

**Tinker Foundation-Latin American and Caribbean Studies Institute (LACSI)
Graduate Field Research Award Proposal
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Project Title:

What factors determine the range of hosts a pathogen can infect? Milkweed butterflies and their protozoan parasites in Latin America as a case study

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Overview and aims: Understanding what factors limit the range of hosts a pathogen can infect remains a central topic of research in ecology and evolution. Its importance stems from the fact that parasites that infect multiple host species are often associated with newly emerging diseases that threaten humans (e.g., Ebola, influenza A and SARS) and wildlife hosts (e.g., white nose syndrome in bats, chytridiomycosis in amphibians)^{1,2}. Determining the relative contribution of evolutionary and ecological factors that limit parasite distributions and infection prevalence can allow researchers to establish scenarios under which shifts to new host species might occur. In some parasite groups, the evolutionary similarity between host species predicts whether parasites will cross species boundaries. Despite this, shifts between distantly related hosts also occur^{3,4}, especially when cross-species exposures are frequent. Several recent studies (on systems including bat-rabies and plant fungal interactions) analyzed host-parasite associations to show that both host evolutionary relatedness and the extent of geographic overlap between pairs of hosts can predict host-parasite interactions⁵⁻⁹. More importantly, intensive field studies, comprehensive experiments, and mathematical modeling approaches are needed to tease apart the underlying mechanisms that determine the ability of pathogens to infect different hosts, and to predict the resulting ecological outcomes¹⁰.

My proposed dissertation research focuses on a debilitating protozoan parasite that infects a group of insect hosts, namely the milkweed butterflies. Milkweed butterflies include the monarch (*Danaus plexippus*), famous for its long-distance migrations in North America, in addition to 12 other species in the genus *Danaus*, and many other species in closely-related genera. Monarchs and several other *Danaus* species commonly occur throughout Latin America, and have been reported to be infected by neogregarine protozoan parasites in areas where multiple species overlap. The overarching goal of my work is to examine the ecological, phylogenetic and immunological barriers to infection by these obligate parasites. I will conduct field sampling, laboratory experiments, and develop mathematical models of cross-species transmission to accomplish the following goals:

(Aim 1) Quantify parasite exposure and cross-species transmission at field sites in Latin America and the Southern US where multiple host species overlap in space and time.

(Aim 2) Test the contribution of immunological barriers to cross-species infection across a subset of hosts and parasites.

(Aim 3) Synthesize ecological and immunological determinants of parasite infection using mathematical models to explore cross-infection dynamics among closely related hosts.

Project outcomes for my dissertation include:

- (1) An approach for predicting the ecological and evolutionary determinants of parasite distributions and host range that could be applied to studies of human infectious diseases.
- (2) A better understanding of disease risks for an important group of pollinators that could inform future efforts to monitor their health.

Background: This project focuses on a well-studied and globally distributed group of butterflies that can be readily captured, identified, and sampled for infection. A recent phylogeny published

for the milkweed butterfly subfamily Danainae includes 43 species across 10 of the 12 described genera¹¹. Migratory monarchs (*Danaus plexippus*) and non-migratory queen (*D. gilippus*) and lesser wanderer (*D. petilia*) butterflies belong to this group. Monarchs host the debilitating neogregarine protozoan parasite *Ophryocystis elektroscirrha* (OE)¹². This parasite has been found in all monarch populations⁴ examined to date, including those from temperate, subtropical, and tropical locations, with prevalence varying from 2% to nearly 100%, and correlating inversely with monarch migratory propensity¹³. Infection of new hosts occurs when caterpillars ingest parasite spores that lyse in the gut and migrate to other parts of the body. Parasites replicate inside the caterpillars, and infected monarchs emerge as adults covered with millions of dormant parasite spores on the outsides of their bodies^{14,15}.

Parasite transmission occurs when adults scatter spores onto milkweed host plants that can be eaten by their own offspring or unrelated caterpillars. This parasite causes substantial harm to monarchs, by inducing smaller body sizes, reduced flight performance, shorter adult life span, and reduced probability of mating¹⁶⁻¹⁸. Although OE has been assumed to specialize on monarchs, OE-like (OEL) parasites have also been found on queens and lesser wanderers¹⁹. Recent field sampling showed that up to 20% of queen and up to 60% of lesser wanderer adults were infected with OEL parasites. Additionally, work is being conducted in the Altizer lab, led by postdoc Paola Barriga, to survey past historical collections in museums, to characterize the host range of parasites across butterfly taxa, and to document the distribution of OE and OEL parasites with holdings dating from 1936-present.

