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Mating system of *Biomphalaria sudanica,* a vector of *Schistosoma mansoni*

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ABSTRACT

Biomphalaria snails are intermediate hosts for schistosome parasites, which cause morbidity and mortality in humans worldwide. We aimed to determine the mating system of *Biomphalaria sudanica*, a hermaphroditic vector of schistosomiasis in the African Great Lakes, with the goal of informing the design of genetic studies such as linkage mapping to improve genome assembly and genetic association studies to identify snail resistance genes. To determine the relative rates of outcrossing *versus* selfing, we assayed the progeny of experimental crosses of snails in the laboratory using a PCR and restriction enzyme digest to determine snail genotype and parentage. Out of 7 experimental crosses and 56 total offspring assayed, 100% were derived from outcrossing rather than inbreeding. These results indicate that *B. sudanica* is primarily an outcrossing species, although previous work has shown that this species retains the capability of self-fertilization.

1. Introduction

Schistosomiasis is a parasitic disease transmitted by specific freshwater snail vectors infecting over 260 million people, 90% of which are in Africa (WHO, 2024). Despite the public health importance of these snail vectors in Africa, there is still much to learn about their biology, including their interactions with schistosomes. Establishing such snail-schistosome interactions is pertinent given the search for novel snail control strategies, a critical component for reducing schistosome infections in humans (Sokolow et al., 2016). Schistosomiasis control strategies in endemic areas primarily consists of mass drug administration of praziquantel to humans; however, reinfection following treatment is common because infected snails persist in freshwater habitats that serve as critical sources of water. Such water sources include some of the largest in Africa, including the African Great Lakes. While experts agree that snail control is critical to sustained control of schistosomiasis (King and Bertsch, 2015; Sokolow et al., 2018), methodology is limited to molluscicides that are indiscriminately toxic and difficult to apply to large areas of snail habitat. Therefore, there is a need to uncover novel ways to control the snail vector to prevent parasite transmission. One such possibility is to utilize gene drive technologies to increase the frequency of schistosome resistance genes in snail populations to make them more refractory to infection, an approach taken in other host-pathogen systems (Famakinde, 2018; Buchthal et al., 2019; Powell, 2022). However, the design of genetic association studies as well as gene drive implementation relies on the underlying mating system of the snails. Snails of the genus *Biomphalaria* are hermaphroditic, and while some species, like *B. glabrata*, have been shown to be predominate outcrossers, others, such as *B. pfeifferi* appear to be predominantly selfers (Table 1).

This study focuses on the mating strategy of *Biomphalaria sudanica*, an important intermediate host of *Schistosoma mansoni* in East Africa. This species inhabits the marshy shorelines of the African Great Lakes and the tributaries of the White Nile, and is considered the primary vector in these habitats (Loker et al., 1993; Brown, 1994). Inbred lines of *B. sudanica* have been developed *via* multiple generations of self-reproduction in the laboratory, indicating that this species is self-compatible and will self-reproduce when no mates are available (Spaan et al., 2023). An allozyme based population genetic study of *B. sudanica* in Lake Victoria reported a mixed mating system of both outcrossing and self-reproduction due to a deficiency of heterozygotes in some populations, although the deficiency could be explained by other factors such as null alleles or hidden population structure (Bandoni et al., 1995, 2000). Thus, our main goal was to determine the relative

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rates of self-reproduction and cross-reproduction in progeny arrays derived from crosses of snail lines in the laboratory. The results of this study will inform the design of genetic studies such as linkage mapping analysis and genome wide association studies that associate genotypes to phenotypes of this important vector species.

2. Materials and methods

2.1. Diagnostic marker development

To determine the mating strategy of *B. sudanica* in the laboratory using a DNA based marker, fixed homozygous single nucleotide polymorphisms (SNPs) that differentiated in parental laboratory lines of *B. sudanica* were identified by aligning the regions of the genome surrounding the cytosolic copper/zinc superoxide dismutase 1 (*sod*1) gene from two laboratory lines of *B. sudanica* that originated from Lake Victoria, Kenya (Bs163 and BsKEMRIwu, see Spaan et al., 2023). The sequences, derived from whole genome sequence data (Pennance et al., 2024a), were interrogated for fixed SNPs that could easily be visualized with a restriction enzyme digest by searching for a GGCC palindrome, which can be cut by many enzymes including *HaeIII*. A homozygous T/C SNP that segregated between *B. sudanica* inbred lines (at position 3 in the GGCC palindrome: BsKEMRIwu = TT genotype; Bs163 = CC genotype) was identified in contig sc68, position 1,049,592 (Fig. 1A), in the 5′ untranslated region of gene BSUD.24856 neighboring the *sod*1 ortholog (BSUD.24857). The gene *sod*1 has been implicated as important in the defense against *S. mansoni* for the closely related snail species, *B. glabrata* (Goodall et al., 2006).

