Crowding and disease: Investigating density dependent parasite resistance in monarch butterflies (*Danaus plexippus*)

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Abstract

The purpose of this research project is to examine the effects of population density on the immune defenses and susceptibility of monarch butterflies (*Danaus plexippus*) to infection by a protozoan parasite, *Ophryocystis elektroscirrha*. To accomplish this goal, we will infect host larvae with calibrated doses of parasite spores and rear them in three density treatments. We will measure lethal and sub-lethal effects of disease, infection status, and immune parameters (such as phenoloxidase activity and anti-bacterial activity of hemolymph) of infected and uninfected larvae. Previous research has shown that other insect species express an enhanced immune response when reared in high densities. Different populations of monarch butterflies vary in both larval population density and parasite prevalence; this study will address whether or not there is a direct relationship between larval population density and variation in host susceptibility to infection.

Background

Development of Monarch Butterflies (*Danaus plexippus*)

• There are five larval instar stages. The total time of development is approximately 30 days.

Figure 1. The five developmental stages of larvae

Ophryocystis elektroscirrha

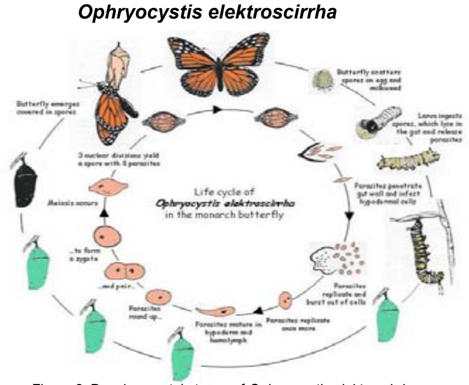


Figure 2. Developmental stages of *Ophryocystis elektroscirrha*

Prevalence of *Ophryocystis elektroscirrha* in Monarch Populations

• There are three populations of monarch butterflies in North America.

• The density of monarchs in wild populations varies throughout North America.

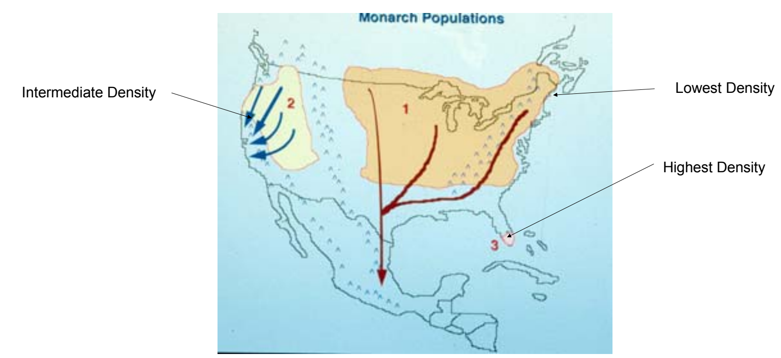


Figure 3. Density variation of wild monarch populations in North America

Effects of larval density on susceptibility to infection and immune response

• The effect of population density on the immune system and disease susceptibility of monarch butterflies.

• Prior studies done on Lepidoptera indicate that larvae reared at high densities show an increased immune response and higher survival rate

• However, if stress is increased due to crowding, then larvae reared at high densities might show increased susceptibility to infection.

Methods

Experimental Design

Table 1. The total number of larvae used in the experiment for each experimental group. Number of replicate counts shown in parenthesis.

	Density treatment – number of larvae per container		
Parasite treatment	*Low (1 larva)*	*Moderate (5 larvae)*	*High (10 larvae)*
Control	40 larvae, 40 containers	60 larvae, 11 containers	110 larvae, 11 containers
Parasitized	40 larvae, 40 containers	60 larvae, 11 containers	110 larvae, 11 containers

Breeding

• Mated 17 families of monarchs from Eastern populations.

Figure 4. Mating cages used to breed monarch butterflies.

Inoculation

• Randomly assigned larvae to treatment group

• Inoculated 210 larvae with 300 spores, and 210 larvae with 0 spores.

Figure 5. Petri dishes of inoculated and control larvae

Rearing

• Growing larvae in densities of 1, 5 and 10 per container.

