Test for Transgenerational Immune Priming in the Snail, *Biomphalaria glabrata*

by
Sonal Anand

A THESIS

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

Presented May 5, 2016
Commencement June 2016

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Abstract approved:

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Michael Blouin

Transgenerational immune priming occurs when an adult is exposed to a parasite or other pathogen and then transmits information to its offspring through protective phenotypes to cope with the same pathogen (Moret, 2006). There has been evidence for transgenerational immune priming in various invertebrates (Tidbury et al. 2011). Here we tested for the presence of transgenerational immune priming in the *Biomphalaria glabrata* snails when challenged by exposure to the *Schistosoma mansoni* parasite. The experimental parent generation was challenged with a parasitic environment, while the control parent generation was not. The results were then determined based on the resistance or susceptibility of the F1 offspring generation. We found that parental challenge did not enhance offspring immunity through transgenerational immune priming.

Key Words: Transgenerational immune priming, Schistosomiasis, *Biomphalaria glabrata, Schistosoma mansoni*

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Sonal Anand, Author

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Sonal Anand

Introduction:

Schistosomiasis

Currently, over 207 million people are estimated to be infected with schistosomiasis (Magaisa et al, 2015). Schistosomiasis is a parasitic disease transmitted by a snail intermediate host through contaminated water. Chronic schistosomiasis affects many people living in rural areas with poor sanitation throughout Africa and South America. Children have shown greater infection rates than adults in affected regions as they spend more time swimming and bathing in water containing infectious cercariae (Magaisa et al, 2015).

The species *Biomphalaria glabrata* are freshwater snails that act as the intermediate host of the *Schistosoma mansoni* parasite in the new world. Human contact with water containing infected snail hosts can lead to the contraction of schistosomiasis. The infectious form of the parasite, cercariae, emerges from the snail host into the water. Cercariae are able to swim in water as well as penetrate human skin. Once cercariae has entered the body, it will move through blood vessels and lymphatic system to mature in the body, specifically in the liver and small intestine

(Magaisa et al., 2015). This disease is difficult to control in rural areas as the parasites are able to enter the water sources when infected humans and animals defecate in the water. The parasitic eggs then hatch in the water as miracidia and infect the intermediate host: *Biomphalaria glabrata.*

The current drug of choice to treat schistosomiasis is Praziquantel. It works by killing the adult worms in infected individuals and is highly effective. It can be successful in reducing the burden of the disease on communities when given out in mass quantities to school age children. However, mass treatment programs can be difficult to administer to all at-risk populations (Magaisa et al., 2015). Also, there is concern about parasites developing resistance to the drug (Melman et al., 2009). Many people are still at risk of being infected by schistosomiasis and much more research still needs to be done in order to successfully control and decrease the spread of this disease. Breaking the cycle of transmission through the intermediate snail host is an alternate way to control the disease compared to treating humans with Praziquantel. Therefore, it is important to learn more about the *Biomphalaria glabrata* immune system and what controls resistance to infection for the snail.

Transgenerational Immune Priming

Not all heritable traits are written in the DNA sequence. Epigenetics and other mechanisms can also lead to a form of "immune memory" inherited by future generations (Harper, 2010). Following exposure to a parasite or virus, some invertebrates have been found to exhibit increased immune protection in the next

generation, this is called transgenerational immune priming. A protective phenotype can be transmitted to offspring by cytoplasmic factors, priming effects, histone modification, DNA methylation, and other mechanisms that have not been fully studied (Mousseau and Fox, 1998).

Transgenerational immune priming has been found in a number of invertebrates proving that invertebrate immune systems are more sophisticated than previously thought. For example there has been evidence that transgenerational immune priming exists in the *Plodia interpuctella* (Lepidoptera) species, increasing its future generation's resistance to its natural DNA virus, *Plodia interpunctella granulosis* virus (Tidbury et al. 2011).

In 2006 Transgenerational immune priming was discovered in the mealworm beetle, *Tenebrio molitor*. There was Transgenerational phenotypic plasticity, which increased immunity in the following generation. This was shown by increased levels of anti-microbial activity in progeny of those parents that received a microbial challenge (Moret, 2006).

