, $\overline{C_l}$ is the average coefficient of lift and $\overline{(d\hat{\phi}/d\hat{t})^2}$ is the average square of the non-dimensional angular velocity. The mechanical power required for flapping is given by:

$$P_{mech} = \frac{1}{16} \rho S_3 n^3 \phi^3 \overline{C_d} \overline{|d\hat{\phi}/d\hat{t}|^3} \quad \text{Eqn. 2}$$

where S_3 is the 3rd moment of wing area, $\overline{C_d}$ is the average coefficient of drag and $\overline{|d\hat{\phi}/d\hat{t}|^3}$ is the average of the absolute value of the cube of the non-dimensional angular velocity of flapping. A reduction in wing area will also reduce the 2nd and 3rd moments of wing area. Thus, if all other parameters remain unchanged, a moth with reduced wing area will produce less lift and require less muscle power to flap its wings. In this case the lift following clipping, L' , is given by:

$$L' = L \left(\frac{S_2'}{S_2} \right) \quad \text{Eqn. 3}$$

where S_2' is the 2nd moment of wing area following clipping. Note that in this case the power required to flap the wings also decreases even though clipping the wing tip is not expected to reduce the available muscle power. If the moth were to increase its flapping amplitude or frequency such that power output before and after clipping were identical, lift following clipping, L' , is equal to:

$$L' = L \left(\frac{S_2'}{S_2} \right) \left(\frac{S_3'}{S_3} \right)^{-2/3} \quad \text{Eqn. 4}$$

where S_3' is the 3rd moment of wing area following clipping. This equation allows prediction of the effect of clipping on maximum load lifted, assuming that load lifted is limited by muscle power before and after clipping.

An increase in flapping frequency or amplitude increases both lift (Eqn. 1) and mechanical power (Eqn. 2) but alters their ratio; increases in frequency or amplitude decrease efficiency (i.e. lift to power ratio). This forms the basis for a prediction of the effect of wing clipping on the energetic cost of hovering flight. If L' is to be maintained at its original value as in a hovering moth supporting its own weight, then P'_{mech} , the mechanical power of hovering following wing clipping and compensation by changing flapping frequency or amplitude, is given by:

$$P'_{mech} = P_{mech} \frac{S_3'}{S_3} \left(\frac{S_2'}{S_2} \right)^{-3/2} \quad \text{Eqn. 5}$$

If the fractional reductions in 2nd and 3rd moment of wing area are assumed to be equal, and they are often similar in practice, then Eqns. 4 and 5 reduce to:

$$L' \cong L \left(\frac{S_2'}{S_2} \right)^{1/3} \quad \text{Eqn. 6}$$

$$P'_{mech} \cong P_{mech} \left(\frac{S_3'}{S_3} \right)^{-1/2} \quad \text{Eqn. 7}$$

To estimate the mechanical power required for hovering under the three experimental conditions, we solved Eqn. 1 for the flapping frequency η required to support body weight, assuming ϕ of 115 degrees, ρ of 1.22 kg m^{-3} , \bar{C}_l of 1.4 and $\overline{(d\hat{\phi}/d\hat{t})^2}$ of 19.74 characteristic of sinusoidal motion (Ellington, 1984a; see Appendix A) then used the resulting η with Eqn. 2 to estimate power, with \bar{C}_d of 1.6 and $|d\hat{\phi}/d\hat{t}|^3$ of 105.29 (i.e. sinusoidal flapping; see Appendix A). These results were then adjusted for clipping using Eqn. 5. Note that other compensatory mechanisms exist such as changes in \bar{C}_l and none of the aerodynamic models can accommodate the effect of wing asymmetry. In calculating the expected L' and P'_{mech} for asymmetric moths we used the total wing area and moments, summing the clipped and unclipped wings. Thus, if asymmetry itself has a cost, the models would tend to underestimate the effect of asymmetric area loss on performance. For these reasons we expect the models to furnish no more than a general prediction of effect magnitude and direction rather than exact predictions of changes in maximum load or flight efficiency.

The above equations do not include any costs for the inertial power requirements of flapping because the degree to which these costs are additive with aerodynamic costs and not recovered elastically or by conversion to aerodynamic forces is unknown. Current models of flight power commonly bracket the possible range of outputs by considering cases with 0% and 100% of inertial costs included in the final result, e.g. Cheng et al. (2016). However, comparison of recent detailed computational fluids simulations of hawkmoth flight with costs measured by respirometry suggest that hawkmoths are likely closer to the 0% than 100% case (Zheng et al., 2013). Thus, we do not include inertial costs in the above analysis but do discuss them below.

Following work by Ellington (1984b), the inertial cost of flapping is given by

$$P_{inertial} = 2nm_2(d\phi/dt)_{max}^2 \quad \text{Eqn. 8}$$

where m_2 is the 2nd moment of wing mass and is proportional to the second moment of wing area for a wing of constant density and thickness. The inertial power required for the virtual mass, i.e. fluid accelerated with the wing, is also proportional to the 2nd moment of area. In both the actual and virtual mass cases, if flapping frequency is increased to maintain lift following wing damage, the inertial power requirement as a whole increases such that:

$$P'_{inertial} \cong P_{inertial} \left(\frac{S_2'}{S_2}\right)^{-1/2} \quad \text{Eqn. 9}$$

assuming that the wing is of constant thickness and density in the actual mass case. Real hawkmoth wings are thicker toward the root by a degree difficult to include in analytic expressions such as Eqn. 9 and this tendency will make $P'_{inertial}$ due to actual mass greater than predicted by Eqn. 9. However, in general, this analysis suggests that for the flight efficiency case inertial power and aerodynamic power vary similarly.

