Rev. Brasil. Genet. 13, 3, 459-467 (1990)
(Brazil. J. Genetics)

SUSCEPTIBILITY OF *Biomphalaria tenagophila* HYBRID DESCENDANTS TO TWO STRAINS OF *Schistosoma mansoni*

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ABSTRACT

There is little information in the literature about the genetics of snail susceptibility to *Schistosoma mansoni*, especially in relation to *Biomphalaria tenagophila*. Freitas *et al.* (Rev. Inst. Med. Trop. 27: 6-12, 1985), studying the susceptibility of *B. tenagophila* hybrids to the LE strain of *S. mansoni* (from Belo Horizonte, MG), noted that the maternal variety factor seemed to affect the results. To clarify this phenomenon, the present study was undertaken with the following objectives: a) to determine differences during the juvenile and adult phases between the albino variety of *B. tenagophila* from Joinville, SC, and the pigmented variety from Taim, RS, in terms of susceptibility to the LE and SJ strains of *S. mansoni* (from São José dos Campos, SP); and b) to determine the susceptibility of juvenile and adult F_1 hybrids resulting from crosses between the varieties Joinville and Taim to the LE and SJ strains of *S. mansoni*. Snails were mass-exposed to about 10, 25 and 50 miracidia per snail. When exposed to about 10 miracidia, juvenile and adult Taim snails were not susceptible to the LE and SJ strains. Juvenile and adult snails of the Joinville variety showed little (7.1% juvenile) or no susceptibility (adult) to the LE strain; however 86.1% of the juvenile snails and 74.0% of the adults were susceptible to the SJ strain. Two different F_1s were considered: one resulting from crossing female Joinville snails with male Taim snails (Joinville ♀ x Taim ♂) and the inverse (Taim ♀ x Joinvile ♂). Juvenile and adult snails from both crosses were all refractory to infection by the LE strain as well as by the SJ strain. Another group of snails, 500 juvenile and 278 adult F_1 hybrids of the two crosses, were exposed to 25 or 50 SJ miracidia per snail, and all presented the same negative susceptibility results.

INTRODUCTION

Schistosomiasis induced by *Schistosoma mansoni* is currently expanding in Brazil due to human migration from endemic areas to areas where susceptible snails occur. Three species are responsible for the transmission of this parasitic disease in

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Brazil: *Biomphalaria glabrata, B. tenagophila* and *B. straminea. B. glabrata* is the major intermediate host of the parasite. Studies by Piza *et al.* (1960), Ramos *et al.* (1961, 1965) and Piza and Ramos (1970) have also confirmed the important role of *B. tenagophila* in the maintenance of focal points of schistosomiasis, especially in regions of the Paraiba Valley, Ribeira Valley, "Baixada Santista", and near the city of Campinas. Recently, Carvalho *et al.* (1985) and Melo and Pereira (1985) found *B. tenagophila* naturally infected with *S. mansoni* in Minas Gerais.

Little information is available on certain genetic aspects of intermediate host susceptibility, especially in *B. tenagophila*. Studies initiated by Newton (1953) demonstrated that the susceptibility of *B. glabrata* is an inheritable trait, probably involving several genes. Paraense and Correa (1963) noted variation in the susceptibility of 23 *B. glabrata* populations to a *S. mansoni* strain and concluded that the degree of population susceptibility depends on the relative frequency of resistant and susceptible genotypes in each population. Richards and Merrith (1972), in a study of juvenile *B. glabrata* susceptibility, noted that several genetic factors determine juvenile susceptibility but that the real number of traits, their interactions and the level of penetrance of each still need to be clarified. Richards (1973) demonstrated for some stocks that the non-susceptibility of *B. glabrata* adults is due to a dominant gene. The derivation of non-susceptible descendants from susceptible adults suggested the presence of other factors that modify adult susceptibility. These modifications start with the development of the changes related to maturation occurring soon before oviposition and need to be studied in more detail. Freitas *et al.* (1985), in a study of the susceptibility of *B. tenagophila* hybrids to the LE strain (Belo Horizonte, MG) of *S. mansoni,* noted that maternal variety seemed to affect the results.

