Parasite-host associations: An experiment on fecundity and infection success

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Vocabulary

- Parasitism - The act of an organism (the parasite) diverting resources from a host for their own growth and fitness without rewarding that host.
- Fecundity - Fecundity is the reproductive rate and quantification of the number of individual offspring produced.
- Fitness – Fitness is a quantitative depiction of the success an organism has in passing its genes. The likelihood that an organism is able to survive and live longer to reproduce, the higher is the fitness of that organism.
- Filial generations – Filial generations refer to the sets of offspring which come from observed or controlled reproduction. First set of offspring would be referred to as F1 and future offspring of F1 would be labelled sequentially thereafter (F2, F3, etc...)

Abstract

Parasitylenchus *nearcticus* are a rare nematode parasite that infects Drosophila species. The affects of Parasitylenchus *nearcticus* has been previously observed on the eastern Drosophila *recens*, however, for our experiment we are concerned with Drosophila *subquinaria*, a western species, since the host range of Parasitylenchus *nearcticus* is understudied. Using the host range, it is possible to describe the niche of parasites. In this experiment, we question the effects of the parasite on host fecundity and we also observe the parasite performance according to host sex. Fly larvae were infected with parasites, separated by sex and kept alive for one week after pupae emergence before being dissected under a microscope. Once dissected, the number of worms were counted and categorized depending on their filial generation. We also compared the mature eggs of females to see if the number of eggs vary according to infection status. Infected female Drosophila *subquinaria* had no mature eggs in their body therefore had been deprived of their fecundity. When compared, males had a much higher parasite density than females indicating more reproductive success of the mother worm in males. Our results suggest an immunological difference between male and female flies and implies the male's susceptibility to parasite reproduction within the fly body.

The host

The fly that is investigated in our experiment is of subgenus Drosophila and species *subquinaria*. Drosophila *subquinaria* are related to Drosophila *melanogaster* which is the common fruit fly that would be found in a household that feeds on fruit and compost. Drosophila *subquinaria* on the other hand, is not a household pest but rather a woodland fly that feeds on mushrooms. Plenty of Drosophilidae species such as *melanogaster* and the eastern Drosophila *recens*, are identifiable by stripes that cover their body from their abdomen downwards, however, Drosophila *subquinaria* are characterized by their particular spotty patterns (as seen in images below). Images below were obtained using a microscope and a cellphone camera at the insect and parasitism lab at Uvic (August 2022).

Figure 1., Female Drosophila *subquinaria,* as a feature, have two prongs on its rear end.

Figure 2., Male Drosophila *subquinaria,* as a feature, have a lack of prongs at the rear end. Instead, there are two black lines on the underside of its rear end.

The parasite

Parasitylenchus *nearcticus* is a rare understudied worm parasite that infects Drosophilidae. Parasitylenchus *nearcticus* falls under the phylum Nematoda (also referred to as nematodes) and is not well known in the west coast. Parasitylenchus *nearcticus* performs parasitism by infecting the fly and draining its resources and nutrients. Through this process, the nematode is able to grow and reproduce within the fly. The existence of this nematode within a fly body is a very negative consequence for the fly as it hosts the worm without gaining anything in exchange while having to endure the worm's harmful growth. The life cycle of Parasitylenchus *nearcticus* consists of laying eggs within the host. The aim is for the offspring worms to be excreted or shed out of the body of the fly and onto the mushroom where Drosophila *subquinaria* are situated to infect more flies upon emergence. A host, as a source of nourishment, is a finite resource, however, the worm doesn't completely rely on the fly's success since the offspring that the worm produces will eventually be shed out.

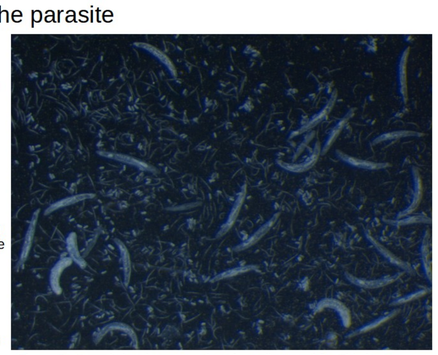

Figure 3., Parasitylenchus *nearcticus* from dissected fly. This image was obtained using a microscope at the insect and parasitism lab at Uvic (August 2022).

