

## Selection for *Schistosoma mansoni* Isolate with High Infectivity

ARIJO, A.G.<sup>1</sup>, N.M. SOOMRO AND M.J. DOENHOFF<sup>†</sup>

Sindh Agriculture University, Tandojam, Pakistan

<sup>†</sup>University of Wales, Bangor, UK

<sup>1</sup>Corresponding author's e-mail: [a\\_arijo@hotmail.com](mailto:a_arijo@hotmail.com)

### ABSTRACT

A parasite line with enhanced infectivity potential has been selected and its infectivity to both infection resistant snails and unselected control snails has been tested. When the selected parasite line was used to infect infection-resistant snails and unselected Bg-Gp control snails, the frequency of patent snails producing large number of cercariae was found to increase when compared with patent snails infected with un-selected Sm-Gp isolate of *S. mansoni*.

**Key Words:** *S. mansoni*; *B. glabrata*; Schistosomiasis

### INTRODUCTION

During selection for an infection-resistant snail host after infection with the *Schistosoma mansoni* gene pool [Sm-Gp] parasite isolate (Arijo *et al.*, 2001), some snails hosts that became patent with infection were found to have shed a large number of cercariae. An attempt was made to select a parasite line from the cercariae shed by in-susceptible snails, and the parasite line, which emerged, has been designated as, Sm-Hc. The Sm-Hc parasite line (that gives a high percentage of snails with patent infection shedding large number of cercariae when compared with that of un-selected Sm-Gp) was intentionally selected to use it as means of selection pressure.

Host-parasite relations between intermediate host snail and trematode parasites are reported to be influenced by the genetic variations in susceptibility in the snail hosts and the infectivity of the trematode parasites. Studies on variations in infectivity in *S. mansoni* and on hybridization in schistosomes have been reported (Files, 1951; Barbosa & Barreto, 1960). Inter-breeding natural populations of *S. mansoni* and inter-breeding natural populations of intermediate snail host may vary qualitatively in the genetic alleles present or absent, and quantitatively in gene frequencies. These variations may 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 any where from 0 to 1000/0, subject also to various environmental factors, and both the infectivity of parasite and susceptibility of snail host may be changed through genetic selection.

### MATERIALS AND METHODS

The procedure of maintenance, infection, screening and selection for infection resistance is already reported by Arijo *et al.* (2001).

Selection for a parasite line with enhanced infectivity potential commenced during production of the F3 generation of the infection-resistant snail line. Offspring of the F2 snails being selected for infection resistance was infected with the Sm-Gp isolate and screened for patency approximately 35 days after exposure to miracidia. Cercariae from 5-10 snails that had shed the largest number of cercariae were pooled and used to infect mice. The method of infection in mice was same as described by Arijo *et al.* (2001). Eggs from the infected mice were hatched and used to mass-infect both the infection-resistant snail line and un-selected control snails. Subsequently this line of parasite was maintained by infecting mice with cercariae shed only by infection resistant snail hosts and the infection of the snail host was done from the eggs that were recovered from mice infected with Sm-Hc cercariae. The respective timings of the snail and mouse infections allowed us to infect at least 3 batches of infection-resistant snails in each generation.

### RESULTS

Tables Ia and Ib illustrate infection of selected in-susceptible snails with the Sm-Gp isolate and the Sm-Hc isolate and Table IIa and IIb illustrates infection of un-selected control snails for Sm-Gp and Sm-Hc isolates. Fig. 1 uses data extracted from Table Ia and Ib and shows the difference in the percentage of infected snails in the selected snail line when infected with Sm-Hc and Sm-Gp isolates. In terms of the percent infected snails, there was no significant difference in the infectivity of Sm-Gp and Sm-Hc during the F3 ( $P=0.38$ ) and F4 ( $P=0.09$ ) generations. However, the Sm-Hc isolate gave a significant difference when compared with Sm-Gp isolate in term of the percentage resistant line snails infected during F5 ( $P<0.05$ ), F6 ( $P<0.01$ ) and F7 ( $P<0.001$ ) when exposed to miracidia as young adults. In terms of the mean number of cercariae per infected snail, the Sm-Hc isolate gave a significant