This is also true for the case where flapping frequency is increased to restore mechanical power output following clipping. Here $P'_{inertial} \cong P_{inertial}$ given the aforementioned assumption of constant thickness and density as well as the assumption equal reduction in 2nd and 3rd moment of wing area as was used in Eqn. 6 and 7. Thus, at the level of detail we believe simple models such as these capture, our model and predictions are not influenced by whether or not inertial power is included in the metabolic or muscle costs.

Results

Asymptotical load-lifting

Overall mean and standard error values for maximum vertical aerodynamic force production (F_{Vmax}), and wingbeat kinematics (η and θ) are given in Table 1 for each experimental treatment and also separated by small and large area clipped in Table 2.

As expected wingbeat frequency (η) increased following wing clipping. The linear mixed-effects model with random effects based on individual moths, showed that wingbeat frequency was significantly different between the unclipped and symmetric clipping treatments (lme, $P = 0.036$, $n = 8$, Table 3). However, the unclipped and asymmetric treatments did not differ nor was the asymmetric treatment different from the symmetric wing clipping (lme, $P = 0.15$ and $P = 0.42$ respectively, $n = 8$, Table 3).

In the case of wing stroke amplitude (θ) the linear mixed-effects model showed that the asymmetric treatment was significantly different from the symmetric clipping treatment (lme, $P = 0.02$; Table 3). The model also showed a marginal significant difference between the unclipped and the symmetric wing clipping treatments (lme, $P = 0.05$, $n = 8$, Table 3). However, we found no significant difference between the asymmetric and unclipped treatments (lme, $P = 0.64$, $n = 8$, Table 3).

As expected, the reduction in wing 2nd moment of area was associated with a decrease in maximum vertical aerodynamic force production. We found that F_{Vmax} decreased significantly between the unclipped and clipping treatments (lme, $P < 0.001$, $n = 8$, Table 1, Fig. 3). However, the asymmetric clipping was not significantly different from the symmetric treatment (lme, $P = 0.39$, $n = 8$, Table 3).

We also found that F_{Vmax} decreased more than expected as per Eqn. 3 when clipping wings asymmetrically (lm, $F_{Vmax} = 1.32 * \Delta 2^{nd}$ moment of area, $r^2 = 0.56$; Fig. 3). In contrast, clipping wings symmetrically showed a decrease in F_{Vmax} , but had a smaller effect than asymmetric clipping as indicated by the differences in the $F_{Vmax}/\text{wing } 2^{nd}$ moment of area slope for the two treatments (lm, $F_{Vmax} = 0.78 * \Delta 2^{nd}$ moment of area, $r^2 = 0.77$). In addition, the interaction between both factors, treatment and $\Delta 2^{nd}$ moment of area, was significant (lm, $P = 0.02$ $n = 8$, Fig. 3). To further examine the effect of wing area loss on maximum vertical force we categorised the magnitude of the wing clipping. We found a significant reduction in F_{Vmax} depending on the clipping magnitude. When clipping a larger area of the wing we found a significant decrease in force in the asymmetric treatment compared to unclipped (lme, $P = 0.0001$, $n = 4$, Table 2; Fig. 4), indeed a further decrease from what is expected from the aerodynamic model ($F_{Vmax} \propto 2^{nd}$ moment of area, Eqn. 4). The symmetric treatment also produced a significant decrease in F_{Vmax} (lme, $P = 0.0001$, $n = 4$), but there was no significant difference between the asymmetric and symmetric treatments for large clipping group (lme, $P = 0.49$, $n = 4$). In comparison, when we clipped only a small percentage of the wing, we found an approximately linear reduction of maximum vertical force production in the asymmetric treatment (lme, $P = 0.13$, $n = 4$) and a significant further decrease in force when clipping wings symmetrically compared to the unclipped treatment (lme, $P = 0.02$, $n = 4$, Fig. 4). Similar to the large clipping magnitude we did not find a significant difference between the asymmetric and symmetric treatments (lme, $P = 0.30$, $n = 4$, Table 2).

Respirometry and flight efficiency

Overall mean and standard error values for metabolic power (P_{met}), mass-specific carbon dioxide production ($\dot{V}CO_2$), mass-specific oxygen consumption ($\dot{V}O_2$), respiratory quotient (RQ) and the predicted mechanical power (P_{mech}) are given in Table 4 for each experimental treatment.

Metabolic power was affected by the asymmetric and symmetric treatments, clipped moths exhibited greater metabolic power compared to moths with unclipped wings. The linear mixed-effects model showed a significant difference between the unclipped and the clipping treatments (lme, $P = 0.005$; $n = 6$; Table 5; Fig. 5). However, there was no significant difference between asymmetric and symmetric treatments (lme, $P = 0.98$; $n = 6$; Table 5; Fig. 5).