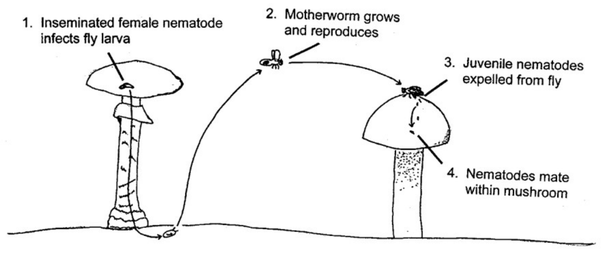

Figure 4., nematode life cycle within fly host.

Jaenike, J & Perlman, S. J., (2002). Ecology and Evolution of Host-Parasite Associations: Mycophagous Drosophila and Their Parasitic Nematodes. The American naturalist. 160 Suppl 4. S23-39. 10.1086/342137.

Images were obtained using a microscope at the insect and parasitism lab at Uvic (August 2022).

Figure 5. Dissected Drosophila *subquinaria* with nematode infection.

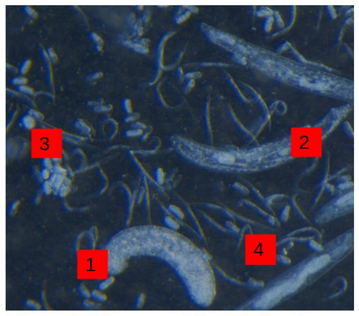

Figure 6. Close up of Parasitylenchus *nearcticus.* Four maturity stages are pres

1: F0 mother worm 2: F1 offspring

3: Eggs layed by F1 4: F2 offspring from eggs

Objectives

- Does Parasitylenchus *nearcticus* infection affect the fecundity of Drosophila *subquinaria*?
- What are the differences between male and female Drosophila *subquinaria* when infected with Parasitylenchus *nearcticus*?

Experiment and Methods

Vials with cheese cloth bases and slices of mushroom have been prepared to accommodate the flies and to facilitate their good health in order to ensure pregnancy. Once approximately 20 eggs have been collected, these flies were exposed to 250 nematodes. At this stage, flies that have been infected will not be dissected but grinded with a mortar. The ground up infected flies were mixed with a saline solution. 1 micro-litre of ground up fly was used to measure how many worms per 1 micro-litres were present. With 19.5 being the average amount of worms per 1 micro-litre, **250 worms equated to 12.8 micro-litres which was the amount of worms that were added to the mushrooms with the fly eggs with the aim to infect the offspring**. Flies were collected as they emerged daily. They needed to be separated by sex; it was crucial for females to remain unmated so that no more eggs were made. After the date of emergence of any particular fly, they were kept healthily alive for a week before being frozen. The flies collected had emergences from the dates July 7-9. The frozen flies were thawed, and dissected. If infected, the number of F0 mother worms as well as F1 offspring worms were measured and recorded for each dissected fly. For the females in particular, the ovaries were inspected and the number of eggs were measured and recorded. For our experiment, if the yolk of the egg is anywhere above half full, it has been marked as mature. The data has been visualized using the program R, the standard error and mean of the comparison of the axes were showcased.

How host fecundity depends on infection status

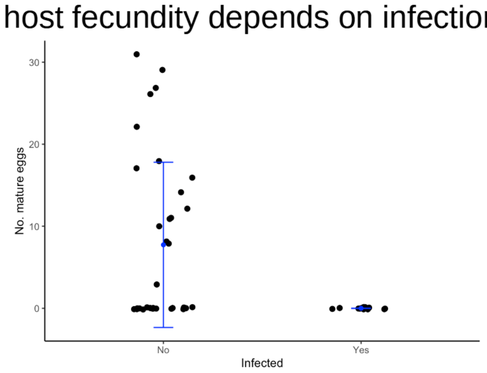

Figure 7. Comparison of the amount of mature eggs produced according to infection status of the fly.

