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Selection for Resistance to *Schistosoma mansoni* Infections in *Biomphalaria glabrata*

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Abstract.: Commencing with parental stock of *Biomphalaria glabrata* snails, the majority of which were initially susceptible to infection with *Schistosoma mansoni*, selectively bred a line of *Biomphalaria glabrata* snails that displays substantially reduced susceptibility to infection with this parasite. Approximately only 2% of young adult snails of the 7th generation of selected line became infected after exposure to large numbers of miracidia, compared with infection rates of 90 % or more in control batches of un-selected parental snails.

Key words: *Schistosoma mansoni, Biomphalaria glabrata*, resistance

Introduction

The snail intermediate host is an essential link in schistosome life-cycle. Effective control of schistosomiasis requires an understanding of biology of the snail host and also its interaction with the parasite. Susceptible intermediate hosts have been described as snails which provide a suitable habitat and are supportive for the metabolic needs of the parasites. Resistance implies that there are attributes of the host, both innate and acquired, which can impose limitations upon the parasite at any stage of its relationship with host. Individuals of the same species of snails show remarkable variation in their level of susceptibility to schistosome parasites. 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).

The idea of infection-resistant host is not very new. This idea of selection was first put forward in Paris in 1954 at the meeting of the WHO study-group on Bilharzia snail vector identification and classification. Newton (1953) probably was the first who established a highly susceptible snail line after manipulating the genetic makeup of a highly naturally infection-resistant pigmented snail host and a highly susceptible albino coloured snail host. The first selection for an infection-resistant snail host was done by Richards (1972a) who obtained susceptible albino snails from a colony of snails originally established by Newton (Brown, 1978) and crossed them with pigmented refractory stock which he obtained from Dr. Ernest Bueding of Hopkins University. In selecting for refractory stock, juvenile snails that remained uninfected after two exposures of 5 miracidia per snail for 8 hours were isolated and reared singly for selfing. The line of infection-resistant snail established by Richards is known as 10-R2 which was found to have developed resistance against the specific Puerto Rican *S. mansoni* isolate that was passaged in the NIH laboratory, whereas 48% of these snails became infected when exposed to Lac-1 miracidia (a) sub-strain generated by Cooper (1992).

Susceptibility and resistance may be absolute (Wakelin, 1994) and the infectivity of parasite and the susceptibility of host may be altered by deliberate or inadvertent selection (Santana *et al*., 1978). The main aim of this study was to breed and characterize isolates of *Biomphalaria glabrata* that display a high degree of insusceptibility to the infection with *Schistosoma mansoni*.

Materials and Methods

Selection for infection-resistant snail host: Selection for an infection-resistant snail host originally commenced with *Biomphalaria glabrata* Gene Pool (Bg-Gp) snails which remained uninfected after exposure to the *Schistosoma mansoni* Gene Pool (Sm-Gp) parasite isolate.

The Bg-Gp is an interbred pool of 3 snail isolates, i.e. *Biomphalaria glabrata* Puerto Rico isolate (Bg-Pr), Brazil isolate (Bg-Br), and Egyptian isolate (Bg-Eg). The Sm-Gp parasite is pool of 4 geographically distinct *S. mansoni* isolates i.e. Sm-Pr from Puerto Rico, Sm-Br from Brazil, Sm-Ken from Kenya and Sm-Eg from Egypt.

The *Schistosoma mansoni* Gene pool (Sm-Gp) parasite was the main isolate used to infect snails during selection for insusceptibility. Successive batches of about 100 Bg-Gp snails (10-12mm) were mass-infected with Sm-Gp miracidia. After approximately 35 days, snails were screened for infection patency. The non-shedding snails were kept and re-screened 3 times at 14 days intervals. Any snails that were found to have a patent infection were culled.

Representative young adults from above mentioned 3 snail isolates were allowed to interbred and hybridize in an aquarium tank as evidenced by pigmentation of offspring. The young adult offspring (now called Bg-Gp) were tested for their susceptibility to all the above mentioned parasite isolates.

After the 4 screens, 16 non-shedding snails were randomly selected to breed the next generation to be subjected to selection. From this breeding tank 60-100 snails of 5 ± 2mm shell diameter were harvested and left for approximately 4 weeks to grow into adults. These snails were then mass-infected with Sm-Gp miracidia. A tank of unselected Bg-Gp snails was also infected as a control. 35 ± 2 days post infection, both the selected and control snails were screened for patency and the percentage of snails infected and was recorded mean number of cercariae per infected and per total snail.. The non-shedding snails from the selected stock were isolated and subjected to the same protocol of 4 screens at intervals of approximately 14 days, with all patent snails being culled.

