Genetic Markers from *Biomphalaria tenagophila* (Gastropoda: Pulmonata: Planorbidae) Obtained by the Double Stringency Polymerase Chain Reaction Technique

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Biomphalaria tenagophila, *one of the intermediate hosts of the trematoda* Schistosoma mansoni, *is a simultaneous hermafrodite snail species. In order to analyse the genetic structure of these populations, we performed a double-stringency PCR technique to obtain genetic markers with microsatellites and arbitrary primers in a single reaction.*

Key words: *Biomphalaria* - DNA polymorphism - PCR

Biomphalaria are simultaneous hermaphrodite freshwater snails, which can breed mainly by cross (Paraense 1955) but also by self-fertilization (Tuan & Simões 1998). Analysis of the effects of reproduction in freshwater snails using genetic markers such as isozymes (Bandoni et al. 1995) shows heterozygote deficiencies indicating that selfing could be a regular mating strategy creating complex patterns of population structure exhibiting genetic differences that can cause differences in susceptibility to *Schistosoma mansoni* (Hofman et al. 1998).

Molecular markers are in large use for freshwater snails diagnosis, mainly PCR based protocols which show a great power for quick and simple characterization of genetic variation within and among populations (Avise 1994). DS-PCR described here combines the specificity of microsatellites and the simplicity of amplification of RAPDS markers in a double stringency condition leading to the amplification of selective population of DNA. The technique produced genetically informative co-dominant markers in *Drosophila mercatorum* (Matioli & Brito 1995).

We applied DS-PCR to laboratory strains *B. tenagophila* from Tremembé, São José dos Campos and Bananal, all from endemic areas in São Paulo, SP; and a strain from Taim, RS, a non-endemic area. In order to observe intra-population variation we used 12 *B. tenagophila* snails collected from rice paddies at Tremembé, SP, a site with a long history of *S. mansoni* transmission (Silva 1992). The hepatopancreas-ovotestis complex were extracted from snails previously identified by morphological reproductive traits (according to Paraense 1975), and free from *S. mansoni* infection. We used a standard phenol-chloroform protocol for DNA extraction (Sambrock et al. 1989) which produced a clear pattern of DNA (Fig. 1).

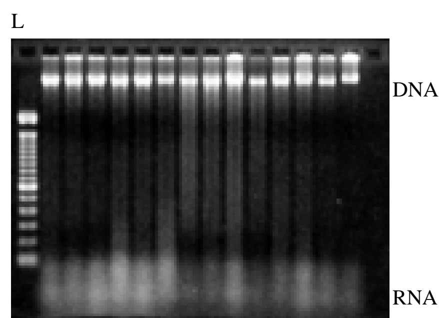

Fig. 1: DNA from *Biomphalaria tenagophila* (n=14) shows high molecular weight.

The reactions were done in a 25 µl volume containing: 2.5 µl buffer, 1.25 µl MgCl2 50 mM, 5 µl DNTPs mix, 0.25µl of microsatellites primer, 1 µl of RAPD primer, 0.25 µl of Taq DNA polymerase and 2 µl of extracted DNA, and 12.75 µl of distilled water. The microsatellites and RAPD combinations used were 4C [$(CAG)_4$ and GAACGGACTC], 5C [$(GAG)_4$ and GAACGGA CTC], 6C [$(AACG)_4$ and GAACGGACTC], 7C [$(ATCG)_4$ and GAACGGACTC], 10C [$(CGGA)_4$ and GAACGGACTC], 14C [$(TTTG)_4$ and GAA CGGACTC]. All reactions were performed with a Minicycler Termocycler (MJ Research).

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The temperature profile was the following: a first denaturation step at 94ºC for 2 min+15 cycles at 94ºC for 30s-52ºC for 45s and 72ºC for 60s + 25 cycles at 94ºC for 30s- 35ºC for 45s- a ramp of 60s to 72ºC for 60s. Finally extension at 70ºC for 10 min. The products were analyzed by agarose gel electrophoresis (1.4% in TBE buffer) and stained with ethidium bromide (EtBr).

The interpopulational profiles of the six primer combinations show a significant degree of genetic variation, suggesting heterogeneous genetic population patterns (Fig. 2).

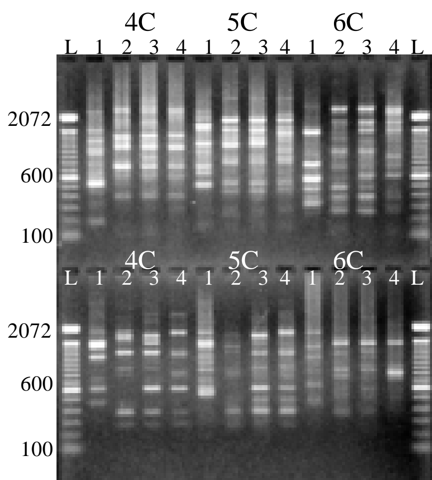

Fig. 2: bands produced with six primers combinations by DS-PCR in four *Biomphalaria tenagophila* population.

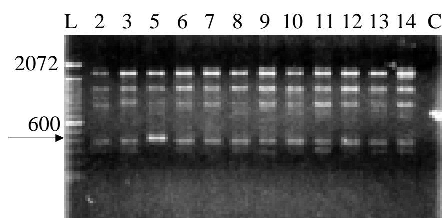

Fig. 3: bands produced by primer 7C (the arrow indicates the polymorphic markers, L indicates the DNA Ladder, the numbers in the left indicate the approximate sizes in pb, and C indicates control).

At the intrapopulational level DS-PCR generates polymorphisms that can contribute with the comparison of populations derived from different environmental conditions (Fig. 3).

We concluded that DS-PCR is an excellent tool that can be applied to identify genetic polymorphisms within and among hermaphrodite snail populations. DS-PCR employed here is a method which has the large applicability of RAPD but also can generate codominant markers that are more informative for population analyses.

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