Behavioral and environmental determinants of parasite transmission in a butterfly host

Chastity Ward[1], Paola Barriga[2], Sonia Altizer[2], Richard Hall[2]

[1]Department of Biological Sciences, Fayetteville State University, [2]Odum School of Ecology, University of Georgia

Conceptual Background

- Many pathogens of humans and wildlife have transmission stages that are shed into the external environment by infected hosts, where they are encountered by susceptible hosts. Examples include human diarrheal diseases like cholera, and emerging fungal diseases of wildlife (Fig. 1).
- The dynamics of environmental transmission require further investigation to better understand environmental and behavioral determinants of parasite shedding, which are important for modeling and predicting pathogen spread.
- In particular, some pathogens show evidence for hosts that act as superspreaders, which has been well documented for contact-transmitted diseases, but is less well known for pathogens with environmental transmission.

Fig. 1. A-B. Many human diarrheal diseases, including cholera and rotavirus, are transmitted when water contaminated with human waste is consumed. **C.** Chytrid fungus induced mortality in wild frogs

Study System

- Monarch butterflies *Danaus plexippus* are infected by the obligate, debilitating protozoan parasite *Ophryocystis elektroscirrha (*OE*)* throughout their global range.
- Environmental transmission occurs when infected adult butterflies shed spores onto milkweed (*Asclepias* spp., food plants for caterpillars), which are consumed by larvae and develop internally.
- Quantifying the rate of environmental transmission is important for modeling infection dynamics at the population level, but has not been rigorously estimated in the field. Previous models assume monarchs contaminate 1-300 leaves/day with infectious spore doses.
- The goals of this project are to estimate adult monarch visitation rates on milkweed as a proxy for environmental transmission, and to examine behavioral and environmental factors that cause variation in adult visitation rates.

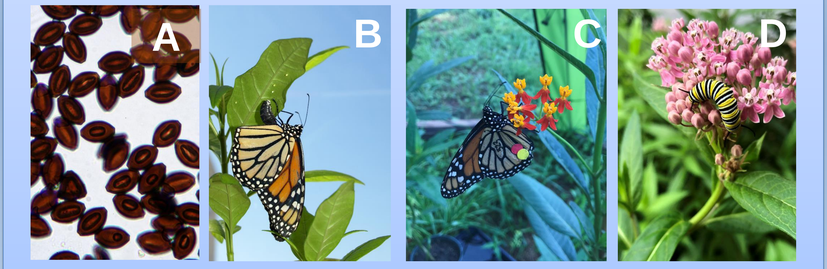


Fig. 2 A. OE spores viewed at 1000x. **B-C.** Infected monarchs can shed spores onto milkweed during oviposition, or when landing on milkweed to nectar or for other purposes. **D.** Caterpillars become infected when they ingest spores dropped onto milkweed by infected adults.

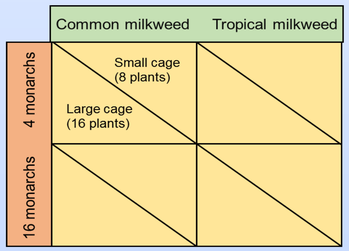
Research Questions

1. How much individual variation do we observe among monarchs in plant visitation behavior, and what monarch traits might predict superspreaders of infection?
2. How do environmental factors, including milkweed species identity and monarch density, influence plant visitation rates and potential pathogen shedding onto plants?

Experimental Design and Methods

- We set up 8 outdoor flight cages that experimentally varied host plant species, and monarch and host plant numbers (Figs. 3, 4A).
- Captive-raised monarchs from distinct genetic lineages were marked and added to each cage (Fig. 4B).
- The monarch sex (M:F) ratio per cage was fixed at 50:50.
- During 20 min intervals, observers recorded the identity of plants and number of leaves visited (for egg-laying, mating, nectar-feeding or resting) and monarch IDs involved in each visit (Fig. 4C-D).

Fig. 3. Experimental design. Each outdoor cage contained either common milkweed (the most abundant native milkweed for migratory monarchs) or tropical milkweed (an exotic species planted by humans and used by monarchs in the southern US). Large cages (10'-cubed) had 16 plants, and smaller cages (6' cubed) had 8 individually numbered plants.