Mass-specific $\dot{V}O_2$ and $\dot{V}CO_2$ increased with clipping treatments. When looking at mass-specific $\dot{V}O_2$ the linear mixed-effects model showed a significant difference between the unclipped and clipping treatments (lme, $P = 0.008$ for asymmetric and $P = 0.007$ for symmetric treatment; $n = 6$; Table 5) but no significant difference between both clipping treatments (lme, $P = 0.93$; $n = 6$; Table 5). Identical patterns of significance were found for mass-specific $\dot{V}CO_2$ (lme, $P = 0.0005$ between unclipped and asymmetric treatments; $P = 0.002$ between unclipped and symmetric treatments and $P = 0.42$ between clipping treatments; $n = 6$; Table 5).

The ratio of $\dot{V}CO_2$ to $\dot{V}O_2$ varied among moths and there was a trend toward higher RQ values in the clipped-wing moths. The linear mixed-effects model showed a significant difference between the unclipped and asymmetric clipping treatment (lme, $P = 0.022$; $n = 6$; Table 5) but no significant difference between unclipped and symmetric clipping (lme, $P = 0.414$; $n = 6$) and both clipping treatments (lme, $P = 0.094$; $n = 6$; Table 5). The average RQ among all moths and treatments was 0.84 with a standard error of 0.017 (Table 4).

Mechanical power (P_{mech}) was estimated using Eqn. 1, Eqn. 2 and Eqn. 5 to look at the effects of wing clipping on efficiency (in this case the ratio between the estimated mechanical power and the measured metabolic power). We found that estimated P_{mech} increased with wing clipping (Table 4). The linear mixed-effects model showed a significant difference between the unclipped and both clipping treatments (lme, $P = 0.003$ for asymmetric and $P < 0.001$ for symmetric treatment; $n = 6$; Table 5) and also between moths with asymmetrically clipped wings and symmetrically clipped wings (lme, $P = 0.006$; $n = 6$; Table 5). Note that this P_{mech} estimate from our simplified aerodynamic model does not indicate that P_{mech} actually varied in the way described, the model cannot estimate any additional costs due to asymmetry. Instead it shows that the three treatments (unclipped, asymmetric clip and symmetric clip) produce enough variation in expected power requirements that they should be distinguishable even given the small sample size, variation in morphology and variation in clipping treatment magnitude. Finally, the model operates by computing a flapping frequency for each specific moth from the

morphological data and aerodynamic assumptions; these were 26.4 ± 1.4 , 28.2 ± 2.5 , 31.1 ± 1.4 Hz (mean \pm s.d., $n = 6$) for the unclipped, asymmetric and symmetric treatments respectively.

When we looked at the effects of wing clipping on efficiency we found, as expected, that efficiency decreased with clipping treatments, being the lowest in the asymmetric treatment. The linear mixed-effects model showed a significant difference between the unclipped and the asymmetric treatments (lme, $P = 0.02$; $n = 6$; Table 5) but no significant difference between the unclipped and symmetric treatments (lme, $P = 0.24$; $n = 6$; Table 5) and also between clipping treatments (lme, $P = 0.16$; $n = 6$; Table 5). The absence of a significant difference in efficiency between unclipped and symmetric treatments was surprising since the components of efficiency, P_{mech} and P_{met} , do exhibit a significant difference. The result appears to be due to the decline in signal to noise ratio brought about by the combination of the two results.

Discussion

Costs of damage and asymmetry

Our investigation of the costs of asymmetric and symmetric wing damage to the maximum load lifting performance and metabolic cost of hovering flight in hawkmoths demonstrated substantial and significant negative effects of wing damage, especially asymmetric damage, in the hawkmoth *Manduca sexta*. To a first approximation, the effect of asymmetric damage was twice that of symmetric damage on a per-area basis, demonstrating a substantial additional cost of asymmetry and suggesting that in most cases flight performance is determined by the capability of the most damaged wing, not the average of all wings in these functionally two-winged insects. Thus, the cost of providing flight stability with asymmetric wings appears to be similar in magnitude to the benefit obtained from the additional area of the undamaged wing (Fernández et al., 2012).

This overall result is contrary to our initial hypothesis that insects might have evolved effective coping mechanisms for the stability problems of wing asymmetry or damage, as suggested by a number of studies finding little or no effect of wing damage on different aspects of flight performance. The opposing results of different studies of wing wear or damage may arise from different experimental protocols which produced qualitatively and quantitatively

different amounts of damage. To examine this possibility, we divided our load-lifting data into large and small damage classes and found that for only small amounts of damage (< 12% of wing area), the effect of the damage was linearly proportional to the total loss of wing area with no additional penalty for wing asymmetry. This is consistent with prior results from *Manduca sexta* which showed that neuromuscular compensation for wing asymmetry begins once >10% of wing area is lost (Fernández et al., 2012) and supporting a non-neural, biomechanical compensation mechanism in those cases where area loss is < 10%.

When is wing damage costly?