The present study was undertaken to clarify some of the possible genetic factors of susceptibility such as maternal influence and the level of susceptibility of juveniles and adults of different *B. tenagophila* varieties to the LE and SJ strains of *S. mansoni*. The specific objectives were: 1) to determine the degree of susceptibility of adult and juvenile *B. tenagophila* snails of the Joinville-SC (albino) and Taim-RS (pigmented) varieties to the LE and SJ strains of *S. mansoni*, 2) to determine the maternal influence of the Joinville variety on the susceptibility of juvenile and adult hybrids resulting from the Joinville x Taim cross to the LE and SJ strains of *S. mansoni*, and 3) to determine the maternal influence of the Taim variety on the susceptibility of juvenile and adult hybrids resulting from the Taim x Joinville cross to the LE and SJ strains of *S. mansoni.*

MATERIAL AND METHODS

Two *B. tenagophila* varieties were used: an albino one from Joinville, SC, and a pigmented one from Taim, RS, both maintained in the laboratory of the Ecology Sector, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

(ICB/UFMG). In addition, a *B. glabrata* variety maintained in the Interdepartamental Group of Studies on Schistosomiasis (GIDE), ICB/UFMG, was used as a control.

The *S. mansoni* strains used were: LE from the Belo Horizonte, MG, region, and SJ, from the São José dos Campos, SP, region, both maintained in the laboratory in hamsters and in *B. glabrata.*

For multiplication of the parental lines, 50 specimens of each mollusk variety were placed in containers with natural (dechlorinated) water to obtain spawns. Spawns of approximately three days were transferred to aquaria (one for each variety) and the snails were reared according to the standard technique of Freitas *et al.* (1975).

F_1 was obtained as follows: 65 snails of each *B. tenagophila* variety measuring 3 to 4 mm in diameter were placed in individual double glasses containing approximately 200 ml natural water and a small amount of sterilized earth mixed 9:1 with $CaCO_3$, and they were periodically fed fresh lettuce. The water in the glasses was changed when it became turbid. When the snails reached sexual maturity, determined by the presence of spawn, the Taim snails were allowed to mate with the Joinville ones for 4 days (one pair to a glass). After this period, the pairs were separated and returned to individual glasses to obtain spawn. The spawns of each variety were transferred to an aquarium to obtain the F_1 resulting from cross-fecundation.

To determine the occurrence of cross-fecundation, two alleles were used as genetic markers: *C,* for pigmentation (dominant), and *c* for the absence of pigmentation or albinism (recessive).

The animals were infected at two different phases: juvenile (snails measuring 3 to 4 mm in diameter and averaging 23 days of age), and adult (snails measuring 6.5 to 16.0 mm in diameter and averaging 73 days of age). Miracidia were obtained and used for mass infection (approximately 10, 25 and 50 miracidia per snail) by the technique used at GIDE for the maintenance of the *S. mansoni* cycle in the laboratory (Pellegrino and Katz, 1968).

After infection, each group of snails was transferred to an aquarium kept in a room at a temperature of approximately 26°C, during the prepatent period. To determine the elimination of cercariae, the snails were examined as described below 30, 40, 50, 60, 70 and 90 days after infection: each specimen was placed in a separate small flask containing 4 ml of natural water kept under artificial light and examined with a stereoscopic microscope three hours later.

Susceptibility rates were determined for a) generation P_1 in the juvenile and adult phase, b) juvenile and adult pigmented F_1 obtained from ♀ albino mollusks, c) juvenile pigmented F_1 obtained from ♀ pigmented mollusks, d) adult pigmented F_1 which presented an albino F_2 after self-fecundation. This F_1 was also obtained from ♀ pigmented mollusks.

Mortality was calculated as the number of snails that died from the day of infection with *S. mansoni* and was determined on the days when the snails were examined for cercarial elimination.

