

## ENZYME POLYMORPHISM AND GENETIC STRUCTURE OF *Biomphalaria tenagophila* (GASTROPODA, PLANORBIDAE) POPULATIONS: FOUNDER EFFECT

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### ABSTRACT

The genetic variation in 15 enzymatic loci was studied among nine populations of *Biomphalaria tenagophila*, the principal vectors of esquistossomiasis in the State of São Paulo. The polymorphism of the populations was manifested at seven of the 15 loci analyzed. Low heterozygosity values among populations was found ( $H = 0.00$  to  $0.086$ ). The Sorocaba samples showed heterozygosity for the HBDH loci ( $H = 0.461$ ), elevated in relation to the remaining populations. In the present study, it was possible to identify fixed alleles in *B. tenagophila*. The probable origin of distribution of the polymorphism in these populations is discussed and the hypothesis of a founder effect is proposed to explain colonization and genetic variability.

### INTRODUCTION

Several studies have been carried out to justify the level of genetic variability observed in natural populations. However, the adaptative significance of variation continues to be a controversial topic in Evolutionary Biology.

The neutralist interpretation proposes that the level of polymorphism observed in a population is the result of the frequency of random mutations which occur in the genome of individuals. According to Kimura (1983), most of the mutations fixed

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in the genome have no effect on the fitness of a population and therefore are selectively neutral. In contrast, the selective characteristic of genotypic variation is reaffirmed in Ohta and Dover (1984) and Lewontin (1985). The individual variation confers differences in fitness and therefore provides raw material for adaptation to new situations (Futuyma, 1986).

The hypothesis that polymorphism may be correlated with the occupation of multiple niches, has been advanced by Powell (1975) and Nevo (1978). They showed that species of wide geographic distribution and intense mobility, which exploit a large number of ecological niches, exhibit greater genetic variability when compared with species of restricted geographic distribution. However, other experimental evidence has been obtained (Morgante and Malavasi, 1985), that reaffirms the principles formulated by Levins (1968). This author suggested that the lower variability observed in generalist species may be correlated with homeostatic mechanisms which would permit these species to utilize the different types of environments to which they are exposed.

Study of the amount of variation in natural populations has permitted correlation of the distribution of the polymorphic patterns of genomes with the dynamics of colonization and dispersal of populations and species (Mulvey *et al.*, 1988). Woodruff *et al.* (1985) used electrophoresis for the study of the origin and colonizing process of introduced species. When populations of the pulmonate snail *Biomphalaria straminea* were analyzed in Hong Kong for enzyme polymorphism, it was possible to demonstrate a multiple origin for the populations in the region as well as to delimit the areas occupied by each of the existing genetic lines.

In the present study, natural populations of the pulmonate snail *B. tenagophila* were analyzed electrophoretically with the initial objective of surveying the level of genetic variation in geographically isolated populations.

## MATERIAL AND METHODS

This work was carried out in the Mollusk Cytogenetics Laboratory of the Department of Biology, Biosciences Institute, University of São Paulo.

### *Specimens*

The species *B. tenagophila* is found in Central Brazil to Northern Argentina, commonly living in lakes, lagoons, permanent or temporary streams, small irrigation ditches and even domestic sewage canals. In the State of São Paulo, *B. tenagophila* breeding sites are seldom contaminated with *Schistosoma mansoni*. Collections of *B. tenagophila* were made between April 1986 and May 1987.

### Samples

Samples were collected from nine localities in the State of São Paulo (Figure 1). The number of breeding sites sampled in each locality ranged from a minimum of two to a maximum of six according to the frequency of occurrence of the species at each site. The Sorocaba specimens (three samples) were obtained from two breeding sites. Samples denoted S01 and S03 were obtained from breeding site I, a 400 m<sup>2</sup> lagoon formed by natural damming of rainwater. Sample S02 was obtained on the same day from breeding site II, located 500 meters from site I. Both reservoirs are located within the urban perimeter of Sorocaba.