Polymerase chain reaction (PCR) primers were developed in conserved sites using the Primer3 plugin (Untergasser et al., 2012) in Geneious v.2022.0.2 (Biomatters Ltd.). A forward and reverse primer (sod1_1F: 5′-CCC ATG ATG GTT GCT ATG ACA ACA G-3′ and sod1_2R: 5′-CTT TCC CTC CTC ACA CTG TTC TGA G-3′, respectively) that amplify a 208 bp region including the T/C SNP (at bp position 65 of the 208 bp amplicon) were selected (Fig. 1A). Using BLAT (Kent, 2002) (options: stepSize = 1; minScore = 15), > 20 bp of forward and reverse primer sequences were confirmed to not match other non-target regions in the *B. sudanica* genome (GenBank: GCA_036873155) or the *S. mansoni* genome v9 (GenBank: GCA_000237925).

Using these primers in combination with *HaeIII* digestion, for a snail that is homozygous for the T allele we expected to see one band on the gel at 208 bp, and for a snail homozygous for the C allele we expected to see two bands after restriction digest with *HaeIII,* one at 144 bp and one at 64 bp. Snails that are heterozygous and contained both the T and C allele would be expected to have all three bands present on the gel (Fig. 1B).

2.2. Snail crosses and DNA extraction of parents and offspring

Seven snail families were developed by isolating, per family, a single pre-reproductive *B. sudanica* snail (< 4 mm in diameter) from both an inbred line Bs163 and line BsKEMRIwu, and placed into 1-liter aquaria that were aerated. Snails were fed green leaf lettuce and Aquatic Fresh Water Snail Mix ABF2 (Aquatic Blended Foods, Hagerman, ID, USA) *ad libitum*. Snails of line Bs163 are incompatible with two *S. mansoni* lines, UNMKenya and NMRI. BsKEMRIwu snails are compatible with UNMKenya, but not NMRI (Spaan et al., 2023). Tanks were checked bi-weekly to monitor egg laying and hatching, from which eight randomly chosen juvenile offspring (< 3 mm) and parent snails were selected per family and DNA extracted from soft tissue (headfoot tissue only from parent snails) following a modified Qiagen Blood and Tissue Kit as described previously (Pennance et al., 2024b).

2.3. PCR and enzyme digest

For the PCR amplification, Promega (Madison, WI, USA) GoTaq® Master Mix was adopted. Each PCR was performed in 20 µl reactions: 10 µl of GoTaq® Master Mix; 1.5 µl of forward (sod1_1F) and reverse (sod1_2R) primer (10 µM); 5 µl water and 2 µl of diluted (either 1:100 for parents and 1:10 for offspring) gDNA. Thermocycling conditions followed the GoTaq® Master Mix manufacturer's instructions: 95 °C 2 min followed by 30 cycles of 95 °C for 45 s, 60 °C for 30 s, 72 °C for 45 s, and a final elongation step at 72 °C for 5 min.

Restriction digest of PCR products was performed using the New England Biolabs (Ipswich, MA, USA) restriction endonuclease *HaeIII*, following the manufacturer's protocol. This involved per PCR product to be digested, mixing 0.4 µl of *HaeIII* enzyme with 2 µl of the provided rCutSmart™ Buffer and adding this 2.4 µl mixture to a separate tube containing 6 µl of PCR product (~1 µg) and 11.6 µl of DNA/RNA-free water. The 20 µl mixture was then gently mixed by pipetting and then incubated at 37 °C for 30 min in a heat block. Half the incubated product (10 µl) was then mixed with 1 µl of Invitrogen 10 × Blue Juice™ (Thermo Fisher Scientific, Waltham, MA, USA) gel loading buffer and loaded into an Invitrogen SYBR™ Safe DNA stained 3% agarose gel and

Table 1
A summary of the findings of previous studies (and the present study) investigating the predominant mating systems of hermaphroditic species of *Biomphalaria* in natural populations or in the laboratory when mates were available, either as predominantly cross- (CF) or primarily self-fertilization (SF), or where a predominance was not possible to infer (SF/CF).

