A PCR-based diagnostic to detect reproduction between snails that vector schistosomiasis: a tool for genetic mapping studies

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Objectives

- **Long term: Determine the genetics underlying snail host resistance to schistosomiasis.**
- **Short term: Develop a protocol to easily genotype snails and assess their outcrossing rates.**
- **Short term: Use the protocol to determine the role of superoxide dismutase in resistance of snails to schistosomes.**

Introduction

Schistosomiasis is a chronic inflammatory disease affecting humans in 78 countries with over 250 million people infected and requiring treatment. An estimate of 90% of those were people living in Africa (1). The blood fluke *Schistosoma mansoni*, causes intestinal disease and is found in areas of South America and sub-Saharan Africa (1). *Biomphalaria sudanica* is one of the freshwater snail intermediate hosts for *S. mansoni*, in which the parasite produces the larval form, cercariae (**Figure 1**). People become infected through skin contact with cercariae that are shed from infected snails in freshwaters (1).

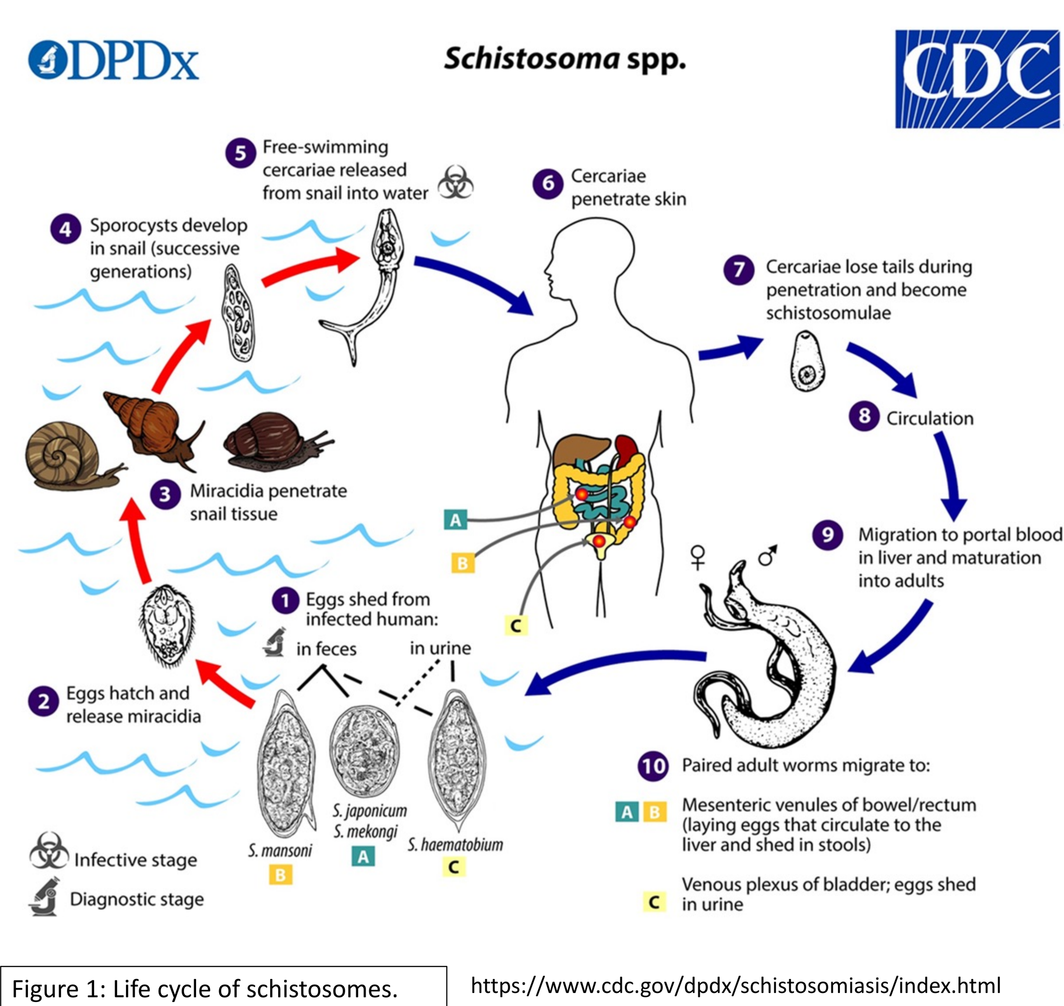

Figure 1: Life cycle of schistosomes. https://www.cdc.gov/dpdx/schistosomiasis/index.html