Different studies examining wing damage in insects have come to different conclusions as to the effect of wing damage on flight performance or costs. Results from artificial wing area reduction experiments in hawkmoths and dragonflies (Combes et al., 2010) demonstrated costs to wing area loss while artificial damage to bumblebees has revealed no metabolic cost (Hedenström et al., 2001) and only minimal flight biomechanics effects (Haas and Cartar, 2008). Damage to butterfly wings was not found to increase field mortality (Kingsolver, 1999), but the biomechanical consequences are unknown. The hawkmoth, dragonfly and bee studies (Combes et al., 2010; Hedenström et al., 2001) used similar methods, applied qualitatively and quantitatively similar amount of damage and measured similar aspects of performance but reached opposing conclusions. This raises the possibility that differences in the flight physiology of the species in question account for the different results; moths and dragonflies use synchronous flight muscle while bumblebees use asynchronous muscle. Asynchronous flight muscle might facilitate biomechanical accommodation of wing damage by allowing for greater increases in flapping frequency in response to damage. Alternatively, maximum-effort experiments such as the asymptotic load-lifting tests may also more easily identify effects of wing damage on flight performance than the metabolic approach which found no effect of wing damage in bumblebees (Hedenström et al., 2001). Such an experiment has demonstrated that wholesale removal of the hindwing does reduce flight performance in bumblebees (Buchwald and Dudley, 2010). Similar results have been shown in honey bees flying in hypodense gases, where they show that reduced wing area and high asymmetry produced lower maximum wingtip velocities than bees with intact or symmetric wings, causing a greater impairment in maximal flight capacity (Vance and Roberts, 2014).

Comparison to theoretical aerodynamic models

Our aerodynamic model of the effect of wing damage on maximum load lifting capability predicted that the moths would increase flapping frequency or amplitude following wing clipping, allowing them to restore their aerodynamic power output to the level of an undamaged moth and making the reduction in load lifting performance proportional to the cube root of the reduction in the second moment of area of the wings (Eqn. 6). The underlying assumption of this model, that frequency or amplitude would change in response to clipping, was not supported, with only small and non-significant increases in frequency and amplitude observed. This result is in contrast to many other studies of wing damage in insects, including our prior result from hovering hawkmoths (Fernández et al., 2012). The difference in this experiment is that the flapping frequency of unclipped moths engaged in maximum load lifting was ~30 Hz, already elevated above the ~25 Hz commonly observed during hovering flight in this species. Wing clipping did not facilitate a further increase, suggesting that other factors such as minimum neuromuscular activation timing may ultimately limit flapping frequency in hawkmoths. This may not be the case for insects with asynchronous flight muscle. In keeping with the small and largely non-significant changes in flapping frequency and amplitude, the hawkmoth load-lifting results agree more closely with an aerodynamic model (Eqn. 3) where flapping frequency and amplitude are constrained, which predicts a direct relationship between the proportional reduction in wing second moment of area and load-lifting capability. This suggests that the changes in frequency and amplitude, statistically significant or not, were insufficient to fully restore muscle power output to its original value or came at an additional cost to muscle contractile efficiency.

With regard to the metabolic power requirements of hovering flight, our results for moths following symmetric wing clipping were a close match to our simplest aerodynamic model (Eqn. 7) where the change in the cost of hovering is proportional to the inverse square root of the change in the 3rd moment of wing area. More complex modelling efforts such as re-solving equations 1 and 2 repeatedly for the different conditions in an attempt to include the interplay between the capacity for lift production and its cost were a poor fit to the data. These efforts predicted a much lower increase in hovering flight costs but also large increases in flapping frequency and thus might better fit results from asynchronous insects since bumblebees were

found to experience no detectable metabolic cost penalty from artificial wing area reduction (Hedenström et al., 2001).

The agreement between the metabolic data and our simplest aerodynamic model (Eqn. 7) should not necessarily be taken as support for the mechanism – variation in flapping frequency only – used by the model. Many other changes in kinematic parameters such as variation in angle of attack or the timing of wing rotation could also increase the lift per unit area produced by a flapping wing and help compensate for wing area lost due to damage. However, as a general principle increasing force per unit area decreases the lift to power ratio, so all these mechanisms would produce trends similar to those from Eqn. 7.

Summary

We found highly significant evidence of biomechanical performance consequences for wing wear or damage in hovering hawkmoths, in contrast to studies performed in bumblebees where the consequences of damage to flight metabolism appear to be slight. We also found a significant cost of asymmetry in damage for cases in which the damage exceeds ~10% of wing area, smaller amounts of asymmetric damage produced no additional effect beyond total wing area loss. This varied response suggests that small asymmetries such as those typically implicated in fitness loss via sexual selection against fluctuating asymmetry (Watson and Thornhill, 1994) may have little to no functional consequence, even for structures as closely associated with flight costs as the wings of an insect. Natural losses in wing area such as those due to moulting in birds could also avoid any asymmetry penalty by remaining small.

Data accessibility

All data presented and analysed here are available in the electronic supplementary material, tables S1-S3.

Competing interests

We have no competing interests.

Authors' contributions

MJF developed the protocol, performed experiments, analysed data and drafted the manuscript. MED performed experiments, analysed data and produced figures for the manuscript. TLH developed the project, analysed data, performed the modelling and drafted the manuscript.

Acknowledgements

We wish to thank Dwight Springthorpe for assistance with the experiments.

Funding

Funding was provided by the National Science Foundation (NSF IOS-0920358) to TLH.

