Heritability of Resistance to a Protozoan Parasite (*Ophryocystis elektroscirrha)* in Monarch Butterflies (*Danaus plexippus)*

Elizabeth Friedle[1] and Sonia Altizer[1,2]

[1]Graduate Program in Population Biology, Ecology & Evolution, [2]Department of Environmental Studies, Emory University, 400 Dowman Dr., Atlanta, GA

Abstract

Monarch butterflies (*Danaus plexippus*) occur in South and Central America, North America, some Caribbean Islands, Australia, New Zealand, Hawaii, and other Pacific Islands. The protozoan parasite, *Ophryocystis elektroscirrha*, infects monarchs from all populations examined to date. However, parasite prevalence varies within and between populations. The non-migratory populations (Florida, Hawaii) have the highest parasite prevalence, the Western population which migrates a moderate distance shows intermediate parasite prevalence, and the Eastern population which migrates the longest distance shows the lowest parasite prevalence. The goal of this study was to examine heritability of resistance to *O. elektroscirrha* among eastern migratory monarchs, and to explore different ways of measuring resistance (via parasite loads, lethal, and sub-lethal effects of infection). Factors such as migration, climate differences, genetic variation, and density of hosts differ between existing monarch populations and could cause variation in parasite prevalence. Genetic variation within isolated host and parasite populations could be another key component of differences in parasite prevalence and could influence host response to infection by novel parasite strains. For resistance to be a trait under selection, it must be heritable, i.e. determined through additive genetics and therefore at the level which selection on the expressed trait results in the selected phenotype being passed from parent to offspring and then expressed in the offspring. Understanding the genetic basis for host resistance and predicting evolutionary responses in wild monarchs requires at least a minimal understanding of the heritability and costs of host resistance and the potential strength of parasite mediated selection. Uninfected monarchs were collected as adults and larvae from Georgia, New York, and Minnesota. The designed mating schemes crossed males and females from different regions, allowed males to each mate with 3-5 females, and formed full and half sib family groups. Offspring from each family group were exposed to one of three different inoculation treatments, control (no parasite spores), low (50 parasite spores), or high (500 parasite spores). Preliminary results indicate no difference in pupal mass or survival pre-adult (defined as oviposition to emergence) between all three treatments. A significant difference in presence/absence of infection was found between all three treatment groups. These initial results indicate that pupal mass and survival pre-adult are not measurements indicative of resistance to infection with low and moderate parasite doses, and therefore will not be used to addresses varying resistance to parasitism.

Biology of the Host-Parasite Interaction

Monarch Butterfly (*Danaus plexippus*)

Larval Development
Five Instar Stages (1st – 5th)
Egg to adult: 30 days
Adults mate multiply
Females produce hundreds of eggs

Ophryocystis elektroscirrha

Neogregarine Apicomplexan
First recovered from monarch and queen butterflies in Florida in the late 1960's

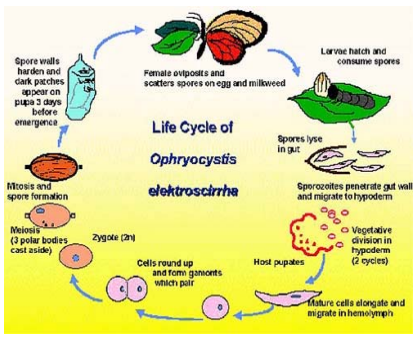
Spore size: 14 µm
Approximately 30 times smaller than a scale from monarch

Effects of Infection

Infected pupa

Heavily infected adult monarch

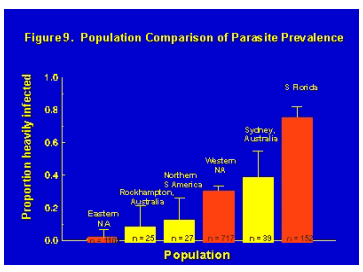
- Heavily infected adults
  - difficulty emerging and expanding wings
  - smaller and decreased body mass
  - shorter-lived than uninfected
- Spore load on adults directly related to
  - Dose of spores
  - Larval stage
  - Age of spores
- Similar fecundity observed for Infected and Non-infected females

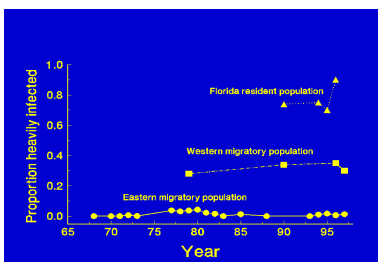


Vertical and Horizontal Transmission:

Parasites can be transmitted maternally (mother to offspring), paternally (father to mother to offspring) or horizontally (via spore accumulation on milkweed leaves)
**Larvae must ingest spores to become infected; parasites not directly infectious to adults

Parasite prevalence varies among populations and over time





Figures at left from Altizer et al. 2000. Ecol. Ent. 25: 125-139

Estimating Heritability and Measuring Resistance

Estimating Heritability

Heritability (h_2)– the proportion of phenotypic variance due to the additive effect of alleles

Phenotypic Variance (V_p) = Genetic Variance (V_g) and Environmental Variance (V_e)

$V_g = V_a + V_d + V_i + V_{me}$

V_a – variance due to additive genetic effect
V_d – variance due to dominance genetic effect
V_i – variance due to epistatic genetic effect
V_{me} – variance due to maternal effect

Phenotype of any given offspring
$P_{ijk} = \mu + s_i + d_{ij} + w_{ijk}$

Paternal Half-Sib (HS)
Full-Sib (FS)
COV(HS) = ¼ Va
COV(FS) = ½ Va + ¼ Vd

Heritability = V_a / V_p

Measuring Resistance

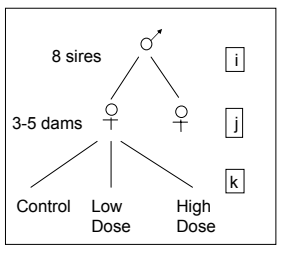
Four ways to quantify resistance:

- **baseline immunity**; haemocyte count and encapsulation response
- **lethal effects**; probability of survival to adult and probability of adult survival
- **sub-lethal effects**; mass, wing morphology, and developmental rate
- **parasite load**; presence/absence of infection and a quantitative estimate of spore load

Experimental Design – Nested Sib Analysis

Wild parents collected as larvae and adults from Eastern North America (GA, NY, MN) in September 2003

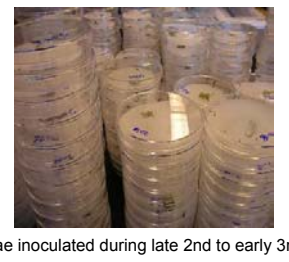
30 Full-Sib Groups
8 Paternal Half-Sib Groups



10 progeny per dam per treatment

Mating Cages in Greenhouse

Treatment and Inoculation
- Control: no spores per larva
- Low: 50 spores per larva
- High: 500 spores per larva



Larvae inoculated during late 2nd to early 3rd instar

Larvae reared at low density in plastic containers

Data Measured
- Pupal mass, survival
- Time to pupation and eclosion
- Adult size and wing morphology

Quantifying Parasite load:

- Tape method -- records presence/absence of infection
- Q-tip method -- quantitative measure of parasite loads based on digital image analysis

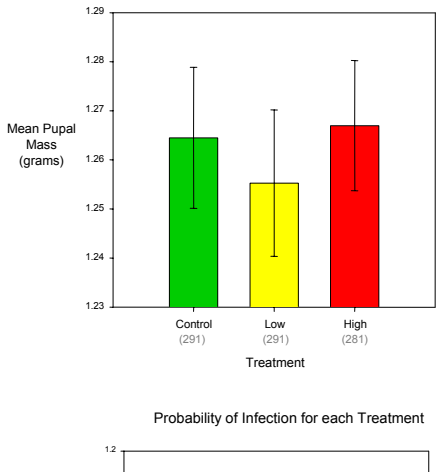
Preliminary Results

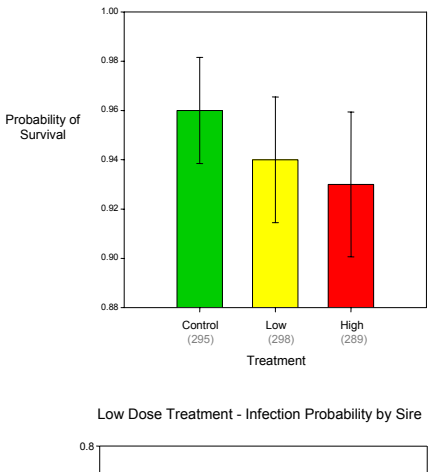
Predictions:
- Monarch survival should decrease with increasing infection dose
- Size and pupal mass should correlate negatively with parasite load, and should be significantly different among the 3 infection treatments (infected individuals should weigh less and have reduced wing area)
- Monarchs parasite loads (and proportion heavily infected) should increase with increasing infection treatment
- Heritable variation might be observed for lethal effects of infection (survival), sub-lethal effects (size and pupal mass), overall probability of infection, and mean parasite loads