Currently, praziquantel is given for treatment of chronic infection and mass drug administration (MDA) has been implemented for prevention and control in endemic areas (1). However, this treatment allows for reinfection of the parasite once medication has ended (1). Given this, more efforts are looking into how to control the snail vector to prevent parasite transmission (2,3). While several genes influencing resistance have been identified in other snail species, there is still little understood of genetic factors in determining resistance or susceptibility of *B. sudanica* to *S. mansoni* despite its importance in transmission of *S. mansoni* in Africa (5).

We will take a genetic mapping approach to identify genes important in snail resistance to *S. mansoni* infection. Genetic mapping first requires a cross between two different strains and phenotypes of snails (2). Since *B. sudanica* is hermaphroditic, the present study aims to confirm progeny from crosses between snails that are resistant and susceptible to *S. mansoni* are the result of outcrossing and not simply self-reproduction (**Figure 5**).

Primer for Association Mapping

Association mapping aims **to associate regions of the genome with a phenotype (Figure 2)**.

Example of an association mapping study:

1. Parents with two distinct phenotypes are crossed. For example, a susceptible parent (SS) is crossed with a resistant parent (RR). Rectangles represent one set of chromosomes.
2. F1 progeny are heterozygous for each parental gene (SR).
3. F2 progeny will comprise SS, RR, and SR/RS phenotypes. By sampling several offspring, the phenotype can be associated with genomic regions coding for a trait typically at the level of the chromosome.
4. Intercrossing the F2 for several generations (FN) allows for recombination of chromosomes, allowing the trait to be associated with a smaller genomic region.

- **Association of phenotype with genome wide genomic markers locates the region involved in disease resistance as noted by the asterisk.**

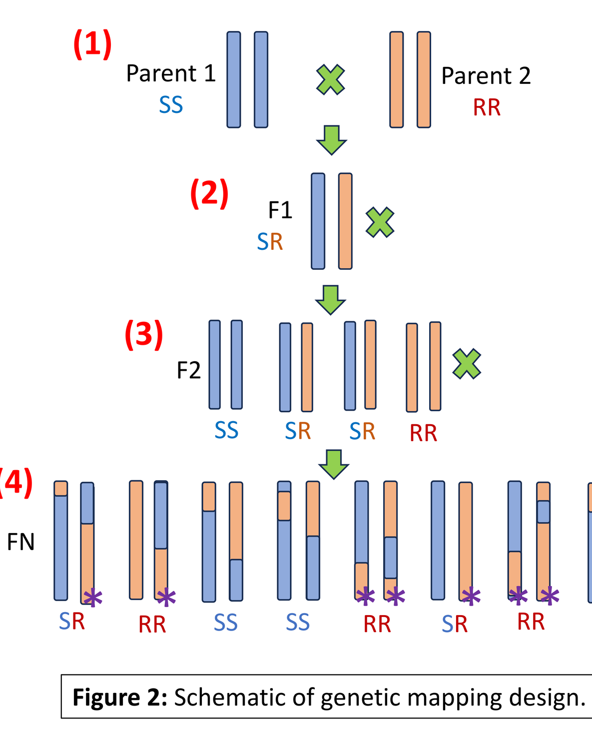

Figure 2: Schematic of genetic mapping design.

Methods

Marker Choice and PCR Optimization

- We interrogated the genome sequences of the 163 and KEMRI genetic lines for fixed differences in a single nucleotide polymorphism (SNP) that could easily be visualized with restriction enzyme digest.
- A T/C SNP was identified near the superoxide dismutase type 1 gene (*sod1*) that differs between the lines 163 (C) and KEMRI (T) and occurs at an enzymatic cut site (*HaeIII* - GGCC).
- The gene, *sod1* is a candidate resistance gene. It codes for cytosolic copper/zinc superoxide dismutase and has been implicated as important in the defense against *S. mansoni* for the closely related snail species, *B. glabrata*. Increased expression levels are based on allelic variation and high levels promote resistance to infection (6).

