

Schistosome/mollusk: genetic compatibility

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ABSTRACT. Schistosomiasis remains one of the most prevalent parasitic infections and has significant economic and public health consequences in many developing countries. Economic development and improvement in standard of living in these countries are dependent on the elimination of this odious disease. For the control of Schistosomiasis, understanding the host/parasite association is important, since the host parasite relationship is often complex and since questions remain concerning the susceptibility of snails to infection by respective trematodes and their specificity and suitability as hosts for continued parasite development. Thus, the long term aim of this research is to learn more about the genetic basis of the snail/parasite relationship with the hope of finding novel ways to disrupt the transmission of this disease. In the current research, genetic variability among susceptible and resistant strains within and between *Biomphalaria glabrata* and *B. tenagophila* was investigated using RAPD-PCR. The results indicate great genetic variations within the two snail species using three different primers

(intrapopulational variations), while specimens from the same snail species showed few individual differences between the susceptible and resistant strains (interpopulational variation).

Key words: *Biomphalaria*; Schistosomiasis; RAPD-PCR; Genetic variability; Polymorphism

INTRODUCTION

Biomphalaria snails are the most common freshwater intermediate host snail prevailing in developing countries and have been recognized as the intermediate host for *S. mansoni*, which is responsible for wide spread schistosomiasis infections in humans. *B. glabrata* and *B. tenagophila* play an increasingly important role in transmission of *S. mansoni* in Brazil, and they are considered to be potential hosts of the schistosome parasite. The understanding of the genetic basis for schistosome resistance/or susceptibility in these snail species is especially important, because schistosome eradication programs may benefit from the knowledge of the genetic polymorphism of these snails since association between hosts and parasite tend to generate and maintain genetic polymorphism (May, 1985), as has been predicted in models of schistosome-snail interactions (Morand et al., 1996).

Several molecular biology techniques for studies of genetic variability in schistosomiasis vectors have been used (Miller et al., 1996; Spatz et al., 1999; Knight et al., 1991, 2000; Jannotti-Passos and Souza, 2000; Vidigal et al., 2000, 2001; Caldeira et al., 2000, 2001; Souza and Passos, 2001; Tuan and Bortolato, 2001).

Many attempts have been made to define markers for susceptibility/resistance in either *B. glabrata* (Mulvey and Vrijenhoek, 1982; Mulvey and Woodruff, 1985; Sire et al., 1999; Knight et al., 1999) or *B. tenagophila* (Abdel-Hamid et al., 1999). Most attempts involved linkage analysis by examining pigmentation and allozyme phenotypes in resistant and susceptible snails. To date, no single association between such markers and linkage to the resistant phenotype has been identified in these snail species.

Recently, several investigators showed that random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is useful for distinguishing between and within different *Biomphalaria* snail species (Abdel-Hamid et al., 1999; Knight et al., 1999; Kristensen et al., 1999; Sire et al., 1999).

Due to the high applicability of RAPD-PCR and the quality of the results obtained, we have used this technique to investigate the intra- and interpopulational variability related to susceptibility and resistance to *S. mansoni* infection of both *B. glabrata* and *B. tenagophila* and to detect genetic markers associated with resistant/or susceptible snails which may be responsible for schistosome resistance in *Biomphalaria* snails.

MATERIAL AND METHODS

Intermediate host snails

The separation of schistosome resistant and susceptible strains from both *B. glabrata* (BH strain) and *B. tenagophila* (SJ strain) was performed according to the method described by Zanotti-Magalhaes et al. (1997). The snails originated from Campinas (São Paulo, Brazil)

and had been maintained in our laboratory under suitable environmental conditions.

In selecting for resistant stocks, snails that remained uninfected after two parasitic exposures (10 miracidia/snail) were isolated and reared singly for selfing. Also, in selecting for susceptible stocks, unexposed progeny of snails from which exposed test groups yielded high infection frequencies, were isolated and reared singly for selfing. Resistant and susceptible snails were used in the present study to identify RAPD fragments that could be associated with resistance.

Molecular analysis

DNA was extracted from the tip of the head foot region of snails individually, using lysis buffer containing 2% CTAB (Winnepenninckx et al., 1993 and modified by Abdel-Hamid et al., 1999). DNA concentration and purity was determined spectrophotometrically (Spectrophotometer Ultrospec III - Pharmacia, UK) based on absorbance at 260 and 280 nm, and by 2.0% agarose gel electrophoresis using the gel photodocumentation system (EDAS) DC 120 Zoom Digital Camera (Eastman Kodak, NY, USA).