In 2013 it was shown that *Biomphalaria glabrata* snails possess the ability for *within-generation* immune priming to the *Schistosoma mansoni* parasite (Portela, 2013). This means that a snail that was previously exposed to a parasite undergoes some form of genotype-dependent immune priming causing the snail to be resistant to a subsequent homologous infection. The resistance of the snail was dependant on which strain of parasite they were exposed to in the subsequent challenge (Portela, 2013). However to our knowledge there have been no studies on *transgenerational* immune priming in *Biomphalaria glabrata.*

In nature *Biomphalaria glabrata* snails can be faced with attack from the *Schistosoma mansoni* parasite multiple times within a generation as well as across consecutive generations. In general, the parental environment predicts the general quality of the progeny's environment, so it would make sense that parents may provide their offspring with phenotypes to cope with pathogens in their specific environment (Moret, 2006). A specific advantage of transgenerational immune priming is that it allows organisms to rapidly adapt to new pathogens in their environment. We are interested to see whether exposure of parents to the parasite will change the rate of infection in their progeny. This experiment will provide important information about host-parasite dynamics of the disease schistosomiasis.

Here we assess whether exposure to the *Schistosoma mansoni* parasite leads to immune priming transgenerationaly in *Biomphalaria glabrata* snails. Specifically we examined the effect of parasite exposure in the parent generation on the rates of subsequent infection in the progeny after being challenged by the same parasite, *Schistosoma mansoni*.

Methods:

Ethics Statement

Animals that were used in this research at Oregon State University are covered by our Institutional Animal Care and Use Committee (IACUC). We also use an approved Animal Care and Use Proposal (ACUP), which is approved by the IACUC.

Maintaining the Schistosome parasite

We used both mice and hamsters to maintain the Schistosome parasite and to produce miracidia for challenge experiments. The mice were infected through contact with water containing cercariae. The infected rodents were then euthanized in order to dissect and remove livers containing parasitic eggs. The miracidum then hatched out of the parasitic eggs when exposed to light. The miracidia were then collected in order to challenge the *Biomphalaria glabrata* snails.

Inbred lines of Biomphalaria glabrata

We used Line 121 and 125 of previously established inbred lines of *Biomphalaria glabrata* to conduct the experiment and duplicate it. These lines have been maintained in separate populations for a number of generations. Twenty-four snails from each line were chosen based on their size and were between 4-6 mm in

diameter. Twenty-four snails of each line were exposed to the *Schistosoma mansoni* parasite. Another 24 snails from each line were kept unchallenged to provide a control. The unchallenged snails were kept separated but in identical tanks. Snails from each group, line 121 exposed and control and line 125 exposed and control, were divided into 2 groups of 12 snails each and kept in identical tanks. The challenged parent snails began laying eggs approximately 7 days after the challenge. The next generation of snails was collected and separated from the parent generation 6 weeks post parental challenge, in order to eliminate any breeding between snails and offspring. These F1 offspring were then challenged to test whether offspring of challenged parents were more resistant than offspring of control parents.

Challenging of Biomphalaria glabrata

Each of the 48 challenged snails, 24 from line 121 and 24 from line 125, were placed in separate wells at the time of the challenge. They were placed in wells that contained 5 miracidia each. They were exposed to miracidia for 24 hours then placed back into separated tanks.

Twenty-one snails from the F1 generation of line 121 exposed and control parents, and 21 snails from the F1 generation of line 125 control parents and 22 snails from line 125 exposed parents were collected. The separated F1 offspring of snails were then challenged when they reached the ideal size of 4-6mm using the same challenging format described above.

Normal living conditions of Biomphalaria glabrata

The *Biomphalaria glabrata* snails were kept in a room at 26 degrees Celsius to provide normal living conditions. They were separated into identical containers filled with de-chlorinated water and supplemented with calcium. The snails were fed ad libitum fresh lettuce 3 times a week.

Assay for infection

The F1 offspring were checked for infection 3 times: once each during the 5^{th}, 6^{th}, 7^{th}, and 8^{th} week after challenge. The snails were checked by placing all collected offspring in individual wells of de-chlorinated water then placing them under light for an hour, which would cause cercariae to emerge from any infected snails. Then each well was checked under the microscope for evidence of emerged cercariae. After this check all snails were placed back into original tanks. During week 6 and 7 the snails were also tested for infection strength. At each date of checking the total number of surviving snails was noted as well as the number of snails infected at that point.

Strength of infection test

During the 6^{th} and 7^{th} week post challenge, the infected F1 generation snails were tested for the strength of their infection. 2 drops of iodine were added to the well of

each infected snail. The numbers of cercariae were then counted under the microscope. The cercariae were counted twice for each infected snail and these numbers were averaged to show a more precise count. This test was conducted in order to compare the difference in number of cercariae that emerged from the infected progeny of the exposed parents compared to the infected progeny of the control parents.

