Effects of Parasite Infection on the Flight Performance of Monarch Butterflies *(Danaus plexippus)*

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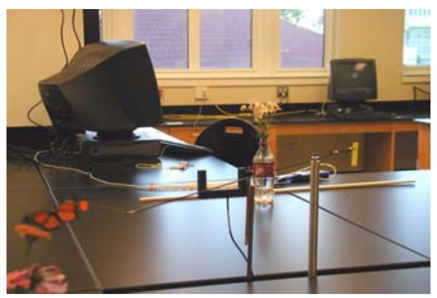
OBJECTIVES: 1) Quantitatively describe the flight performance of monarch butterflies using a flight mill; **2)** Explore the relationship between parasite infection and monarch flight performance

EMORY UNIVERSITY

ABSTRACT

North American monarch populations are parasitized by the neogregarine protozoan parasite, *Ophryocystis elektroscirrha*. The prevalence of infection within and among populations is inversely related to the migratory distance flown. One hypothesis for this observation is that *O. elektroscirrha* reduces the flight ability of the host, limiting the probability that infected hosts will successfully reach overwintering sites or remigrate to breeding grounds in the spring. This study explores the relationship between parasite infection and flight performance in a lab-reared group of monarchs. A flight mill and photogate were used to measure the total distance and duration of each flight, as well as initial velocity, average velocity, and average rate of deceleration. The initial velocity (average speed over the first two minutes of flight) and the maximum speed of infected monarchs were significantly lower than the same measures for healthy monarchs. Principle Components Analysis (PCA) explained 69% of the variation across 4 flight parameters, and showed that the flight performance of infected monarchs was significantly lower than that of healthy monarchs. Studies examining the flight performance of insects typically measure the total time and total distance of the flight. The use of flight parameters not commonly examined in captive insects (initial speed, maximum speed, rate of decline) were essential to the conclusions of this study, and suggest that traditional measurements of flight characteristics may not be sufficient to fully describe the flight performance of monarch butterflies.

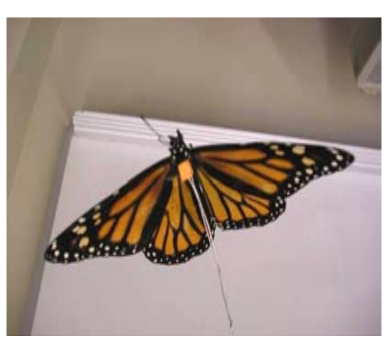
METHODS



A flight mill was constructed as shown in the view to the left, and in the above diagram (Figure 3). The photogate was connected to a PASCO PS-2000 datalogger, which recorded the time elapsed between rotations and the cumulative flight time.

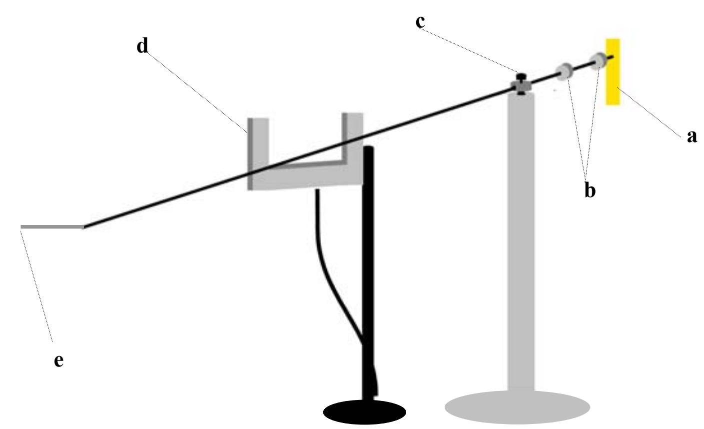
Monarchs used in this study were lab-reared at Emory University in the fall of 2003. One-half of the larvae were inoculated with a moderate dose (500 spores per larva) of *O. elektroscirrha* between late second instar and early third. Upon emergence, abdominal samples were examined to identify infected individuals. Fifty pairs of full siblings (one healthy, one infected) from different family groups were chosen for the flight mill study. Forty-three healthy and thirty-two infected individuals made recorded flights. Adult butterflies were held in a 12°C cooler until 48 h before flying, and were fed every fourth day with a honey water solution.

Digital wing scans were made for every individual so that morphological data could be analyzed later (including wing size, shape, and color). Ultra-light wire attachments were glued to the dorsal side of the thorax using rubber cement (see view). Mass was recorded immediately before and after attachment [average weight of wire =0.245g (min.=0.169g, max=0.358g)].