Table 1: Primers used for PCR amplification of SNP near *sod1* gene in *B. sudanica* lines and amplification size (amplicon products shown in **Figure 3**).

Primer	Primer Sequence	Size
F	CCATGATGGTTGCTATGACAACAG	208
R	CTCAGAACAGTGTGAGGAGGGAAAG	

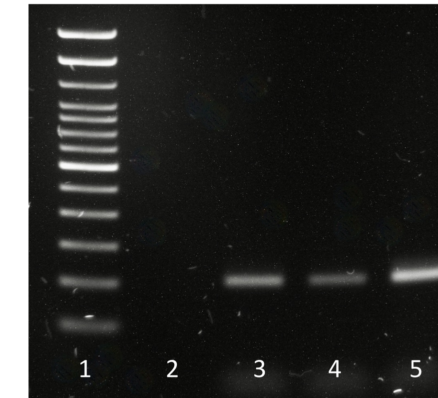

Figure 3: PCR results using primers from **Table 1. Lane 1**, 100bp DNA ladder, **lane 2** negative control, **lane 3** *B. sudanica* 163, **lane 4** *B. sudanica* KEMRI, **lane 5** F1 progeny.

Restriction Digest Protocol

- At the T/C SNP a *HaeIII* restriction enzyme cut site present (GG|CC) (**Table 2, Figure 4**).
  - T allele = GTCC
  - C allele = GGCC
- PCR products were incubated (30 min at 37°C) with the *HaeIII* restriction enzyme at manufacturers recommended concentration and the products visualized on an agarose gel (**Figure 4**).
- Predicted number of bands and their sizes are given in **Table 2**.

Table 2: Expected number and size of bands visualized on a gel and genotype interpretation for SNP near *sod1* gene.

Number of Bands	Band Size	Interpretation
1	208	Homozygous T = **163**
2	144, 64	Homozygous C = **KEMRI**
3	208, 144, 64	Heterozygous T/C = **F1 progeny of 163/KEMRI**