DNA amplification by RAPD-PCR

The genotypes of the resistant and susceptible strains of the two snail species *B. glabrata* and *B. tenagophila* were determined with 5 arbitrary 10-mer primers by RAPD-PCR as in the method of Simpson et al. (1993), with some modifications. An amount of 2 ng genomic DNA was amplified with a PTC 200 Peltier Thermal Cycler (MJ Research, USA) using RAPD-PCR. Each reaction was carried out in a final volume of 20 μ L containing 1 unit Taq DNA polymerase (Gibco-BRL, MD, USA), 1X PCR buffer, 0.2 mM of each dNTP, and 7 pmol of each random primer (Gibco-BRL) [1 (5'-GTGCTACGT-3'), 2 (5'-CAGGCCCTTC-3') and 3 (5'-GGTCCCTGAC-3')]. The amplification conditions were as follows: 1 cycle at 95°C for 5 min, 2 cycles at 95°C for 30 s, at 30°C for 2 min and at 72°C for 1 min, and 33 cycles during which the annealing temperature was changed to 40°C and the time of the extension step was increased to 5 min during the final cycle. As control, PCR were run without DNA genome. After DNA thermal cycle, PCR products were analyzed by using polyacrylamide gel electrophoresis and silver stained to resolve amplified fragments as described by Santos et al. (1993).

Intra- and interpopulational genetic variability among *B. glabrata* and *B. tenagophila* was evaluated by analyzing the electrophoretic band patterns obtained on the gels and by determining the similarity coefficient as described by Dice (1945).

RESULTS

Susceptibility or resistance to *S. mansoni* infection within *Biomphalaria* snails was studied starting 30 days post-infection and continuing thereafter weekly up to 10 weeks after miracidia exposure. The results point out that about 9 and 93% of the examined *B. glabrata* and *B. tenagophila* snails were resistant, in contrast to 91 and 7% of these snails yielding high infection frequencies, respectively (Figure 1). The curves representing percentages of susceptible snails showed notable levels starting at the 6th week post-infection, which reached the highest peaks by approximately the 8th week. On the other hand, the curves of refractory snails gradually decreased toward the 8th week and up to the 8th week post-infection no changes in percent-

ages of refractory snails occurred, with no differences being noted up to the 10th week. The ratio of susceptibility/resistance within the same species at a time was fixed, although the number of snails could change depending on the increasing of mortality within susceptible ones.

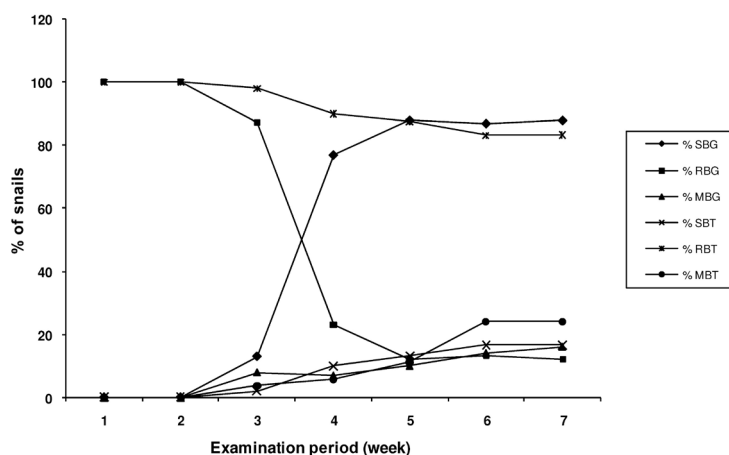


Figure 1. Diagrammatic representation of susceptibility/resistance of *Biomphalaria glabrata* and *B. tenagophila* to *Schistosoma mansoni*. SBG = susceptible *B. glabrata*; RBG = resistant *B. glabrata*; MBG = mortality of *B. glabrata*; SBT = susceptible *B. tenagophila*; RBT = resistant *B. tenagophila*; MBT = mortality of *B. tenagophila*.

The results also indicate that despite the low efficiency of *B. tenagophila* as a host (experimental infection rates are less than 9% as shown in Figure 1, in contrast with high infection rates of *B. glabrata*), this species is an important schistosomiasis vector in Brazil and plays a potential role together with *B. glabrata* in transmission of *S. mansoni* in different regions.

