

***Biomphalaria tenagophila*: dynamics of populations of resistant and susceptible strains to *Schistosoma mansoni*, with or without pressure of the parasite**

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Resistant (Taim, RS) and susceptible albino (Joinville, SC) Biomphalaria tenagophila populations were kept together, at different proportions, throughout a 18-month-period. Some of the snail groups were submitted to Schistosoma mansoni infection. The targets of this study were (a) to analyze the populational dynamics among resistant and susceptible individuals to S. mansoni; (b) to study the resistance phenotype in descendants of cross-breeding; (c) to observe whether the parasite could exert any kind of selection in those snail populations. Throughout the experiment it could be observed that the susceptible B. tenagophila strain (Joinville) underwent a selective pressure of the parasite that was negative, since the individuals showed a high mortality rate. Although B. tenagophila (Taim) population presented a higher mortality rate without pressure of the parasite, this event was compensated by a reproductive capacity. B. tenagophila Taim was more fecund than B. tenagophila Joinville and was able to transmit the resistance character to their descendants. F₁ generation obtained by cross-breeding between resistant and susceptible lineages was completely resistant to S. mansoni infection, irrespective of the Taim proportion. Moreover, less than 5% of F₂ progeny were susceptible to S. mansoni infection.

Key words: *Biomphalaria tenagophila* - dynamics of populations - *Schistosoma mansoni* - resistant and susceptible strains

Distribution of *Biomphalaria tenagophila* snails has considerably been spread through the Brazilian territory, and its importance in the transmission of schistosomiasis has been increased as well, mainly in the Southern and Southeastern regions of Brazil (Paraense & Corrêa 1987). This is the only transmitter species in extensive areas of the state of São Paulo, and it is responsible for isolated focuses in the states of Minas Gerais, Santa Catarina, and Rio de Janeiro.

Paraense and Corrêa (1978) demonstrated that *B. tenagophila* from different localities presented diversified susceptibility levels, when submitted to the SJ strain (São José dos Campos, SP) of *Schistosoma mansoni*. Santos et al. (1979) carried out a study with *B. tenagophila* dealing with two interesting susceptibility profiles: a 100% susceptible strain (Cabo Frio, RJ) and another one highly resistant (Taim, RS). This resistant strain proceeds from the Ecological Station at Taim, state of Rio Grande do Sul, and has been the target of several studies. These snails, at different ages, have continuously been resistant to various strains of *S. mansoni*, as well as to different bur-

dens of miracidia. The detected resistance was not due to the result of a physiological incompatibility between host-parasite, rather than to the innate defense system of the mollusc itself (Coelho et al. 2004).

Resistance in *B. tenagophila*, as well as in *B. glabrata*, is also associated with genetic factors. Previous studies dealing with cross breedings between *B. tenagophila* from Taim/RS and susceptible specimens of *B. tenagophila* from BH/MG and Joinville/SC showed that F₁ descendants, as a result of such cross-breedings, presented low susceptibility levels to *S. mansoni* (Santos et al. 1979, Freitas et al. 1985). Recently, several cross-breedings were carried out using a resistant strain (*B. tenagophila* Taim) and a susceptible albino one (*B. tenagophila* Joinville). Studying the phenotype of F₁ and F₂ descendants of those cross-breedings, it was observed that the resistance character could be determined by two dominant genes, and probably some genetic and environmental factors could modulate this response (Rosa et al. 2005).

Since the resistance in *B. tenagophila* has a dominant character, and also thinking in obtaining a possible model for transmission control, we initiated another kind of study. Populations of resistant (Taim, RS) and susceptible albino (Joinville, SC) *B. tenagophila* were maintained together, at different proportions, with and without *S. mansoni* pressure.

The idea is to analyze the genic flux of the alleles involved in the resistance character, and to evaluate the hypothesis related to the selective advantage for the re-

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sistant lineage when compared with the susceptible one, when both are under *S. mansoni* pressure.