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Appendix A

The aerodynamic model used here includes the average square of non-dimensional angular velocity $\overline{(d\hat{\phi}/d\hat{t})^2}$ and $\overline{|d\hat{\phi}/d\hat{t}|^3}$, the average absolute value of the cube of non-dimensional angular velocity. Numerical values of 19.74 and 105.29 are given, respectively, for these two quantities and noted in the text to represent sinusoidal motion. Non-dimensional angular velocity was described in Ellington (1984a), and represents normalization of angular motion to a range of -1 to 1 and time to a range of 0 to 1. Values of 19.74 and 105.29 were generated numerically in MATLAB by constructing a time sequence t from 0 to 2π , calculating $\phi(t) = \sin(t)$ which is already limited to a range -1 to 1, so $\hat{\phi} = \phi$. Non-dimensional time is $\hat{t} = \frac{t}{2\pi}$ and from these values we numerically differentiated $\hat{\phi}$ and calculated the average of the squared or absolute value of the cubed derivative. These were 19.74 and 105.29 and thus represent the non-dimensional angular velocity for sinusoidal flapping.

Tables

Table 1. Maximum vertical aerodynamic force production (F_{Vmax}) and wingbeat kinematics (wingbeat frequency (η), stroke amplitude (θ), stroke amplitude difference between both wings (θ_{l-r})) during treatments (unclipped, asymmetric wings and symmetric wings).

	<i>Unclipped</i>	<i>Asymmetric wings</i>	<i>Symmetric wings</i>
F_{Vmax} (N)	0.0256 ± 0.001	0.0212 ± 0.001	0.0203 ± 0.001
η (Hz)	28.78 ± 0.492	29.38 ± 0.521	29.71 ± 0.195
θ (degrees)	116.98 ± 2.105	115.85 ± 1.629	121.99 ± 1.490
θ_{l-r} (degrees)	6.31 ± 1.898	15.29 ± 6.031	5.71 ± 1.563

Note: We calculated θ_{l-r} , from the difference between left-right wings.

Values were generated using all individuals (n= 8). Data are means \pm s.e.m.

Data used in this table are available in supplementary material Table S1.

Table 2. Load lifting data by small and large area clipping group, including maximum vertical aerodynamic force (F_{Vmax}), wingbeat frequency (η) and wingbeat amplitude for both wings (left and right; θ) during each experimental treatment (unclipped, asymmetric, and symmetric wing clipping).

	F_{Vmax} (N)		η (Hz)		θ (degrees)			
	<i>S</i>	<i>L</i>	<i>S</i>	<i>L</i>	<i>S</i>	<i>L</i>	<i>S</i>	<i>L</i>
Unclipped	0.027 ± 0.0013	0.024 ± 0.0013	28.21 ± 0.44	29.34 ± 0.78	117.71 ± 2.32	120.78 ± 3.20	111.62 ± 2.73	118.94 ± 4.37
Asymmetric wings	0.025 ± 0.0005	0.017 ± 0.0007	28.44 ± 0.23	30.19 ± 0.70	$118.52 \pm 4.10\ddagger$	116.05 ± 3.13	$127.16 \pm 2.69\ddagger$	102.40 ± 5.01
Symmetric wings	0.024 ± 0.0006	0.017 ± 0.0012	29.45 ± 0.31	30.08 ± 0.32	122.75 ± 4.98	117.71 ± 1.28	125.08 ± 2.75	121.57 ± 0.81

\ddagger indicates data from the wing clipped in the asymmetric treatment

Note: *S*, small clipped area; *L*, large clipped area.

Values were generated using all individuals, n = 8, with 4 in *S* and *L* each. Data are means \pm s.e.m.

Table 3. Linear mixed-effect model table showing the effect of treatment on maximum vertical aerodynamic force and wingbeat kinematics (frequency and stroke amplitude). Individuals are used as random effect, n = 8 moths.

Maximum vertical Force (F_{Vmax})	t-value	P-value
Unclipped vs asymmetric	4.432	0.0006 ***
Unclipped vs symmetric	5.303	0.0001***
Asymmetric vs symmetric	0.871	0.398
Frequency (η)		
Unclipped vs asymmetric	1.528	0.152
Unclipped vs symmetric	2.353	0.036*
Asymmetric vs symmetric	0.825	0.425
Average amplitude (θ)		
Unclipped vs asymmetric	0.485	0.636
Unclipped vs symmetric	2.164	0.051
Asymmetric vs symmetric	2.649	0.021*

Data used in this table are available in supplementary material Table S1.

* $P < 0.05$; *** $P < 0.001$

Table 4. Mass-specific oxygen consumption, mass-specific carbon dioxide production, respiratory quotient, metabolic power, estimated mechanical power and flight efficiency (lift to power ratio) for each experimental treatment (unclipped, asymmetry, and symmetry).

