HYBRIDIZATION OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA JAPONICUM* IN MICE

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Abstract. Crossing experiments in mice with two human species of *Schistosoma japonicum* (Taiwan strain) and *Schistosoma mansoni* (Puerto Rican strain) were performed. The hybrid miracidia from the cross between female *S. japonicum* and male *S. mansoni* infected both *Biophalaria glabrata* and *Oncomalania h. chiui*. However, those from the reciprocal crossing could infect only *B. glabrata*. *B. glabrata* infected with hybrid miracidia of female *S. mansoni* x male *S. japonicum* survived up to 30 days while those infected with miracidia of *S. mansoni* remained alive for more than 100 days after the first shedding of cercariae. Relatively few hybrid eggs reached maturity either in tissues or in the feces of infected mice. A low percentage of F1 eggs hatched and the infectivity of F1 miracida was also low. Morphology and behavior of hybrid eggs, miracidia, cercariae, and adults were similar to the maternal species. The daily egg production of the hybrid worm pair was less than that of the normal one. The observations in the present study may be attributed to the maternal effects. However, the phenomenon of parthenogenesis in schistosomes cannot be confirmed.

INTRODUCTION

Schistosoma japonicum, *S. mansoni*, and *S. haematobium* are the schistosomes of medical importance. *S. japonicum* is distributed in the Far East, *S. mansoni* in Africa, South America, and islands of Caribbean Sea, and *S. haematobium* in Africa and Middle East (Beaver *et al*, 1984). Since the latter two species have common endemic areas in Africa, it is possible that hybridization of these human schistosomes occurs in this part of the world. According to Ali and Eissa (1984), *S. mansoni* eggs have been observed more frequently in the urine than in the stool specimens of the patients in certain foci in Egypt. These findings are contrary to the well-known fact that eggs of *S. mansoni* are often in the stool and those of *S. haematobium* in the urine (Beaver *et al*, 1984). In Mali, schistosomiasis due to hybrids of schisotosomes has been reported in the Dogon country (De Clercq *et al*, 1994). In Cuba, a patient from the eastern region of Africa were found to pass characteristic eggs of *S. intercalatum* in feces and urine by the modified technique of Ziehl-Neelsen. This patent was considered to be infected by the hybrid between *S. intercalatum* and *S. haematobium* (Villaverde Ane *et al*, 1997). Khalil and Mansour (1995) accomplished the hybridization of these two species and examined the effects of species and sex interactions on the development of these schistosomes, egg laying capacity and the excretion routes of eggs.

Although interspecific hybridization between several schistosome species has been reported (Taylor, 1970; Wright *et al*, 1974; Wright and Ross, 1980; Kruatrachue *et al*, 1987; Bremond *et al*, 1989; Theron, 1989), there is little information on the hybridization between the human schistosomes of medical importance (Vogel, 1941, 1942; Khalil and Mansour, 1995). The present study was designed to investigate the hybridization of *S. mansoni* and *S. japonicum* in mice.

MATERIALS AND METHODS

Parasites and snails

In the present study, *S. japonicum* (Taiwan strain) and *S. mansoni* (Puerto Rico strain) em-

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ployed in the hybridization experiments were maintained in albino mice in our laboratory. The intermediate host for the former species was *Oncomelania h. chiui* and that of the latter species was *Biomphalaria glabrata*.

Experimental infection of snails

Albino mice infected with *S. japonicum* or *S. mansoni* were sacrificed and the liver was then removed and placed in a Warning blender. The sediment was washed with normal saline until clear and put into a Petri dish (15 x 1.5 cm) with dechlorinated water. The Petri dish was placed in a Sargent incubator at 30°C for 1 hour.

Miracidia were collected from the dish using a capillary pipette under a dissecting microscope. After placing one miracidium into each of 50 vials (2 x 2 cm), one snail of *O. h. chiui* or *B. glabrata* was placed in one vial, then filled with dechlorinated water. A cover glass (22 x 22 mm) was placed on each vial and then placed under a 40-watt incandescent lamp overnight. *B. glabrata* were then maintained in aquaria (21 x 26 x 35 cm) and fed with fresh lettuce. *O. h. chiui* were reared in Petri dishes containing disinfected soil. Examination for cercariae was carried out 3 weeks after miracidial exposure in *B. glabrata* and 1.5 months in *O. h. chiui*.

Infection of mice

Determination of sex of cercariae. Cercariae from each infected snail were used to infect two albino mice. The mice were anesthetized by percutaneous injection of pentobarbital and fixed on a small operation pan (40 x 29 x 2.5 cm) fixed with adhesive tape. Hairs on the abdomen were shaved and the belly wetted with dechlorinated water. A specified number of cerceriae was placed with a capillary pipette on a cover glass (18 x 18 mm) which was inverted and placed on the abdomen and left for 1 hour. The abdomen was kept wet throughout by adding a few drops of dechlorinated water every 15 minutes. The mice were kept in a cage and fed with a regular diet. Four to five weeks after infection, the mice were sacrificed and worms collected with a perfusion technique (Duvall and DeWitt, 1967). Sex of cercariae was then determined.