MATERIALS AND METHODS

Snails - The pigmented *B. tenagophila* Taim strain, that was originally collected at the Ecological Station at Taim, state of Rio Grande do Sul, Brazil, has been completely resistant to experimental infection with *S. mansoni* (Santos et al. 1979). This strain has been maintained at the Laboratory of Schistosomiasis, Department of Parasitology, UFMG, Brazil, for more than 27 years, without any kind of selecting favoring the resistance character, since these snails are naturally resistant to *S. mansoni*. The albino *B. tenagophila* strain was originally collected at Joinville area, state of Santa Catarina, Brazil, by Dr Lobato Paraense that kindly provided the Joinville snail strain to be used in our experiments. In previous experiments, we observed that *B. tenagophila* Joinville was highly susceptible to different *S. mansoni* strains, such as *S. mansoni* SJ – isolated from infected snails from São José dos Campos/SP and *S. mansoni* LE – isolated from an infected patient from Belo Horizonte/MG.

Parasite - The LE strain of *S. mansoni*, isolated from a patient in Belo Horizonte, Minas Gerais, Brazil, was used in all the experiments described bellow. This parasite strain has been maintained by successive passages through *B. glabrata* and hamsters (*Mesocricetus auratus*).

Cross-breeding - Ten parental groups were assembled with 50 young snails, measuring 3-4 mm of shell-diameter, placed into plastic vessels with approximately 30 l dechlorinated water and fed with lettuce. Each group consisted of *B. tenagophila* Taim and/or *B. tenagophila* Joinville in the following proportions: Group 1: 50 pigmented *B. tenagophila* Taim (only parasite resistant snails); Group 2: 50 albino *B. tenagophila* Joinville (only parasite susceptible snails); Group 3: 25 resistant snails and 25 susceptible snails; Group 4: 40 susceptible snails and 10 resistant snails; Group 5: 40 resistant snails and 10 susceptible snails. The groups 1-5 were not infected. The groups 6-10 were assembled with the same snail proportion than groups 1-5, however the groups 6, 7, 8, 9, and 10 were submitted to the parasite pressure, as detailed bellow. The cross-breedings are summarized in Table I.

Each group was kept for 60 days, so the snails could reach sexual maturity. After this period, the snails of groups

6 to 10 were infected with *S. mansoni* miracidia every week, for 8 weeks, and the fecundity parameters and survival rates of each group were analyzed for 10 consecutive weeks. Fifty pigmented F₁ snails, randomly obtained from each parental group, were assembled in 10 new groups to obtain the F₂ generation. This F₂ progeny was kept and analyzed as described for the parental group.

Fecundity parameters - After the snails had reach sexual maturity, styrofoam strips were placed on the water surface of each plastic vessel, to be used as support for snails egg-masses deposition. The styrofoam strips were recovered weekly, and replaced by new ones to evaluate the group fecundity. The strips were analyzed under stereomicroscope and the number of egg-masses, the number of eggs/egg masses and number of eggs/snail were estimated for each group during 10 consecutive weeks of examination. In case of need, the results were statistically compared, the Wilcoxon test being used for the non-parametric data, and the paired Student's "t" test for the parametric ones. The total number of the surviving snails in each experimental group and the phenotypic parameter (pigmentation) were analyzed 30 days after eclosion.

Snail infection - The snails were infected with miracidia of *S. mansoni* isolated from livers of hamster infected for 45-50 days, according to Pellegrino and Katz (1968). For the infection of breeding groups 6 to 10, the miracidia were counted and added directly to the vessels where the snails were kept. Each group received 200 miracidia/week and the infection was accumulative, since this procedure was performed for 8 consecutive weeks.

To test susceptibility level of F₁ and F₂ progenies, 50 young individuals (3-4 mm) of each cross-breeding were exposed to *S. mansoni* infection. For this purpose, each snail was individually exposed to 30 miracidia.

Methods for analysis of snail infection - Aiming at verifying the emergence of *S. mansoni* cercariae, the F₁ and F₂ infected snails were individually observed under a stereomicroscope, every week between 40 and 90 days after exposure to miracidia. For examination of cercarial shedding, each snail was kept in small Snap-cap glass recipients, with dechlorinated water, and exposed to artificial light for 2 h (Pellegrino & Katz 1968). After this period, the negative snails were squeezed between slide and coverslip in search for sporocysts.

RESULTS

In this study, it was verified that *S. mansoni* is a negative factor of selective pressure for the susceptible population. As can be observed in Fig. 1A, the individuals pertaining to *B. tenagophila* Taim (Group 1) or Joinville (Group 2) populations had a high survival rate, 90 and 97 % respectively, when the populations were kept isolated and not exposed to parasite infection. In contrast, the survival rate of *B. tenagophila* Joinville dropped to 60% when the snail population was exposed to the parasite (Group 7), whereas no difference in the survival rate of *B. tenagophila* Taim was detected after parasite infection (Group 6).