Snail species	Snail origin	Method[a]	Predominant mating system: Cross-fertilization (CF), Self-fertilization (SF)
Neotropical species			
B. glabrata	Brazil, Venezuela, Lesser Antilles	PopGen, Ped	**CF** (Mulvey and Vrijenhoek, 1984; Mulvey and Woodruff, 1985; Vianey-Liaud, 1992; Vernon, 1997; Mavárez et al., 2002; Wethington et al., 2007)
B. tenagophila	Brazil	Ped	**SF** (Guimarães et al., 2016)
B. straminea	Hong Kong [b]	PopGen	**CF** (Mulvey and Woodruff, 1985)
African species			
B. pfeifferi	Madagascar, Côte d'Ivoire, Cameroon	PopGen, Ped	**SF** (Mimpfoundi and Greer, 1990a; Charbonnel et al., 2005; Tian-Bi et al., 2008, 2013, 2019; Escobar et al., 2011; Kengne-Fokam et al., 2016)
B. alexandrina	Egypt	PopGen	**CF** (Vrijenhoek and Graven, 1992)
B. sudanica	Kenya	PopGen, Ped	**SF/CF** (Bandoni et al., 1995, 2000) **CF** (Present study)
B. choanomphala	Kenya	PopGen	**SF/CF** (Bandoni et al., 1995, 2000)
B. cameroonensis	Cameroon	PopGen	**CF** (Mimpfoundi and Greer, 1990b)

[a] There are two broad methods by which mating systems have been characterized: through population genetics (PopGen) or pedigree (Ped) studies. Population genetics infers mating systems in natural populations by quantifying F_{IS}, which is estimated as the deficiency of heterozygotes compared to a similar, randomly outcrossing population in Hardy-Weinberg equilibrium. Pedigree studies rely on measuring the inheritance of phenotypic traits or genetic markers in the offspring of paired mates.

[b] *Biomphalaria straminea* was introduced to Hong Kong sometime during the 1970's (Meier-Brook, 1974).

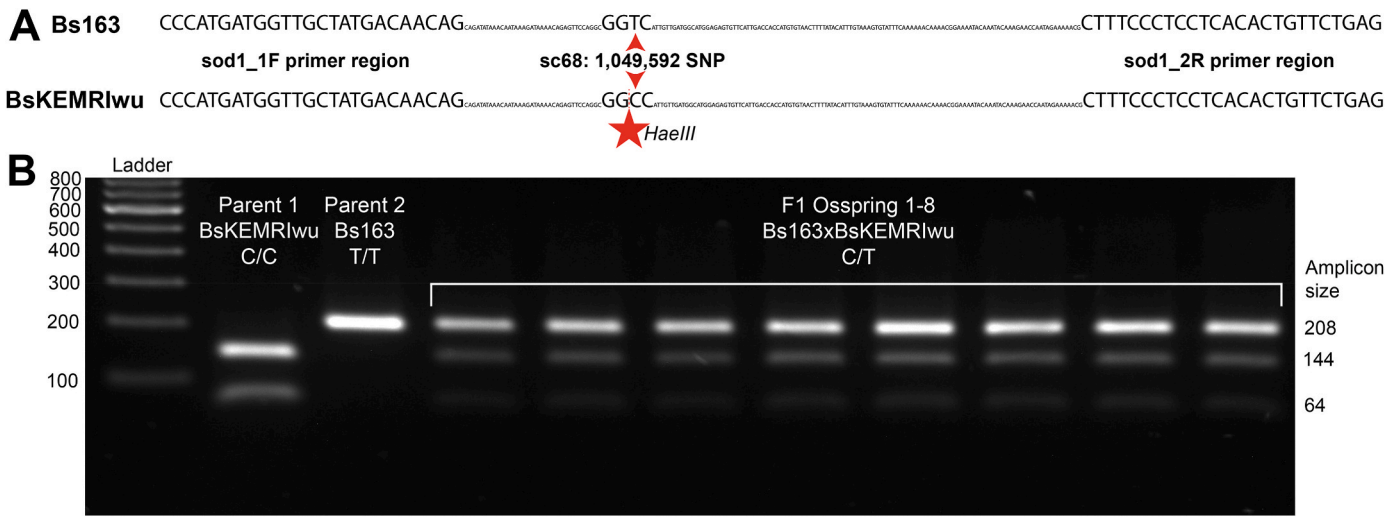

Fig. 1. **A** *Biomphalaria sudanica* nuclear DNA region (contig sc68:1,049,528–1,049,735) amplified and site of enzyme cut site in GGCC palindrome of BsKEMRIwu line snails only. **B** Gel electrophoresis results for one of the Bs163 × BsKEMRIwu hybrid family (six other families had identical results), showing the banding patterns following PCR amplification and enzyme digest of parent homozygous genotypes (C/C and T/T) and F1 offspring heterozygotes (C/T).

viewed following electrophoresis at 50 V for 90 min. Amplicon band sizes were compared to an Invitrogen 100 bp DNA ladder.

