Genetic and Environmental Determinants of Host Susceptibility and Parasite Infection in Monarch Butterflies

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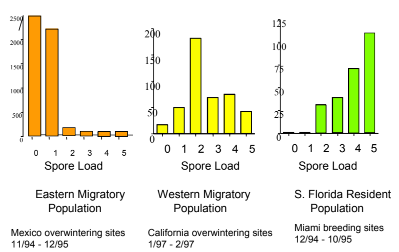
Background and Study System

Premise for Research

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 sharply within and between populations.** The non-migratory populations (Florida, Hawaii) have the highest parasite prevalence, the Western population that migrate moderate distance shows intermediate parasite prevalence, and the Eastern population which migrates the longest distance shows the lowest parasite prevalence. Factors such as migration, climate differences, 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. The objective of my dissertation is to explore and identify the factors determining variation in parasite prevalence within and between wild populations of monarch butterflies.

Variation in Parasite Prevalence Within and Between Three North American Populations



Cluster of monarch at over-wintering site

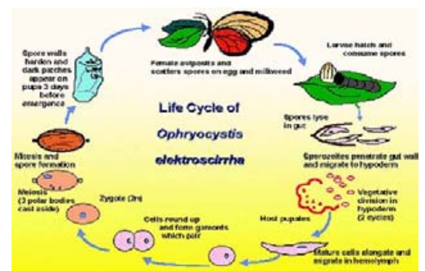
Migratory and non-migratory patterns and locations of North American monarch. 1: Eastern North American; migrate to over-wintering sites in Mexico, 2: Western North American; migrate to costal regions of California, 3: Southern Florida; non-migratory

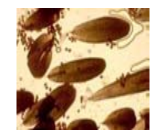
Biology of the Host-Parasite Interaction

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





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

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

Transmission Dynamics
Infection **ONLY** occurs by larvae ingesting spores. Modes of Transmission: strict maternal, paternal (combination of frequency dependent and vertical), horizontal

Effects of Infection

Heavily infected adult monarch

Infected pupa

- 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

Research Questions

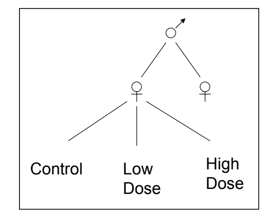
1. What is the genetic basis for variation to host resistance and how does this differ within and between monarch butterfly populations?
2. How genetically diverse are *Ophryocystis elektroscirrha* within and between populations of infected monarch butterflies?
3. Does host evolutionary response differ for native versus novel parasite strains?
4. How do environmental factors affect host susceptibility and parasite development? (Specifically, temperature and host density)

Research Plans

1. Genetic Basis for Host Resistance

- Four ways to quantify resistance:
  - **baseline host 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



Collect Wild Monarchs
Repeat for multiple monarch populations:
Eastern N. A. – long distance migratory
Western N. A. –short distance migratory
Florida or Hawaii –non-migratory

Experiment conduct Fall 2003 with Eastern monarchs

Results: No difference in probability of survival to adult, no difference in pupal mass, and no difference in developmental rate between infection treatments

Significant difference in pupal mass and development time between males and females.

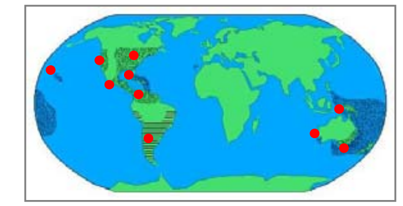
Significant dam effect on wing area, pupal mass, and development time.

Mating Cages

Inoculation of larvae (during 2^{nd} & 3^{rd} instar. By administering 10 µl of solution containing spores on to 1 cm^2 piece of milkweed.

Future Development: estimate heritability for infection and morphological variables and repeat experiment to collect immune defenses and adult survival.

2. Parasite Genetics



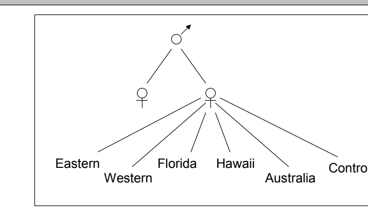
The shaded regions are areas where monarchs persist and the red dots signify the occurrence of parasites. Note the occurrence of parasites at every known location of monarch butterflies.

I will examine parasite genetic variability at three levels: within an individual monarch, within a population, and between populations

I expect to find a high degree of variability between populations of *Ophryocystis elektroscirrha* due to extreme isolation by distance.

Variation in parasite genetics could influence the ability of hosts to evolve resistance to native and novel parasite strains.

3. Resistance to Native and Novel Pathogens



Goal is to determine the difference in host response to multiple parasite strains, including those from natal, neighboring, and distant populations.

Conduct experiment similar to nested sib analysis.

I predict that hosts will be more resistant to native versus novel parasite strains and that resistance will decline with increasing geographic and genetic distance.

For all experiments, I will measure host defenses, parasite replication, and effects on host fitness.

Environmental Determinants of Susceptibility and Infection

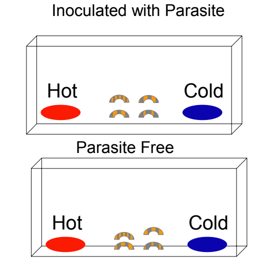
Temperature

Explore the effects of temperature on host immune defenses, behavior, parasite development, and pathogenicity.

Treatment Groups

	Control Temperature 26º C	Low Temperature 20º C	High Temperature 30º C
Control (no infection)	30 monarchs	30 monarchs	30 monarchs
Low Dose (50 spores per larvae)	30 monarchs	30 monarchs	30 monarchs
Medium Dose (500 spores per larvae)	30 monarchs	30 monarchs	30 monarchs
High Dose (1000 spores per larvae)	30 monarchs	30 monarchs	30 monarchs

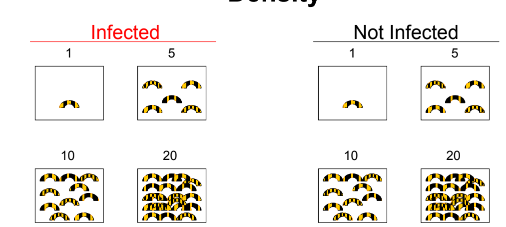
Behavior Experiment



Predictions

- Increasing temperature could affect host immune defense by altering:
  - haemocyte count
  - phenoloxidase activity
  - melanism (percentage black on individuals)
  - parasite load
  - pathogenicity (including lethal and sub-lethal effects)
- If behavioral response through thermal regulation is possible, infected individuals should spend more time at and seek temperatures that improve host immune defenses

Density



Predictions

- Increased host density should result in elevated immune defenses in the absence of parasites
  - Increasing density in the presence of parasites could increase parasite loads and negative effects on host fitness if monarchs are stressed
  - If high density elevates host defenses, parasite loads among innoculated monarchs should decrease with increasing density or show a non-linear relationship