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Bio 490s: genetics of interactions
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Sub-lethal nematode infection impacts the mortality rates of snowshoe hares via an interaction with predation

Introduction

Parasitism has long been perceived as a relevant influence upon changes in animal communities, for example parasite induced changes in host survival causing reduction in size, rate of growth and demography of animal populations (Holmes, 1982). Negative impacts of parasitism on snowshoe hares may be the direct result of parasite-induced mortality, but indirect negative impacts of parasitism on host populations include an increase in vulnerability to predation (Yuill, 1987). The snowshoe hare is an apt case study for the effects of nematodes on the rate of predation. The snowshoe hares undergo a cyclic population fluctuation over the course of 8-11 years in the boreal forests of North America (Keith, 1990) and similarly previous studies have shown that some nematode population cycles synchronize with the natural fluctuations in hare population. When the hare population is beginning to decrease the nematode infection levels of the hares are at their peak suggesting that as the nematode infection level rises, the hare population begins to experience a decrease in population. This could be due to the population cycles of the snowshoe hares and their predators such as the lynx. However, there is a hypothesis that parasitism plays an indirect role in predation due to its hampering effects on the hares making them easier prey.

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Few studies have specifically examined the potential interactive effects of parasitism and predation. However, due to the primary role predation plays in many natural systems, such interactions are potentially important to the snowshoe hare population dynamics (Aanes & Anderson 1996).

There are three ways infection by helminthes in general can alter host behavior which would increase vulnerability to predation.

1. Biochemical changes in behavior. This is typical of helminthic parasites where behavioral changes enable transmission from host to host (Hurd, 1990). If their behavior is changed in a way that results in an increase of time spent out in the open, slower reaction time or a decrease in predator awareness then this could result in a higher level of predation for infected hosts.

2. Causing debilitating pain. This is a common side effect of infection by lethal or debilitating parasite but does not have strong affiliation with nematodes. (Lefcort & Blaustein 1995).

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3. Increasing energy needs of the host through consumption of host reserves (Poulin, 1994). This reduces the nutritional status of the host, which could lead to malnutrition of the host or alternatively the host spending more time foraging for food, which increases their vulnerability to predation as they are exposed for longer.

Main question

Does sub-lethal parasitism affect the mortality rates of snowshoe hares via an interaction with predation?

Specific aims

Aim 1- is the main cause of snowshoe hare mortality due to predation?

Aim 2- what genes are involved in the stress caused by nematode infection? How do these genes relate to the hosts interaction with predation?

Aim 3- does the anthelminthic treatment reduce prevalence and intensity of nematodes in snowshoe hares and thus result in less susceptibility to predation?

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Experimental approach

We will establish a 30-ha area in the Narcisse Wildlife management area near Chatfield Manitoba, Canada. We will place 30 live traps on hare runways and bait them with food. We will trap hares for 10 days bimonthly and handle in accordance with Canadian animal care (1984). We will tag, weigh and sex the hares during this time. Once tagged the hares will be split in half with the first group being given 0.3mg/kg of Ivermectin (anthelminthic drug- parasite reduced) suspended in propylene glycol and the other half of the group will be injected with an equal volume of just propylene glycol (parasite- normal) (Murray et al, 1997). After the initial treatment we will maintain equal numbers of hares in each group by adding newly caught hares to replace those lost to predation, dispersal or natural causes of death etc.

They will be tagged and released over the course of two years from April 2017 to May 2019.

Aim one- is the main cause of snowshoe hare mortality due to predation?

10 hares from each group will be tagged with radio collars. The hares will be monitored via the collar to check for vital signs and location. Data will be collected from each of the groups and comparisons will be made between each group's rates of predation and differences in causes of death (Murray et al, 1997). The collar will

give off radio pulses if the collar has not moved for over 3 hours, which will allow us to track and find potential carcasses. Proximate causes of death will be determined by evidence left at the site such as the state of the carcass, track marks from potential predators, feces at the site, state of the collar with bite marks etc. If a carcass can not be recovered due to predator's eating it before we were able to track the collar, we will aim to recover the collar and predict to the best of our abilities the predator involved and at the very least note that the hare was killed due to predation. As with tagging and treatment the hares lost from the collared groups will also be replaced by newly caught and treated hares. Any carcasses found in a substantial state will be taken back to the lab for analysis.

Aim two- what genes are involved in the stress caused by nematode infection? How do these genes relate to the hosts interaction with predation?

We will investigate the transcriptional changes induced by parasitism. We will take RNA samples from the hares in each group every 2 months, sequence them, and compare the sequences of up or down regulated genes between each group. We will then use a BLAST search to look for the homologous genes in the European rabbit, which is the closest species with a published genome. This should indicate which genes or at least QTL regions are being differentially expressed. This would indicate the genes involved in the stress caused by parasitism response. The selected hares

Comment [RK1]: Experimentally, this aim works. But why is it included? (aside from the fact that I told you to have genetics in it). What additional information does knowing the genes tell you? The second question in this aim seems like it would be making connections between the genes and the traits, but you never really discuss it further.

