

OBSERVATIONS ON TWO BIOLOGICAL RACES OF *SCHISTOSOMA MANSONI*

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A comparative study of the BH strain of Schistosoma mansoni from Belo Horizonte, Minas Gerais state, infective to Biomphalaria glabrata from the same locality, and the SJ strain from São José dos Campos, São Paulo state, infective to B. tenagophila from the latter locality, showed the following differences:

- 1. Length of adult worms and size of eggs significantly larger in the BH strain.*
- 2. Higher infection rates in the B. glabrata-BH strain association than in the B. tenagophila-SJ strain association, following exposure of each snail to 1 or 10 miracidia.*
- 3. Longer prepatent period (from penetration of miracidium to first shedding of cercariae) in the B. tenagophila-SJ strain association.*
- 4. Infection of both Biomphalaria species when exposed to hybrid miracidia from crosses between the two strains, at lower levels than those resulting from exposure of each snail species to miracidia of the pure sympatric strain. (Both Biomphalaria populations are practically refractory to infection with the allopatric strain).*

These results are interpreted as pointing to a better host-parasite adjustment in the B. glabrata-BH strain association than in the B. tenagophila-SJ association. The inter-fertility between the two strains, which produced viable hybrids infective to both Biomphalaria species, supports the conclusion that the observed differences are merely intraspecific, and that the two strains may be considered distinct biological races of Schistosoma mansoni.

In two preceding papers, we have shown that a strain of *Schistosoma mansoni* from the river Paraíba valley, in the Brazilian state of São Paulo (SJ strain) was highly infective to the local planorbid snail *Biomphalaria tenagophila* (Paraense & Corrêa, 1963) and, with variable infection rates, to 15 other populations from the whole range of that snail in Brazil (Paraense & Corrêa, 1978). On the other hand, attempts to infect a highly

susceptible strain of *Biomphalaria glabrata* from Belo Horizonte with the SJ strain were unsuccessful (Paraense & Corrêa, 1963). Additional experiments carried out in this laboratory with large numbers of snails confirmed the last-mentioned results.

Furthermore, only rarely did we succeed in infecting *B. glabrata* and *B. tenagophila* from other areas with the SJ and BH strains, respectively. These experiments will be reported elsewhere.

The demonstration of so marked host discrimination on the part of two strains of apparently the same parasite led us to investigate the problem of their taxonomic status. In fact, the possibility of that difference in behavior reflecting some degree of taxonomic diversity seems reasonable, requiring a definition of the nature of such diversity.

In the present study a comparison between the two strains is made with respect to some morphological and biological characteristics.

MATERIAL AND METHODS

Baby mice, one-week old, were infected by transcutaneous route, each with 40 cercariae from a group of 5 *B. glabrata* from Belo Horizonte (BH *B. glabrata*) or *B. tenagophila* from São José dos Campos, river Paraíba valley (SJ *B. tenagophila*), which had been exposed to miracidia of the sympatric schistosome (BH and SJ strains, respectively). The mice were killed 50–60 days later, their portal system was perfused with saline, and 5 couples of worms were collected from each mouse that yielded more than that number, summing up 100 couples from each origin (BH and SJ). The worms, transferred to a small petri dish with clean saline, were killed by gradual heating up to about 50°C on a hot plate. They were then gently pressed between glass slides, fixed in Railliet-Henry's fluid, and stained with Mayer's hydrochloric carmine. The stained worms were carefully examined under the microscope in search of intergroup differences. Mensurations were made on camera lucida drawings ($\times 10$ magnification).

One-hundred eggs from each origin (BH and SJ) were measured on camera lucida drawings ($\times 480$ magnification) from petrolatum-sealed preparations of freshly passed feces of mice infected 55–60 days before. The material was suspended in a drop of isotonic saline large enough to prevent deformation of the eggs by coverglass pressure. The eggs were sketched with the spine clearly projecting sideward on the horizontal plane.

Unisexual infections were produced by exposing 259 BH *B. glabrata* and 377 SJ *B. tenagophila*, 4–6 mm in shell diameter, each to a single sympatric miracidium. The snails were dealt with as described by Paraense & Corrêa (1978), and those that survived for 70 days without shedding cercariae were dissected and examined. The resulting cercariae from each positive snail were used to infect, as described above, 3 baby mice which were perfused 6 weeks later in order to sex their worms.