For the first 7 generations of selective breeding of snails for resistance to *S. mansoni* infection, 10 snail batches were harvested approximately every 2 weeks, and infected, screened and selected in each generation.

Each generation thus took approximately 20 weeks to complete, I-e., 5 weeks for maturation of the parasite in the snail, 6 weeks between the first and the fourth screening and 8 -9 weeks between initiation of reproduction and offspring being infected.

Results

Before the commencement of the selection program, the above-mentioned snail and parasite isolates were tested for

compatibility and it was found that more than 80% of all three snail isolates became patent when mass-infected with all 5 parasite isolates (Tables 1a, b and c).

Table 2 shows the infection of inter-breed snails (Bg-Gp) with *S mansoni* isolates from different geographical isolates. More than 90% of the Bg-Gp interbred offsprings became infected when exposed to above mentioned 5 parasite isolates.

Table 3 shows the results of infection of the gene-pooled Bg-Gp snail line with Sm-Gp parasite isolate (i.e. the bottom line of Table 2 in more detail). A total of 11 batches of the Bg-Gp snail line were mass-infected with Sm-Gp parasite isolate and an average of 93% of snails were found with patent infection.

Since the 'gene-pooled' parasite line gave an infection pattern that was not different from that of geographically-distinct parasite isolates, it was therefore decided that selection for infection-resistant snail line could commence with the 7% of snails which remained uninfected after exposure to Sm-Gp miracidia.

A total of seven generations were selected after infection of young adult snails. Approximately 10 batches were infected in generation. At the completion of the seventh generation, an average of 2% snails were found with patent infection (Table -4)

Table 4, however, shows that the successive cycles of breeding from snails which failed to become patent after exposure to miracidia has resulted in a progressive decline in susceptibility to infect from 93% in unselected snail stock in F_0 to 2% in selected F_7 generation. Statistical analysis showed an extremely significant difference ($P < 0.001$) in % infected snails in unselected and selected snails.

The mean number of cercariae at the first time of screening also decreased from 2039 ± 731 cercariae / infected snail in unselected F_0 to 42 ± 42 cercariae / infected snail in selected F_7 generation. The ANOVA showed a significant difference in the mean number of cercariae / infected snail in that of unselected and selected snails except in the F_4 generation.

Fig. 1 shows the decline in susceptibility of selected snails in successive generations, whereas susceptibility remained more or less stable i.e. 90 ± 5% in unselected snail stock.

Selected snails that were exposed to miracidia, but did not shed cercariae at the time of first screening, were kept for a further six weeks before a representative group of 16 non-shedder snails was isolated and allowed to breed the next generation. Therefore, snails were re-screened at least four times after the first screening.

Fig. 2a shows the results from the 4 screens that were made approximately at 2 week intervals after first screening. It is observed that a proportion of snails that were negative at the first time of screening became cercarial-shedders later; indeed, some snails first produced cercariae only 10 weeks after the 'normal' pre-patent period. The reduction in number of patent snails at the first time of examination was not due to delayed maturation of the larvae in this host-parasite relationship in all generations (Fig. 4).

A cumulative total of snails found to have a patent infection in all screens as a percentage of total number of snails examined at the first screening (Fig. 2b). The proportion of snails with patent infection appeared to decrease with each successive generation. Mean number of days between first screen of successive batches of snails in the F_1-F_7 generations. (Fig. 3a). Mean number of snails screened in each batches of the F_7 generations(Fig.3b). The combination of results in Fig. 3a and 3b indicated that there was no marked impairment in fecundity of the selective-bred snails, since there was no large decrease in the number of snails screened, nor increase in interval between infection of successive batches.

Discussion

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

The base line experimental work during the present study suggest that an interbred snail line is highly susceptible as its parental lines (Table 1 a, b, and c and Table 2). Files (1951) and Wright and Southgate (1981) showed that hybrid snails are not equally compatible with both parental intermediate host snail stocks, whereas Richards (1975b) in his later work supported the idea that in some cases hybrids are more compatible with the parental, in others (Files, 1951) with the maternal schistosome host snail stock. This may be due to hybrid vigor.

During present study we have found that the percentage infected snails, mean number of cercariae per infected snails and mean number of cercariae per snail decreased as the selection program proceeded to future generations (Table 4). Our findings are in agreement with those of Richards (1972a) and Cooper *et al.*, (1994) in terms of percentage infected snails. There are no published reports available to compare the cercarial output.