RESULTS AND DISCUSSION

Parental and hybrid susceptibility to the LE strain of S. mansoni

Table I, which presents the data for the parental lines, shows that *B. tenagophila* from Taim was fully resistant to infection both during the juvenile and the adult phase. Santos *et al.* (1979), using snails of the same variety measuring 4 to 10 mm in diameter, and Freitas *et al.* (1985), using specimens measuring 5 to 10 mm in diameter, obtained similar results. *B. tenagophila* from Joinville showed 7.1% susceptibility during the juvenile phase and 0.0% susceptibility during the adult phase. A higher susceptibility was expected since Santos *et al.* (1981) reported 20.6% susceptibility for the same variety. The susceptibility of *B. glabrata* (control) was 64.5% during the juvenile phase and 70.8% during the adult phase.

Table I - Parental *Biomphalaria tenagophila* susceptibility to the LE strain of *Schistosoma mansoni.* (150 individuals exposed to infection in each test).

	B. tenagophila from Taim		*B. tenagophila* from Joinville		*B. glabrata* (control)	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
Dead (%)	6.0	6.0	6.7	0.0	58.7	20.0
Examined (no.)	141	141	140	150	62	120
Positive (no.)	0	0	10	0	40	85
Susceptibility (%)	0.0	0.0	7.1	0.0	64.5	70.8

Table II, which presents the data for F_1, shows that the Joinville ♀ x Taim ♂ and Taim ♀ x Joinville ♂ hybrids were totally resistant to infection with the LE strain. These results do not show any evidence of an effect of maternal susceptibility on F_1 susceptibility. Santos *et al.* (1981) observed 24.1% susceptibility for Joinville x Taim hybrids. Freitas *et al.* (1985), using specimens measuring 5 to 10 mm in diameter and individual infection with 10 miracidia/mollusk, detected 4.5% and 0.0% susceptibility for Taim x Joinville hybrids, and 0.0% susceptibility for the hybrids resulting from the pigmented and resistant Cabo Frio variety and the Joinville variety. These susceptibilities are close to those of the maternal variety, although Freitas *et al.* (1985) also obtained 12.5% for crosses between Curitiba specimens (pigmented and having 0.0% susceptibility) and Joinville specimens. The susceptibility of *B. glabrata* was 90.3% for the juvenile phase and 85.5% for the adult phase.

Table II - Susceptibility of F_1 hybrids of two *Biomphalaria tenagophila* varieties during the juvenile and adult phase to the LE strain of *Schistosoma mansoni.*

	B. tenagophila F_1 (Taim ♀ x Joinville ♂)		*B. tenagophila* F_1 (Joinville ♀ x Taim ♂)		*B. glabrata* (control)	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
Exposed to infection (no.)	150	61	150	100	300	100
Dead (%)	0.0	1.6	0.0	5.0	7.3	10.0
Examined (no.)	150	60	150	95	278	90
Positive (no.)	0	0	0	0	251	77
Susceptibility (%)	0.0	0.0	0.0	0.0	90.3	85.5

The data in Tables I and II show that the Joinville and Taim varieties and their hybrids had low mortality, possibly because of low susceptibility or total resistance to infection.

Parental and hybrid susceptibility to the SJ strain of S. mansoni

Table III shows that *B. tenagophila* from Taim was resistant to infection both in the juvenile and the adult phase. Santos *et al.* (1979), in a study of snails of the same variety 4 to 10 mm in diameter, obtained the same result. The susceptibility of *B. tenagophila* from Joinville was 86.1% in the juvenile phase and 74.0% in the adult phase. These rates are higher than those reported by Freitas *et al.* (1983) for snails measuring 5 to 10 mm in diameter (17.0% and 33.3%). These discrepancies may be linked to gene alterations that occurred in the *B. tenagophila* stocks during the time that elapsed between the experiments of these authors and the present ones, or to modifications in *S. mansoni* due to the change in host (*B. tenagophila* or *B. glabrata*). The susceptibility of *B. glabrata* (control) was 90.0% for juveniles and 50.5% for adults. Mortality was higher in the more susceptible varieties.