Multiple factors could determine parasite host range in this butterfly clade and other host-parasite systems. First, geographic overlap among host species' ranges and use of the same local habitat patches provides ecological opportunities for cross-species transmission and parasite sharing among hosts^{6,20}. Second, morphological, behavioral, and ecological similarity among potential host species can affect the ability of parasites to infect multiple host species²¹. Host species that use the same resources, for example, might share similar opportunities for parasite transmission, and parasites might face challenges in adapting to host species with distinct physical or behavioral traits^{22,23}. Host evolutionary relatedness should also influence parasite infection in part because related hosts have similar physiological, immunological, and molecular traits that facilitate pathogen colonization^{9,24,25}. Experimental studies are needed to explore potential immunological barriers to parasite infection across host species. My proposed PhD work will advance knowledge of host-parasite ecology and evolution by exploring the biological and ecological factors that determine parasite host range at multiple scales, and by considering a combination of ecological and immunological variables.

Work plan: LACSI/Tinker funding will support an initial field trial to address Aim 1 of my dissertation, to indicate the extent to which multiple milkweed butterfly species use the same habitat patches in Latin America might be exposed to parasite strains from alternate host species.

Specifically, requested funds will support pilot field observational studies at a research station in San Luis, Costa Rica, a site where milkweed is abundant and where three *Danaus* species overlap in habitat use (monarchs, queens and soldiers, *D. plexippus*, *gilippus*, and *eresimus*), in

addition to representatives of three other genera of milkweed butterflies. OE infections were previously observed on monarchs and queens at this location¹⁹.

I will identify 10 replicate milkweed patches within a 10-km radius of the field station. In each patch, we will quantify spatial overlap in habitat use between butterfly species by recording the species identity of each butterfly observed, their behavior (e.g., mating, foraging, ovipositing, patrolling), and contact or proximity to milkweed plants. Adults will be captured and sampled for the presence of infection, and leaves will be randomly sampled for the presence of parasite spores. I will scan plants and record the presence of eggs and caterpillars for each host species. The host frequency use and patch use will be used as a proxy to calculate the likelihood of exposure each butterfly species to its own versus novel parasite strains.

For all *Danaus* species captured, non-destructive sampling of the exterior of butterfly abdomens will be used to obtain samples of parasite spores and determine infection status. Samples will be viewed at 1000x to visualize parasite spores. I will score samples for the presence of infection (0/1) and an index of parasite load, based on the presence and numbers of parasites spores²⁶. I will also collect a subset of caterpillars observed on milkweed plants, rear them to the adult stage, and determine infection status prior to release. Finally, I will mark and follow individual adults, and conduct a capture-mark-release-recapture study, to examine site fidelity of individual butterflies, and to estimate population sizes of each butterfly species.

Feasibility: I have previous experience studying tropical butterflies. My co-mentors at the Odum School of Ecology at the University of Georgia, Dr. Sonia Altizer and Dr. Nicole Gottdenker, are experts in studying host-pathogen ecology in natural populations, including the OE parasite in monarchs, and other protozoal infections in Latin America. UGA has a track record of collaborations and field studies in Costa Rica that will allow access to a field research station in San Luis and will facilitate obtaining research permits. Previous field trips to the San Luis study site have confirmed the presence of milkweed butterflies and milkweed plants location that are critical for a successfully carrying out this research plan. (I also note that the UGACR campus will remain under UGA management during Summer 2019, when this initial study will take place)

Budget: I request \$2,000 from Tinker Foundation-LACSI to use towards travel, field supplies, and permits needed to record butterfly and parasite data at field sites near San Luis, Costa Rica. Additional field supplies will be supplemented by other funding sources.

Budget item	Unit value (\$)	Total (\$)
Airfare to Costa Rica	400	400
Ground transportation	115	115
San Luis field station daily fees for food and lodging	80 per day x 14 days	1120
Costa Rican research permits	240	240
Field supplies (gloves, butterfly nets, sampling supplies)	125	125
Total		2,000

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