	$\dot{V}O_2$ (ml g ⁻¹ h ⁻¹)	$\dot{V}CO_2$ (ml g ⁻¹ h ⁻¹)	RQ	P_{met} (W kg ⁻¹)	P_{mec} (W kg ⁻¹)	Efficiency (N W ⁻¹)
Unclipped	51.60 ± 2.08	41.36 ± 2.32	0.80 ± 0.03	288.25 ± 11.90	54.72 ± 0.81	19.18 ± 0.97
Asymmetric wings	67.28 ± 2.50	58.94 ± 2.41	0.88 ± 0.03	381.09 ± 14.46	59.27 ± 1.84	16.01 ± 0.72
Symmetric wings	67.73 ± 6.50	56.02 ± 5.69	0.83 ± 0.02	380.73 ± 36.84	63.33 ± 1.37	17.71 ± 1.45

Values are generated using all individuals (n = 6). Data are means ± s.e.m.

Data for each individual are available in Tables S1, S2 and S3.

Table 5. Linear mixed-effect model table showing the effect of treatment on metabolic power, predicted mechanical power, mass-specific $\dot{V}O_2$, mass-specific $\dot{V}CO_2$ and efficiency. Individuals are used as random effect.

<i>Metabolic power (P_{met})</i>	<i>t-value</i>	<i>P-value</i>
Unclipped vs asymmetric	3.527	0.005 ***
Unclipped vs symmetric	3.513	0.005 ***
Asymmetric vs symmetric	0.013	0.989
<i>Mechanical power (P_{mech})</i>		
Unclipped vs asymmetric	3.854	0.003 ***
Unclipped vs symmetric	7.298	<0.001 ***
Asymmetric vs symmetric	3.443	0.006 ***
<i>Mass-specific $\dot{V}O_2$</i>		
Unclipped vs asymmetric	3.273	0.008 ***
Unclipped vs symmetric	3.366	0.007 ***
Asymmetric vs symmetric	0.093	0.928
<i>Mass-specific $\dot{V}CO_2$</i>		
Unclipped vs asymmetric	5.093	<0.001 ***
Unclipped vs symmetric	4.245	0.002 ***
Asymmetric vs symmetric	0.847	0.416
<i>Efficiency</i>		
Unclipped vs asymmetric	2.737	0.021 *
Unclipped vs symmetric	1.233	0.245
Asymmetric vs symmetric	1.503	0.163
<i>RQ</i>		
Unclipped vs asymmetric	2.704	0.022 *
Unclipped vs symmetric	0.851	0.414
Asymmetric vs symmetric	1.852	0.093

Figures

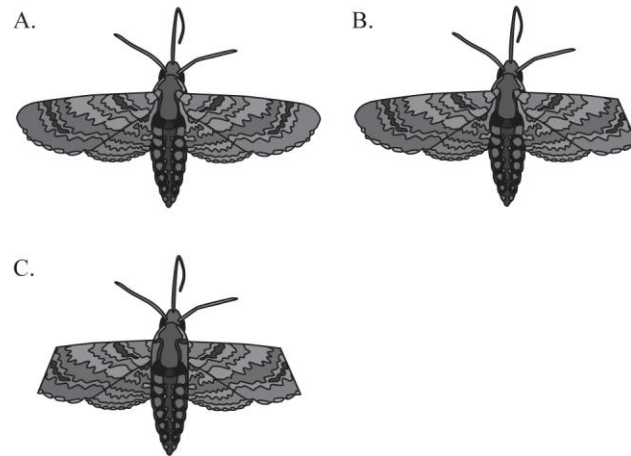


Fig. 1. Schematic representation of the treatments applied to each individual moth during asymptotical load-lifting and respirometry measurements. (A) Control or fully intact wings. (B) Asymmetric wing clipping, where the moth has only one forewing tip clipped. (C) Symmetric wing clipping, where both forewings have been clipped.

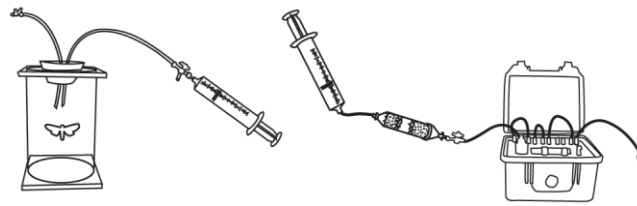


Fig. 2. Schematic representation of the hovering flight chamber (a) and the portable oxygen and carbon dioxide analyser (b).

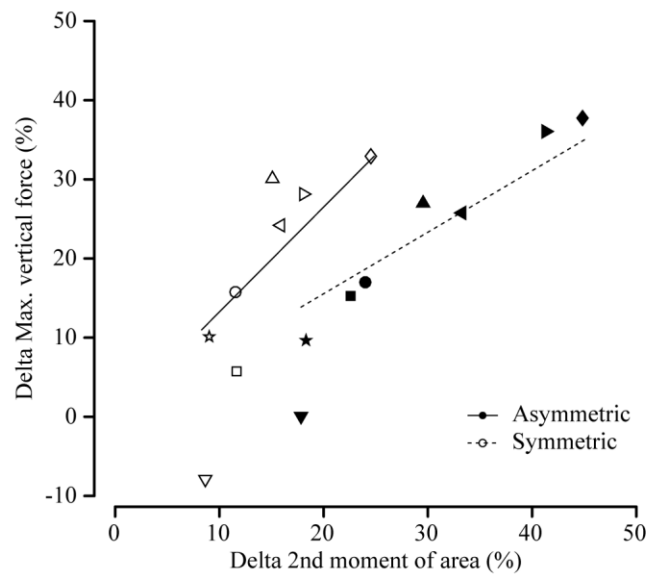


Fig.3. Effect of clipping treatment (asymmetric, filled markers and symmetric, open markers, clipping) on maximum vertical force produced by eight moths, individually indicated by marker shape. Here we show a linear fit to the data for each treatment. Symmetric or both wings symmetrically clipped has a slope of 0.78 ($r^2 = 0.77$, $P = 8.31 \times 10^{-7}$) and asymmetric clipping 1.32 ($r^2 = 0.56$, $P = 5.23 \times 10^{-6}$).

