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VARIATION IN SUSCEPTIBILITY OF POPULATIONS OF *AUSTRALORBIS GLABRATUS* TO A STRAIN OF *SCHISTOSOMA MANSONI*

W. L. PARAENSE and Lygia R. CORRÊA

SUMMARY

A great majority of 23 populations of *A. glabratus* were highly susceptible to a strain of *S. mansoni* from Belo Horizonte, showing infection rates above 50%. Six populations, however, proved less susceptible or even highly resistant to infection. The negative rates were observed in three population from Salvador, one of which gave a 20% rate on exposure of each snail to 1,000 miracidia. Hybrids between Salvador and the highly susceptible strains from Santa Luzia and Recife showed intermediate rates in relation to the parental strains.

No correlation was observed between the degree of susceptibility and the distance separating the site of each snail population from that of the schistosome strain.

Variation in susceptibility of *A. glabratus* to infection with *S. mansoni* must be considered as an aspect of intraspecific variation. Therefore, degree of populational suspectibility depends upon the relative frequency of resistant and susceptible genotypes in each population.

The differences in susceptibility, as observed in this study, are obviously related to the genotype of the snail populations.

INTRODUCTION

Particular attention has been given to the subject of the susceptibility of freshwater snails to infection with human schistosomes. Most investigations have concerned the infectivity of *Schistosoma mansoni* to either usual or unusual planorbid hosts.

Australorbis glabratus is the most suitable transmitting host of *S. mansoni* in the Neotropical region, showing the highest infection rates both in nature and experimentally. An exception to this rule was pointed out by FILES & CRAM [7], who showed that a Puerto Rican, a Venezuelan and an Egyptian strain of *S. mansoni*, infective to Puerto Rican and Venezuelan strains of *A. glabratus*, were completely uninfective to a strain of this snail from Recife, Brazil. Another Puerto Rican strain of *S. mansoni*, also infective to local and Venezuelan strains of that snail, showed a low degree of infectivity (6%) to the Brazilian *A. glabratus*.

FILES [6], exposing *A. glabratus* from Puerto Rico, Venezuela, Dominican Republic, Surinam, Salvador and Egypt, observed dif- to *S. mansoni* from Puerto Rico, Venezuela, Surinam, Salvador, and Egypt, observed differences in susceptibility of the snails to the parasites of the different geographical areas. The most striking of such differences consisted in the absolute insusceptibility of *A. glabratus* from Salvador to seven strains of *S. mansoni* from Egypt, Puerto

Centro Nacional de Pesquisas Malacológicas (of the Instituto Nacional de Endemias Rurais and the Instituto Oswaldo Cruz). Caixa Postal 2113, Belo Horizonte, Minas Gerais, Brasil.

Rico, Surinam, and from crossings Egypt × Puerto Rico, Salvador × Puerto Rico, Salvador × Recife and Salvador × Egypt.

WRIGHT [15] also found that *A. glabratus* from Salvador was insusceptible to *S. mansoni* from Puerto Rico and from Paulista, Brazil.

There is a tendency to explain the variation in susceptibility as the expression of an adjustment between local strains of the snail and of the parasite. This interpretation, however, can not be generalized, since KUNTZ [9] observed that *A. glabratus* from Venezuela and Puerto Rico were equally susceptible to *S. mansoni* from Egypt, the infection range being the same as in the Egyptian *A. boissyi*.

NEWTON [10], working with strains studied by FILES [6], confirmed the resistance of the snails from Salvador, even when exposed to large numbers of miracidia. He showed that susceptibility is a heritable character influenced by the age of the snail, seeing that a fair proportion of the Brazilian snails could be infected when exposed at the ages of 1 to 24 days.

The factors determining susceptibility are not yet well understood. Since a host-parasite interaction is implied, it is obvious that both genotypes must be concerned.

In a preliminary approach to the problem, we got information on the variation in susceptibility, to a single strain of *S. mansoni*, of a number of populations of *A. glabratus* sampled over the whole species range.