Hybridization. Thirty male cercariae of *S. japonicum* were placed on a cover glass and 30 females of *S. mansoni* on another. The two cover glasses were then placed upside down on the abdomen of an anesthetized mouse for 1 hour. Mice were similarly infected with 30 male cercariae of *S. mansoni* and 30 female cercariae of *S. japonicum*.

Stool examination

One month after the exposure, stool samples were collected and examined by concentration method for schistosome eggs (PUMC, 1934) twice a week to determine the first appearance of eggs. In addition, a magnetic stirrer technique (Fan, 1971; Fan *et al,* 1972) was used to determine the daily output of eggs by the hybrids in the stool. Developmental stages of eggs in the stool were also determined by the method described by Pellegrino *et al* (1962) and Hsu and Fan (1973).

Tissue examination

From the liver, small intestine, large intestine and other viscera (spleen, lungs, and lymph glands, etc), 10 pieces of tissue from each organ were taken and placed on a large slide (75 x 36 mm). The tissues were then pressed hard with a small slide (50 x 50 mm). The pressed specimens were examined under a dissecting microscope and the stage of maturation in 100 schistosome eggs were determined using the method of Hsu and Fan (1973).

Hatching rates of hybrid eggs

Hybrid eggs were separated from the liver, small intestine, and large intestine. Hatching rates of 100 hybrid eggs from each organ were compared under the same conditions.

Adult morphology of Schistosome hybrids

For the measurement of the position of cecal union of male adults, the worms were placed on a Turtox metric-inch slide. The distance between the posterior margin of oral sucker and the cecal union was measured under a dissecting microscope. At the same time, the tegument of males and eggs in the uterus were observed.

Longevity of *B. glabrata* infected with Schistosome hybrids

B. glabrata infected with miracidia of hybrid schistosomes were separately reared in 250-ml beakers (with a cover) containing

Table 1
Susceptibility of *Biomphalaria glabrata* and *Oncomelania h. chiui* to F1 miracidia produced by schistosomes.

Hybrid (Female x Male)	No. of miracidia/ snail	No. of snails				Prepatent period (days)	
		Exposed	Survived	Infected	% infected	Mean	Range
Oncomelania h. chiui							
S.m. x *S.j.*	3	79	79	0	0		
S.j. x *S.m.*	1	74	74	3	4	133	126-148
S.j. x *S.m.*	5	79	35	16	17	116	106-126
Biomphalaria glabrata							
S.m. x *S.j.*	1	227	158	3	2	29	27-34
S.m. x *S.j.*	3-5	143	96	13	14	45	35-89
S.j. x *S.m.*	1	31	22	2	9	127	127-127
S.j. x *S.m.*	5	11	10	1	10	94	94

dechlorined water with fresh lettuce. Dechlorined water was changed every five days and the conditions of the snails were observed.

RESULTS

Susceptibility of *O. h. chiui* and *B. glabarata* to hybrid miracidia

O. h. chiui was susceptible to F1 miracidia between female *S. japonicum* and male *S. mansoni*, showing infection rates of 4% and 17% given 1 and 5 miracida/snail, respectively. The prepatent periods were 126-148 days and 106-126 days, respectively. However, this snail was not susceptible to F1 miracidia produced by the crossing of female *S. mansoni* and male *S. japonicum* (Table 1).

B. glabrata was susceptible to F1 miracidia from female *S. mansoni* and male *S. japonicum* and those from the crossing of female *S. japonicum* with male *S. mansoni*. The rates of susceptibility and prepatent period for the former hybrid were 2% (1 miracidium/snail) and 14% (3-5 miracidia/snail) at 27-34 days and 35-89 days, respectively, and those for the latter hybrid were 9% (1 miracidium/snail) and 10% (5 miracidia/snail) at 94 and 127 days (Table 1), respectively.

Life-span of *B. glabrata* after cercarial emergence

B. glabrata infected with F1 hybrid miracidia from female *S. mansoni* and male *S. japonicum* survived for 30 days after the first emergence of cercariae. However, snails infected with *S. mansoni* survived for more than 100 days (Table 2).

Table 2
Life-span of *Biomphalaria glabrata* infected each with one miracidium of *Schistosoma mansoni* and hybrid miracidia (female *S. mansoni* x male *S. japonicum*).