Table IV shows that the Joinville ♀ x Taim ♂ and Taim ♀ x Joinville ♂ hybrids were totally resistant to infection with the SJ strain. Again, no maternal influence on F_1 susceptibility was observed. Santos *et al.* (1979), after performing crosses between the varieties Taim and BH (albino with 35.0% susceptibility) and the varieties CF (pigmented and 97.4% susceptibility) and BH, obtained F_1 susceptibilities of 4.1% and 56.7%, respectively. The present results diverge from those reported by these investigators. *B. glabrata* showed 80.2% susceptibility for juveniles and 40.9% for adults. Mortality was low both in the two *B. tenagophila* varieties and in *B. glabrata*.

Table III - Parental *Biomphalaria tenagophila* susceptibility to the SJ strain of *Schistosoma mansoni.*

	B. tenagophila from Taim		*B. tenagophila* from Joinville		*B. glabrata* (control)	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
Exposed to infection (no.)	110	110	310	110	110	110
Dead (%)	1.8	2.7	41.9	12.7	72.7	2.7
Examined (no.)	108	107	180	96	30	107
Positive (no.)	0	0	155	71	27	54
Susceptibility (%)	0.0	0.0	86.1	74.0	90.0	50.5

Table IV - Susceptibility of the F_1 hybrid of two *Biomphalaria tenagophila* varieties during the juvenile and adult phase to the SJ strain of *Schistosoma mansoni.*

	B. tenagophila F_1 (Taim ♀ x Joinville ♂)		*B. tenagophila* F_1 (Joinville ♀ x Taim ♂)		*B. glabrata* (control)	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
Exposed to infection (no.)	76	47	149	110	110	110
Dead (%)	2.6	0.0	2.0	0.0	8.2	0.0
Examined (no.)	74	47	146	110	101	110
Positive (no.)	0	0	0	0	81	45
Susceptibility (%)	0.0	0.0	0.0	0.0	80.2	40.9

Five hundred additional juveniles and 278 adults, all of them F_1 hybrids resulting from Joinville ♀ x Taim ♂ and Taim ♀ x Joinville ♂ crosses, were exposed to 25 or 50 miracidia per mollusk, and all yielded negative results in terms of susceptibility. The control (*B. glabrata*) showed 94:1% susceptibility (juveniles) and 73.1% susceptibility (adults) after exposure to approximately 25 miracidia/mollusk, and 100% susceptibility (juveniles) and 45.8% susceptibility (adults) after exposure to approximately 50 miracidia/mollusk.

Newton (1953), in a study of the susceptibility of F_1 hybrids of *B. glabrata* from Brazil (albinos with 0.0% susceptibility) and Porto Rico (pigmented with 95.0% susceptibility) measuring 7 to 9 mm in diameter, to the *S. mansoni* strain from Porto

Rico (individual infection with 10 miracidia/mollusk), detected 12.1% susceptibility. Paraense and Correa (1963) obtained 13.3% susceptibility for F_1 from *B. glabrata* from Salvador (pigmented and resistant) x Santa Luzia (albino with 94.7% susceptibility); 14.6% for Santa Luzia x Salvador, and 1.4% for Salvador x Recife (albino with 96.1% susceptibility). All were exposed to the Belo Horizonte *S. mansoni* strain (individual infection with 10 miracidia/mollusk). Richards and Merrith (1972), in a study of the susceptibility of pigmented *B. glabrata* juveniles from Porto Rico and Brazilian albinos measuring 1 to 4 mm in diameter to the Porto Rico strain of *S. mansoni* (individual infection with 5 miracidia/mollusk), obtained the following results: when resistant mollusks were crossed with susceptible snails, approximately half the progeny of each parent was susceptible; in two other series of crosses in which the snails functioning as males were resistant and those functioning as females were susceptible or resistant, the susceptibility of the descendants varied widely. The authors concluded that several genetic factors determine juvenile susceptibility. Working with the same snails as used by Richards and Merrith (1972) but in the adult phase (5 to 16 mm), and mass infection with *S. mansoni* from Porto Rico (25 miracidia/mollusk), Richards (1973) performed several crosses and obtained the following results: 1) a susceptible female whose descendants by self-fecundation showed 70% (juveniles) and 100% (adults) susceptibility was crossed with a resistant male whose descendants by self-fecundation showed 90% (juveniles) and 0% (adults) susceptibility, produced F_1 juveniles with 88% susceptibility and F_1 adults with 0% susceptibility; 2) a susceptible female whose descendants by self-fecundation showed 80% (juveniles) and 100% (adults) susceptibility was crossed with a resistant male whose descendants by self-fecundation showed 0% susceptibility (juveniles), producing F_1 adults with 0% susceptibility.