Infection success for each sex

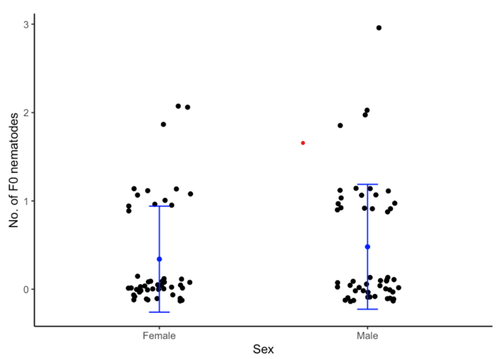

Figure 8. Comparison of the physical infection success of F0 mother worms in both sexes. Non-infected flies at value zero have no mature eggs whereas Infected flies at value zero have a complete absence of eggs altogether.

Reproductive success of mother worm for each sex

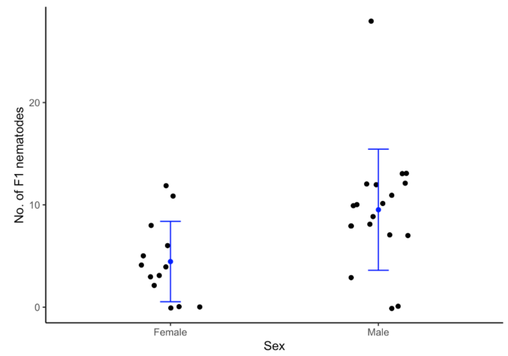

Figure 9. Comparison of the reproductive success of F0 mother worms (producing F1 offspring as consequence) in both sexes.

Results

- There is an absence of mature eggs in female flies that have been infected.
- There is a very minimal difference in the mean when comparing male and female. The physical infection success is not significantly greater in one sex or the other.
- The F0 mother worm was able to perform more successfully in males than in females as there are more offspring found in the dissected males.

Discussion

As seen in Perlman and Jaenike (2001), Parasitylenchus *nearcticus* has this fascinating ability to completely sterilize its female host which consequentially leads to no production of eggs. **The data extrapolated in our experiment shown in Figure 7, are consistent with their findings as the sterilization of the female host caused by the nematodes was observed.** If there is infection, then there were no eggs observed. Conversely, if there is no infection, numerous eggs were observed in a majority of the females. Interestingly, the Drosophila species that were observed in Perlman and Jaenike was an eastern species known as Drosophila *recens* whereas the fly we observed was western. The result we have obtained exhibits the inclusion of Drosophila *subquinaria* as a candidate in our nematode's host spectrum.

For the F0 mother worms, the difference between the main values for the sexes (0.5-0.4=0.1) is observed to be insignificant. However, for the F1 offspring, the difference of the main values for the sexes is about 5 (10-5=5). **Therefore, the data indicates that physical infection success doesn't vary according to sex. But rather, how well the infecting F0 mother worm performs is dependent on sex.** This dependency raises the question whether the sexes could have inherent immunological differences. In fact, Belmonte et al., (2020) surveyed the literature of the studies on the immunity of Drosophilidae and concluded that there is a strong sex dependency in the immune system responses of Drosophila *melanogaster*.

References

- Perlman, S. J., & Jaenike, J. (2001). Competitive interactions and persistence of two nematode species that parasitize Drosophila recens. Ecology Letters, 4(6), 577–584. https://doi.org/10.1046/j.1461-0248.2001.00270.x
- Belmonte, R. L., Corbally, M.-K., Duneau, D. F., & Regan, J. C. (2020). Sexual Dimorphisms in Innate Immunity and Responses to Infection in Drosophila melanogaster. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.03075

Acknowledgements

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