**Table I. Infection of selectively bred young snails with (a) Sm-Gp and (b) Sm-Hc isolates**

**(a)**

Generation	Parasite isolate	No. batches	Number of snails	% positive snails	Mean cercariae / snail	Mean cercariae / infected snail
F3	Sm-UP	5	78±27	35±6	290±93	844±321
F4	Sm-GP	4	69±9	12±5	51±40	624±757
F5	Sm-GP	7	69±12	9±4	80±79	588±586
F6	Sm-GP	5	78±10	8±2	60±38	825±405
F7	Sm-GP	8	67±18	2±2	2±3	42±42

**(b)**

Generation	Parasite isolate	No. batches	Number of snails	% positive snails	Mean cercariae / snail	Mean cercariae / infected snail
F3	Sm-HC	3	62±11	46±9	404±246	834±338
F4	Sm-HC	4	81±27	29±16	89 ± 35	337±176
F5	Sm-HC	4	63±3	23±14	113±139	369±244
F6	Sm-HC	4	79±5	20±8	195±86	969±297
F1	Sm-HC	3	19±2	15±11	838±126	4016±3516

**Table II. Infection of unselected control snails with (a) Sm-Gp and (b) Sm-Hc isolates**

**(a)**

Generation	Parasite isolate	No. batches	Number of snails	% positive snails	Mean cercariae / snail	Mean cercariae / infected snail
F3	Sm-GP	5	80±26	91±10	2030±831	2211±792
F4	Sm-GP	4	70±21	93±5	1315±728	1395±715
F5	Sm-GP	7	67±28	95±4	1253±481	1338±523
F6	Sm-GP	5	56±6	95±7	2346±857	2454±838
F7	Sm-GP	8	47±16	89±12	2624±612	3229±1011

**(b)**

Generation	Parasite isolate	No. batches	Number of snails	% positive snails	Mean cercariae / snail	Mean cercariae / infected snail
F3	Sm-HC	3	65±36	97±4	2206±1327	2252±1285
F4	Sm-HC	4	66±77	95±4	1137±614	1194±630
F5	Sm-HC	4	80±8	96±3	1894±387	1906±541
F6	Sm-HC	4	69±5	95±2	3193±1190	3408±1337
F7	Sm-HC	3	70±11	98±4	4213±2516	4558±2028

difference only in the F7 generation ( $P<0.001$ ) of the selected snail line. In unselected control snails there was no significant difference either in the percent snails infected or the mean number of cercariae per infected snail.

Fig. 1a-j show the frequency distribution of cercariae produced in 3 h during the first screening for patency. There was a trend towards production of a large number of cercariae in snails infected with Sm-Hc parasite line compared with Sm-Gp both in the selected line of snails and in unselected control snails. Fig. 2 shows that from the F3 through to the F7 generation of selected line snails, there was a significant difference ( $P<0.001$ ) in the infectivity of Sm-Hc to infection-resistant snail hosts when compared with the un-selected Sm-Gp isolate.

## DISCUSSION

The results described above indicate that a parasite line with enhanced infectivity for both the resistant line and un-selected control snails has been produced. Our data showed that the selected Sm-Hc parasite line was more infective for infection-resistant snail line when compared with the infectivity of the un-selected Sm-Gp line. Our data is consistent with the infectivity of *S. mansoni* being genetically determined and that it is amendable to alteration by selection in the laboratory.