Statistical Analysis

A fisher's exact test was conducted to compare the proportion of infected snails between the control and exposed progeny of each line. An ANOVA was done to study whether infection strength differed between F1s from exposed vs. control parents.

ANOVA model:

We used a two-way ANOVA to test the effects of snail treatment and line as the two independent variables on the number of average cercariae count produced as the dependent variable. This analysis was done separately on snails that were shedding on week 6 and on snails that were shedding on week 7. This is because individual snails were not marked and were housed in groups, so I could not know which snails that were observed on week 6 were the same as snails observed on week 7.

Results:

(a) Infection rate results

	Offspring of Exposed Parents- Line 125 [N=19]	Offspring of Control Parents- Line 125 [N=20]
Infected	13	8
Uninfected	6	12

P value*= 0.1110

	Offspring of Exposed Parents- Line 121 [N= 20]	Offspring of Control Parents- Line 121 [N=19]
Infected	5	3
Uninfected	15	15

P value*= 0.6968

*The P value was calculated using a Fisher's exact test comparing exposed and control groups

The two-tailed P value of both the lines show that the association between control and exposed groups is considered to be not statistically significant. This shows that there is no overall effect of parental exposure. Not only is the result not what we predicted, the trend is in fact in the opposite direction from what we predicted. Slightly more F1 snails were infected from exposed parents than from control parents in both lines.

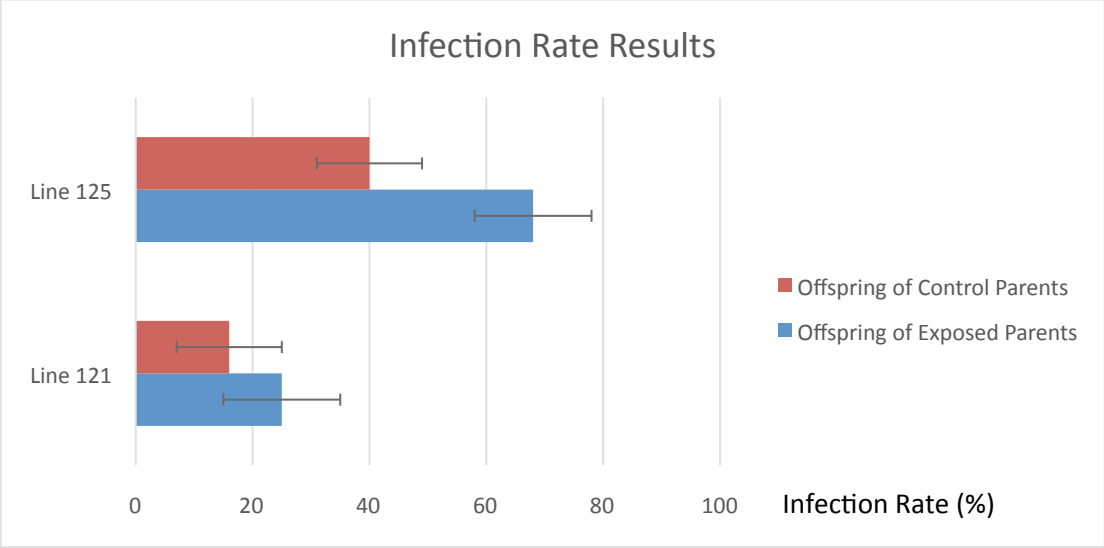

Fig. 1. This graph shows the Infection rates of 2 independent lines of *Biomphalaria glabrata* snails during week 8. Error bars are one standard deviation of the estimate of the proportion. The control group was the progeny of snails that were not previously challenged with *Schistosoma mansoni*. The experimental group was the progeny of snails that had been challenged with *Schistosoma mansoni*.

(b) Strength of Infection Results:

	Average Parasite Count from infected F1 offspring –Week 6	Average Parasite Count from infected F1 offspring –Week 7	Average Parasite Count from infected F1 offspring- Week 6 and 7 averaged
Line 125 Control Progeny	214	150	182
Line 125 Exposed Progeny	271	301	286
Line 121 Control Progeny	230	293	262
Line 121 Exposed Progeny	274	459	367