MATERIAL AND METHODS

Specimens from 23 populations scattered over the range of *A. glabratus* (Fig. 1) were used in this study. The experimental snails

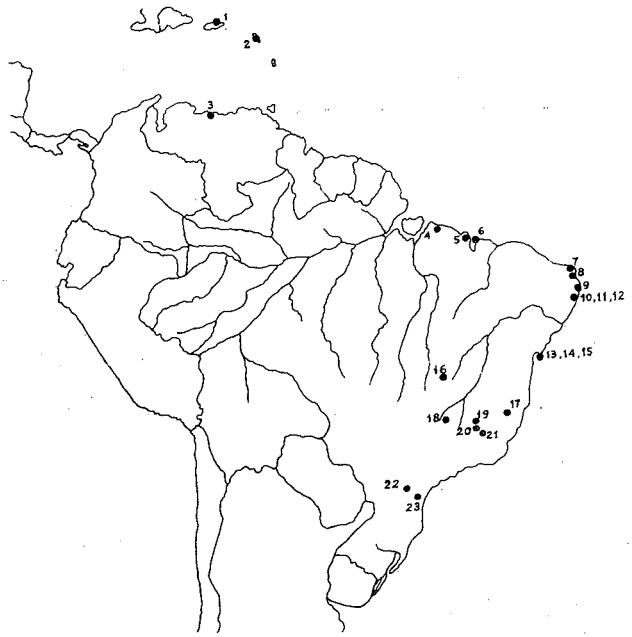

Fig. 1 — Distribution of the populations of *A. glabratus* mentioned in the text. 1 — Puerto Rico. 2 — St. Kitts Island. 3 — Caracas (Venezuelas). 4 — Capanema (Pará). 5 — Cururupu (Maranhão). 6 — São Luiz (Maranhão). 7 — Estremoz (Rio Grande do Norte). 8 — Papari (Rio Grande do Norte). 9 — João Pessoa (Paraíba). 10 — Paulista (Pernambuco). 11 — Peixinhos (Pernambuco). 12 — Recife (Pernambuco). 13 — 14 — 15 — Salvador (Bahia). 16 — Lagoa Feia (Goiás). 17 — Mascarenhas (Espírito Santo). 18 — Araxá (Minas Gerais). 19 — Santa Luzia (Minas Gerais). 20 — Belo Horizonte (Minas Gerais). 21 — Alvinópolis (Minas Gerais). 22 — Jacarèzinho (Paraná). 23 — Curitiba (Paraná).

were reared in the laboratory, descending from large samples of those populations. Unless otherwise stated, the snails were individually exposed to 10 miracidia of a size of 8 to 10 mm in diameter. Miracidia were collected as described by CHAIA [3], from feces of a chronic patient who contracted the infection on a single exposure to cercariae at Belo Horizonte. The water containing the concentrate of miracidia was taken with a pipette and dropped, under a dissecting microscope, into a small Petri dish, 4 cm in diameter, until the required number of miracidia was gathered. Only freshly hatched, fast moving miracidia were taken into account. A snail was then placed in the dish and just enough spring water was added to cover it. The dish was covered and the snail left overnight in it. On the next morning, the exposed snails were removed to small aquaria, where they were kept under observation, being watched at least four times daily. If any specimen happened to die, it was dissected and examined for the presence of developing stages of the schistosome.

On the 28th and 30th days after exposure to miracidia, and then every five days, the snails were placed singly in Borrel tubes with spring water and exposed to the light of electric lamps. The water in each tube was examined for the presence of cercariae, and the infected snails were isolated. Those which remained negative till the 60th day were dissected for microscopic examination. All the snails that showed developing parasites were considered positive, even in cases of retarded development in which the early cercarial stage was not attained.