From a genetic point of view, the results obtained by Dice's coefficient point out that, by using different primers, the mean percentages of shared bands among all pairs from both susceptible and resistant strains within the same species *B. glabrata* or *B. tenagophila* ranged from 93 to 95% and from 96 to 97%, respectively, in contrast to 85 to 87% and 80 to 83% among all the pairs of the two different snail species. From Tables 1, 2 and 3, it is also noted that the most related individuals belong to the same snail species either *B. glabrata* or *B. tenagophila* with the similarity coefficient ranging from 0.93 to 0.1, while snails from different species showed great genetic variations with a similarity coefficient ranging from 0.67 to 0.77.

Table 1. Dice's similarity coefficient (*S) of the profile bands between susceptible and resistant strains of both *Biomphalaria glabrata* and *B. tenagophila* using primer 1 (5'-AGTGCTACGT-3').

Snail strain	<i>B. tenagophila</i> susceptible	<i>B. tenagophila</i> resistant	<i>B. glabrata</i> susceptible	<i>B. glabrata</i> resistant
<i>B. tenagophila</i> susceptible	1.0	0.94	0.81	0.72
<i>B. tenagophila</i> resistant	0.94	1.0	0.72	0.85
<i>B. glabrata</i> susceptible	0.81	0.72	1.0	0.95
<i>B. glabrata</i> resistant	0.72	0.77	0.95	1.0

*S = $2a / 2a + b + c$, where: a = the number of shared bands between two individuals; b = the bands present in the 1st and not in the 2nd, and c = the bands present in the 2nd and not in the 1st.

Table 2. Dice's similarity coefficient (*S) of the profile bands between susceptible and resistant strains of both *Biomphalaria glabrata* and *B. tenagophila* using primer 2 (5'-CAGGCCCTTC-3').

Snail strain	<i>B. tenagophila</i> susceptible	<i>B. tenagophila</i> resistant	<i>B. glabrata</i> susceptible	<i>B. glabrata</i> resistant
<i>B. tenagophila</i> susceptible	1.0	0.93	0.80	0.67
<i>B. tenagophila</i> resistant	0.93	1.0	0.78	0.82
<i>B. glabrata</i> susceptible	0.80	0.78	1.0	0.94
<i>B. glabrata</i> resistant	0.67	0.82	0.94	1.0

*S = $2a / 2a + b + c$, where: a = the number of shared bands between two individuals; b = the bands present in the 1st and not in the 2nd, and c = the bands present in the 2nd and not in the 1st.

Table 3. Dice's similarity coefficient (*S) of the profile bands between susceptible and resistant strains of both *Biomphalaria glabrata* and *B. tenagophila* using primer 3 (5'-GGTCCCTGAC-3').

Snail strain	<i>B. tenagophila</i> susceptible	<i>B. tenagophila</i> resistant	<i>B. glabrata</i> susceptible	<i>B. glabrata</i> resistant
<i>B. tenagophila</i> susceptible	1.0	0.93	0.79	0.71
<i>B. tenagophila</i> resistant	0.97	1.0	0.76	0.77
<i>B. glabrata</i> susceptible	0.79	0.76	1.0	0.95
<i>B. glabrata</i> resistant	0.71	0.77	0.95	1.0

*S = $2a / 2a + b + c$, where: a = the number of shared bands between two individuals; b = the bands present in the 1st and not in the 2nd, and c = the bands present in the 2nd and not in the 1st.

The results also show that the data analyzed using primer 2 displayed more variation than with both primer 1 and primer 3. Despite the homogeneity of most bands, among the susceptible and resistant strains within the *B. glabrata* snail species, the genomic DNA amplified with primer 1 showed a polymorphic band of nearly 4060 bp only in the susceptible strain, as shown in Figure 2, while the amplification results of genomic DNA of the same snail species with primers 2 and 3 pointed out a polymorphic marker of 1100 bp in the susceptible strain also (Figures 3 and 4).