The results obtained for F_1 descendants in the present study using the LE and SJ strains are similar to those obtained by Richards (1973) for adult descendants, but disagree with those reported by Newton (1953), Paraense and Correa (1963) and Richards and Merrith (1972) for *B. glabrata.*

CONCLUSIONS

1. The *B. tenagophila* variety from Taim and all the hybrids resulting from the Joinville ♀ x Taim ♂ and Taim ♀ x Joinville ♂ crosses were resistant to both the LE and SJ strains.

2. The *B. tenagophila* variety from Joinville was more susceptible to the SJ than the LE strain.

3. There was no maternal effect on F_1 susceptibility to the LE or SJ strain.

4. In general, juveniles were more susceptible than adults to both strains, except for *B. glabrata* juveniles infected with 10 LE miracidia/snail.

ACKNOWLEDGMENTS

We are grateful to the technicians José Tomé de Almeida and Jefferson do Carmo Bernardes for their collaboration.

This research was supported by CNPq-PIG.

RESUMO

São poucas as informações na literatura sobre a genética da susceptibilidade, principalmente em relação à *Biomphalaria tenagophila*. Freitas *et al.* (1985), estudando a susceptibilidade de híbridos de *B. tenagophila* à cepa LE (de Belo Horizonte-MG) de *Schistosoma mansoni*, verificaram que o fator variedade materna pareceu ter influência nos resultados. Buscando maiores esclarecimentos a respeito foi feito este trabalho que teve como objetivos: a) determinar diferenças entre as variedades albina de Joinville-SC e pigmentada de Taim-RS, nas fases juvenil e adulta quanto à susceptibilidade às cepas LE e SJ (de São José dos Campos - SP) de *S. mansoni*; b) determinar a susceptibilidade de seus híbridos (F_1) juvenis e adultos (resultantes dos cruzamentos da variedade de Joinville com a de Taim) às cepas LE e SJ de *S. mansoni*. A criação dos moluscos foi feita segundo técnica de Freitas *et al.* (1975). A infecção foi feita em massa com cerca de 10, 25 e 50 miracídios por molusco, segundo técnica de Pellegrino e Katz (1968). Na infecção com cerca de 10 miracídios por caramujo, juvenis e adultos da variedade de Taim foram insusceptíveis às cepas LE e SJ; da variedade de Joinville ou foram pouco susceptíveis (7,1% juvenis) ou insusceptíveis (adultos) à cepa LE; à cepa SJ os juvenis foram susceptíveis em 86,1% e os adultos em 74,0%. Foram considerados dois tipos diferentes de F_1: a) aquele resultante dos caramujos de Joinville funcionando como mãe e dos de Taim, como pai (Joinville ♀ x Taim ♂); b) vice-versa (Taim ♀ x Joinville ♂). Juvenis e adultos de ambos os tipos foram totalmente refratários à infecção tanto pela cepa LE quanto pela cepa SJ. Outros 500 juvenis e 278 adultos, híbridos (F_1) dos dois tipos, foram expostos a 25 ou 50 miracídios SJ por molusco, tendo todos também apresentado resultado negativo quanto à susceptibilidade.

