Quantifying Infection Rates in Natural Populations (*Drosophila-Howardula* System)
(prepared by Michael Hood, Amherst College – Draft of September 2017)

Model system: mycophagous *Drosophila* and parasitic *Howardula* nematodes
The exercise take two 3-hour classes; tractable for maintenance and experimentation in the lab.

Introduction

Wild *Drosophila* that feed on mushrooms are a very widespread system that is easy to work with, and there are easy connections to the biology that students have seen in other contexts (e.g. the lab model fruitflies). Their parasitic nematodes are also fascinating and occur a high enough prevalence to help engage student with discovering and quantifying infected individuals. The host-pathogen system has been best developed by John Jaenike (University of Rochester), who helped advise during creation of this teaching module.

The disease cycle of the *Drosophila*-nematodes system involves infected flies depositing larval nematodes on mushrooms as the flies come to feed, mate or lay eggs. The nematodes mate, and then females penetrate and colonize the fly larvae. Infection, which fills the abdomen with a "motherworm" and her larval nematode offspring, often results in partial or complete sterility of female flies, while there can be some effects on males and on mortality. The flies and infecting nematodes overwinter as adults.

(Photos show flies on surface of bolete mushroom, and observation of an infected fly, with large mother worm [bottom center] and many larval worms released from the abdomen.)

Aims and Objectives

This model has been used as the first exercise in an undergraduate field course on disease ecology. The goal is to have the students learn about the quantification of infection rates in natural populations. We emphasize the importance of species determination (of the flies).

Beforehand

Collect local mushrooms to use as bait for the Drosophila (even months before the class)
Try particularly for bolete and stinkhorns.

Commercial mushrooms also work, as flies will both feed and oviposit on them in the wild.
The mushrooms can be frozen and thawed for use.
Scout areas where mushrooms are found; wooded areas work well.
Set out small piles of mushrooms one to several day in advance.

Have students each find a primary research paper in which infection rates were quantified in natural populations. Guide them on Web of Science or Google Scholar

Have students summarize and briefly present to the class:
What was the host-pathogen system?
How was population size estimated?
How were the boundaries of the population defined?
What was the proportion infected or diseased?
How many populations were assessed?

Have students read and discuss Jaenike 1992 Am Nat 139: 893-906.
(There are other papers to read, but this is a good one to start with, without giving away too much of what's already known about the system.)

Materials and Resources

Fly guide to local mycophagous species (see link below)
Insect aspirators (Forestry Suppliers has a nice one)
Collection tubes
Freezer
Stereo microscope, perhaps access to a compound microscope
Insect pins and or very fine forceps
Glass microscope slides
Water

Activities

Day 1:

Discuss reading and student questions.
Go out and collect;
Aspirating flies off the mushrooms and surrounding vegetation. Note that flies have predators and can thus be skittish. Be stealthy in approaching them for collection. This also allows students to observe aspects of the flies' behavior in natural conditions.
(Others have used fine sweep nets)
Freeze flies until next lab.
(In the period of an hour or so, each student might collect more than 50 flies)

(In between Days 1 and 2, it is helpful to determine the species for lots of flies because this takes the most time in the lab. We leave some flies for students to sort out. We also see the invasive *D. suzukii* in the collections, which is most associated as a pest of fruit, but also frequently feeds on mushrooms.)

Day 2:

Students group flies by species and sex.

Dissect flies by pulling open the abdomen with insect pins or forceps.
Can confirm sex base on internal reproductive anatomy.
Assess the presence and numbers of mother worms and larval worms.
We use rough quantification of larval worms (few/intermediate/many)
Assess the condition the ovary and eggs, again as a rough score.

Points of Discussion

Comparison of infection rates among species.
Comparison of infection rates between sexes.
Data considerations:
Sample size and confidence intervals for proportional data.
Contingency tables and chi-square test statistic for comparing multiple proportions.
By counting the number of motherworms per fly, students can determine whether the motherworms per fly frequency distribution matches a Poisson distribution.
Possible problems or biases in collection or estimates of infection rates.
Effects of infection on survival or behavior.
Temporal or spatial variation in infection rates.
Community-level interactions and impacts on infection rates.
Possible further studies:
Test for effects on fly mortality (e.g. fly survival affected by infection status).
Test for effects on fly behavior.
Among site or temporal variation in infection rates.
Predicting impacts on host populations.
Read and discuss recent paper(s) on this system.

Resources and References

Species Identification: "Drosophilids of the Midwest and Northeast" website
by Thomas Werner and John Jaenike
http://humanities.lib.rochester.edu/drosophilaguide/
Jaenike, J. "Mycophagous Drosophila and Their Nematode Parasites."
The American Naturalist 139 (1992): 893-906.
Jaenike, J., and S. Perlman. "Ecology and Evolution of Host-Parasite Associations:
Mycophagous Drosophila and Their Parasitic Nematodes."
The American Naturalist 160 (2002): S23-S39.

Example of infection rate data from Amherst College, BIOL 201 course, fall 2017

	infected	uninfected	Tot	Prop
D. falleni	23	44	67	0.343
D. neotestacea	27	29	56	0.482
D. putrida	6	17	23	0.261
D. recens	5	15	20	0.250
D. tripuncta	0	10	10	0.000

(interestingly, these results look very similar to the among species pattern in Jaenike 1992)