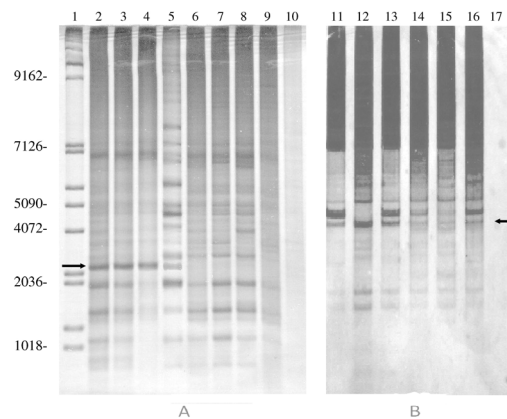


Figure 2. Random-amplified PCR of genomic DNA of two different snail strains (resistant and susceptible) within *Biomphalaria glabrata* and *B. tenagophila* species using arbitrary primer 1 (5'-AGTGCTACGT-3'). **A.** Lane 1, 1-kb DNA Ladder; lanes 2-4, individual of susceptible *B. tenagophila*; lane 5, Pool S, S1, S2 and S3; lane 6, Pool R, R1, R2 and R3; lanes 7-9, individual of resistant *B. tenagophila*; lane 10, negative control. **B.** Lanes 11, 12, individual of resistant *B. glabrata*; lane 13, pool of resistant *B. glabrata*; lane 14, pool of susceptible *B. glabrata*; lanes 15, 16, individual of susceptible *B. glabrata*; lane 17, negative control.

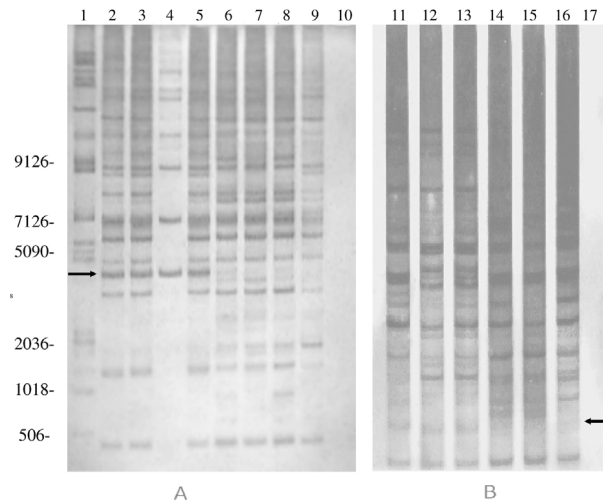


Figure 3. Random-amplified PCR of genomic DNA of two different snail strains (resistant and susceptible) within *Biomphalaria glabrata* and *B. tenagophila* species using arbitrary primer 2 (5'-CAGGCCCTTC-3'). **A.** Lane 1, 1-kb DNA Ladder; lanes 2-4, individual of susceptible *B. tenagophila*; lane 5, Pool S, S1, S2 and S3; lane 6, Pool R, R1, R2 and R3; lanes 7-9, individual of resistant *B. tenagophila*; lane 10, negative control. **B.** Lanes 11, 12, individual of resistant *B. glabrata*; lane 13, pool resistant of *B. glabrata*; lane 14, pool susceptible of *B. glabrata*; lanes 15, 16, individual of susceptible *B. glabrata*; lane 17, negative control.

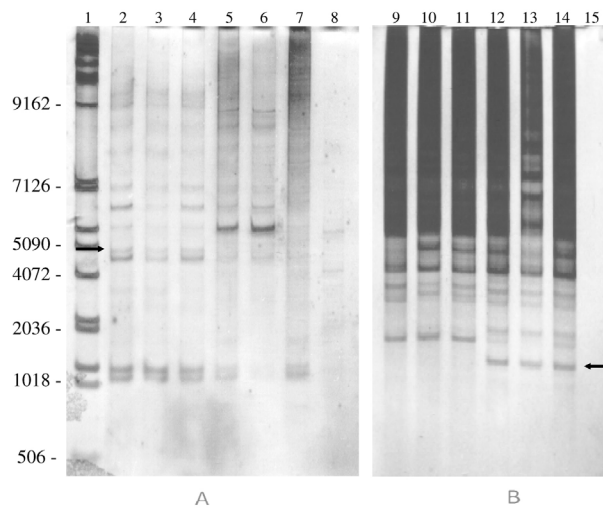


Figure 4. Random-amplified PCR of genomic DNA of two different snail strains (resistant and susceptible) within *Biomphalaria glabrata* and *B. tenagophila* species using arbitrary primer 3 (5'-GGTCCCTGAC-3'). **A.** Lane 1, 1-kb DNA Ladder; lanes 2, 3, individual of susceptible *B. tenagophila*; lane 4, pool of susceptible *B. tenagophila*; lane 5, pool of resistant *B. tenagophila*; lanes 6, 7, individual of resistant *B. tenagophila*; lane 8, negative control; **B.** Lanes 9, 10, individual of resistant *B. glabrata*; lane 11, pool of resistant *B. glabrata*; lane 12, pool of susceptible *B. glabrata*; lanes 13, 14, individual of susceptible *B. glabrata*; lane 15, negative control.