Survival interval (days)	With one hybrid miracidium		With one *S. mansoni* miracidium	
	No.	%	No.	%
1-10	5[a]	33	2[b]	3
11-20	9	60	18	29
21-30	1	7	13	21
31-40	0	0	3	5
41-50	0	0	4	7
51-60	0	0	4	7
61-70	0	0	4	7
71-80	0	0	3	5
81-90	0	0	0	0
91-100	0	0	1	2
Over 100	0	0	10	16
Total	15	100	62	100

[a]Two snails died on day 1, two on day 2, and 1 on day 5 of the beginning of cercarial emergence.
[b]Two snails died 9 days after first cercarial emergence.

Development of hybrid eggs

The percentage of viable eggs from female

Table 3
Development of *japonicum* and F1 hybrid (female *Schistosoma japonicum* x male *S. mansoni*) eggs in Swiss albino mice.

Parasite (female x male)	No. of mice	Age of worms (weeks)	Organs	Viable eggs (%)			Dead eggs
				Immature (1^{st}-4^{th})	Mature (5^{th})	Total	%
S. japonica x *S. mansoni*	2	15	Liver	22	2	24	76
			Small intestine	27	2	29	71
			Large intestine	25	2	27	73
			Others	26	1	27	73
	Mean			25	2	27	73
S. mansoni x *S. japonica*	2	14	Liver	7	3	10	90
			Small intestine	17	2	19	81
			Large intestine	9	1	10	90
			Others	22	1	23	77
	Mean			14	2	16	84

Table 4
Number and developmental stage of schistosome eggs in the feces of normal and F1 hybrid infected Swiss albino mice.

No. of mice used	Age of female (weeks)	No. of stool samples exam	Number and development of eggs				Total no.
			Viable		Dead		
			Immature (1^{st}-4^{th})	Mature (5^{th})	Shell	Others	
S. mansoni x *S. japonica*							
4[a]	12-17	22	9	1/10 (10.0%)	5	369/374 (97.4%)	384
S. mansoni							
4[b]	12-36	12	3	53/56 (90.4%)	0	6/62 (9.7%)	62

[a]Each infected with many pairs of worms.
[b]Each infected with one pair of worms.

S. japonicum x male *S. mansoni* in the liver, small intestine, large intestine, and other organs was 27%. However, only 2% of the hybrid eggs developed to maturity. In the mice infected with a hybrid of female *S. mansoni* and male *S. japonicum*, the percentage of viable eggs was 16%, only 2% of these eggs reach maturity (Table 3).

Number and developmental stage of eggs

Although 384 eggs were found in the feces of mice infected with many pairs of hybrids of female *S. mansoni* x male *S. japonicum*, only one (10.0%) was found to be mature, 9 were in the 4th stage and 374 were dead. However, 90.3% eggs recovered from mice infected with one pair of *S. mansoni* were found to be mature (Table 4).

Hatching of hybrid eggs recovered from tissues

From four mice infected with a total of 14 hybrids of female *S. japonicum* x male *S. mansoni*, 64 miracidia hatched from the liver, 4 from the small intestine and 6 from the large intestine. However, in the reciprocal crossing (female *S. mansoni* x male *S. japonicum*) only 6 miracidia hatched from eggs recovered from the liver of 2 mice (Table 6). For the hybrid eggs (female *S. japonicum* x male *S. mansoni*) from

Table 5
Hatching test of F1 hybrid eggs of schistosomes from the tissues of Swiss albino mice.

No. of mice used	Age of worms (weeks)	No. of mature females	Miracidia hatched from whole organs of			
			Liver	Small intestine	Large intestine	Total
S. japonica x *S. mansoni*						
4	11-15	14	64	4	6	74
S. mansoni x *S. japonica*						
2	14	2	6	0	0	6

Table 6
Morphology of parent and F1 hybrids from schistosmes.

Male worm						Female worm					
No. exam	Tegumental tubercule and spines		Cecal union and ovary location			No. exam	Morphology of intrauterine eggs		Cecal union and ovary location		
	Present	Absent	Ant	Mid	Pos		S. m.	S. j.	Ant	Mid	Pos
Female *S. mansoni* x male *S. japonica*											
19	19	0	0	2	17	10	10	0	10	0	0
S. japonica											
110	0	110	107	1	2	100	100	0	99	1	0
Female *S. japonica* x male *S. mansoni*											
19	0	19	0	0	19	3	0	3	0	3	0
S. mansoni											
84	84	0	0	0	84	40	0	40	0	40	0

the liver, 7 of 100 hybrid eggs hatched. For the hybrids of female *S. mansoni* x male *S. japonicum*, 22 eggs hatched from 6 g of hepatic tissues (Table 5).

