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# Establishment of a Gene Pool Strain of *Schistosoma mansoni*

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## ABSTRACT

A Gene-pool line of *Schistosoma mansoni* was established after allowing four isolates of *S. mansoni* from Brazil, Egypt and Puerto Rico and Kenya. The infectivity test of these four parental isolates in three *Biomphalaria glabrata* isolates from Brazil, Puerto Rico and Egypt gave > 90% infection rates. The infectivity test of newly established parasite (now called Sm-Gp [*Schistosoma mansoni* gene pool]) gave par-higher rates of infection when compared with parental parasite isolates.

**Key Words:** *Schistosoma mansoni; Biomphalaria glabrata;* Susceptibility

## INTRODUCTION

It is estimated that 200-300 million people in tropics are infected with schistosomiasis, and several hundred million more are at risk. This risk is increasing because of development of water resources for industry and agriculture, with little provision of public health measures to prevent spread of this water-borne disease. 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 relationship.

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

## MATERIALS AND METHODS

The materials and methods used are same as already described by Arijo and Doenhoff (2000).

**Snail culture.** Plastic aquarium tanks of 12 litre volume filled with 10 litres water were used for snail culture. Mains water was supplied through copper, and was stored and aerated for > 24 h before use. The snail culture room was kept at 26-28°C, and 12 h 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 (0.75-1g/day per tank of 100 snails of 10-15 mm shell diameter).

**Infection of snail with miracidia.** Snails with shell diameter of 12-14 mm were subjected to mass infection of *S. mansoni* miracidia. Infected livers were obtained from up to five 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 excess copper-free water, poured into a petri dish, and illuminated and warmed gently under a 60 watt light bulb. The preparations were examined after 10-20 minutes to confirm that miracidia had hatched, and the material poured into a tank containing approximately 100 snails in 2-3 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 20-60 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 5 mL 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 50, 40 and 20 µl 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 *S.mansoni* Gene-pool isolate.** Four geographical isolates of *S. mansoni* those from Puerto Rico, Brazil Pernambico, Egypt and Kenya were being routinely maintained in the laboratory in the *B. glabrata* snail and randomly bred laboratory mice.

In order to pool the genetic material of the parasites, prior to selective breeding for particular traits, a further

parasite line was established. Cercariae of four isolates were mixed in approximately equal numbers and six random-bred Taylor's Original strain mice were infected with 200 cercariae per mouse according to the method described above. The mice were killed 42 days after infection and snails were infected with the miracidia obtained from the infected livers.

## RESULTS AND DISCUSSION

The results of infection of four parasite isolates i.e. *S. mansoni* Puerto Rico isolate (Sm-Pr), Brazilian isolate (Sm-Br), Egyptian isolate (Sm-Eg), Kenyan isolate (Sm-Ken) and Gene pool (Sm-Gp) are shown in Tables I, II and III. Results revealed that all four parental *S. mansoni* isolates were highly infective to three *B. glabrata* isolates, and so was new parasite line i.e. Sm-Gp.

Table I shows the infection of Puerto Rican snail with four *S. mansoni* isolates including newly established *S. mansoni* (Sm-Gp) parasite line. The percentage of Puerto Rican snails that became patent was 97±3, 97±3, 88±13, and 99±3 and 99±2 with Sm-Pr, Sm-Br, Sm-Eg, Sm-Ke and Sm-Gp isolates of *S. mansoni,* respectively. Table II shows the patency of Brazilian isolate of *Biomphalaria glabrata* snail with above mentioned five *S. mansoni* isolates. The percentage of the snails that became patent was 96±3, 84±8, 82±7, 96±1 and 99±2, when infected with Sm-Pr, Sm-Br, Sm-Eg, Sm-Ke and Sm-Gp, respectively.

Table III shows the rate of infection of Egyptian isolate of *Biomphalaria glabrata* with five *S. mansoni* isolates. 90±12, 89±11, 94±5, 93±3 and 94±2% snails shed cercariae, when infected with above mentioned four *S. mansoni* isolates, respectively. Table I, II and III also reveal mean number of cercariae per snail and per individual infected snail. Table I shows that the lowest number of cercariae per infected snail (973) 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*. This table also indicates that in terms of mean number of cercariae per infected individual snail, the new line of Sm-Gp gave par higher number of cercariae as parental isolates.

Genetics play important role in the host-parasite relationship and both infectivity and susceptibility may be altered by deliberate or inadvertent selection (Woodruff, 1985). A large body of data in literature is available regarding selection of insusceptible and susceptible snail lines. Studies on variations in infectivity in *S. mansoni* and hybrid schistosomes have also been reported (Barbosa & Barreto, 1960; Files, 1951). These variations have been reported to be influenced by maturation rates and selection pressures (Richards, 1975). The host-parasite relationship between snail and trematode populations may, therefore, result in infection frequencies ranging anywhere from 0 to 100%, subject also to various environmental factors, and both the infectivity of parasite and susceptibility of snail host may be changed through genetic selection.

The idea of establishing a new line of parasite (during our study) was based on the selection of infection-resistant snail line of *Biomphalaria glabrata*. Since there are quite a few geographical isolates of *S. mansoni* parasite and its intermediate host *B. glabrata* snail and there is difference in the infectivity of parasite isolates and susceptibility of snail lines, belonging to various geographical localities. Hence, idea was to pool the genetic potential of available snail lines in one line called Bg-Gp (*Biomphalaria glabrata* gene pool)

**Table I. Infection *B. glabrata* Puerto Rico isolate 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/infected snail infected snail** |
| Sm-PR | 5 | 36±2 | 30±7 | 97±3 | 1843±812 | 1898±809 |
| Sm-Br | 4 | 36±2 | 31±3 | 97±3 | 1883±644 | 1954±743 |
| Sm-Eg | 3 | 38±3 | 30±11 | 88±13 | 934±1023 | 973±990 |
| Sm-Ke | 4 | 38±2 | 21±102 | 99±3 | 1612±445 | 1637±463 |
| Sm-Gp | 4 | 36±2 | 28±8 | 99±2 | 1734±983 | 1743±974 |

**Table II. Infection of *B glabrata* Brazil isolate with different geographical isolates of *S mansoni***

| Snail | | | Bg-Br (pigmented) | | | |
|---|---|---|---|---|---|---|
| **Parasite isolates** | **No. of batches** | **Mean days after infection** | **Mean No. of snails** | **% of snails infected** | **Mean cercs/ snail** | **Mean cercs/ infected 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 | 46±11 | 99±2 | 1263±1263 | 1271±589 |

**Table III. Infection of *B glabrata* Egyptian isolate 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/ infected 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 |

line and that of parasite isolates also in one line called Sm-Gp (*Schistosoma mansoni* gene pool) line. The hybridisation of schistosome isolates is not new. Wrights *et al*. (1974) made hybridisation between *S. mansoni* and *S. interclatum* cercariae, and Wrights *et al.* (1976) published their work on hybridisation of schistosomes and some of its implications.

Our work on establishment of a new line of *S. mansoni* parasite revealed that inter-breeding of different *S. mansoni* lines from various geographical localities resulted into a new line, which was equally infective for snails (Table I-III). However, in some cases, the infectivity of Sm-Gp line was slightly higher than parental lines, which may be due to hybrid vigour. This is in agreement with the findings of Richards (1975) and Wrights *et al.* (1974). They also demonstrated that in some cases hybrids are more compatible with parental stock.

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