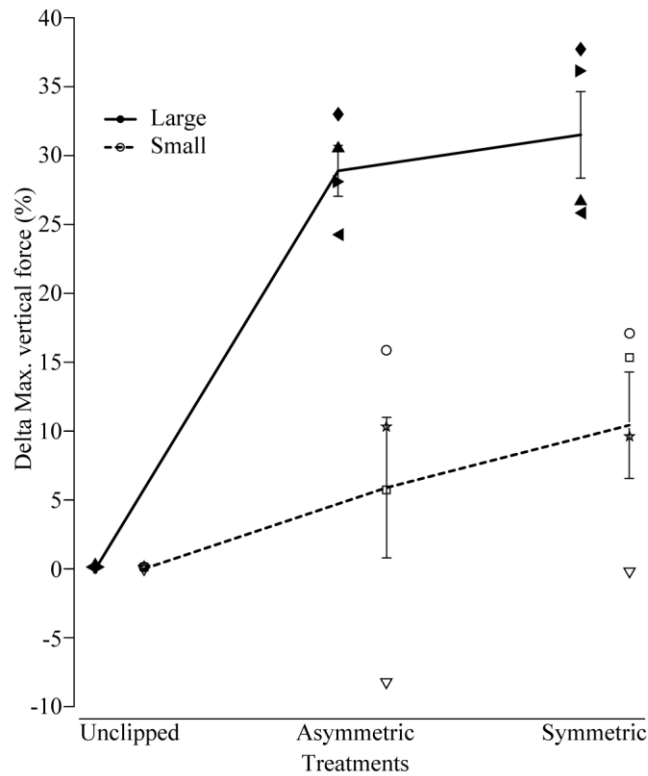


Fig. 4. Effect of experimental treatments (unclipped, asymmetric, and symmetric) on the percent decrease in maximum vertical aerodynamic force. Filled markers represent large area clipped (12-23 %), and open markers represent small area clipped (7- 10%). Marker shape corresponds to Fig. 3. Data are means \pm s.e.m.

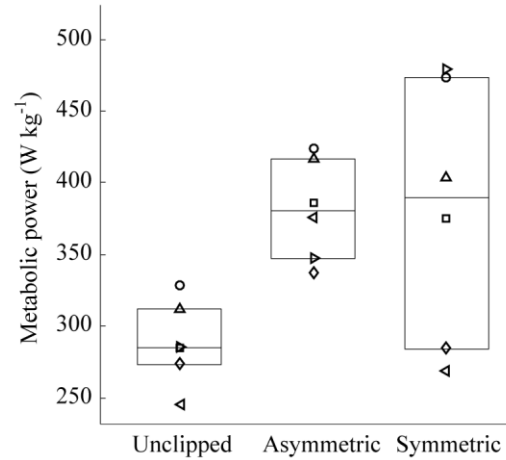


Fig. 5. Effect of experimental treatment on metabolic power measured by oxygen consumption and carbon dioxide production. Boxes show the median, 25th and 75th percentile. Markers show moths, marked individually by shape, but shape does not correspond to Fig. 3 and Fig. 4 as the load-lifting experiments were conducted on a separate set of moths. Complete data on individual moths is provided in the supplementary tables, marker shape to moth ID is as follows: circle = moth #9, square = #10, diamond = #11, left-pointed triangle = #12, right triangle = #13, upward triangle = #14.

Supplementary Data

Table S1. Morphological parameters for each individual during control (full wings) and clipped wing treatments. Moths 1- 8 were used for the load-lifting experiment and moths 9 – 15 were used for the respirometry experiment. Asymmetric trials were recorded from moths 1, 2, 10, 11, 12, 13, 14 after clipping the right wing (R) and in moths 3, 4, 5, 6, 7, 8, 9 after clipping the left wing (L).