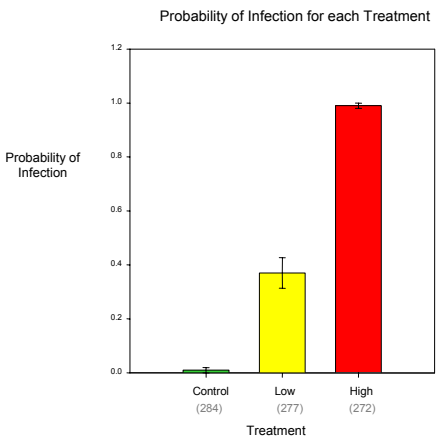
Results:
- Survival and pupal mass were similar across all treatments (no significant difference between control, low dose, and high dose)
- Infection probability increased significantly with parasite treatment (control < low dose < high dose)
- Significant dam and sire-level effects were observed for pupal mass and infection probability
  *Significant differences among half-sib groups in infection probability indicate that resistance to low parasite exposure could be heritable

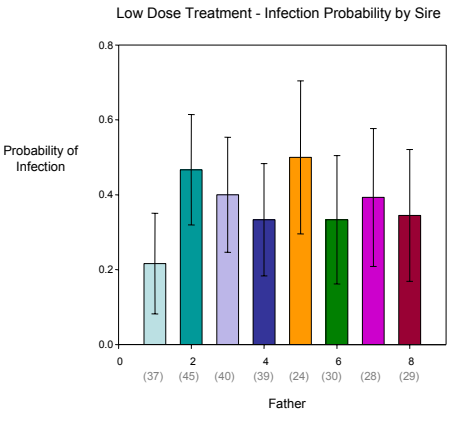
- Next analyses will examine quantitative (continuous) parasite loads, adult deformities, and adult wing area
- Heritability will be measured within each treatment using both traditional components of variance (ANOVA) and restricted maximum likelihood (REML) approaches

Initial conclusions:
- Pupal mass and pre-adult survival were not useful indicators of resistance to low and moderate parasite doses
- Differences in presence/absence of infection indicate a high degree of variability in response to low dose treatment
- Preliminary data from quantitative parasite loads indicates even greater variability in this continuous measure of infection in both low and high dose treatment groups
- Next step is to examine heritability of resistance characters









Future Studies

- Heritability will be calculated for pupal mass, parasite load, survival, and wing morphology
  - no single variable provides a perfect measure of resistance

- Repeat experiment for eastern, western and non-migratory monarchs
  - Increase to 30 offspring per treatment
  - Measure adult survival (longevity) post-eclosion
  - Measure baseline immunity as haemocyte counts and encapsulation response

- Predict that heritability and response to selection will be higher among eastern monarchs than either western or non-migratory monarchs, due to the smaller population sizes, potentially lower genetic variability, and stronger parasite mediated selection operating in resident or short-distance migrant populations.

Measure Cost of Resistance
Energetic costs: wing morphology, pupal mass
Fitness costs: survival, fecundity, developmental rate

By quantifying resistance, family groups can be categorized into increasing levels of resistance. Traits representative of fitness costs due to resistance can be compared between control groups in families with low levels of resistance and families with high levels of resistance. This is important because resistance is assumed to be costly. If resistance is not costly and is a heritable trait, the expectation is that all individuals should evolve to be highly resistant. High levels of variation in resistance points to a potential cost of this character.

Quantify resistance and underlying heritability to native (from same population) vs. novel pathogen genotypes

Significance

Parasites are ubiquitous in natural monarch butterfly populations, but prevalence and impacts differ among populations. Heritability and evolution of resistance could affect variability in parasite occurrence in this natural host system. Heritability of resistance will determine the ability and rate at which hosts can respond to selection pressures dictated by environmental determinants and parasite mediated selection.

Little is known about host resistance to disease in wild animal populations or about the rate and ability of host response to parasite mediated selection. It is important to understand the potential effects of natural selection on host resistance and parasite virulence, especially when considering natural systems of conservation interest and when interested in the potential host response to novel parasites introduced or re-introduced to a system.

These issues arise from a major over-arching question: What factors account for the variability to parasitism within and between populations of monarch butterflies? This dissertation research will focus on the genetic and environmental difference between and within migratory and non-migratory populations to better understand factors causing variation in parasite prevalence among monarch butterflies.

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