Primers 1, 2, and 3 revealed a single polymorphic band of approximately 5056, 3136, and 5041 bp, respectively (Figures 2-4) in the susceptible strain of the *B. tenagophila* snail species.

Although, the specimens from different strains within the same species showed few individual differences, the amplified bands with primers 1, 2, and 3 of the corresponding strains between the two different snail species *B. glabrata* and *B. tenagophila* showed many polymorphic markers (Figures 2-4).

These results represent a starting point for the identification and isolation of genes specifically related to susceptibility and supporting the previous hypotheses that RAPD-PCR is a suitable and efficient methodological approach to the analysis of genetic variability of schistosomiasis vectors.

DISCUSSION

The results show that *B. tenagophila* is not so efficient as intermediate host, compared to *B. glabrata*, since the experimental infection rates are less than 7% as seen in Figure 1, in contrast to high infection rates of *B. glabrata*. These results support the data obtained by Brasio et al. (1985) who found that *B. glabrata* has a more efficient power of attraction to miracidia than *B. tenagophila*. Paraense et al. (1983) also pointed out that “the presence of *B. glabrata* is coincident with the schistosomiasis transmission”. Thus, these observations have important epidemiological implications, as Zanotti-Magalhães et al. (1997) demonstrated that the human disease caused by *S. mansoni* that use *B. glabrata* as an intermediate host is more dangerous than that involving *B. tenagophila*. Despite all the epidemiological implications and also despite the low efficiency as a vector of *S. mansoni*, *B. tenagophila* plays a potential and important role together with *B. glabrata* in transmission of schistosomiasis in different Brazilian regions. In view of all these findings, both *B. glabrata* and *B. tenagophila* snail species were chosen in the current study for investigating intra- and interpopulational genetic variation and to detect some genetic markers associated with resistant snail species.

We also observed in the present study that susceptibility/resistance to *Schistosoma mansoni* infection within both *B. glabrata* and *B. tenagophila* was only considered at 8th week post-infection and up to this time point for selecting resistant snail stock. It is also well recognized that, with time, refractory snails to *S. mansoni* infection may gradually decrease and vice versa, where susceptible ones increase up to the 8th week post-infection. The possibilities explaining this situation could be a delay in development of the parasite within some snail strains, since susceptibility or resistance of the snails to *S. mansoni* has mainly been claimed to be dependent on host genetic background or results from the interaction between the gene products of the mollusk and the parasite as declared previously by both Richards and Merritt Jr. (1972) and Richards and Shade (1987).

In the present study, the identification of polymorphic bands was based on the comparison of the band patterns on the same gel for the two strains, and only those detected in all individuals of the same strain and in the pool were considered polymorphic (Larson et al., 1996).

A possible existence of transposable elements in the snails opens new perspectives in the approach for transfection of sequences with incorporation into the snail genome (Richards et al., 1992). The markers or genomic sequences associated with resistance could be useful in the production of transgenic snails. The perspective of the acquisition of transgenic snail strains resistant to *S. mansoni* infection implies overcoming many theoretical difficulties, especially the experimental ones. In spite of that, such methodological approach presents interesting perspectives (Lardans and Dissous, 1998) that, in association with other measures,

will allow the elaboration of new strategies for the control of schistosomiasis.

The amplified markers that were found only in resistant strains within *B. tenagophila* and segregate between the two snail species could be responsible for the resistance of these snails to schistosome parasite infection, and should contribute to a better understanding of the correlations between vectors, parasites and transmission of the disease. This notion supports the previous results obtained by Brasio et al. (1985) who declared that *B. tenagophila* has low efficiency as an intermediate host for *S. mansoni*. In contrast, the absence of such markers in the corresponding individuals of *B. glabrata* could be responsible for the high efficiency of these snails in transmission of schistosomiasis in humans. These suggestions are in line with the finding of both Paraense et al. (1983) and Zanotti-Magalhaes et al. (1997) who reported that *B. glabrata* has a high efficiency in the transmission of schistosomiasis.

This approach has indeed attempted to add more to our knowledge about the genetic diversity of both snail species in the field and their roles in transmission of schistosomiasis in different regions in Brazil. It is anticipated that the development of molecular markers associated with resistant/susceptible phenotypes should lead to a thorough strategy for the control of schistosomiasis disease.

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