Genetics play an 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 the literature is available regarding selection of in-susceptible and susceptible snail lines, but very little information is available on selection of parasite lines with reduced infectivity or with enhanced infectivity potential. Numerous reports of variation in the infectivity of individual miracidia from a single strain of parasite are largely unexplained, though genetic differences between miracidia have been suspected. Our results are in agreement with Kagan and Geiger (1965) who showed that infectivity of the parasite may be altered by repeated passaging it through a resistant snail host. They selected a highly infective parasite line of *S. mansoni* by repeatedly passaging the parasite through a resistant strain of *B. glabrata* and reported a doubling in the average infection rate. Richards (1975) derived two sub-strains designated Lc and Lt lines from the parental L strain of *S. mansoni*. After infection with Lc sub-strain 0% infection was seen by single miracidial penetrations and 30% snails became infected with Lt sub-strain; whereas, the rate of infection with parental L strain was 4%.

One reason for selection of a parasite line with enhanced infectivity potential was that it may be useful in increasing the pressure of selection for resistance in the snail selection program against *S. mansoni* infection (Arijo *et al.*, 2001) Table Ia and Iib and Fig. 3 confirm that, as the selection proceeded, susceptibility of infected snails decreased and from Table IIa and Iib, a trend can be seen where the infectivity of the selected parasite is increasing. Clarke (1979) reported that host and parasite co-evolve in a mutually aggressive manner, with selection favoring greater virulence in the parasite and greater resistance in the snail host. After surviving infection with a high virulent parasite, the resistant potential of snail host is increased and this may enable them to survive the infectivity potential of parasite. Likewise, the infectivity potential of the parasite may increase in particular when a parasite can manage to escape the strong defensive immune system of a resistant snail host. Thus, a higher percentage of resistant line snails became patent when infected with the Sm-Hc parasite when compared with Sm-Gp isolate. Evidence of an increase in the infectivity potential of Sm-Hc isolate may be seen in terms of a greater number of cercariae produced by

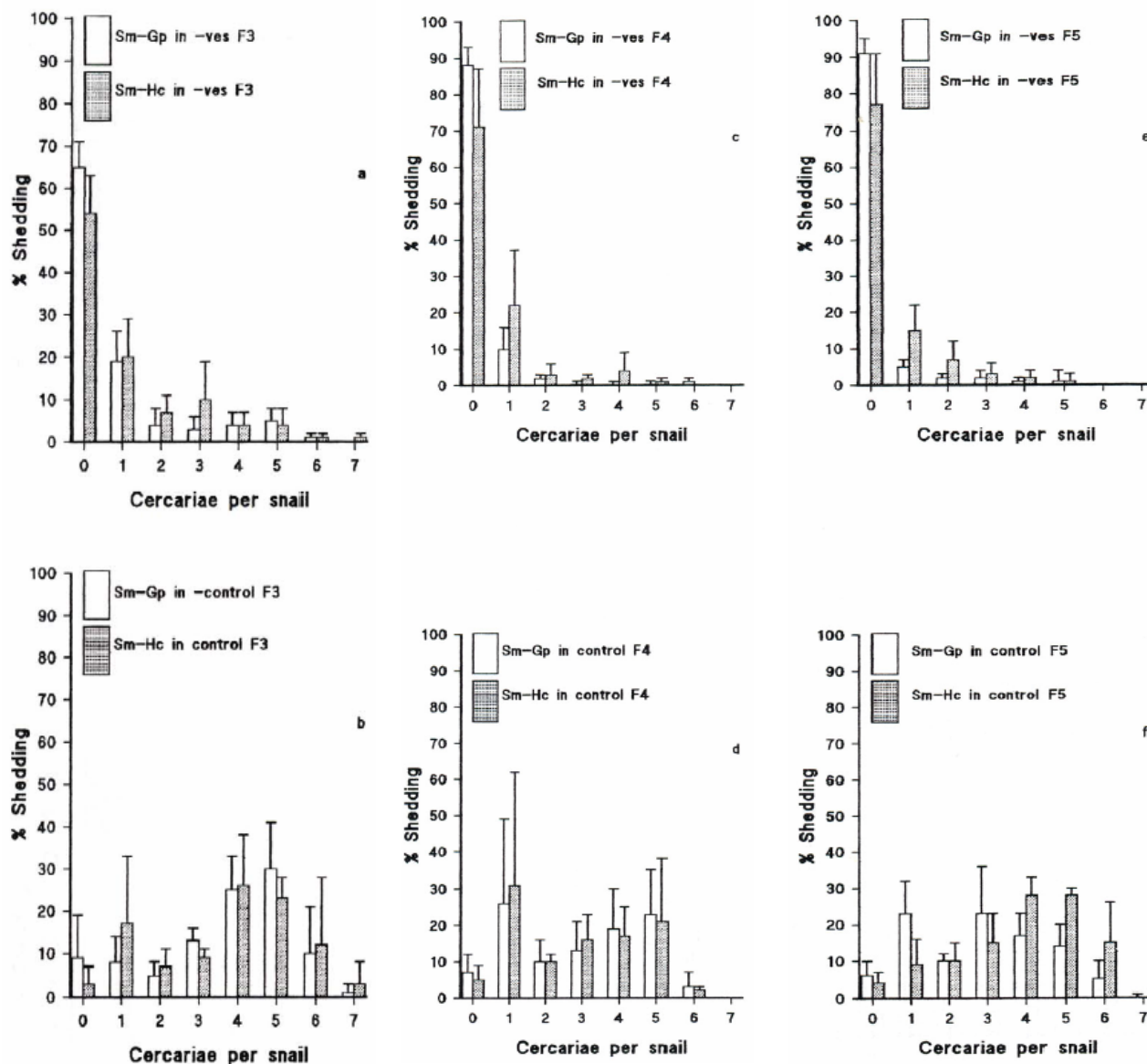
Fig. 1a-j. are the frequency distribution of cercariae shed by individual snail