In addition to the snails from the 23 populations listed in Table I, four groups of hybrids were also exposed to miracidia. The hybrids resulted from crossings between pigmented specimens from Salvador III and albinos from Santa Luzia or Recife (for crossing methods, see PARAENSE [11]). The four groups were distributed as follows: 98 F_1's from Salvador fertilized by Santa Luzia (10 miracidia per snail), 116 F_1's from Santa Luzia fertilized by Salvador (10 miracidia), 73 F_1's from Salvador fertilized by Recife (10 miracidia), and 85 F_2's (66 pigmented and 19 albinos) from Salvador × Santa Luzia (50 miracidia).

RESULTS

As shown in Table I, of the 23 studied populations, 17 were highly susceptible to the schistosome strain, with an infection rate above 50%. Of the remaining six populations, those from João Pessoa and Lagoa Feia showed moderate degrees of susceptibility (48 and 24.1%, respectively), that from Mascarenhas was remarkably resistant (1%), and the three ones from Salvador were quite refractory to a challenge of 10 miracidia per snail. Additional specimens from Salvador III (descended from a sample collected in a lake near the beach, at Amaralina district) were exposed to 50 and 100 miracidia, with negative result. Exposure to 1,000 miracidia was tried, but many snails of 8-10 mm died soon after the massive penetration of miracidia. Therefore, we used larger specimens, 15-20 mm in diameter, of which 19.8% became infected under such unusual conditions.

Of the 31 positive snails from the group exposed to 1,000 miracidia, nine showed unusually retarded infection at dissection, between 40 and 60 days. In eight of the latter, only immature sporocysts were found in the head, foot and mantle collar, no parasites being present in the internal organs. Another specimen showed only immature sporocysts confined to the areolar tissue behind the apex of the ovotestis.

The hybrids were intermediate in susceptibility between the parent strains, which confirms NEWTON's observations [10] on susceptibility as a heritable character. They showed the following infection rates: F_1's from Salvador fertilized by Santa Luzia, 13.3%; F_1's from Santa Luzia fertilized by Salvador, 14.6%; F_1's from Salvador fertilized by Recife, 1.4%; F_2's from Salvador × Santa Luzia, 28.2% (pigmented 25.7%, albinos 31.6%).

In all the experiments the mortality during the period corresponding to the early development of the parasite was negligible, as well as the number of autolysed specimens which could not be examined.

TABLE I

Populational variation in susceptibility of *A. glabratus* to infection with a strain of *S. mansoni* from Belo Horizonte, Brazil (10 miracidia per snail 8-10 mm. in diameter).

Origin of snail	No. Exposed	No. Positive	% Positive
1. Puerto Rico	23	21	91.3
2. St. Kitts Island	11	10	90.0
3. Caracas	37	34	92.0
4. Capanema	31	30	96.8
5. Cururupu	25	18	72.0
6. São Luís	33	23	69.7
7. Estremoz	24	23	95.8
8. Papari	30	20	66.6
9. João Pessoa	50	24	48.0
10. Paulista	47	46	97.9
11. Peixinhos	97	97	100.0
12. Recife	52	50	96.1
13. Salvador I	103	—	—
14. Salvador II	98	—	—
15. Salvador III	102	—	—
Salvador III — 50 miracidia	71	—	—
Salvador III — 100 miracidia	109	—	—
Salvador III — 1000 miracidia *	96	19	19.8
16. Lagoa Feia	29	7	24.1
17. Mascarenhas	101	1	1.0
18. Araxá	33	28	84.8
19. Santa Luzia	113	107	94.7
20. Belo Horizonte	102	97	95.1
21. Alvinópolis	25	25	100.0
22. Jacarèzinho	27	23	85.2
23. Curitiba	26	17	65.4

* Snails 15-20 mm. in diameter.

