

The effect of wing wear on flight metabolic rate of the Mormon Fritillary butterfly

(Speyeria mormonia)

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Abstract

Nearly every aspect of a butterfly's life is dependent on flight. Flight metabolic rate (FMR), which is the amount of CO₂ produced during flight, serves as an indicator of flight capacity and performance. High flight metabolic rate has been found to be correlated with greater dispersal distance, and longer lifespan. Additionally, FMR has been found to decrease with age in the Mormon Fritillary butterfly (*Speyeria mormonia*) as well as in *Colias eurytheme* (Niitepõld *et al.* in review). Wing damage was experimentally facilitated by removing a small portion of the distal edge of butterflies' wings in order to elucidate the effect of wing damage on the flight metabolic rate of butterflies. The FMR of the control group was not significantly different than the FMR of the experimental treatment group. Thus, the decrease in FMR with age in *S. mormonia* is likely not due to a mechanical defect of the wing (i.e. wing damage) but more likely due to an innate physiological process involving senescence at some level.

Introduction

Nearly every aspect of a butterfly's life is dependent on flight. Flight capacity can potentially influence a butterfly's fitness when considering important life history events such as escaping from predators, foraging, and oviposition (Niitepõld 2010). Flight capacity may also indirectly impact the individual, restricting the capacity to perform other energy-demanding functions, such as reproduction. Flight metabolic rate (FMR), which is the amount of CO₂ produced during flight, serves as an indicator of flight capacity and performance. Additionally, resting metabolic rate (the amount of CO₂ produced while motionless) can function as an indicator of the cost of maintenance (Niitepõld 2010). A study on flight metabolic rate in the Glanville fritillary butterfly

revealed no significant correlation between peak flight metabolic rate and resting metabolic rate, suggesting only a minimal cost of high metabolic capacity (Niitepõld 2010). In assessing the effect of flight metabolic rate on dispersal, a study found a significant association between variation in flight metabolic rate and variation in dispersal distance (Niitepõld *et al.* 2009). In further studies, a significant correlation between lifespan and peak flight metabolic rate was found (Niitepõld and Hanski 2013). Additionally, flight metabolic rate has been found to decrease with age in both *S. mormonia* as well as *Colias eurytheme* (Niitepõld *et al.* in review). In a study on bumblebees, Hedenström *et al.* (2001) assessed the effect of wing wear on flight energetics and kinematics by experimentally removing a portion of bumblebees' wings. They found no significant correlation between flight metabolic rate and the wing-clipped experimental treatment (Hedenström *et al.* 2001). Thus, they suggested that wing wear is likely to increase mortality rate in bumblebees due to decreased maneuverability when escaping from predators (Hedenström *et al.* 2001). Due to the general lack of studies on wing wear in butterflies and their fundamental reliance on flight, I aimed to assess the effect of wing wear on the flight metabolic rate of the Mormon Fritillary butterfly (*Speyeria mormonia*).

Methods

Study species and design

Speyeria mormonia (Nymphalidae) is commonly found in the North American montane grasslands. The species follows a univoltine life cycle and females typically lay eggs singly (Boggs 2003). After hatching, larvae spend winters as unfed first instars and then feed on *Viola* spp. (Violaceae) in the spring following snowmelt (Boggs 2003).

Adult individuals have an average lifespan of five days and take flight between the months of July to September (Boggs 2003). Thus, I collected (X NUMBER) *S. mormonia* males and (X NUMBER) females throughout July and early August 2013 at the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, Colorado.

In order to assess the influence of wing wear on flight metabolic rate, I subjected approximately half of the collected individuals to the experimental treatment and the other half to the sham control. The experimental treatment consisted of removing approximately (X NUMBER) percent of the total wing area by clipping the distal edge of the forewings and hindwings with scissors. In clipping the wings, a near equal amount of wing was removed from the edges of both wings as to not change the aspect ratio of the wings. In the sham control group none of the wing area will be removed. Both before and after experimental treatment, flight metabolic rates will be measured for the butterflies in using the respirometry chamber.