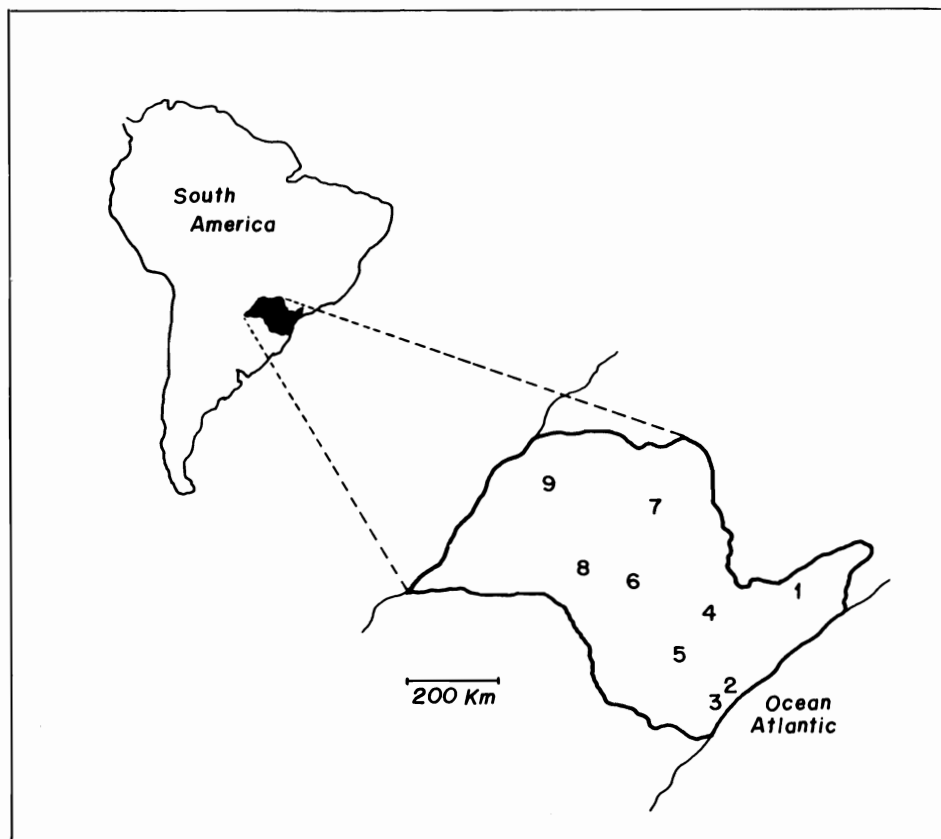


Figure 1 - Map showing the collecting sites of the specimens investigated: 1 - Taubaté; 2 - Itariri; 3 - Pedro de Toledo; 4 - Campinas; 5 - Sorocaba; 6 - Bauru; 7 - Marília; 8 - Bebedouro and 9 - Araçatuba.

### *Electrophoresis*

The specimens were taken to the Mollusk Cytogenetic Laboratory of the Department of Biology, where they were maintained in 25-liter aquaria under normal environmental conditions up to the time of electrophoretic analysis.

Digestive gland homogenates were prepared in 0.5% saline, adsorbed with Whatmann No. 3 filter paper and applied to the electrophoretic gel. Starch and acrylamide gels were used in a horizontal system according to the technique of Malavasi and Morgante (1982).

The following enzyme systems were analyzed: alcohol dehydrogenase (ADH), glycerol-3-phosphate dehydrogenase (alfa-GPD),  $\beta$ -hydroxybutyrate dehydrogenase (HBDH), malate dehydrogenase (MDH), glutamic oxalacetic transaminase (aminotransferase aspartate) (GOT), phosphoglucomutase (PGM), esterase (EST), alkaline phosphatase (APH), leucine aminotransferase (LAP) and phosphoglucoisomerase (PGI).

### *Analysis of similarity*

The levels of genetic identity and distance (Nei, 1972) among the populations from the various locations were calculating using the TAXON-1 program of the University of California. The gene frequency values were analyzed by the unweighted pair-group centroid clustering (UPGMC) method of Sneath and Sokal (1973).

## RESULTS

The degree of genetic polymorphism and heterozygosity of populations from nine different locations was estimated by electrophoretic analysis of ten enzyme systems. Fifteen isozyme loci were identified, seven of which were polymorphic in at least one of the populations analyzed (Table I).

The polymorphism observed in the Sorocaba population was manifested at six of seven loci that showed variation in the population analyzed, nevertheless no heterozygous specimens were detected in the population from Campinas (Table I). The allele frequencies calculated for the polymorphic loci are presented in Table II. The polymorphic loci observed in each population never exceeded two alleles. Two of these alleles can be observed in Figure 2. It was possible to identify heterozygotes on the electrophoretic gel. However, the level of heterozygosity observed in *B. tenagophila* populations was quite low.