REFERENCES

Carvalho, O.S., Souza, C.P. and Katz, N. (1985). Primeiro encontro de *Biomphalaria tenagophila* (d'-Orgigny, 1835), naturalmente infectada com *Schistosoma mansoni*, em Itajubá, sul do Estado de Minas Gerais, Brasil. *Rev. Saúde Públ. 19*: 88-91.

Freitas, J.R., Boschi, M.M. and Santos, M.B.L. (1985). Susceptibilidade de "híbridos" de *Biomphalaria tenagophila* à cepa LE (BH) do *Schistosoma mansoni*. *Rev. Inst. Med. Trop. São Paulo 27*: 6-12.

Freitas, J.R., Resende, E.S., Junqueira, D.V., Costa, A.M. and Pellegrino, J. (1975). Criação em massa e ritmo de crescimento de *Biomphalaria glabrata*. *Ciênc. Cult. 27*: 968-974.

Melo, A.L. and Pereira, L.H. (1985). On the finding of *Biomphalaria tenagophila* naturally infected with *Schistosoma mansoni* in state of Minas Gerais, Brazil. *Rev. Inst. Med. Trop. São Paulo 27*: 99-101.

Newton, W.L. (1953). The inheritance of susceptibility to infections with *Schistosoma mansoni* in *Australorbis glabratus*. *Exp. Parasitol. 2*: 242-257.

Paraense, W.L. and Correa, L.R. (1963). Variation in susceptibility of populations of *Australorbis glabratus* to a strain of *Schistosoma mansoni*. *Rev. Inst. Med. Trop. São Paulo 5*: 15-22.

Pellegrino, J. and Katz, N. (1968). Experimental Chemotherapy of *Schistosomiasis mansoni*. In: *Advances in Parasitology* (Ben Dawes ed.), Acad. Press, London, 6: 233-290.

Piza, J.T. and Ramos, A.S. (1960). Os focos autóctones de esquistossomose no Estado de São Paulo. *Arq. Hig. Saúde Públ. 25*: 261-271.

Piza, J.T., Ramos, A.S., Brandão, C.S.H., Figueiredo, G.C. and Camargo, L.S.V. (1960). Vale do Paraíba. Foco endêmico de esquistossomose. *Arq. Hig. Saúde Públ. 25*: 35-40.

Ramos, A.S., Piza, J.T. and Camargo, L.S.V. (1961). Observações sobre *Australorbis tenagophilus*, transmissor da esquistossomose mansônica. *Arq. Hig. Saúde Públ. 26*: 121-124.

Ramos, A.S., Piza, J.T., Pinto, G.H., Pion, T., Fleury, G.C., Morais, L.V.C. and Campos, L.L. (1965). Focos ativos de esquistossomose no Vale do Ribeira, Estado de São Paulo, Brasil. *Rev. Saúde Públ. 3*: 59-65.

Richards, C.S. (1973). Susceptibility of adult *Biomphalaria glabrata* to *Schistosoma mansoni* infection. *Am. J. Trop. Med. Hyg. 22*: 748-756.

Richards, C.S. and Merrith Jr., J.M. (1972). Genetic factors in the susceptibility of juvenile *Biomphalaria glabrata* to *Schistosoma mansoni*. *Amer. J. Trop. Med. Hyg. 21*: 425-434.

Santos, M.B.L., Freitas, J.R. and Correa, M.C.R. (1981). Susceptibilidade de híbridos descendentes de *Biomphalaria tenagophila* de Joinville, SC, com os de Taim, RS, Curitiba, PR, e Cabo Frio, RJ, à cepa LE de *Schistosoma mansoni*. *Ciênc. Cult.* (Suppl.) *33*: 495.

Santos, M.B.L., Freitas, J.R., Correa, M.C.R. and Coelho, P.M.Z. (1979). Susceptibilidade ao *Schistosoma mansoni* de híbridos de *Biomphalaria tenagophila* do Taim, RS, Cabo Frio, RJ, e Belo Horizonte, MG. *Rev. Inst. Med. Trop. São Paulo 21*: 281-286.

(Received September 13, 1988)