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Establishment of a Gene Pool Strain of *Biomphalaria glabrata* Snail Host of *Schistosoma mansoni*

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Abstract: A Gene-pool line of snail was established after allowing three *Biomphalaria glabrata* isolates from Brazil, Egypt and Puerto Rico. The susceptibility test of three parental isolates against four *Schistosoma mansoni* isolates from Brazil, Puerto Rico, Egypt and Kenya gave > 90% infection rate. The susceptibility test of newly established snail line with all four above mentioned *S. mansoni* isolates gave par-higher rates of infection when compared with parental snail lines.

Key words: *Biomphalaria glabrata- Schistosoma mansoni*-susceptibility

Introduction

Schistosomiasis is an important human disease in tropical countries. *Schistosoma mansoni* is the most wide-spread of the trematode species responsible for infection, being found throughout Africa, on many Caribbean Islands and in South America. The schistosome life-cycle is complex, requiring a snail intermediate host for asexual reproduction in addition to the vertebrate host for sexual reproduction. The planorbid snail *Biomphalaria glabrata* serves as intermediate host for *S. mansoni*. Predictably, the interaction of three genomes (that of the parasite and its two hosts) in a wide range of environmental conditions in the tropics results in considerable biological diversity of host-parasite relationships.

It has been shown that not all individuals of the compatible species of snail are susceptible to infection. Hence, susceptibility to infection is thought to be inheritable and is largely regulated by genetic factors (Newton, 1953). Susceptibility and resistance may be absolute (Wakelin, 1994) and the infectivity of parasite and the susceptibility of the host may be altered by deliberate or inadvertent selection (Santana *et al.*,1978).

The purpose to establish a gene pool line of snail was to pool the genetic potential of snails from various geographical isolates and later on use that line of snail for the selection of infection-resistant snail line.

Materials and Methods

Snail culture: Plastic aquarium tanks of 12 litres volume filled with 10 litres water were used for snail culture. Mains water was supplied through copper ,and was stored and aerated for > 24 hours before use. The snail culture room was kept at 26-28 °C, and 12 hours alternating light / dark cycle was maintained. The water in each tank was aerated by an airline connected to a compressed air supply, and each tank contained a population of water fleas (Daphnia spp). Snails were fed *ad libitum* daily on commercial rabbit food, The amount given being related approximately to the relative density of the snail population in the tank (0.75 to 1g/day per tank of 100 snails of 10 to 15mm shell diameter).

Infection of snail with miracidia: Snails with shell diameter of 12-14mm were subjected to mass infection of *S. mansoni* miracidia. Infected livers were obtained from up to 4 mice that had been given percutaneous infections of 200 *S. mansoni* cercariae 42 days previously, and the liver tissue disrupted by maceration through copper wire mesh. The resulting parasite egg suspension was washed once in 1.8% saline solution, divided into five equal volumes and if necessary, stored at 4°C. When miracidia were required, the egg suspension was centrifuged lightly, re-suspended in execs copper-free water, poured into a perti dishs and illuminated and warmed gently under a 60watt light bulb. The preparations were examined after 10 to 20 minutes to confirm that miracidia had hatched, and the material poured into a tank containing approximately 100 snails in 2 to 3 litres of clean water. The snails were exposed to miracidia as above on two successive days. Representative counts of miracidial densities indicated that each snail was being routinely subjected to 20 to 60 miracidia on each of the two days.

Cercarial counting: Approximately 35 days after infection, snails were screened for cercarial out put. For this purpose, individual snails were placed in small tubes holding 5ml of water, and kept under tube light. After three hours, individual snails were inspected for cercarial movement by holding them in front of light. The snails that shed cercariae were divided into three categorise viz. low shedders, medium shedders and high shedders.

For calculating the number of cercariae per individual infected snail, aliquots of 50ul, 40ul and 20ul were taken from low, medium and high shedders respectively. The aliquots were placed on glass slide, and a drop of iodine added to kill and stain cercariae, which were counted under dissecting microscope.