Table I - Mean heterozygosity for loci and for samples among populations of *Biomphalaria tenagophila*. See "Material and Methods" for locations. P = % polymorphic loci; H = mean heterozygosity by direct count.

Population	APH	HBDH	MDH <sub>2</sub>	EST <sub>1</sub>	EST <sub>2</sub>	EST <sub>3</sub>	EST <sub>4</sub>	H	P
Taubaté n = 38	-	-	-	-	0.021	-	0.039	0.004 ± 0.011	13.3
Itariri n = 35	-	-	0.226	-	-	-	-	0.016 ± 0.058	6.7
Pedro de Toledo n = 35	0.343	-	-	-	-	-	-	0.025 ± 0.088	6.7
Campinas n = 40	-	-	-	-	-	-	-	-	-
Sorocaba n = 68	0.255	0.461	0.180	0.058	-	0.039	0.211	0.086 ± 0.123	40.0
Bauru n = 45	-	-	-	-	-	0.077	-	0.006 ± 0.019	6.7
Bebedouro n = 35	0.077	-	0.039	-	-	-	0.130	0.018 ± 0.036	20.0
Marília n = 40	-	0.013	0.095	-	-	-	-	0.015 ± 0.035	6.7
Araçatuba n = 30	-	0.269	-	-	-	-	0.255	0.037 ± 0.088	6.7

In the Sorocaba sample, there was a mean of 0.086 heterozygous genotypes (H). However, the levels of heterozygosity (H) for the *B. tenagophila* populations, excluding the values for the Sorocaba samples, was only 0.015.

Table II - Allele frequencies for the polymorphic loci among populations of *Biomphalaria tenagophila*.

Loci	Alleles	Bauru n = 45	Taubaté n = 38	Itariri n = 35	Sorocaba n = 68	P. de Toledo n = 35	Campinas n = 40	Marília n = 40	Bebedouro n = 35	Araçat. n = 30
APH	1.50	1.00	-	1.00	-	0.22	-	1.00	0.04	-
	1.20	-	-	-	0.15	-	-	-	-	-
	1.00	-	1.00	-	0.85	0.78	1.00	-	0.96	1.00
HBDH	1.00	-	1.00	1.00	0.64	1.00	1.00	0.06	1.00	0.84
	0.76	1.00	-	-	0.36	-	-	0.94	-	0.16
MDH <sub>2</sub>	1.12	-	-	-	-	-	-	0.05	-	-
	1.04	-	-	0.13	0.10	-	-	-	0.02	-
	1.00	1.00	1.00	0.87	0.90	1.00	1.00	0.95	0.98	1.00
EST <sub>1</sub>	1.00	1.00	-	1.00	0.97	1.00	-	1.00	1.00	1.00
	0.67	-	1.00	-	0.03	-	1.00	-	-	-
EST <sub>2</sub>	1.08	-	0.01	-	-	-	-	-	-	-
	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
EST <sub>3</sub>	1.20	-	-	-	0.02	-	-	-	-	-
	1.00	0.96	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00
	0.90	0.04	-	-	-	-	-	-	-	-
EST <sub>4</sub>	1.00	-	0.98	1.00	0.88	1.00	1.00	-	0.07	0.85
	0.95	-	0.02	-	0.12	-	-	-	-	0.15
	0.90	1.00	-	-	-	-	-	1.00	0.93	-

Allele frequency and mean heterozygosity were determined for the samples from Sorocaba (Table III). Heterozygosity was similar in samples S01 and S03, though the allele frequency for the HBDH, EST3 and EST4 loci differed between samples. The S02 sample had reduced genetic variability. Patterns observed for this monomorphic population were significantly different in relation to the remaining populations. The allele frequencies presented in Table II for the Sorocaba population are the result of the consolidated values for the three samples.