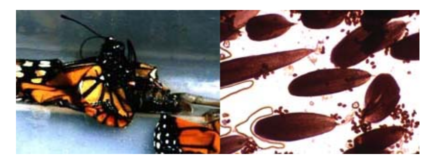


Flights were performed in a laboratory space maintained at 24°C and controlled for light and air movement. Individuals were removed from the cooler and placed in a 2' cubed netting cage with 20% honey water for 48 hours prior to flight. Mass was recorded immediately before flight. The monarch was attached to the flight mill with its abdomen parallel to the table surface.

Because butterfly flight is characterized by both soaring (passive) and flapping (active), a count was made of the number of pauses in the butterfly's flight in the first ten rotations to allow for a comparison in the amount of active wing flapping between individuals. A flight was terminated when the monarch remained still (no wing movement and rotations completely halted) for ten full seconds. Immediately after the flight, each monarch was weighed a final time. Infected and healthy monarchs were flown on alternate days to minimize spore contamination in the lab. Between 2 and 8 individual monarchs were flown per day. Flights for this experiment were performed between November 10, 2003 and December 5, 2003.



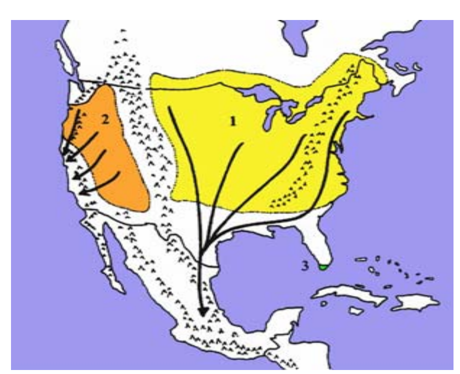
Flight Mill diagram. a. Flag passes through the photogate to interrupt the infrared beam. b. Counter-weights balance graphic rod to minimize friction at the center bearing. c. Nearly frictionless pivot. d. Photogate e. Site of wire attachment. (Flight mill constructed by Horace Dale in the Emory Physics Department)

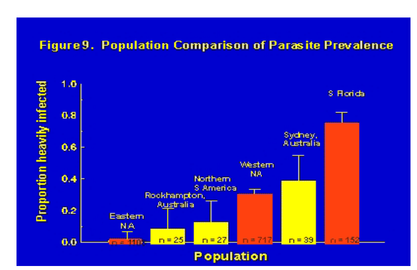


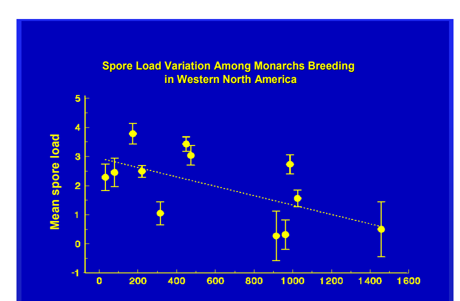
BACKGROUND

O. elektroscirrha is a ubiquitous parasite in monarch butterfly populations. Transmission typically results from spores deposited onto milkweed leaves by ovipositing females. Larvae consume the spores, which then migrate to and replicate within the larval hypoderm, finally residing on the outer cuticle of the butterfly upon emergence from the pupal case. Spore lysis can cause damage to the insect gut, making infected individuals more susceptible to desiccation. In addition, infected monarchs often have difficulty emerging from the pupal case and expanding their wings upon emergence.

Field studies have shown that the prevalence of *O. elekstroscirrha* infection in monarch populations varies with the migratory distance flown by that population. Monarchs of the Eastern United States, which can migrate up to 2000km to overwintering sites in central Mexico have infection rates between 0 and 5%. In contrast, the non-migratory monarch populations of south Florida have a much higher prevalence, reaching up to 80 % or more of the population. Several hypotheses have been suggested to explain these observations. Eastern monarchs may have acquired a higher resistance to disease infection or the parasitic strains present in the Florida population may be more virulent. Ecological differences in climate could explain the relationship. Florida populations are at a much higher density year round in comparison to Eastern populations. In this project, I test the hypothesis that parasitic infection reduces the flight ability of monarchs, thus allowing for higher prevalence in resident populations and direct selection against infected monarchs in migratory populations.