DISCUSSION

The results of these experiments point to several degrees of susceptibility, between 0 and 100%, of the concerned populations of *A. glabratus* to infection with a strain of *S. mansoni.*

There is no apparent correlation between the degree of susceptibility and the distance separating the site of the snail population from that of the schistosome strain. Infection rates above 90% were observed in the original area of the schistosome strain, as well as in far removed areas. The intermediate values of infection rates (between 50 and 80%) are probably not significant in expressing true gradations in susceptibility, owing to the small number of snails in each experiment. Of unquestionable significance, however, are the results indicative of lower degrees of susceptibility of the three populations from Salvador and

those from Mascarenhas, Lagoa Feia and João Pessoa. In Table II the results of exposure of some batches from these populations are shown, in comparison with simultaneous exposure of batches from highly susceptible strains which served as controls for the infectivity of the employed miracidia.

exposed at the ages of 1 to 24 days showed moderate infection rates. As referred to above, the mentioned authors used *S. mansoni* from localities other than Salvador.

Only recently have experiments been made on the susceptibility of *A. glabratus* from Salvador to a local strain of *S. mansoni*. BARRETO [2], examining 434 snails exposed to 20 miracidia per snail, observed the infection rate of 0.92%.

TABLE II

Results of simultaneous exposures of batches from resistant and highly susceptible strains of *A. glabratus* to a strain of *S. mansoni* from Belo Horizonte, Brazil (10 miracidia per snail 8-10 mm. in diameter).

Snail strain	Date of Exposure	No. Exposed	No. Positive	% Positive
Salvador II	25-10-60	47	—	—
Belo Horizonte	25-10-60	22	21	95.4
Mascarenhas	11-12-59	25	—	—
Capanema	11-12-59	21	21	100.0
Caracas	11-12-59	8	8	100.0
Puerto Rico	11-12-59	8	8	100.0
Mascarenhas	12-01-60	6	1	16.6
Papari	12-01-60	30	20	66.6
Lagoa Feia	15-01-60	12	1	8.3
Alvinópolis	15-01-60	25	25	100.0
Puerto Rico	15-01-60	4	3	75.0
Lagoa Feia	26-04-60	14	6	42.8
Jacarèzinho	26-04-60	4	4	100.0
João Pessoa	19-04-60	20	11	55.0
Caracas	19-04-60	7	7	100.0
Peixinhos	19-04-60	12	12	100.0

Previous studies have shown that *A. glabratus* from Salvador is quite refractory to several strains of *S. mansoni* (FILES [6], WRIGHT [15], CRAM [5], RUIZ [12]) or gives only a very low infection rate (1.7% of 233 specimens: BARBOSA & BARRETO [1]). It should be noted that FILES [6] discussed insusceptibility of the Salvador strain not as a localized phenomenon, but as a general characteristic of *A. glabratus* from the Brazilian endemic area. NEWTON [10] observed that the specimens over 7 mm in diameter were nonsusceptible, but that those

The low susceptibility of *A. glabratus* from Salvador has also been observed in the field: 3% in 20,981 snails (COUTINHO [4]), 0.41% in 120,281 (TRAVASSOS [14]), and 0.83% in 2,217,638 (BARRETO [2]). Despite so low infection rates, the local populations of *A. glabratus* are responsible for the maintenance of an important endemic focus of schistosomiasis. This is a discouraging handicap to the practicability of biological

control of schistosomiasis by competition between susceptible and resistant strains of snails, as suggested by HUBENDICK [8].

The aforesaid infection rates show that adjustment between local strains of schistosomes and host snails, expressed in terms of susceptibility of the latter to the former, is by no means a general rule, and, moreover, that very low host susceptibility is not a limiting factor to high endemicity.

It will be of interest to undertake a comprehensive survey of the populations of *A. glabratus* in the region concerned, with regard to susceptibility, to get information on their dispersion area. Such a survey will show whether the resistant populations occupy a definite area or intergrade with more and more susceptible populations.