TABLE I

Schedule of the parental groups submitted or not to *Schistosoma mansoni* pressure

Uninfected group	Infected group
1-50R	6 50R
2-50S	7-50S
3-25S+25R	8-25S+25R
4-40S+10R	9-40S+10R
5-40R+10S	10-40R+10S

R: resistant pigmented (*B. tenagophila* Taim); S: susceptible albino (*B. tenagophila* Joinville).

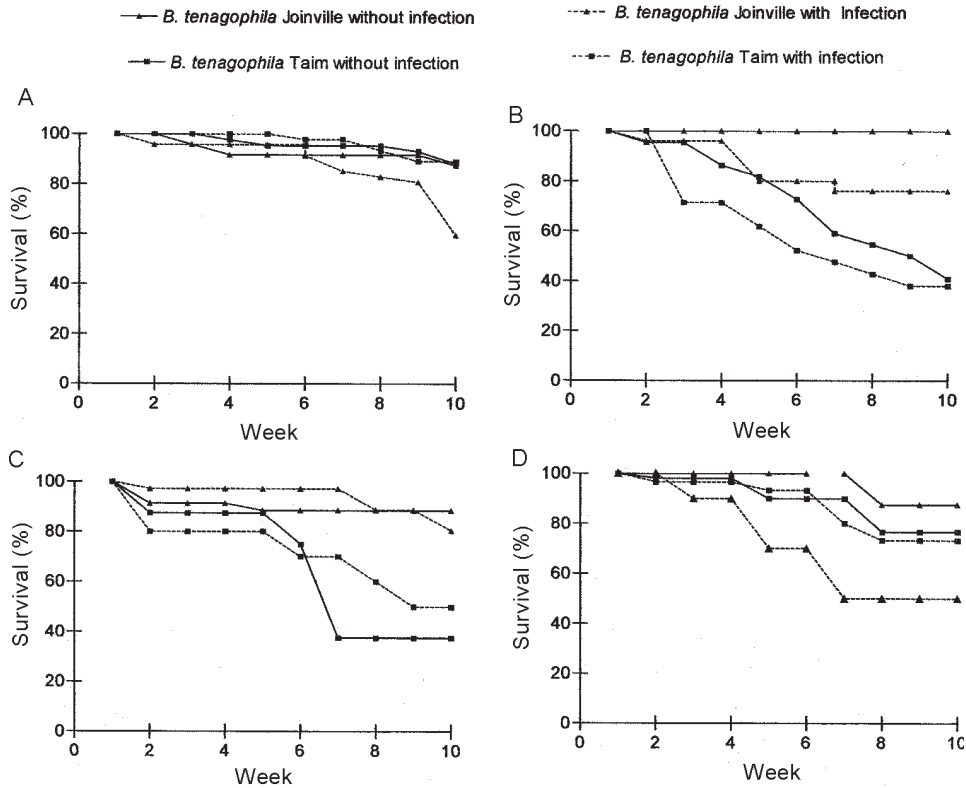


Fig. 1: surviving rate of *Biomphalaria tenagophila* Taim (R) and *B. tenagophila* Joinville (S) populations, submitted or not to *Schistosoma mansoni* pressure. A: G1-50 R and G2-50 S without infection; G6-50 R and G7-50 S with infection; B: G3-25 R + 25 S without infection; G8-25 R + 25 S with infection; C: G4-10 R + 40 S without infection; G9-10 R + 40 S with infection; D: G5-40 R + 10 S without infection; G10-40 R + 10 S with infection.

In breeding groups composed by mixed population of *B. tenagophila* Taim and Joinville, parasite infection also resulted in decreased survival rate of *S. mansoni* susceptible snails (Fig. 1B, C, D). However, in this mixed snail population, where the proportion of Taim and Joinville was similar or had more Joinville (Fig. 1B, C), Taim snails had survival rate inferior to 50%, irrespective of the parasite infection. In contrast, in the mixed snail population with higher proportion of Taim stain, the survival rate of the resistant snail was elevated (70-80%), while only 50% of the *B. tenagophila* Joinville population were able to survive after parasite exposition.