Sm-Gp= Gene pool line of *S mansoni* parasite

Sm-Hc= A parasite line established for higher infectivity potential

-ve= Infection-resistant snail line

Control= Unselected snail line



unselected control snails (Fig. 1, j & k). The data however, showed a non-significant difference but the trend showed that the infectivity potential of parasite was increasing.

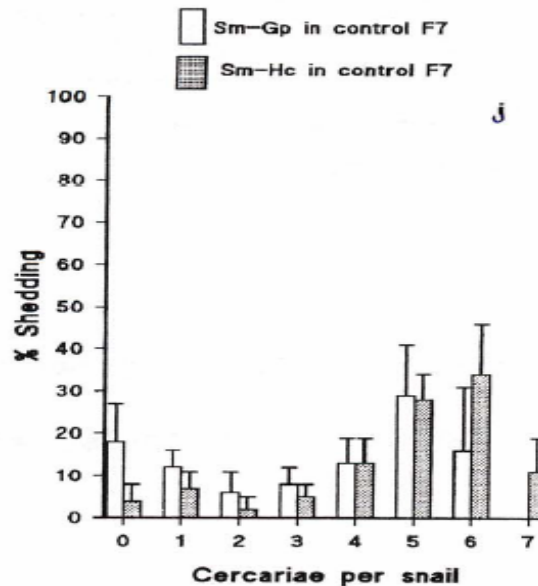
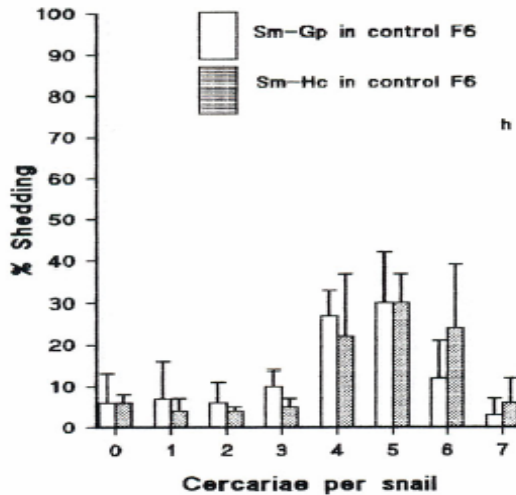
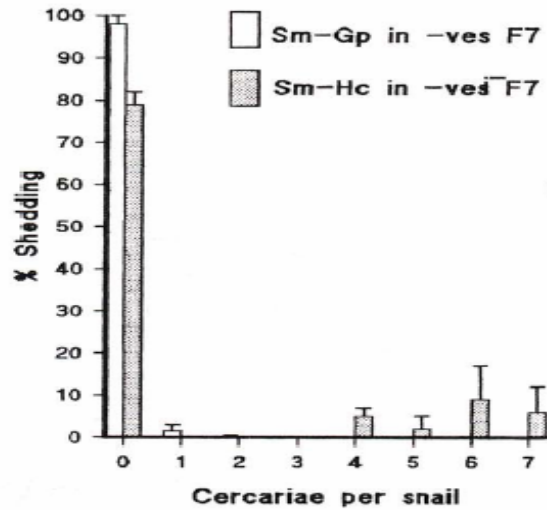
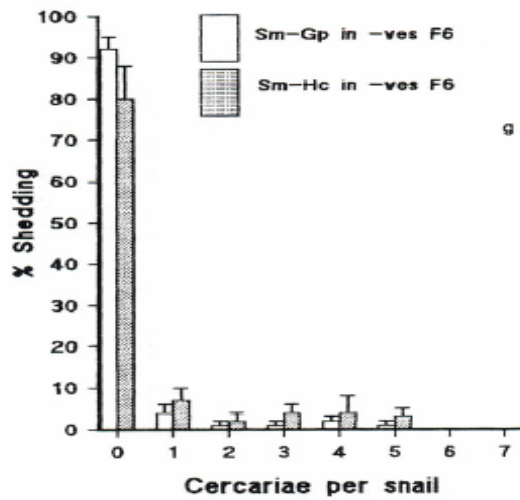
Woodruff (1985) concluded that there is a little merit in selecting for resistance against only a small fraction of the parasite genome. He suggested that it is important that snail lines being selected for infection-resistance are exposed in a standard manner to as much of the spectrum of local *Schistosoma* infectivity as possible. There are indeed published reports that resistance in a selected line of snail

may remain more specific than general if it has been induced by the use of single parasite isolate (Lie *et al.*, 1978).

## REFERENCES

- Arijo, A.G., M.J. Doenhoff and N.M. Soomro, 2001. Selection for resistance to *Schistosoma mansoni* infections in *Biomphalaria glabrata*. *Online J. Biol. Sci.*, 7: 651-5