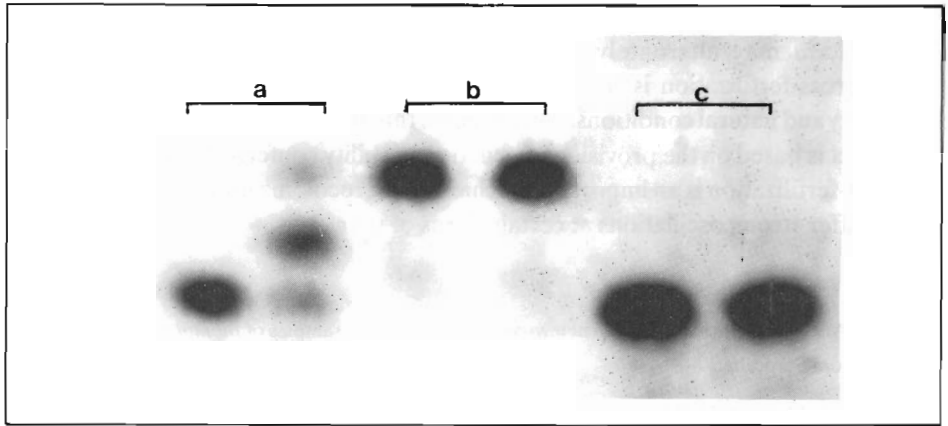


Figure 2 - Hydroxybutyrate dehydrogenase isoenzyme pattern of digestive gland extracts of *Biomphalaria tenagophila*. Samples: a, Sorocaba; b, Bebedouro; c, Marília.

Distinct enzyme phenotypes for four analyzed isozyme loci were identified (Table IV). The phenotypes denoted *A* and *C* correspond to two populations that are monomorphic for two different alleles. Phenotype *B* refers to a population in which the presence of both alleles (*A* and *C* phenotypes) was detected. The values observed are compatible with a model of panmixia. However, allele fixation in populations from different sites did not present a geographic distribution that would permit us to infer some kind of regularity in polymorphism distribution. On the contrary, at the neighboring sites of Pedro de Toledo and Itariri it was possible to obtain greater isozyme differences than observed between distant locations (Table II).

The results of analysis of similarities, are depicted in the dendrogram presented in Figure 3. It can be seen that neighboring populations (P. de Toledo, Itariri, S01, S02 and S03) clustered in a different manner.

## DISCUSSION

The level of polymorphism observed in natural populations have been attributed to several effects. The hermaphrodite trait is common to all pulmonate snails (Tompa, 1984; Geraerts and Joosse, 1984). *Biomphalaria spp.* are classified as simultaneous hermaphrodites since the production of male and female gametes occurs at the same time (Thomas and Benjamin, 1974). The reproductive behavior of

these animals was studied by Paraense (1955, 1956). This author emphasized that *Biomphalaria* may alternately perform self-fertilization and cross-fertilization, though cross-fertilization is the preferential reproductive mechanism, both under laboratory and natural conditions. According to this author, the evolutive significance of this fact is based on the provision of genetic variability conferred by cross-fertilization. Self-fertilization is an important mechanism for recolonization of breeding sites, which suffer strong oscillations at certain times of year.

Table III - Allele frequencies and mean heterozygosity (H) in three samples of *Biomphalaria tenagophila* collected from Sorocaba.

Loci	Alleles	Sorocaba 1	Sorocaba 2	Sorocaba 3
		(S01) n = 28	(S02) n = 20	(S03) n = 19
HBDH	1.00	0.74	-	0.38
	0.76	0.26	1.00	0.62
APH	1.20	0.16	-	0.19
	1.00	0.84	1.00	0.81
MDH <sub>2</sub>	1.04	0.11	0.03	0.06
	1.00	0.89	0.97	0.94
EST <sub>1</sub>	1.00	0.97	1.00	0.94
	0.67	0.03	-	0.06
EST <sub>3</sub>	1.20	-	-	0.13
	1.00	1.00	1.00	0.87
EST <sub>4</sub>	1.00	0.85	1.00	1.00
	0.95	0.15	-	-
Mean heterozygosity		0.083	0.004	0.094

Table IV - Enzymatic phenotypes for four isozyme loci in *Biomphalaria tenagophila* populations.

Loci	Alleles	Phenotype A	Phenotype B	Phenotype C
HBDH		Bauru	Sorocaba	Taubaté
	1.00 = F	-	FF = 0.41	FF
	0.76 = S	SS	FS = 0.46 SS = 0.13	-
APH		Campinas	P. de Toledo	Itariri
	1.50 = F	-	FF = 0.05	FF
	1.00 = S	SS	FS = 0.34 SS = 0.61	-
EST <sub>1</sub>		Taubaté	Sorocaba	Bebedouro
	1.00 = F	-	FF = 0.94	FF
	0.67 = S	SS	FS = 0.06 SS = 0.00	-
EST <sub>4</sub>		Marília	Bebedouro	P. de Toledo
	1.00 = F	-	FF = 0.01	FF
	0.90 = S	SS	FS = 0.13 SS = 0.86	