The *B. tenagophila* Taim was more fecund than *B. tenagophila* Joinville (Table II). Significant differences ($p = 0.0039$) could be observed when the number of eggs/snail in Group 1 (*B. tenagophila* Taim) was compared with Group 2 (*B. tenagophila* Joinville). The same occurred when the number of eggs/snail in those two populations was analyzed, presenting $p < 0.014$ as a result (groups 6 and 7). The number of eggs/egg-laying in Groups 1 and 2 was not significant, conversely Groups 6 and 7 presented highly significant values ($p = 0.0001$). When we compared the fecundity parameters of the infected susceptible population (Group 7) and those ones of the uninfected population (Group 2), a decrease could be observed, mainly in the number of eggs in the infected group, but the values were not significant. It appears that

the parasite was unable diminishing significantly the egg-laying in the infected snails. When both populations were compared, the number of surviving F_1 descendants of *B. tenagophila* Taim was higher than those ones of *B. tenagophila* Joinville. The high proportion of pigmented snails obtained in F_1 and F_2 generations in the groups 3, 4, 5, 8, 9 and 10, suggested that cross-breeding between pigmented *B. tenagophila* Taim and albino *B. tenagophila* Joinville populations occurred (Tables III, IV).

The F_1 and F_2 progeny obtained from cross-breeding group containing only *B. tenagophila* Joinville showed susceptibility rate to *S. mansoni* infection superior to 55%, similarly to the susceptibility rate observed in parental group, while the complete resistance of *B. tenagophila* Taim was confirmed through the generations (Table V). F_1 generation obtained by cross-breeding between resistant and susceptible lineages was completely resistant to *S. mansoni* infection, irrespective of the Taim proportion. Moreover, less than 5% of F_2 progeny were susceptible to *S. mansoni* infection. Therefore, even with higher mortality rate of parental group, *B. tenagophila* Taim snails presented a markedly better reproductive performance and were able to transmit the resistance character to their descendants. The results obtained with F_1 and F_2 generations after the susceptibility trial corroborate this hypothesis.

TABLE II

Mean fecundity per week, in the parental breeding-group and total number of surviving F₁ individuals after 30 days hatching

Group	Number of egg-masses	Number of eggs	Number of egg-masses per snail	Number of eggs/snail	Number of eggs/egg-masses	Total number of surviving F ₁ individuals
1-50R	70.1	1129	2.03	27	15.0	2298
2-50S	62.2	778.4	1.37	17.11	11.5	1050
3-25R+25R	69.1	718	1.71	17.59	9.92	800
4-10R+40S	40.9	539.4	1.08	14.35	12.95	540
5-40R+10S	51	663	1.85	18.9	13.31	1010
6-50R	69	1300	1.52	29.2	20.4	2796
7-50S	52.2	505	1.20	11.94	9.51	922
8-25R+25S	59.4	585	1.69	16.77	9.10	630
9-10R+40S	44.2	443	1.05	10.43	8.8	470
10-40R+10S	44.9	523	2.31	14.94	10.53	932

TABLE III

Percentage of pigmented and albino F₁ progeny snails obtained by cross-breeding between susceptible and resistant adult snails

Parental group	% pigmented	% albino
50-R (Taim)	100	0
2-50S (Joinville)	0	100
3-25R+25S	90	10
4-40S+10R	70	30
5-40R+10S	99	1
6-50R(Taim)	100	0
7-50S (Joinville)	0	100
8-25R+25S	97	3
9-40S+10R	87	13
10-40R+10S	100	0

TABLE IV

Percentage of pigmented and albino F₂ progeny snails obtained by cross-breeding between adult F₁ X F₁

Parental group	% pigmented	% albino
50-R (Taim)	100	0
2-50S (Joinville)	0	100
3-25R+25S	75	25
4-40S+10R	80	20
5-40R+10S	86	14
6-50R(Taim)	100	0
7-50S (Joinville)	0	100
8-25R+25S	79	21
9-40S+10R	81	19
10-40R+10S	85	15

TABLE V

Susceptibility rate of F₁ and F₂ pigmented snails individually infected with *Schistosoma mansoni*

Parental group	Positive surviving snails % (F ₁)	Positive surviving snails % (F ₂)
50-R (Taim)	0	0
2-50S (Joinville)	62.5	68.1
3-25R+25S	0	5.0
4-40S+10R	0	4.5
5-40R+10S	0	0
6-50R(Taim)	0	0
7-50S (Joinville)	50	55
8-25R+25S	0	0
9-40S+10R	0	2.4
10-40R+10S	0	2.4