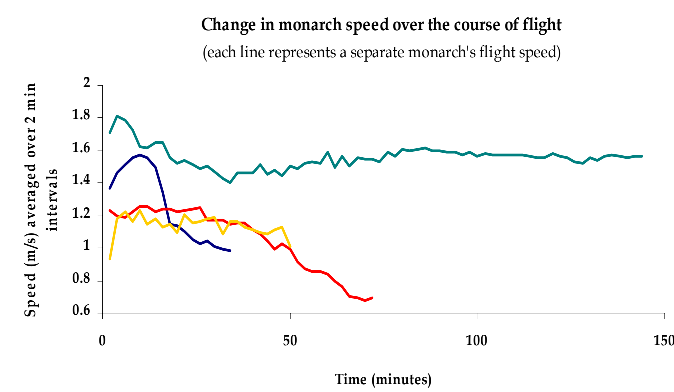






Above: The migratory routes of the major North American populations. Above right: Variation in parasite prevalence between populations. Prevalence is lowest in the longest-distance migrants. Right: In western N. America, prevalence in summer breeding grounds decreases with increasing distance from overwintering sites. *All figures are reproduced from Altizer et al. 2000. Ecol. Ent. 25: 125-139*

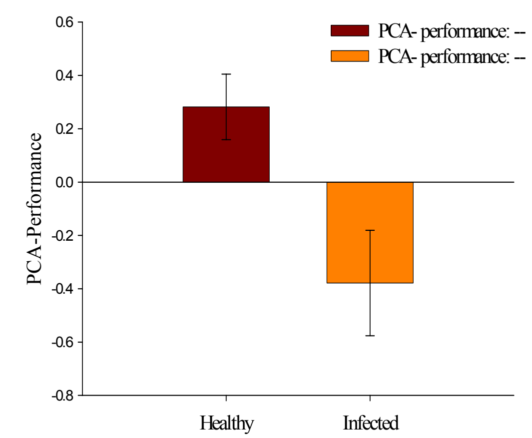
RESULTS AND CONCLUSIONS



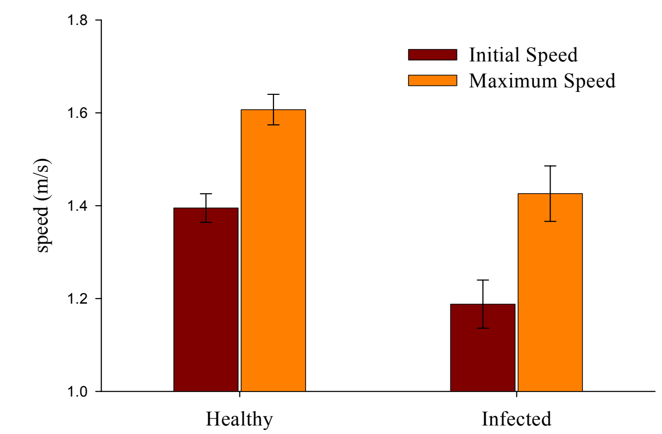
DATA COLLECTED

- **Percent Weight Loss and Percent Weight Loss/Time of Flight**
- **Velocity (averaged over two minute intervals): Initial (1st interval), Maximum, and Average**
- **Total Distance and Total Time of Flight**
- **Rate of Deceleration (difference in the speed of each interval averaged over all intervals)**
- **Wing scans were made for all individuals to examine the efect of morphological differences on flight performance (not discussed here)**

There was large variation in the flight dynamics between individual monarchs. The graph at left shows the change in speed, averaged over two minute intervals, over the course of flight. Two uninfected monarchs, in purple and green, are compared to two infected monarchs, red and yellow.



A principle components analysis (PCA) was performed using initial speed, maximum speed, average speed, and log-transformed total distance. The first principle component explained 69 % of the variation between these four variables. Factor regression scores (first principal component) representing a composite measure of overall flight performance are graphed above.



Both the initial speed and the maximum speed differed significantly between healthy and infected individuals.

	Initial Speed (m/s)		Maximum Speed (m/s)	
	Mean	Standard Error	Mean	Standard Error
Healthy N=43	1.395	0.0307	1.607	0.033
Infected N=32	1.188	0.0519	1.426	0.0598

Results from this study showed significant differences in flight performance between healthy and infected monarchs, with respect to initial speed and maximum speed, as well as using a composite measure of flight performance. This would suggest that *O. elektroscirrha* infection does have a negative effect on the flight performance of the host, supporting the hypothesis that decreased flight performance could explain the striking differences found in the prevalence of infection between migratory and non-migratory populations. Most previous studies of insect flight have examined average speed and total distance flown. Novel parameters, initial speed and maximum speed, were important to the conclusions of this study, and emphasize the need for multiple quantitative measures of flight performance (in addition to automated recording of flight parameters) in monarch butterflies.

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