Paraense (1959) proposed that "genetic mosaics" may exist in planorbid breeding sites, being formed by populations of monomorphic animals. Selander and Hudson (1976), in an analysis of the distribution of the pulmonate snail *Rumina decollata*, reported the existence of varying monomorphic patterns among closely located populations. The breeding sites of these snails represent isolated monomorphic complexes, formed by predominantly homozygous populations. According to these authors, this is due to the fact that self-fertilization is the preferential reproductive mode for this species. McCracken and Selander (1980) strengthened this hypothesis, and added that the self-fertilization of snails is the product of an evolutionary process that tries to fully preserve a genome with wide adaptative tolerance. However, when the number of heterozygotes in a breeding site is elevated, the authors admit the occurrence of cross-fertilization as a preferential mode of reproduction.

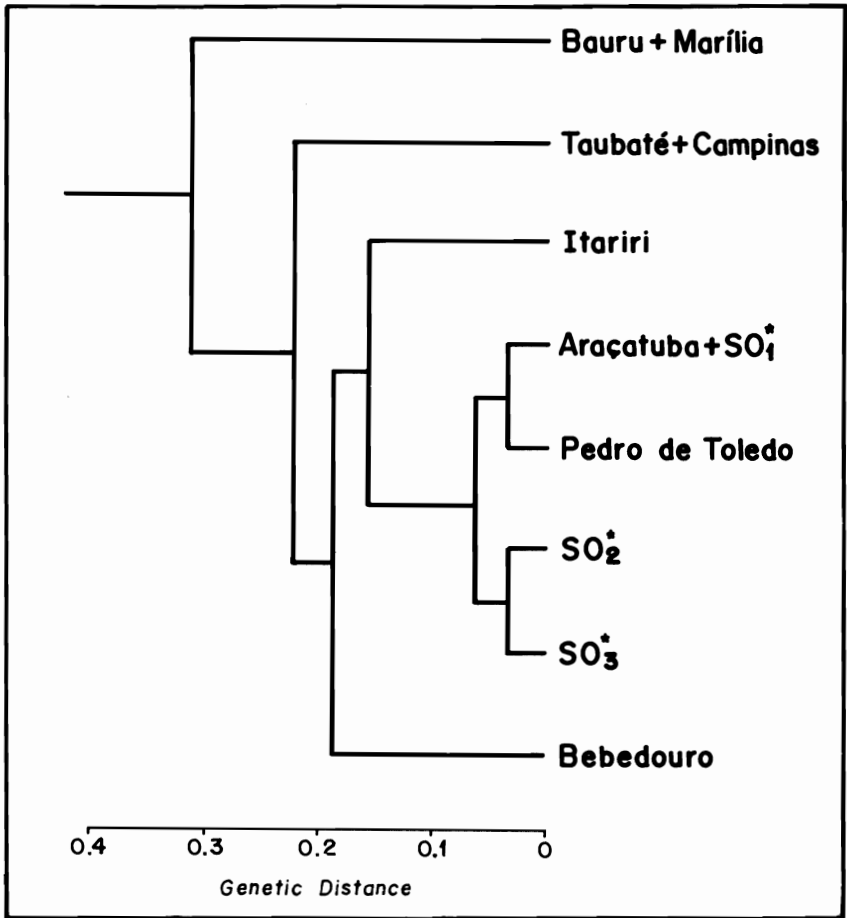


Figure 3 - Dendrogram for the *Biomphalaria tenagophila* populations studied in the State of São Paulo, Brazil.

In the present study, distinctly fixed alleles were found in *B. tenagophila* populations. Different allele frequencies sometimes occur among populations from close locations (Tables III and IV).

As observed by Paraense (1955) and Mulvey *et al.* (1988), the environment in which these freshwater planorbid live is subjected to frequent climatic variations that can cause almost complete destruction of the snail populations. Thus, through self-fertilization the survivors may act as founders of a new population, recolonizing the breeding site.

Electrophoretic analysis showed a mean heterozygosity of  $0.023 \pm 0.025$  in the *B. tenagophila* populations (Table I). Malavasi and Morgante (1981, 1982) and Morgante and Malavasi (1985) reported low heterozygosity levels in *Ceratitis capitata* and *Anastrepha fraterculus*, with values of  $0.046 \pm 0.023$  and  $0.03 \pm 0.08$  for these fruit-fly species, respectively. Another observed effect was the reduced allele number in the *B. tenagophila* populations. Even though several alleles at each locus have been reported for this species (Table II), each population has a maximum of two alleles at each locus. Thus we conclude that in populations of *B. tenagophila* the determining factor of the monomorphic structure of these animals is the abrupt reduction of population density and subsequent recolonization of the breeding sites. This hypothesis is in accordance with the "founder effect" discussed in Mayr (1977).