DISCUSSION

Throughout an 18-month-experimental period, we studied the populational dynamics of resistant and susceptible strains of *B. tenagophila*. The two populations were maintained together, at different proportions of individuals, with and without *S. mansoni* pressure. The first point to be discussed is the question about the natural selection in those two populations. The presence of *S. mansoni* in susceptible snails represents a disease with devastating effects, since it causes a high mortality in the infected snails and a decrease in the egg-laying, thus becoming an element of natural selection, which is highly negative for the potentially transmitter snails of schistosomiasis (Coelho 1995). The lineage of *B. tenagophila* Joinville suffers the effect of infection, and this was observed due to the high mortality rate and a slight reduction in the number of eggs per snail in this population.

It could be observed that the parental populations submitted to pressure of the parasite presented a lower number of albino F₁ individuals, when compared with the uninfected groups. Probably, this result could be related to reduction of the susceptible albino parental population itself. This experiment underwent the interference of another factor, possibly competition (Groups 3, 8, 4, 9). *B. tenagophila* Taim lineage presented on the one hand a drawback, i.e., a higher mortality rate without pressure of the parasite. On the other hand, this lineage presented a markedly a superior reproductive capacity studies carried out in our laboratories confirmed that *B. tenagophila* Taim was more fecund than *B. tenagophila* Joinville. This can be accounted for by the fact that a higher number of F₁ surviving snails from *B. tenagophila* Taim population was obtained.

In all groups, the individuals pertaining to F₁ generation were almost all predominantly pigmented. The proportion between pigmented and albino individuals detected in F₂ generation showed that a cross-breeding occurred between parenteral *B. tenagophila* Taim × albino *B. tenagophila* Joinville. The recessive albino character was used, in this case, as a phenotypic marker, which facilitates distinction between F₁ individuals generated by cross-breeding (all of them being pigmented) and those ones generated by self-fecundation (albino). Paraense

(1955) demonstrated that those individuals performed self-fecundation only when they were isolated, otherwise they always preferred cross-breeding (when in the presence of one or more individuals).

Analyzing the resistance phenotype of F_1 and F_2 individuals, it was observed that, in this experiment, a predominance of resistant individuals occurred in all groups. Lewis et al. (2002) analyzed the genic flux of the resistance character in F_1 descendants generated by cross-breeding between a resistant strain of *B. glabrata* and a susceptible one of the same species. The results obtained showed that the number of resistant F_1 individuals was higher than the number of susceptible ones. Based on the results obtained in this study, and taking into account studies carried out on the resistant *B. tenagophila* Taim strain throughout the last 27 years, our research group proposes a biological control model for schistosomiasis. The idea is to introduce the genetic characters of the resistant strain into the susceptible populations in endemic areas maintained by *B. tenagophila* (Coelho et al. 2004). It is expected that after a drastic reduction of the local population in the transmitter focus as, for example, after the use of molluscicide, the introduction of the resistant Taim lineage on a large scale would force the surviving individuals to perform cross-breeding with the introduced lineage. Their descendants would be endowed with the desired dominant character of resistance to the parasite, and would inherit the adaptive capacity to the local environment of their local ascendants as well.

The molecular marker, which is typical of the Taim lineage, although being not associated to resistance, has a dominant character too (Rosa et al. 2004). This marker is represented by the band with 350 bp of the ITS region of the ribosomal mitochondrial DNA detected by PCR-RFLP technique, using *Ddel* I enzyme. This marker will be of great importance for the follow-up, and to demonstrate the success obtained with the insertion of the genetic characteristics of the Taim lineage into the population after intervention. This model has been already used in focuses located in Bananal and Caraguatatuba – two cities in the state of São Paulo, Brazil, in a collaborative work with Sucen, UFMG, and Fiocruz. In those focuses, molluscicides were not previously used. This experimental approach was approved by Ibama, the Brazilian official agency that control environmental interventions.

The success of introduction of the genetic characteristics of resistance will be confirmed by the presence of the molecular marker of Taim lineage in the snails born after intervention, and by means of tests related to susceptibility to *S. mansoni* carried out with these progenies, when compared with those ones of the local strain, that was isolated before introduction of the Taim lineage.

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