To obtain bisexual infections with BH male and SJ female schistosomes, or SJ males and BH females, other mice were exposed, at the same time as the aforementioned ones, to a mixture of 20 cercariae from one BH and 20 from one SJ snail. Since each mouse-snail association could be precisely identified, it was possible to determine the origin of male and female worms in bisexual infections. Finally additional snails were exposed each to a single hybrid miracidium of bisexual mice infections, as follows:

- 126 BH *B. glabrata* to miracidia from ♂ BH \times ♀ SJ schistosomes;
- 112 BH *B. glabrata* to miracidia from ♂ SJ \times ♀ BH schistosomes;
- 152 SJ *B. tenagophila* to miracidia from ♂ BH \times ♀ SJ schistosomes;
- 149 SJ *B. tenagophila* to miracidia from ♂ SJ \times ♀ BH schistosomes.

Parallel experiments were carried out by exposing, on the same day, 45 *B. glabrata* and 34 *B. tenagophila* each to 10 miracidia of the sympatric strain. In this group the survivors that did not shed cercariae were dissected on days 100, 130 and 135.

The above-mentioned experiments covered a period during which the room temperature ranged between 22° and 26°C.

RESULTS

The BH male schistosomes measured 5.00-11.44 mm in length (mean 9.04 ± 1.16), and the females 7.21-14.45 mm (mean 11.85 ± 1.29). The SJ males measured 5.66-10.55 mm (mean 8.44 ± 0.95), and the females 5.32-12.89 mm (mean 9.76 ± 1.15). These data show that the parasites of the BH strain are significantly longer than those of the SJ strain ($P < 0.01$ for the males, $P < 0.001$ for the females).

The number of testes varied from 4 to 9 (mean 7.12 ± 1.20) in the BH males and from 4 to 10 (mean 7.48 ± 0.94) in the SJ males, with no significant difference between the two strains ($2.0 > P > 1.0$).

As to other taxonomically relevant characteristics respecting the alimentary and reproductive organs, no significant differences were observed.

The eggs measured 135-179 μm (mean 159.74 ± 8.61) in length by 65-76 μm (mean 70.01 ± 2.75) in width in the BH strain, and 137-167 μm (mean 150.75 ± 7.28) in length by 61-76 μm (mean 69.55 ± 2.66) in width in the SJ strain. They were significantly larger in the BH strain ($P < 0.001$ for length, $P > 0.1$ for width).

Of the snails exposed to a single sympatric miracidium, 86 *B. glabrata* (33.20%) and 15 *B. tenagophila* (3.98%) became infected. Some differences in host-parasite relationships are worth mentioning. The length of the prepatent period (from penetration of miracidium to first shedding of cercariae) varied from 30 to 35 days in BH infections and from 30 to 70 days in SJ ones. However, as shown in Table I, on the 35th day a greater proportion (87%) of the BH infections were patent, as compared with the SJ infections (61%). Moreover, all BH specimens which died after the 35th day were negative, whereas, among the SJ specimens, two which died on the 60th and 65th days showed exclusively immature secondary sporocysts in the internal organs.

The results of parallel exposures to 10 miracidia are also recorded on Table I, 35 *B. glabrata* (77.78%) and 23 *B. tenagophila* (67.65%) having become infected. As in the preceding experiment, the prepatent period was longer in SJ infections; 5 specimens that did not shed cercariae up to days 70, 85, 100, 130 and 135 were dissected, showing secondary sporocysts in the internal organs.

The infection rates of the snails exposed to hybrid miracidia were much lower than in the preceding experiments: 15 *B. glabrata* (11.90%) infected with δ BH \times η SJ and 17 *B. glabrata* (15.18%) with δ SJ \times η BH; 2 *B. tenagophila* (1.32%) infected with δ BH \times η SJ and 1 *B. tenagophila* (0.67%) with δ SJ \times η BH. The prepatent period in *B. glabrata* varied from 30 to 35 days in the first group and from 30 to 40 days in the second, all the remaining snails proving negative thereafter. In *B. tenagophila* only 2 specimens of the first group were infected, one of them shedding cercariae on the 35th day and the other dying on the 49th day with mature secondary sporocysts in the internal organs; in the second group a single specimen became infected, and died on the 35th day with immature secondary sporocysts in the internal organs.

TABLE I

Length of prepatent period in infections of *Biomphalaria glabrata* from Belo Horizonte and *B. tenagophila* from São José dos Campos, 4-6 mm in shell diameter, each with 1 or 10 miracidia of the sympatric strain of *Schistosoma mansoni*.