Fig. 1 Continued....



Barbosa, F.S. and A.C. Barreto, 1960. Differences in susceptibility of *Biomphalaria glabrata* to *S. mansoni*. *Exp Parasitol.*, 9: 137-40  
 Clarke, B.C., 1979. The evolution of genetic diversity. *Proc Royl Soc London*, 205: 453-74  
 Files, V.S., 1951. As study of the vector parasite relationships in *S. mansoni*. *Parasitol.*, 41: 264-9  
 Kagan, I.G. and S.J.C. Gieger, 1965. The susceptibility of three strains of *Australorbis glabratis* to *Schistosoma mansoni* from Brazil and Puerto Rico. *J. Parasitol.*, 51: 622-7

Lie, K.J, D. Heynmen and C.S. Richards, 1979. Specificity of natural resistance to trematode infections in *B. glabrata*. *Int. J. Parasitol.*, 9: 529-31  
 Richard, C.S., 1975. Genetic factors in susceptibility of *B. glabrata* for different strains of *S. amsnoui*. *Parasitol.*, 70: 231-41  
 Woodruff, D.S., 1985. Genetic control of Schistosomiasis: A technique based on the genetic manipulation of intermediate host snail populations. *Advance Comp Pathol.*, 8: 41-68

(Received 01 April 2005; Accepted 28 June 2005)