Opposite patterns of schistosome resistance in two snail lines suggests significant intraspecies variability in host immune genes

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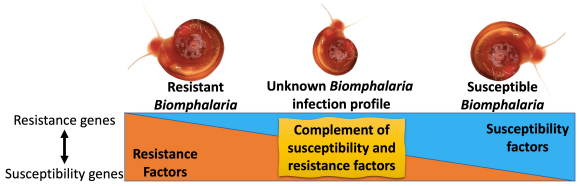
Introduction

Intestinal schistosomiasis is a disease caused by infection with *Schistosoma mansoni,* a parasitic trematode responsible for this chronic disease affecting millions of people in the tropics and sub-tropics (1). The parasite **requires a snail vector host of the genus *Biomphalaria*** for transmission between human hosts (2). Specific species and strains of *Biomphalaria* have been shown to have **varying resistances** to *S. mansoni* (3). One proposed way to reduce *Schistosoma* transmission to humans is to manipulate the resistance of snails to infection in order to break the transmission cycle of the parasite, however this requires a thorough understanding of the genetic and environmental factors influencing infection rates in snails. To date, most of our understanding of snail resistance to schistosomes stems from experiments performed with laboratory infections of a South American vector, *Biomphalaria glabrata*. Virtually nothing is known regarding resistance of African vectors to schistosomes even though 90% of transmission of *S. mansoni* occurs in sub-Saharan Africa.

Model A: Figure depicting different resistance/susceptibility profiles of snails.



Allele matching theory suggests that during the coevolution of snails and schistosomes, there has been selection for susceptible allele combinations that allow the parasite to evade the snail immune defenses (4). Perhaps the snails have evolved immune genes conferring resistance to infection by schistosomes **(Model A).** We predict these allele combinations would present distinct infection profiles during different snail parasite exposure combinations.

Rationale: Our long-term goal is to discover the factors that determine susceptibility and resistance of African vectors to *S. mansoni* so that these can be translated into snail focused interventions in order to reduce and eventually eliminate *S. mansoni* in humans.

Aims

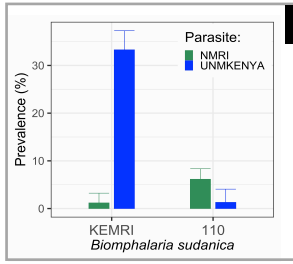
1. Determine the levels of resistance in two of our laboratory *Biomphalaria sudanica* lines originating from Africa to infection with two strains of *Schistosoma mansoni*.
2. Determine the effects of *B. sudanica* development (size) on resistance to *S. mansoni* infection.
3. Determine the factors that influence the reproductive rate of *S. mansoni* within *B. sudanica*.
4. Determine the relative abilities of parasites to find hosts. We hypothesized that host finding ability would correlate positively with infection success.

Methods

Resistance - A total of 2,248 *B. sudanica* snails from Kenya (KEMRI, and 110) were exposed to five miracidia from two strains of *S. mansoni,* one from Puerto Rico (**NMRI**)and one from Kenya (**UNMKENYA**) in a series of infection trials. We measured the effect of parasite source treated with a dose of 5 miracidia on infection status of each species line separately to compare infection rates and intensities among different snail and parasite combinations, and across various juvenile and adult size classes of snails.

Penetrance: 228 (ncontrol=58, nexposed= 170), 4-5 mm *B. sudanica* snails of KEMRI and 110 lines, and also one line of *B. glabrata,* line 7 were used. We exposed snails to 8 miracidia and counted the parasites **(Model B, top right)** remaining in the well after 0.5 and 3 hours to infer how many parasites had penetrated the snail at each time point. All miracidia counts were performed blindly.

Results

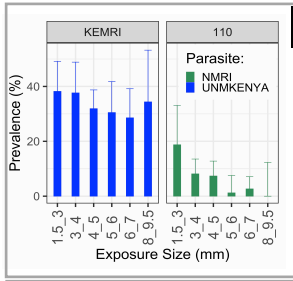


AIM 1: LEVELS OF RESISTANCE OF 2 SNAIL LINES

- For *B. sudanica* KEMRI, exposure to 5 **UNM Kenya** miracidia as opposed to **NMRI** *S. mansoni* significantly increased the odds of infection by 2257% ($P < 0.0001$).
- For *B. sudanica* 110, exposure to 5 miracidia of **UNM Kenya** as opposed to **NMRI** *S.mansoni* significantly decreased the odds of infection by 83% (P = 0.0038). Error bars represent 95% confidence intervals.

Interpretation: KEMRI snails displayed higher susceptibility to the UNMKenya parasite and increased resistance to the NMRI parasite, while the 110 snail line showed a reciprocal resistance pattern.

Statistics: A generalized linear model (GLM) with a binomial family distribution.