Method of establishment of Biomphalaria glabrata Gene-pool isolate: Three geographical isolates of *B. glabrata*, i.e. the Brazilian and Egyptian isolates (both pigmented) and an albino Puerto Rican isolate were in routine culture in laboratory. The susceptibility of these three snail isolates to four *S. mansoni* isolates those of from Brazil, Egypt, Puerto Rico and Kenya was tested and it was found that all three snail isolates were highly susceptible to each of the four parasite isolates. In order to pool the genetic material from all three geographical snail isolates, a further snail line *Biomphalaria glabrata* (Bg-Gp) was established.

To summarise it, representative adults from 3 snail lines were pooled in aquarium tanks and allowed to interbreed. Some hybridisation did occur which was noted due to

Table 1: Infestation of Bg-PR snail with different geographical isolates of *S. mansoni*

Snail			Bg-PR (Albino)			
Parasite	No. of batches	Mean days after infection	Mean No. of snails	% of snails infected	Mean cercs/snail	Mean cercs/infectd snail
Sm-PR	5	36 ± 2	30 ± 7	97 ± 3	1843 ± 812	1898 ± 809
Sm-Br	4	36 ± 2	31 ± 3	97 ± 3	1883 ± 664	1954 ± 743
Sm-Eg	3	38 ± 2	30 ± 11	88 ± 13	934 ± 1023	973 ± 990
Sm-Ke	4	38 ± 2	21 ± 10	99 ± 3	1612 ± 445	1637 ± 174
Sm-Gp	4	36 ± 2	28 ± 8	99 ± 2	1734 ± 983	1743 ± 974

Table 2: Infestation of Bg-Br snail with different geographical isolates of *S. mansoni*

Parasite	No. of batches	Mean days after infection	Mean No. of snails	% of snails infected	Mean cercs/snail	Mean cercs/infectd snail
Sm-PR	5	36 ± 2	30 ± 14	96 ± 3	1349 ± 585	1393 ± 599
Sm-Br	4	36 ± 2	46 ± 6	84 ± 8	1126 ± 203	1332 ± 154
Sm-Eg	3	38 ± 3	44 ± 13	82 ± 7	640 ± 685	739 ± 743
Sm-Ke	4	38 ± 2	39 ± 6	96 ± 1	1886 ± 1886	1977 ± 492
Sm-Gp	4	36 ± 2	99 ± 2	99 ± 2	1263 ± 1263	1271 ± 589

Table 3: Infestation of Bg-Eg snail with different geographical isolates of *S. mansoni*

Snail			Bg-Eg (Pigmented)			
Parasite	No. of batches	Mean days after infection	Mean No. of snails	% of snails infected	Mean cercs/snail	Mean cercs/infectd snail
Sm-PR	5	37 ± 3	49 ± 5	90 ± 12	1560 ± 443	1710 ± 356
Sm-Br	5	37 ± 2	37 ± 10	89 ± 11	1236 ± 508	1353 ± 441
Sm-Eg	5	37 ± 3	42 ± 12	94 ± 5	1626 ± 1083	1688 ± 1065
Sm-Ke	4	37 ± 2	36 ± 7	93 ± 3	1081 ± 573	2236 ± 646
Sm-Gp	3	34 ± 2	51 ± 15	94 ± 2	1712 ± 122	1804 ± 94

Table 4: Infestation of Bg-Gp snail with different geographical isolates of *S. mansoni*

Parasite	No. of batches	Mean days after infection	Mean No. of snails	% of snails infected	Mean cercs/snail	Mean cercs/infectd snail
Sm-PR	11	38 ± 3	120 ± 47	95 ± 5	1974 ± 1138	2061 ± 1123
Sm-Br	6	35 ± 2	97 ± 28	94 ± 7	1782 ± 896	1876 ± 871
Sm-Eg	6	37 ± 4	113 ± 62	94 ± 6	2318 ± 853	2484 ± 923
Sm-Ke	7	38 ± 4	105 ± 40	92 ± 6	1734 ± 529	1899 ± 630
Sm-Gp	11	37 ± 4	105 ± 42	93 ± 6	1880 ± 589	2039 ± 731

presence of the light pigmented colour snails which were different from both pure and dark pigmented parental snails. The offspring were tested for susceptibility to infection by each of the 4 different parasite isolates mentioned above.