Snail species/ schistosome strain	Prepatent period in days												Total
	30	35	40	45	50	55	60	65	70	75	80	≥100	
<i>B. glabrata</i> /BH 1 miracidium	19	41	3	5	0	1	0	0	0	0	0	0	69*
<i>B. glabrata</i> /BH 10 miracidia	19	11	1	2	2	0	0	0	0	0	0	0	35
<i>B. tenagophila</i> /SJ 1 miracidium	1	7	2	1	0	1	0	0	1	0	0	0	13†
<i>B. tenagophila</i> /SJ 10 miracidia	0	4	0	0	1	0	2	2	0	1	2	3¶	15§

* Add: 17 died from days 25 to 35 with secondary sporocysts in internal organs (I.O.).

† Add: 2 died on days 60 and 65 with secondary sporocysts in I.O.

¶ Dissected: with secondary sporocysts in I.O. on days 100, 130 and 135.

§ Add: 6 died from days 22 to 40, and 2 on days 70 and 85, with secondary sporocysts in I.O.

DISCUSSION

When studying the newly isolated SJ strain (Paraense & Corrêa, 1963), we pointed out that a physiological adjustment had been reached between the SJ and BH snails and their sympatric schistosome strains. Comparison between the infection rates of BH and SJ snails exposed to a single miracidium in the present investigation suggests that the SJ schistosome strain is much less adapted to its vector than the BH strain to its own. The still lower infection rates by hybrid miracidia suggests that the level of adjustment reached by the BH and SJ snail-parasite associations is disrupted to some extent by the presence, in the hybrids, of incompatibility components from the heterologous strain. Another fact that seems to be related to a lower degree of host-parasite adaptation is the lengthening of the prepatent period in the SJ snail-parasite association as compared with the BH one.

Data on size of richly muscularized worms like schistosomes (although fixed in apparently good state of relaxation) are unreliable in themselves for taxonomic purpose. Besides having very extensive and contractile bodies, they show appreciable variation in physical development at any stage of the intramammalian life-cycle. The observed differences in body length between the BH and SJ schistosomes, although statistically significant, should be ascribed to intraspecific variation, as well as the difference in size of the eggs. That the two schistosomes merely represent different strains and cannot be regarded as separate species is substantiated by crossbreeding experiments. In fact, the two strains are interfertile, producing viable hybrids infective to both snail species, in contrast with the virtual noninfectivity of each strain to the alternative snail species. Moreover, there is a marked difference in length of the prepatent period of each strain in the respective snail host. All the mentioned characteristics entitle the two strains to be considered distinct biological races of *Schistosoma mansoni*.

A more detailed study of the differences in host-parasite relationships between the two strains will constitute the subject of a further publication.

RESUMO

O estudo comparativo da cepa BH de *Schistosoma mansoni*, oriunda de Belo Horizonte, Minas Gerais, e infectante para *Biomphalaria glabrata* da mesma localidade, e da cepa SJ, oriunda de São José dos Campos, São Paulo, infectante para *B. tenagophila* desta localidade, revelou as seguintes diferenças entre elas:

1. Comprimento dos vermes adultos e tamanho dos ovos significativamente maiores na cepa BH.

2. Índices de infecção mais altos na associação *B. glabrata*-cepa BH do que na associação *B. tenagophila*-cepa SJ, após exposição de cada molusco tanto a 1 quanto a 10 miracídios.

3. Período pré-patente (da penetração do miracídio à liberação das primeiras cercárias) mais longo na associação *B. tenagophila*-cepa SJ.

4. Infecção de ambas as espécies de *Biomphalaria* quando expostas a miracídios híbridos oriundos do cruzamento entre as duas cepas, em níveis mais baixos que aqueles resultantes da exposição de cada espécie a miracídios da cepa simpátrica pura. (As duas populações de *Biomphalaria* são praticamente refratárias à infecção com a cepa alopátrica).

Esses resultados são interpretados como indicativos de melhor ajustamento hospedeiro-parasito na associação *B. glabrata*-cepa BH do que na associação *B. tenagophila*-cepa SJ. A interfertilidade das duas cepas, que produziram híbridos viáveis infectantes para as duas espécies de *Biomphalaria*, permite concluir que as diferenças observadas são meramente intraespecíficas, e que as duas cepas podem ser consideradas como distintas raças biológicas do *S. mansoni*.

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