Comment [RK2]: You have this as part of the aim, but never talk about what you're actually doing with it.

Comment [RK3]: One of the nice things about looking at transcription is that you should actually be able to identify genes, not just regions—you're looking at the RNA sequences, those are specific to a single gene.

from each group will also be tested for their level of nematode infection in order to make a direct comparison of the genes that are being up or down regulated due to parasite infection. Keith et al (1986) and Murray et al (1996) previously described the techniques used to count nematodes.

Aim three- does the anthelminthic treatment reduce prevalence and intensity of nematodes in snowshoe hares and thus result in less susceptibility to predation?

Comment [RK4]: This aim is good, but the emphasis is a little off. The effects of parasite load on predation are the important part here, but they get buried a bit by your methods for testing the effects of the treatment. Those tests are good, you need them to demonstrate efficacy, and I'm glad you thought about them, but they're not what's interesting.

Using the above mentioned laboratory techniques we will compare the nematode infection level results between the two groups. We expect that the parasite-reduced group will have lower levels of nematode infection due to the drug treatment. We will then compare the predation levels of the two groups for each two-month window and overall after the two year period. The predation levels will be calculated based off of the survival rates of the collared hares from each group. To investigate the survival of the parasite reduced group relative to the duration of anthelminthic drug treatment we will also note the time since the first treatment of each hare (Murray et al, 1997). We will compare hares that have had less than 100 days since their first treatment with hares that have had over 100 days since their first treatment. This should indicate if the drug treatment requires a length of time before it is effective in giving the treated hares physiological advantages to parasite

normal hares or if the drug is quick to show substantial result differences between the parasite reduced and parasite normal groups.

Intellectual merit/ broader impacts

Identifying the genes that contribute to the response to parasitism provides a better understanding of the mechanisms of resistance to parasites. Many studies in the past (Reeve Cronin and Strong 1994) have found that parasites in general can destabilize a host population if they show a direct time- delay regulation of survival. However the role of parasitism is also important if it has an indirect effect on population demographics due to parasitism's interactions with other factors such as predation (Gulland et al 1993). Predation has a significant effect on the population dynamics of many vertebrate species especially that of the snowshoe hare and so the tri-tropic interaction between the snowshoe hares, nematodes and predators could pose as a good case study for other tritrophic interactions in many vertebrate-predator systems.

Comment [RK5]: This is true, but how to intepret your results to tell you more about the mechanisms is the sort of thing that would have been good to include in your aims.

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References

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	Below Expectations	Meets Expectations	Exceeds Expectations Meets plus:
Introduction	Background information is irrelevant or incomplete No clear gap in knowledge described, or the proposed study doesn't address it	Clear background on previous work in the system Justification of why this system is appropriate to address this question Background on the theory of the interaction/the genetics Clearly described gap in knowledge that the study will address	Background information ties the theoretical background with previous work in this system or another Includes the gap in knowledge, and why the answer to that gap is important
Central question and aims	The central question is unclear or vague The aims are not relevant to the motivating question	The central question is clearly stated The specific aims are achievable, concrete questions that can be answered experimentally	The central question is framed in the context of the gap in knowledge The link between each aim and the central question is clear and explicit
Experimental Design	Experiments are unfeasible, or won't answer the intended question No discussion of what the results will mean	Clear description of experimental plans that are appropriate to answer the aims Discussion of how the data will be analyzed and interpreted	Sophisticated experimental design Discussion of what the results will mean in the context of the aims and central question
Intellectual Merit and Broader Impacts (optional)	No broader view of the impact of the work is included Disconnected from the rest of the proposal	Discussion of how this proposal addresses a relevant gap in knowledge (Broader Impacts: Description of how this work will have an impact outside the field)	Explanation of why the gap in knowledge is important Discussion of why this study and this system are particularly good to answer this question (Broader Impacts: Makes a strong case for the relevance and importance of this work)
Writing and organization	Disorganized Numerous errors Incomplete or incorrectly formatted citations	Well organized and easy to follow Thoroughly proofread Citations are complete and correctly formatted	

Introduction: Good background, although with a system this well-studied, you might want to include more information about it. I know I know the basic background, but generally you'd want to explain more about

the history of work in the system, and the depth of work on the prey-parasite interation. In a real grant, you'd want to really hammer on the point that there's this really well-studied system, but nobody has looked at this specific question that might be really important in interpreting the rest of it. One thing that is very good in your introduction is how you set up two competing hypotheses (parasites cycling just in response to the predation, or whether parasite load affects predation).

Question and aims: Very clearly stated and organized. Overall, I think the aims could benefit from more connections drawn between them and the main question. I can see how they all fit in, but you never really discuss it. It's particularly an issue for Aim 2. Experimentally, it's a very well-designed aim, but what does it tell you?

Experimental design: Your experimental design is very good, for all three aims. You've clearly thought about not just how to measure the things you're most interested in, but also potential confounding factors and how to control for them.

Intellectual merit: What you have is good, I'd just wish for a little more. Again, this is a very well-studied system, and this is a good place to discuss why you're using it. You did everything you needed to, it would just be even better if it were more fully developed.