Cooper *et al.*, (1994) established an infection-resistant snail line from a highly susceptible snail stock designated as LAC-Line. The first three generations were derived from parasite-negative snails and were allowed to cross-fertilize. All subsequent generations (LACF4-LACF8) were derived from self-fertilized snails. The selection was made from juvenile snails that were individually exposed once to 8-10 miracidia. In the course of 12th generation of selection >90% of snails were non-susceptible. However, in some negative snails, cercarial-laden sporocysts appeared many months after the snails had been scored as negative. In addition to that, insusceptibility was found to have been associated with reduced reproductive potential, because the LAC line of infection-resistant snail host failed to reproduce normally.

During present study, selected snail line reproduced as better as unselected control snails (Fig. 3a and 3b). This may be because, we did not force the snails to breed at our choice i.e. by selfing. The protocol adopted and applied by Richards (1972a) and Cooper *et al.* (1994) may have had some drawbacks. In nature snails live in colonies and experience both self and cross-fertilization and in any endemic area, the snail population is exposed to an unknown number of miracidia. Therefore, the idea of infecting individual snails with a limited number (i.e. 5) miracidia per individual snail and forcing them to self-fertilize may have resulted in a colony of selected snails that was deprived of chance of exchange of genetic characters which could have occurred during cross-fertilization.

Table 1a: Infection of Bg-Br with different geographical isolates of *S. mansoni*

Parasite	Snail			Bg-Br (Pigmented)		
	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 ± 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 1b: Infection of Bg-Br with different geographical isolates of *S. mansoni*

Parasite	Snail			Bg-Br (pigmented)		
	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 1c: Infection of Bg-Eg with different geographical isolates of *S. mansoni*

Parasite	Snail			Bg-Eg (pigmented)		
	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

Table 2: Infection of (interbred snails) with *S. mansoni* isolates from different geographic regions.

Snail Parasite	Bg-Gp					
	No. of batches	Mean days after infection	Mean No. of snails	% positive snails	Mean cercs/ snail	Mean cercs/ infected 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

Table 3: Infection of Bg-Gp snail isolate with Sm-Gp parasite isolate

Batch No.	Parasite	No. of snails	% positive snail	Mean cercs/snail	Mean cercs/infected snail
1	SmGp	90	94	1809 ± 1579	1916 ± 1561
2	SmGp	78	92	1580 ± 1352	1712 ± 1324
3	SmGp	141	97	1482 ± 1352	1536 ± 1474
4	SmGp	110	94	2267 ± 2231	2404 ± 2224
5	SmGp	138	80	3144 ± 3201	3909 ± 3122
6	SmGp	81	90	2360 ± 1697	2390 ± 1687
7	SmGp	98	87	1469 ± 1308	1694 ± 1262
8	SmGp	74	99	2278 ± 1573	2309 ± 1561
9	SmGp	93	94	1370 ± 1021	1465 ± 987
10	SmGp	72	88	1070 ± 1315	1223 ± 1338
11	SmGp	78	99	1852 ± 1834	1876 ± 1833
Mean ± SD	-	95 ± 24	93 ± 6	1880 ± 589	2039 ± 731

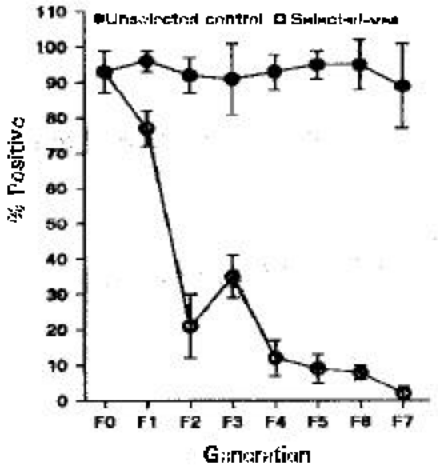

Fig. 1: Showing decrease in susceptibility from zero to 93%

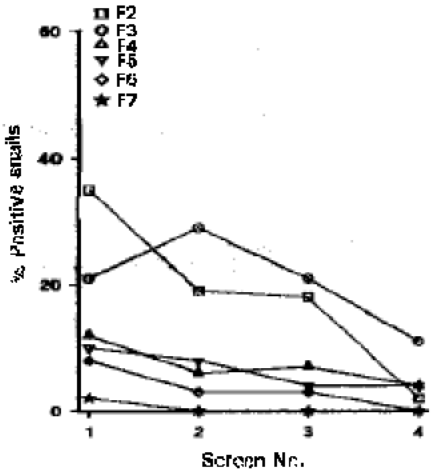

Fig. 2a: Showing % positive snails in 4 screens (F2-F7)