Respirometry

Flight metabolic rate (FMR) was measured for each individual using flow-through respirometry. Individuals were fed honey water (20% honey dilution) the night prior to FMR measurement and kept in envelopes inside a dark box. The following morning, individuals were fed water and then placed in the 1-L respirometry chamber, which was then covered by a dark cloth and kept at a near constant temperature. Before measuring metabolic rate, the individual remained in the respirometry chamber for approximately 25 minutes or until a stable CO₂ baseline was reached. The metabolic measurements were conducted inside an open-faced plywood cabinet in a temperature-controlled room. Three lights attached to the ceiling of the cabinet were used: one 26 W fluorescent bulb

and two 25 W fluorescent backlights. Temperature was measured inside the respirometry chamber using a NTC thermistor probe (Sable Systems International). A SS-4 subsampler (Sable Systems International, Las Vegas, NV, USA) and a mass flow controller (Sierra Instruments, Monterey, CA, USA) were used to pump dried and CO₂ free air at a rate of 1.5 L min⁻¹ through the respirometry chamber. Air was pumped out of the chamber using a LI-7000 differential infrared CO₂ analyzer (Li-Cor Biosciences, Lincoln, NE, USA). After the individual remained stationary and a stable CO₂ baseline was reached, the resting metabolic rate was be recorded. If there was a spike in the CO₂ levels, typically caused by the individual moving in the chamber, I waited until the individual is again motionless and a stable baseline was once again reached before recording the RMR. Due to the large volume of the respirometry chamber, temporary variation in CO₂ readings was reduced. After measuring RMR for 1.5 minutes, the dark cloth was removed from atop of the chamber and the individual was exposed to light. After 30 seconds of exposure to the lights, the chamber was shaken vigorously, forcing the butterfly to fly within the chamber. During the FMR measurements, the individuals were forced to fly as consistently as possible. The chamber was shaken again whenever the individual alighted. If shaking was not enough to force the individual into flight, and the individual is stuck to the side of the chamber, the chamber was tapped. As the FMR measurement continued, some individuals became fatigued and were unwilling to continue flying. In these cases, the shaking and tapping will increase, forcing the individuals to flap their wings, likely without creating any significant lift. FMR experiments were ran for 7 minutes and FMR was be recorded as the total amount of CO₂ produced during the experimental period. After FMR measurements, the identity and sex

of each individual was recorded along with a description of its flight performance and the time of day. The individuals were then weighed, photographed using a Nikon coolpix (CITATION), fed honey water, and returned to their envelopes.

Morphological and statistical analyses

Photographs of the individuals were used to calculate wing area using imageJ (CITATION), both before and after experimental treatment. Linear regressions of body mass versus FMR, as well as peak MR, were completed using JMP 9.0 (JMP 2010) and the subsequent residuals were calculated. A (LINEAR MIXED) model was constructed using JMP 9.0 (JMP 2010) with (BODY MASS) as covariate and (experimental treatment, body mass, and day of FMR measurement) as factor.

Results

Body mass of *S. mormonia* males was strongly correlated with both peak MR ($R^2=0.175141$, $p=0.0002$) and FMR ($R^2=0.178164$, $p=0.0001$). The peak MR of *S. mormonia* males did not differ significantly between the experimental treatment and the control (See Figure 1). Additionally, the FMR of *S. mormonia* males did not differ significantly between the experimental treatment and the control (See Figure 2).

(FEMALE RESULTS)

Discussion

The experimental reduction in wing area had no significant effect on the flight metabolic rate of *S. mormonia* males, which is similar to the findings of Hedenström *et al.* (2001). Because flight metabolic rate is known to decrease with age in *S. mormonia* (Niitepõld *et al.* in review), it can be suggested that this decrease is not due to a mechanical deformity of their wings. Rather, the decrease in FMR with age is likely due

to an inert physiological process involving senescence at some degree. Future studies could further investigate the flight capacity of *S. mormonia* by assessing maximum wing loading and take off velocity. Additional studies could more closely analyze the cellular metabolism of the flight cells of *S. mormonia* to elucidate the cause for the decrease in FMR with age.

Tables and Figures

Figure 1. The residual FMR of *S. mormonia* males on the first and second day of measurement for both the experimental group (n = 23) and the control group (n = 18).

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Figure 2. The residual peak MR of *S. mormonia* males on the first and second day of measurement for both the experimental group (n = 23) and the control group (n = 18).

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Figure 3. The residual peak MR of *S. mormonia* females on the first and second day of measurement for both the experimental group (N=X NUMBER) and the control group (N= X NUMBER).

Figure 4. The residual peak MR of *S. mormonia* females on the first and second day of measurement for both the experimental group (N=X NUMBER) and the control group (N= X NUMBER).

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