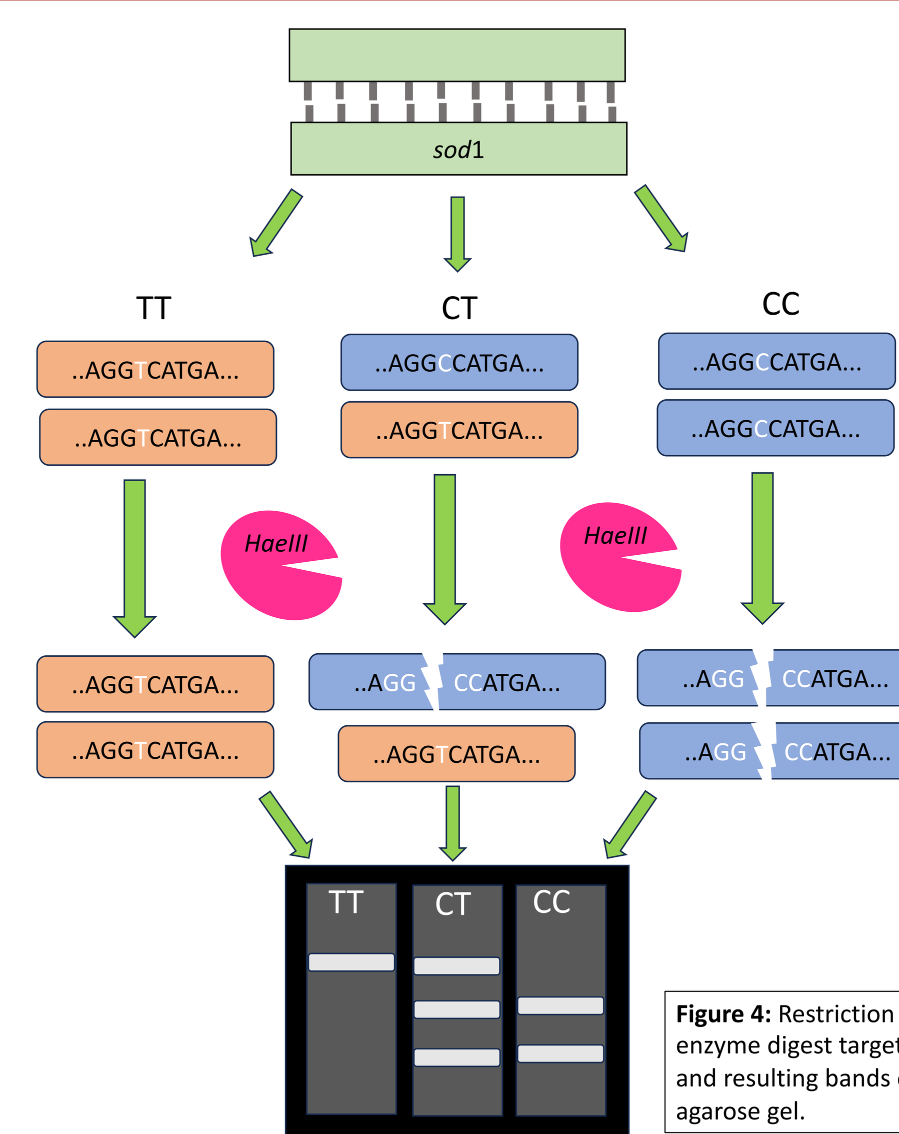

Figure 4: Restriction enzyme digest targets and resulting bands on agarose gel.

Snail Breeding

- 7 families were created by isolating juvenile individuals of the 163 (resistant) and KEMRI (susceptible) genetic lines of *B. sudanica* in aquaria (**Figure 5**).
- The parents and 8 offspring were subsampled from each family and DNA extracted (Qiagen Blood and Tissue Kit) **(Figure 6).**

163 · KEMRI · F1 Progeny · Self-fertilization %? · Cross-fertilization %? · Self-fertilization %?

Figure 5: Genetic cross schematic.

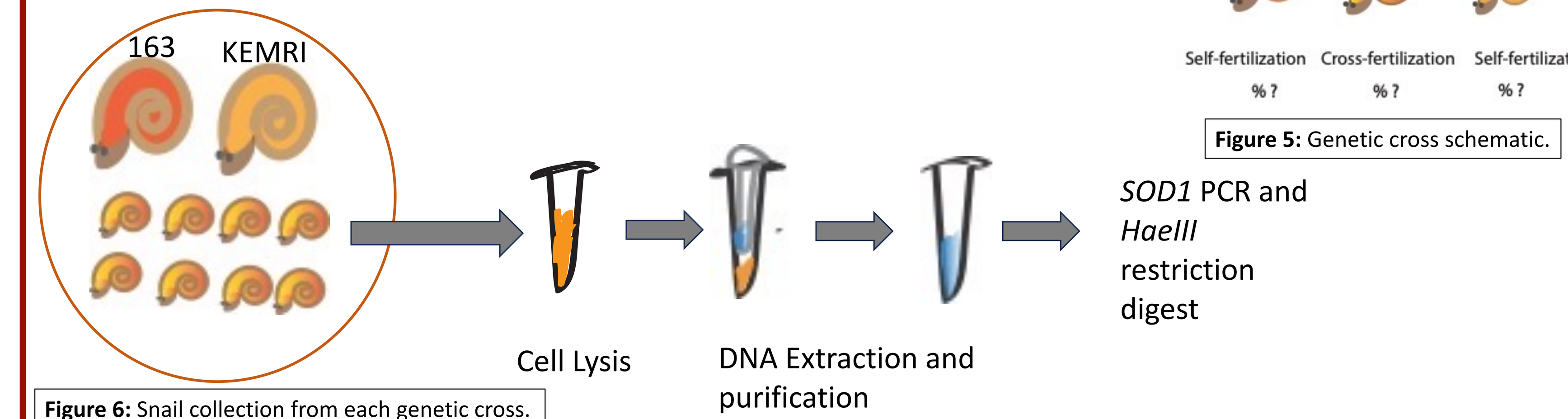

Figure 6: Snail collection from each genetic cross.