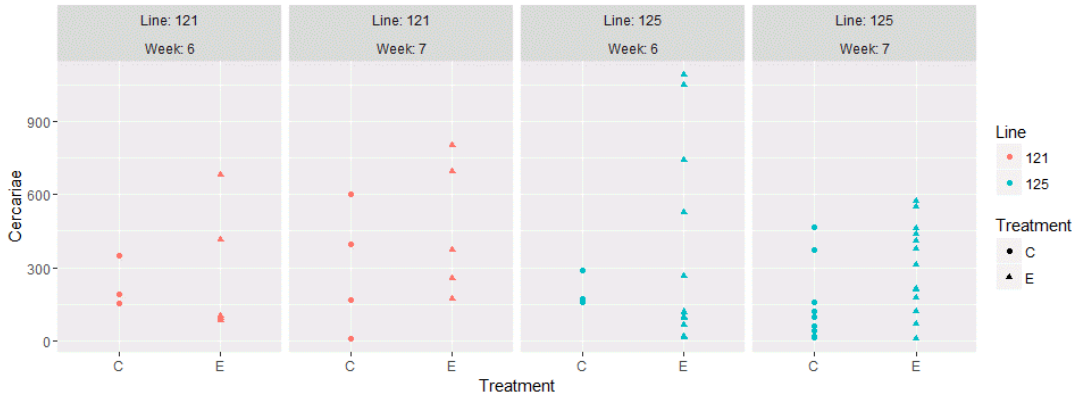

Fig. 2. These graphs show the strength of infection for both lines. Each plot is separated in by treatment (control (C) vs. exposed (E)). The data points are also separated by weeks. Each data point represents an individual snail that was shedding cercariae on the week of observation. Note that points in week 6 cannot be matched with points in week 7 because we did not mark individual snails.

ANOVA statistical test results:

Week 6:

P Value: 0.449 for treatment groups (exposed vs. control)

P Value: 0.691 for lines (line 121 vs. line 125)

Week 7:

P Value: 0.046 for treatment groups (exposed vs. control)

P Value: 0.065 for lines (line 121 vs. line 125)

These P values show that there is no statistical difference between the means of the control and exposed group either within each line or between both the lines during week 6. More snails were observed to be shedding during week 7 than week 6, and

there was a marginally statistically significant difference ($p = 0.046$) in the average cercariae counts when comparing the two treatments groups (progeny of exposed vs. progeny of control parents). Furthermore, the trend is in the *opposite* direction from expectation, showing stronger infections in the progeny of exposed offspring than in the control progeny. This result is not what we predicted, as we thought there would an infection strength *reducing* effect of transgenerational immune priming in the progeny of the challenged parents.

Discussion:

Our results show that there is no evidence of transgenerational immune priming in *Biomphalaria glabrata* snails in response to challenge by the *Schistosoma mansoni* parasite. The low sample sizes of this experiment might have caused low statistical power to detect a difference in the infection rates of the exposed vs. control progeny *Biomphalaria glabrata* snails. However, the trends from this experiment, both in terms of percent infected and in terms of infection intensity per infected snail, are in the opposite direction from what we predicted. This leads us to believe that there really is no strong transgenerational immune priming in this system, rather than a failure to detect it owing to low statistical power. This result suggests that the *Biomphalaria glabrata* snails rely on their own environment and own genetic make up, not their parent's, in order to display resistance to the *Schistosoma mansoni* parasite. However, this does not mean that there is no transgenerational immune priming in the *Biomphalaria glabrata* snails, as this experiment is specific to it's

interaction with the *Schistosoma mansoni* parasite. There may be potential transgenerational immune priming in the snails when exposed to a different parasite, virus, or bacteria.

One possible reason we did not see evidence of transgenerational immune priming could be that the *Biomphalaria glabrata* snails in the parent generation had already formed their eggs before they were challenged. At any time, unpackaged ova are most likely spread throughout the reproductive tract of the snail (Boyle and Yoshino, 2000). Although the eggs are at different stages of development we cannot be sure that epigenetic or other effects caused by parental challenge were inherited by the F1 progeny that we collected for this experiment. If eggs were already present and developed at the point of parental challenge, this would have most likely have not allowed for any epigenetic effects from the parent to occur in the F1 progeny. If this experiment were repeated it would be beneficial to check eggs laid by the parent generation several weeks after challenge. This would allow for the inclusion of new gametes that were created post parental challenge to ensure a better chance of observing any epigenetic or other changes in the offspring.

One interesting result is that we found higher infection rates and a barely statistically significant increase in cercariae counts for the progeny of the exposed parents compared to the progeny of the control parents. This trend in cercarial counts was seen in the data from both week 6 and week 7, although only the data from week 7 had a significant p-value, presumably owing to the larger sample size in week 7. If

this result is a general phenomenon, one possible explanation is a cost of resistance in the parental generation leading them to produce lower quality of eggs. This could have led to weaker progeny of the exposed parents, which would account for the greater strength of infection in those snails.

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