Results

The results of infection of three snail isolates i.e. *Biomphalaria glabrata* Puerto Rico isolate (Bg-Pr), Brazilian isolate (Bg-Br) and Egyptian isolate (Bg-Eg) is shown in Table 1 and 3. The tables reveal that all three snail isolates were highly susceptible to four *S. mansoni* isolates.

Table 1 shows the infection of Puerto Rican snail with four *S. mansoni* isolates. The percentage of Puerto Rican snails that became patent was 97 ± 3, 97 ± 3, 88 ± 13 and 99 ± 3, with Sm-Pr, Sm-Br, Sm-Eg and Sm-Ke isolates of *S. mansoni* respectively.

Table 2 shows the patency of Brazilian isolate of *Biomphalaria glabrata* snail with above mentioned four *S. mansoni* isolates. The percentage of the snails that became patent was 96 ± 3, 84 ± 8, 82 ± 7 and 96 ± 1 when infected with Sm-Pr, Sm-Br, Sm-Eg and Sm-Ke respectively.

Table 3 shows the rate of infection of Egyptian isolate of *Biomphalaria glabrata* with four *S. mansoni* isolates. 90 ± 12, 89 ± 11, 94 ± 5 and 93 ± 3 % snails shed cercariae when infected with above mentioned four *S. mansoni* isolates respectively.

Table 1 and 3 also reveal mean number of cercariae per snail and per individual infected snail. The Table 1 shows that the lowest number of cercariae per infected snail (973 ± 990) was shed by Puerto Rican isolate when infected with Egyptian isolate of *S. mansoni* and the highest number of cercariae (1954 ± 743) were shed when infected with Brazilian isolate of *S. mansoni*.

Table 2 shows that the lowest number of cercariae (739 ± 743) and highest number of cercariae (1977 ± 492) were shed by Brazilian strain when infected with Egyptian and Kenyan isolates of parasite respectively.

Table 3 indicate that the lowest number of cercariae (1353 ± 441) and highest number (2236 ± 646) were shed by Egyptian snail isolate when infected with Brazilian and Kenyan isolates of the parasite respectively.

Table 4 shows the infection rate of newly established Gene-pool (Bg-Gp) snail isolate. It is clear from the table that like parental snail isolates, the established line of snail was also highly susceptible both in terms of percentage of the snails that became infected and number of cercariae shed by individual infected snail.

Discussion

We have begun to breed selectively, a line of infection-resistant snail host and this type of work suggest that it may eventually be possible to reduce the size of the human-infecting schistosome population by genetic manipulation of their intermediate host. Such genetic manipulation of the snail host is possible through selective breeding of an infection-resistant snail host, mass rearing it and returning the descendants to the population from which they were isolated.

In order to select an infection-resistant snail line, the base line experimental work (of which the results are given in this paper) during present study suggest that, an inter-

breed snail line is equally highly susceptible as their parental lines (Table 1 and 3). Files (1951) Richards (1975) and Wrights and South gate (1976; 1981) showed that hybrid snails are not equally compatible with both the parental intermediate host snail stock, whereas Wrights *et al.* (1974) and Richards (1975) in their later work supported that in some cases, hybrids are more compatible with the parental, in others (Files, 1951) with the maternal schistosome host snail stock.

During present study, the established inter-breed snail line gave a slightly higher susceptibility (Table 4) which may be due to hybrid vigour as also supported by Wrights *et al.* (1974) and Richards (1975).

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