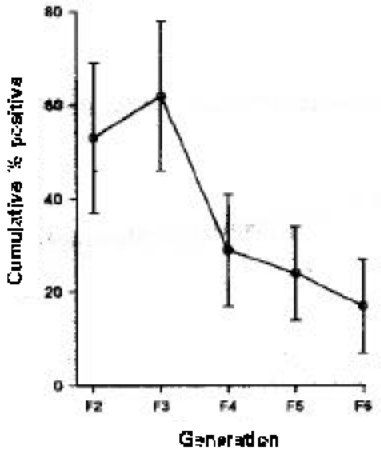

Fig. 2b:Showing total No. of infected snails found in all screens as % of starting population

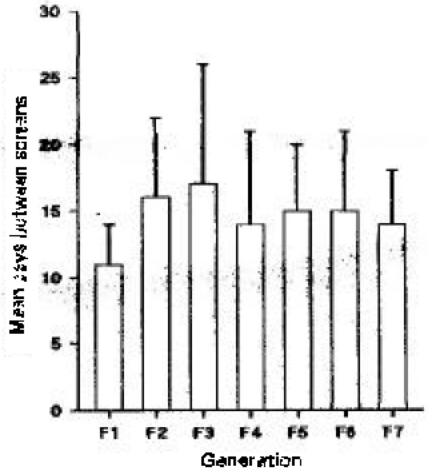

Fig. 3a:Showing mean days between successive screens (F1-F7)

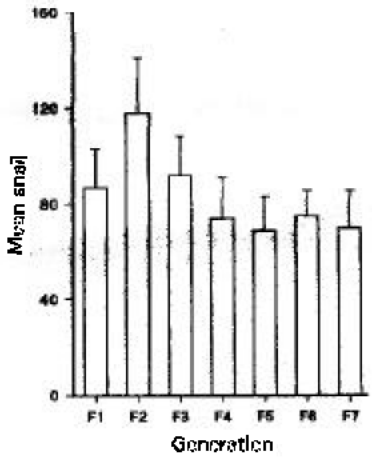

Fig. 3b: Showing mean number of snails screened in batches of F1-F7 generation snails

Both infectivity of the parasite and susceptibility of the snail host may be altered by deliberate or inadvertent selection. Santana *et al.* (1978) showed how deliberate selection procedures involving collection of the self-fertilized offspring of susceptible snails and discarding resistant snails produce very rapid shifts in the overall susceptibility of a population. In one experiment they increased susceptibility of *B. tenagophila* from 7% to 97% in two generations, whereas, we have decreased susceptibility just from 3 to 98 % in course of seven generations of selective breeding program without impairment of fecundity potential. The selective breeding program is continue.

It can be concluded from these results that selective breeding of *Biomphalaria glabrata* spp. snails for insusceptibility to infection with *S. mansoni* has resulted in a significant reduction in the proportion of snails becoming patent after exposure to miracidia, when compared with

Table 4: Infection of seven generations of selectively bred resistant and unselected control snails

Snail	Selected-Ves					Unselected Bg-Gp				
Generation	No. of batches	Mean No. of Snails	% of snails infected	Mean cercs/ snail	Mean cercs/ snail	No. of batches	Mean No. of snail	% of snails infected	Mean cercs/ infected	Mean cercs/ infected snail
F0						11	95 ± 24	96 ± 6	1880 ± 589	2039 ± 731
F1	8	87 ± 16	77 ± 5[a]	1348 ± 525	1754 ± 639	9	90 ± 23	96 ± 3[b]	2834 ± 717	2944 ± 734
F2	8	115 ± 22	21 ± 9[a]	130 ± 108	562 ± 312	8	98 ± 18	92 ± 5[b]	1547 ± 416	1678 ± 449
F3	5	78 ± 27	35 ± 6[a]	290 ± 93 321	844 ±	8	80 ± 26	10[b]	91 ± 831	2030 ± 792	2211 ±
F4	5	69 ± 9	12 ± 5[a]	51 ± 40 757	624 ±	5	70 ± 21	5[b]	93 ± 728	1315 ± 715	1395 ±
F5	7	69 ± 12	9 ± 4[a]	80 ± 79 586	588 ±	5	67 ± 28	4[b]	95 ± 481	1253 ± 523	1338 ±
F6	5	78 ± 10	8 ± 2[a]	60 ± 38	825 ± 405	7	56 ± 6	95 ± 7[b]	2346 ± 857	2454 ± 838	
F7	8	76 ± 18	2 ± 2[a]	2 ± 3	42 ± 42	8	47 ± 16	89 ± 12[b]	2624 ± 612	3229 ± 1011	

Figures in the same row with different superscript show significant difference ($P < 0.05$)

similarly exposed, unselected control snails, and the selected snail line does not show any impairment of reproductive potential.

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