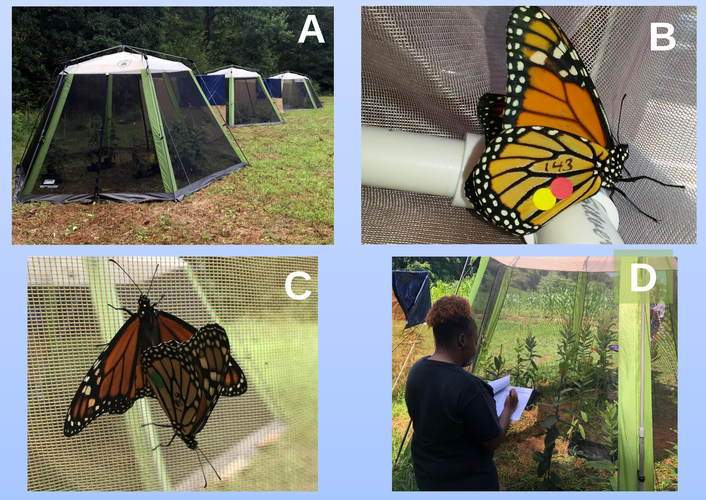


Fig. 4. A. Cage set-up showing large and small alternating cage sizes **B.** Monarchs were marked with unique color combinations for easy identification. **C.** Two monarch butterflies mating on cage net. **D.** Recording observations of plant visitation in the field.

Acknowledgements

I thank the NSF-funded Population Biology of Infectious Diseases Research Experience for Undergraduates program for this opportunity. I would like to thank the Altizer lab for assistance in setting up the experiment and recording observations, and Ania Majewska for help in data analysis and creating figures.

Data Analysis and Results

1. Variation in monarch visitation rate

- Monarchs on average visit 14.5 leaves per hour and show substantial variation in their milkweed visitation rates (variance to mean ratio = 14.7)
- Visitation rate differed by sex, with females visiting 4.7 times more frequently than males.
- Most (73%) female visits to milkweed were for oviposition.

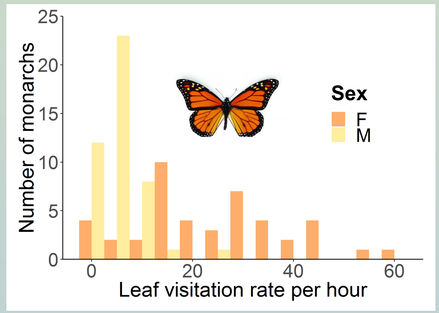


Fig.5. Histogram showing variation in the leaf visitation rate for female and male monarchs.

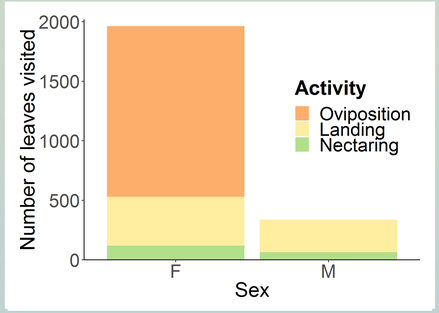


Fig.6. Breakdown of leaf visitation by activity type for each sex.

2. Variation in plant visitation rates

- Plants received on average 5.7 visits per hour, with substantial variation among individual plants (variance to mean ratio = 5.3).
- The only significant factor predicting plant visitation was the number of monarchs per cage.
- Milkweed traits such as flowering status, number of leaves, and species identity did not affect the visitation rate.

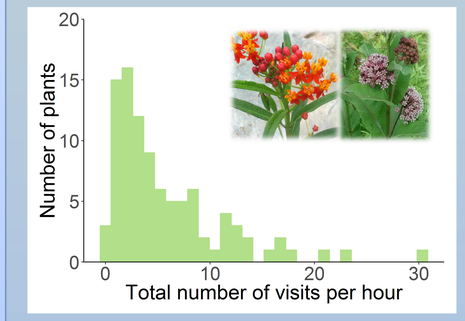


Fig.7. Histogram of the per plant visitation rate

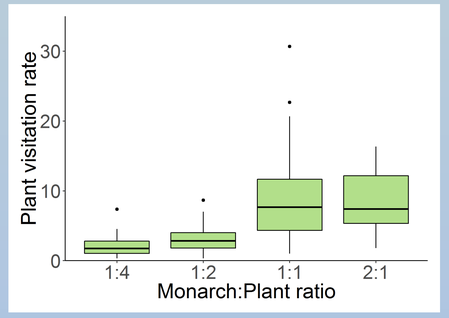


Fig.8. Plant visitation rates based on Plant:Monarch ratio from treatments.

Conclusions and Future Directions

- This experiment provided estimates for environmental transmission rates based on plant visitation behavior.
- High variance in visitation rates suggests the potential for some monarchs to act as superspreaders, and for some plants to be transmission hotspots.
- We found support for monarch sex and density, but not the other design variables, in explaining visitation rates.

Future Studies:

- Further work is needed to determine other factors that contribute to variation in visitation rates among individual plants and monarchs.
- We would like to develop a mathematical model that accounts for variation in visitation rate to understand its role in transmission dynamics.