Nine cases were observed of unusually retarded infection among the positive snails from Salvador exposed to 1,000 miracidia. In eight of them, which showed no parasites in the internal organs, the infection would probably die out, seeing that the immature sporocysts found in the external parts seemed to be impaired by reaction of the host tissues. Observations on retarded development of *S. mansoni* sporocysts in *A. glabratus* were previously made by NEWTON [10]. Such behavior of the parasite was more frequent in less susceptible strains of snails. It occurred not only among Salvador snails, but also in some specimens from Mascarenhas, Lagoa Feia, João Pessoa, São Luiz and Cururupu, and more rarely in highly susceptible strains like Paulista, Belo Horizonte and Santa Luzia. These observations show that, even in highly susceptible populations, there are individuals that react efficiently against the parasite, so as to be completely refractory. One specimen from Paulista exposed to 1,000 miracidia remained negative, whereas another one from Salvador, exposed at the same time, became intensely infected and eliminated abundant cercariae for 54 days. Such extreme intrapopulational variation in susceptibility means that the populations are endowed with the raw material for operation of selective mechanisms towards the building up of more or less resistant strains.

As demonstrated by STANDEN [13], the infection rate is markedly influenced by the prevailing temperature, the optimum for the cultivation of *S. mansoni* in *A. glabratus* lying between 26 and 28°C. In our experiments, however, it seems that the variation in infection rates was not significantly influenced by other factors than the susceptibility of the populations concerned. In some strains it was possible to compare the infection rates in the warmest and coldest months, when the lowest and highest average temperatures in the laboratory were 24.9 and 27.6°C. (December-January), against 19.5 and 22.7°C. (June-July). As shown in Table III, no significant differences were found, either in the resistant populations of Salvador, or in the highly susceptible ones of Paulista, Peixinhos and Re-

TABLE III

Infection rates in one strain of *A. glabratus* resistant to *S. mansoni*, and in three highly susceptible strains, in the warmest and coldest months.

Snail strains	December-January 24.9-27.6°C *		June-July 19.5-22.7°C *	
	No. Exposed	% Positive	No. Exposed	% Positive
Salvador	30	6.6	192	8.8
Paulista	23	95.6	24	100.0
Peixinhos	12	100.0	20	100.0
Recife	22	95.4	30	96.6

* Lowest and highest average temperatures in the laboratory.

cife. The only significant influence of the temperature, observed in our material, was the expected inverse correlation between its variation and the developmental period of the parasite. Thus, in colder months the ripening of sporocysts and production of cercariae were delayed. Such a slow development of sporocysts, however, did not bear on the above-mentioned "unusually retarded infections". In the latter, the sporocysts stop developing at some stage before the production of cercariae, and the infection eventually dies out. Moreover, in our material the frequency of such retarded infections was the same in cold as in warm months.

The data presented in this paper show that there are physiological differences, expressed in degrees of susceptibility to a single strain of *S. mansoni*, between populations of *A. glabratus* from different places within the same general endemic area. Such differences in susceptibility, as observed in this study, are obviously related to the genotype of the snail populations. In this connection, our findings confirm those of FILES & CRAM [7] and FILES [6]. The other side of the question, that is, the existence of physiological differences in parasites of different areas, postulated by the same authors, is being investigated in this laboratory.

RESUMO

Variação na suscetibilidade de populações de Australorbis glabratus *a uma cêpa de* Schistosoma mansoni.

A grande maioria dentre 23 populações de *A. glabratus* foi altamente suscetível a uma cêpa de *S. mansoni* de Belo Horizonte, apresentando índices de infecção superiores a 50%. Entretanto, seis populações mostraram menor suscetibilidade ou mesmo alta resistência. Três populações de Salvador deram índices negativos; porém, em uma, expondo-se os caramujos individualmente a 1.000 miracidios, obteve-se 20% de positividade.

Em híbridos do cruzamento de uma cêpa de Salvador com cêpas altamente suscetíveis de Santa Luzia e Recife, observaram-se índices de infecção intermediários em relação às cêpas parentais.

Não houve correlação entre o grau de suscetibilidade de cada população e a distância entre a respectiva localidade e a do parasito.

A variação na suscetibilidade do *A. glabratus* ao *S. mansoni* deve ser considerada como um aspecto da variação intra-específica. Portanto, os graus de suscetibilidade populacional dependem da freqüência relativa dos genótipos resistentes e suscetíveis em cada população.

As diferenças observadas no presente estudo acham-se relacionadas ao genótipo das populações de moluscos.

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