Moth	Treatment	Wing area (cm ²)		Length (cm)		2 nd moment (cm ⁴)		3 rd moment (cm ⁵)		Total Clip (%)	
		L	R	L	R	L	R	L	R	L	R
1	Control	7.42	7.53	5.34	5.31	73.53	76.94	280.24	295.38	10.17	9.35
	Clipped	6.67	6.83	4.52	4.54	54.78	59.58	188.12	210.07		
2	Control	7.28	7.14	5.13	5.19	64.66	65.57	233.63	239.49	9.40	8.68
	Clipped	6.59	6.52	4.41	4.38	50.42	50.39	167.44	167.80		
3	Control	6.86	6.91	5.27	5.28	65.95	66.60	244.82	247.67	22.86	18.46
	Clipped	5.29	5.63	3.80	4.00	33.42	39.70	99.33	124.85		
4	Control	6.53	6.62	4.94	4.95	57.02	56.42	202.11	197.81	14.23	16.28
	Clipped	5.60	5.54	4.07	3.90	39.25	36.65	123.12	111.87		
5	Control	6.03	6.15	4.57	4.70	44.83	46.26	147.67	153.86	16.16	21.97
	Clipped	5.06	4.80	3.71	3.49	28.26	25.04	79.89	68.20		
6	Control	6.67	6.43	5.19	4.93	59.18	52.44	215.17	181.78	12.06	11.75
	Clipped	5.87	5.67	4.24	4.18	42.34	38.46	136.68	120.54		
7	Control	7.27	7.27	5.06	4.94	62.18	65.65	222.19	238.13	9.18	8.37
	Clipped	6.61	6.67	4.60	4.46	51.13	53.91	171.19	183.72		
8	Control	7.29	7.23	5.37	5.16	74.04	65.67	282.34	239.66	7.52	8.43
	Clipped	6.75	6.62	4.77	4.42	61.44	52.69	220.01	177.74		
9	Control	8.92	9.66	4.35	4.68	49.34	61.18	152.61	202.78	23.22	24.57
	Clipped	6.85	7.28	3.46	3.78	21.94	33.50	51.22	92.51		
10	Control	9.41	9.18	5.32	4.94	70.33	62.57	250.10	209.11	10.01	9.26
	Clipped	8.47	8.32	4.24	3.92	48.16	45.81	147.89	137.48		
11	Control	11.01	10.54	5.46	5.60	85.54	84.18	309.88	317.97	11.33	12.17
	Clipped	9.76	9.25	4.10	4.10	53.22	49.06	159.82	147.56		
12	Control	10.62	10.54	5.34	5.60	76.63	84.88	267.95	317.97	7.62	9.70
	Clipped	9.81	9.52	4.41	4.39	57.57	56.10	180.66	178.43		
13	Control	9.63	9.32	5.25	5.17	70.47	66.61	247.77	230.41	9.90	9.91
	Clipped	8.68	8.40	4.15	4.13	49.78	47.27	152.72	142.26		
14	Control	9.13	8.77	4.99	4.83	58.59	51.68	195.24	167.07	8.40	8.24
	Clipped	8.36	8.05	4.13	3.89	46.81	38.17	140.53	109.89		

Table S2. Maximum load and wing kinematics data

Moth	Treatment	Mass (g)	Max. Force (mN)	Flapping Frequency (Hz)	Flapping Amplitude R – (Deg.)	Flapping amplitude L – (Deg.)
1	Control	1.76	29.11	28.74	114.7	117.6
	Asymmetric	1.69	24.53	28.07	110.4	110.7
	Symmetric	1.45	24.16	29.01	115.6	113.2
2	Control	1.49	26.53	27.34	122.0	121.8
	Asymmetric	1.54	25.01	28.87	123.7	116.0
	Symmetric	1.48	22.48	29.27	117.5	129.9
3	Control	1.46	26.05	31.05	109.3	106.9
	Asymmetric	1.38	17.48	32.27	87.4	134.0
	Symmetric	1.35	16.22	30.32	122.9	131.8
4	Control	1.41	21.75	27.26	115.9	106.9
	Asymmetric	1.27	16.49	29.31	108.0	128.8
	Symmetric	1.24	16.15	29.25	119.8	127.2
5	Control	1.44	22.00	29.49	130.1	117.0
	Asymmetric	1.21	15.82	29.52	107.4	124.0
	Symmetric	1.24	14.07	29.97	123.0	119.9
6	Control	1.54	27.31	29.03	120.5	115.7
	Asymmetric	1.49	19.03	29.27	106.8	121.9
	Symmetric	1.42	20.09	30.01	120.6	121.2
7	Control	1.57	24.23	28.55	125.6	113.8
	Asymmetric	1.86	26.20	28.38	121.6	121.5
	Symmetric	1.66	24.27	30.06	120.0	125.2
8	Control	1.58	27.76	27.01	125.5	127.9
	Asymmetric	1.53	24.93	-	-	-
	Symmetric	1.50	25.10	28.10	131.6	126.9

Table S3. Oxygen consumption and carbon dioxide production data

Moth	Treatment	Mass (g)	$\dot{V}O_2$ (ml hr ⁻¹)	VCO ₂ (ml hr ⁻¹)
9	Control	1.56	90.80	77.07
	Asymmetric	1.56	115.76	102.03
	Symmetric	1.57	134.48	103.70
10	Control	1.67	88.04	59.78
	Asymmetric	1.63	112.14	92.56
	Symmetric	1.63	108.12	92.38
11	Control	1.73	84.34	68.8
	Asymmetric	1.63	96.79	82.84
	Symmetric	1.53	77.30	64.12
12	Control	1.89	83.56	65.49
	Asymmetric	1.63	109.65	87.29
	Symmetric	1.62	78.15	61.08
13	Control	1.69	84.06	75.57
	Asymmetric	1.61	96.78	99.26
	Symmetric	1.50	126.17	111.23
14	Control	1.29	72.32	56.63
	Asymmetric	1.23	89.64	79.37
	Symmetric	1.12	80.25	67.10