• Larvae were fed fresh cutting of greenhouse-reared swamp milkweed

Figure 6. Rearing tubs used to grow larvae at different densities

Low Density Larva

Medium Density Larvae

High Density Larvae

Data Measured

Monarch survival

- Mortality of larvae and pupae
- Adult survival (in days) post-eclosion)

Monarch developmental and growth

- Development time
- Growth rate
- Pupal mass
- Adult mass
- Adult wing area

Immune system parameters

- Hemocyte count
- Phenoloxidase activity

Larval and adult coloration

- Larval melanism
- Adult wing pigmentation

Developmental Time = (Date of Eclosion – Date of Oviposition)

Growth Rate = Pupal Mass / Developmental Time

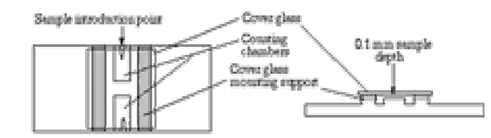


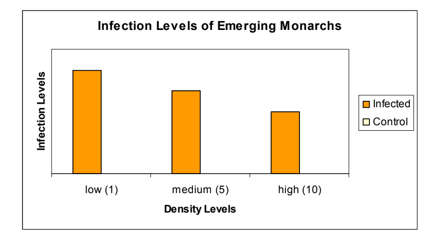
Figure 7. Hemocytometer used to measure immune response in monarch butterflies.

Expected Results

Prediction

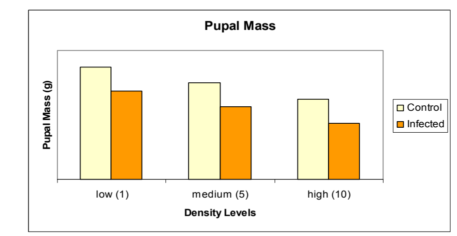
• Based on prior studies indicating an increase in immune response at high densities, our data should show that larvae reared at the highest density will have the greatest immune response, highest survival rate, and the least infection.

• However, if stress due to crowding plays a significant role, the highest density will show the lowest immune response, lowest survival rate, and the most infection.



• Infection levels should decrease as treatment levels increase, due to better immune response by higher density larvae.

• Low infection levels in high density larvae due to larvae's ability to better fight off parasitic infection.



• Pupal mass in the control and infected larvae decreases as density level increases

• Decrease in pupal mass caused by low density larvae's ability to better allocate energy

• Allocating less energy to immune response allow for nomal development

Significance

• Gain a better understanding of the relationship between infectious disease and immune defense to parasitic infection.

• Destruction of milkweed due to urban sprawl forcing monarch to find the same milkweed clusters, thereby increasing larval population densities.

• Global warming creates a temperature increase allowing milkweed to grow in different regions, thereby altering the geographical range and density of monarch populations.

Acknowledgements

We would like to thank these individuals for helping us implement and carry out this project:

• Catherine Bradley
• Andrew Davis
• Laura Gold
• Liz Harp
• Jaap de Roode
• Jane Stout
• Nick Vitone

We would like to thank Emory University SIRE (Scholarly Inquiry and Research at Emory) Grant for giving us the funding that aided in the completion of the project.

Important Terms

Developmental rate – is a measure of the time it takes for a larva to develop into an adult monarch, from egg to adult

Encapsulation – Foreign cells or other material is 'deactivated' by surrounding it with a layer of melanin, essentially walling this off from the rest of the host's cells.

Hemocyte – a component of the innate immune system in monarch butterflies, comparable to white blood cells

Melanism – level of dark pigmentation derived from melanin production. In insects, cuticular melanism (body darkness can be associated with investment in immune defenses.

Melanization – a type of immune defense mechanism in invertebrates whereby foreign material is encapsulated by producing a melanized layer around the invader. This depends on a key enzyme called phenoloxidase and is formally referred to as an encapsulation response and nodule formation.

Phenyloxidase activity – an enzyme (often abbreviated PO) that regulates the production of melanin. This converts phenols into quinones, which subsequently polymerize to melanin, and this melanin is then deposited onto foreign material like parasite cells.