However, the occurrence of cross-fertilization in planorbid population does not lead to increased genetic variability, as would be expected for communities of dioecious animals. The effects of cross-fertilization are "masked" by the genomic monomorphism of the population.

Animals or plants adapted to unstable environments, as is the case for planorbid snails, have a genome capable of providing them with sufficient adaptability, as proposed by Barton and Charlesworth (1984) and Carson and Templeton (1984). In these species, a genome was selected which would confer an ability to equilibrate functions in different environments or ecological niches. Levins' model (Levins, 1968) predicts that the likelihood of polymorphism in organisms decreases with increasing of individual developmental and functional tolerance relative to a range of environmental conditions. The reduction in genetic variability and consequently their uniformly monogenic character must mean that there is strong selection for their specific combinations of alleles. However, the genetic variability of the species is not lost. The present results indicate that variability is not always available to populations. The variation is diluted in isolated monomorphic populations, cross-fertilization has been preserved in these animals as an occasional guarantee of hybridization between different genome sets. Cross-fertilization recombines several genetics pools, while at the same time preventing the loss of certain genes in isolated populations.

The *B. tenagophila* samples from the city of Sorocaba reveal the existence of an elevated mean heterozygosity level, 0.094 (sample S03) (Table III) in relation to the remaining populations. The HBDH locus presented a mean value of heterozygous genotypes of 0.461 (Table I).

McCracken and Selander (1980) have proposed that self-fertilization is the preferential reproductive mechanism when mean population heterozygosity is low, whereas cross-fertilization is the preferential mechanism when mean heterozygosity is high. We believe that alternations in the preferential reproductive mode of a species

do not explain the detected polymorphism levels. The monomorphism and low heterozygosity found in hermaphrodite snails result from a "founder effect". Reproduction by cross-fertilization may be preferred in the populations, even though it does not cause increased genetic variability.

Populations with high heterozygosity levels, such as those collected in the Sorocaba locality, may indicate a multiple "founder effect". The breeding sites where the samples were collected, and therefore the populations that inhabit it, are recent. In this small breeding site (400 m<sup>2</sup>) it was possible to identify two samples (S01 and S03) with different allele frequencies for some loci. In a nearby breeding site we identified another sample (S02) with quite peculiar electrophoretic patterns (Table III). These results indicate the existence of different genetic populations at this location. Cross-fertilization among these populations resulted in the elevated polymorphism detected (Tables I and II).

The present study demonstrates the existence of alleles fixed in several populations. Albinism has been used as a genetic marker in systematic and other studies, as for example in crossing experiments between populations. Nevertheless, albinism is not known for all species, moreover this mutant form may be associated with undesirable characteristics. Thus, monomorphic samples with genetic markers more appropriate experiments may prove to be useful.

The polymorphism of *B. tenagophila* populations does not follow a geographic distribution pattern (Figures 1 and 3). The dispersal of planorbid snails in São Paulo State highlands occurred through westward flowing rivers (Vaz, 1989). This hypothesis was not confirmed by the electrophoretic results obtained. However, it is important to continue research on the mechanism of colonization and dispersal of planorbid species, especially those that, like *B. tenagophila*, are the vectors of endemic diseases in tropical regions.

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### RESUMO

A variação genética em 15 locos enzimáticos foi estudada entre 9 populações de *Biomphalaria tenagophila*, o principal vetor de esquistossomose no Estado de São Paulo. O polimorfismo das populações manifestou-se em 7 dos 15 locos analisados, baixos valores de heterozigidade foram

observados entre as populações ( $H = 0,00$  até  $0,086$ ). A amostra procedente do município de Sorocaba apresentou uma heterozigiosidade para o loco HBDH ( $H = 0,461$ ) bastante elevada em relação as demais populações. No presente estudo foi possível identificar alelos fixados nas populações de *B. tenagophila*. Nós discutimos a provável origem da distribuição do polimorfismo nas amostras estudadas. A hipótese do efeito-fundador foi proposta para explicar a colonização e a variabilidade genética detectada.

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