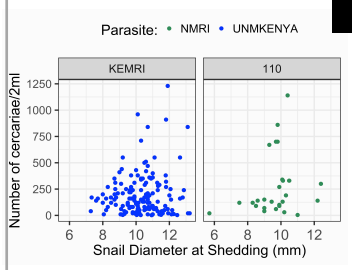


AIM 2: EFFECTS OF SNAIL DEVELOPMENT ON RESISTANCE TO INFECTION

Figure shows the prevalence of infections in KEMRI snails with **UNM Kenya** parasites and in 110 snails with **NMRI** parasites. With 110 snails, prevalence decreased as exposure size of snail increases, with highest prevalence at exposure size 1.5-2.9mm (GLM; Exposure sizes 3-3.9, 4-4.9, 5-5.9, and 6-6.9mm had 75, 76, 79, and 96% lower odds of getting infected compared to 1.5-2.9mm; $P = 0.0062$, 0.0018, 0.0024, and 0.0011, respectively). With KEMRI snails, the exposure sizes 4-4.9, 5-5.9, and 6-6.9mm had 56, 58, and 60% lower odds of getting infected compared to 1.5-2.9mm (GLM, $P = 0.0272$, 0.0369, and 0.0244, respectively). Error bars represent 95% confidence intervals.

Interpretation: Resistance increases with age with youngest snails being most susceptible.

Statistics: A GLM with a binomial family distribution.



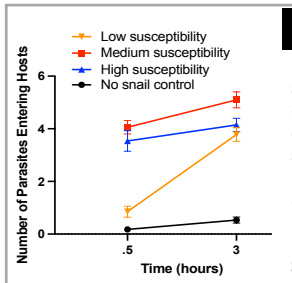
AIM 3: FACTORS INFLUENCING PARASITE REPRODUCTIVE RATE

The reproductive rate of the parasite determines the number of infectious stages released into the environment by the snail.

Figure shows the number of cercariae released by snails in a two-hour interval in relationship to their size at time of shedding. For KEMRI infected with **UNM Kenya**, there was no significant relationship in the expected number of cercariae released with snail diameter ($P = 0.2447$). For **110** infected with **NMRI**, there was a positive relationship between diameter and number of cercariae produced. The number of cercariae produced increased by 119% for every 1 mm increase in snail diameter ($P < 0.0001$).

Interpretation: For 110, bigger snails produce more infective stages, but not for KEMRI.

Statistics: A GLM with negative binomial family distribution was used to account for over dispersed count data (# of cercariae).



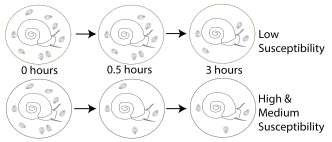
AIM 4: SNAIL-SEEKING ABILITY OF PARASITE

Figure shows the number out of 8 larval parasites that penetrated *B. glabrata* line 7 (low susceptibility), *B. sudanica* 110 (medium susceptibility), and *B. sudanica* KEMRI (high susceptibility) at 0.5 and 3 hours. The mean and standard error are shown. At 0.5 hours there was a significant difference (P <0.0001) in penetration between BG line 7 and both KEMRI and BS 110, and no significant difference between KEMRI and BS 110. At 3 hours there was a significant difference in penetration (P<0.0011) between BG 7 and BS 110, and no significant difference between KEMRI and 110 or BG 7.

Interpretation: At 0.5 hours, more parasites found the high and medium susceptible snails than resistant snails.

Statistics: ANOVA with type III sum of squares followed by multiple pairwise comparisons with a Bonferroni correction for the significant variables (snail line, time, and their interaction).

Model B: Depiction of number of parasites identified in each well at time intervals after exposure for Low and High/Medium susceptibility snail lines.



Discussion

KEMRI snails display higher susceptibility to **UNM Kenya** and increased resistance to the **NMRI** parasite, while the 110 snails showed a reciprocal resistance pattern. This is consistent with the classical allele matching or gene for gene models of pathogen resistance.

Infection prevalence of 110 snails after exposure to **NMRI** parasites appears to be related to snail size and accordingly age at the time of exposure, with higher susceptibility to infection at the smallest exposure size and lower susceptibility at larger sizes. This suggests that snails become more resistant with age, perhaps as the immune system develops. For KEMRI snails exposed to **UNM Kenya**, this relationship was not as distinct across all class sizes but did hold true for 3 class sizes as compared to the smaller size class, suggesting variability in immune system development between different snail species.

For the compatible 110-**NMRI** combination, the number of cercariae produced significantly increased as snail size increased at time of shedding. Snail size at shedding was the most important factor in determining how many parasites were produced by a snail, suggesting that larger snails provide more resources for parasite reproduction, as has been reported for infections in *B. glabrata* 5. Surprisingly, this relationship was not significant for KEMRI-**UNM Kenya** infections.

The host-seeking trials indicated that more parasites were able to find and penetrate the *B. sudanica* snails, both 110 and KEMRI, than the refractory *B. glabrata* snails, although these differences were much smaller at 3 hours compared to 30 minutes. Thus, host finding ability of parasites may, in part, play a role in resistance profiles when considering different snail species. However, because successful finding did not differ between the two lines of *B. sudanica,* it appears that other resistance mechanisms are at play to prevent infections in resistance snails.

Our experiments characterize important models that can be used to further understand the susceptibility/resistance of snails in Africa and how the factors that influence susceptibility and resistance may influence transmission of schistosomes to humans.

References

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