Results

- All 7 parental KEMRI *B. sudanica* were homozygous for the T allele (TT).
- All 7 163 *B. sudanica* were homozygous for the C allele (CC).
- **100% of 56 offspring tested were heterozygous (Figure 7) confirming that outcrossing had occurred in all cases.**

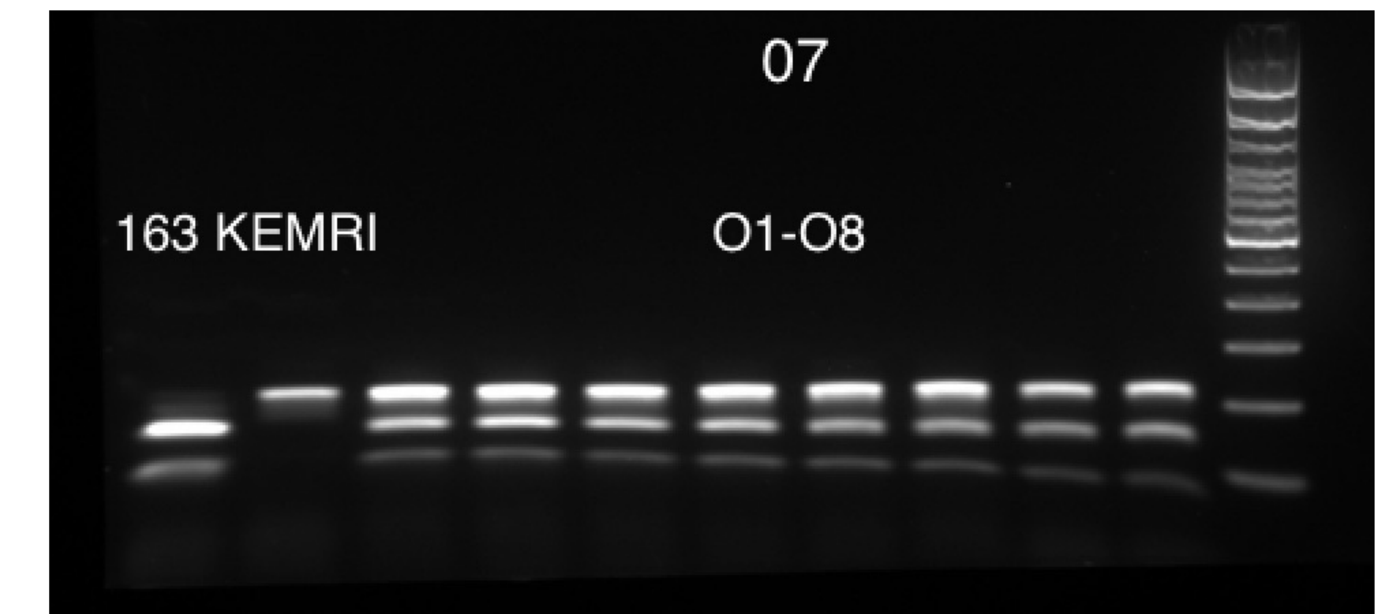

Figure 7: An example of an agarose gel image after restriction enzyme digest for one family. This example is cross #7, with parents 163 (CC) and KEMRI (TT), and 8 offspring (O1-O8). Note the presence of 3 bands in the offspring, confirming heterozygosity.

Discussion

- **100% of tested F1 progeny were heterozygous for T/C, indicating they were outcrossed and not self-progeny.**
- **High rate indicates the snails interbreed readily.**
- **Validated F1 progeny will be used for further linkage analysis and genetic mapping studies (Figure 2):**
  - Progeny and parental genome sequences will be compared to identify SNPs to use for linkage map analysis.
    - This work will improve assembly of the *B. sudanica* genome.
    - Validate/refute associations between genomic regions and resistance.
- **Understanding genetic factors linked with *B. sudanica* resistance to *S. mansoni* infection could lead to novel control mechanisms that utilize naturally occurring resistance. Reduced infections in snails means reduced infections in humans.**

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