From one family, genotypes of each parent snail (Bs163 and BsKEMRIwu) and two F1 offspring were validated using Sanger sequencing. Amplified PCR products were sequenced in the reverse direction using primer sod1_2R. Sequences were trimmed, edited and aligned in Geneious to confirm homozygosity of parents and heterozygosity of offspring (Supplementary Fig. S1).

3. Results and discussion

From the seven Bs163xBsKEMRIwu families, a total of 14 parent snails and 56 offspring were assayed using the described PCR and restriction digest. The results indicated that all BsKEMRIwu parent snails were homozygous for reference allele (T), and all from line Bs163 were homozygous for the C allele as expected (Fig. 1B). In all seven crosses, 100% of these F1 progeny were heterozygous, having T/C allele at the sc68:1,049,592 SNP, indicating that 100% of offspring were derived from outcrossing. There was therefore no evidence of selfing within the progeny sample we collected, suggesting that this species preferentially outcrosses when a mate is available. These results indicated successful crossing and transfer of genes between two genotypically unique parents. Thus, these results are highly favorable that these crosses can be applied in designing genetic association studies for mapping of phenotypic traits such as snail resistance to parasite infection, and for future applications of gene drives for control of schistosomiasis at the level of the snail vector. However, prior to implicating gene drives in wild *Biomphalaria* populations, their mating systems should be further investigated because selfing may occur more frequently when there is lack of access to mates or in the presence of environmental stressors (Coutellec and Lagadic, 2006). These data also add to a more complete understanding of the evolution of the mating strategies of *Biomphalaria* which is of interest in the context of host-parasite co-evolutionary theory. The selective pressure induced by parasites is theorized to drive the maintenance of biparental sex in host populations despite its inherent costs because the genetic diversity resulting from biparental mating can enhance the ability of the host to counter parasite virulence evolution (Van Valen, 1973; Williams, 1975; Jaenike, 1978; Maynard Smith, 1978; Hamilton, 1980; Lively and Lloyd, 1990; Jarne and Charlesworth, 1993; Morran et al., 2011; Gibson et al., 2017).

Biomphalaria colonized Africa ~5 mya and diverged across the continent into 12 species with differing mating systems (Woodruff and Mulvey, 1997; Campbell et al., 2000; DeJong et al., 2001; Bu et al., 2023). The best studied of the African *Biomphalaria* species, *B. pfeifferi*, primarily self-fertilizes (Table 1). Populations are characterized by an excess of homozygosity and selfing rates estimated at 80–100% (Charbonnel et al., 2005; Tian-Bi et al., 2008, 2013, 2019; Escobar et al., 2011). The species of *Biomphalaria* in Africa also differ in their susceptibility to schistosomes, with *B. sudanica* being among the most resistant and *B. pfeifferi* being among the most susceptible, which is in line with the theoretical prediction that outcrossing species retain the genetic diversity that enables resistance to infection, while selfing species do not (Mutuku et al., 2017, 2021). Therefore, *Biomphalaria* species could serve as an excellent model to understand how coevolution with parasites influences the evolution of mating systems as well as the evolution of resistance and tolerance strategies to overcome infection. Further work could determine the individual variation in the propensity to self-fertilize *versus* outcross, which may be genetically determined and could be influenced by environmental factors other than the presence or absence of mates (Felmy et al., 2023). Ultimately, a deeper understanding of the evolutionary interactions between hosts and parasites will lead to improvements in implementation of genetic manipulative technologies aimed at infection control.

4. Conclusion

Our findings demonstrate that genetically distinct *B. sudanica* snails preferentially outcross when mates are available. It is therefore possible to design genetic association studies to map phenotypic traits such as parasite resistance, as well as explore future applications for gene drive in schistosomiasis control in this species.

CRediT authorship contribution statement

Jenessa Olson: Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Tom Pennance:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Johannie M. Spaan:** Investigation, Writing – review & editing. **Maurice R. Odiere:** Supervision, Funding acquisition, Writing – review & editing. **Jacob A. Tennessen:** Conceptualization, Formal analysis, Writing – review & editing, Supervision. **Michelle L. Steinauer:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Funding acquisition.

Ethical approval

This project was undertaken following approval from the relevant bodies, including Kenya Medical Research Institute (KEMRI) Scientific Review Unit (Approval # KEMRI/RES/7/3/1 and KEMRI/SERU/CGHR/035/3864), Kenya's National Commission for Science, Technology, and Innovation (License # NACOSTI/P/15/9609/4270 and NACOSTI/P/22/14839), Kenya Wildlife Services (permit # 0004754 and # WRTI-0136–02-22), and National Environment, Management Authority (permit # NEMA/AGR/46/2014 – Registration # 0178 and NEMA/AGR/159/2022 – Registration # 201).

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2025.100241.

Data availability

The datasets supporting the conclusions of this article are included within the article and its supplementary file.

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