Morphology of worms

All of the 19 male hybrids resulting from female *S. mansoni* x male *S. japonicum* had tubercules and spines on their tegument and the cecal union was located in the anterior part of 13 of them. However, no tubercules and spines were observed on the tegument of 110 male *S. japonicum* and their ceca united in the posterior part of most (107) worms. Moreover, intrauterine hybrid eggs were *S. mansoni*-like and the ceca of all females united in the anterior part of the body (Table 6).

In the 19 male hybrids from female *S. japonicum* x male *S. mansoni*, none had tegumental tubercules and spines and ceca were united in the posterior part of the body. However, the tubercules and spines were observed on the tegument of 84 males of *S. mansoni* and the cecal union was in the anterior part of most (67) worms. Moreover, intrauterine hybrid eggs were *S. japonicum*-like and the ceca of all female worms united in the posterior part. All intrauterine eggs were *S. japonicum*-like and their cecal union was in the posterior part of all (40) worms (Table 6).

Egg production capacity between normal and hybrid (F1) Schistosomes

Although eggs of *S. japonicum* were first found in the liver of the mice on day 25 after infection, those of female *S. japonicum* x male *S. mansoni* worm pairs were found on day 40. However, eggs of female *S. mansoni* x male *S. japonicum* worm pairs were found in the liver on day 29 and those of *S. mansoni* on day 36.

Table 7
Egg production capacity between normal and hybrid (F1) schistosomes in mice.

	S. j.	*S. j.* x *S. m.*	*S. m.*	*S. m.* x *S. j.*
1. Age of worms	95	82	172	165
2. No. of mature females	12	9	6	7
3. Eggs found in liver at 1st day after infection	25	40	36	29
4. Total no. of eggs deposited in tissues/female	86,408	61,569	21,760	15,912
5. Eggs found in feces at 1st day after infection	33	46	45	57
6. Total no. of eggs passed in feces/ female	70,618	14,300	20,574	1,389

Table 8
Comparison of early egg finding and egg production by one pair of normal and hybrid of schistosomes.

Species	Days after infection		No. of eggs/ day/pair
	Eggs in liver	Eggs in feces	
S. j. (1 F + 1 M)	26	40	3,000
S. j. (1 F) x *S. m.* (1 M)	33	46	1,060
S. m. (1 F + 1 M)	34	50	300
S. m. (1 F) x *S. j.* (1 M)	37	54	15

The total number eggs deposited in tissue per female were 86,408 by *S. japonicum*, 61,569 by female *S. japonicum* x male *S. mansoni* worm pairs, 21,760 by *S. mansoni*, and 15,912 by female *S. mansoni* x male *S. japonicum* worm pairs. In addition, eggs of *S. japonicum* were first found in the feces on day 33, those of female *S. japonicum* x male *S. mansoni* worm pairs on day 46, those of *S. mansoni* on day 45, and those of female *S. mansoni* x male *S. japonicum* worm pairs on day 57. The total number eggs passed in the feces per female were 70,618 by *S. japonicum*, 14,300 by female *S. japonicum* x male *S. mansoni* worm pairs, 20,574 by *S. mansoni*, and 1,389 by female *S. mansoni* x male *S. japonicum* worm pairs (Table 7).

Daily output of eggs between normal and hybrid (F1) Schistosomes

The daily output of female *S. japonicum* x male *S. mansoni* worm pairs (1,060 eggs/day/pair) was less than that of *S. japonicum* (3,000 eggs/day/pair). Similarly, the daily output of female *S. mansoni* x male *S. japonicum* worm pairs (15 eggs/day/pair) was also less than that of *S. mansoni* (300 eggs/day/pair) (Table 8).

DISCUSSION

The bionomics, sites of infection, and pathogenicity of *S. japonicum* and *S. mansoni* are very similar. However, there are significant differences in their morphological characteristics, geographic distribution, intermediate hosts, and reservoir hosts. The tegument of male worms of *S. japonicum* is non-tuberculate and the intestinal ceca unite in the posterior region of the body. The average number of testes is 7. The intestinal ceca of female worms unite in the middle portion of the body and the uterus egg number is more than 30. The egg is oval in shape and has a small lateral spinose process. This species is prevalent in Mainland China, Taiwan, Japan, the Philippines, and Indonesia. Its intermediate hosts include various subspecies of the amphibious Oncomelanian snails (*O. h. hupensis*, *O. h. formosana*, *O. h. chiui*, *O. h. quadrasi*, and *O. h. lindoensis*. Domestic animals (dogs, cats, cattle, water buffaloes, horses, hogs, sheep, goats), rodents and primates are the reservoir hosts (Beaver *et al*, 1984).

There are conspicuous tubercules on the tegument of male *S. mansoni* and the intestinal ceca unite anterior to midbody. The intestinal ceca of female worms also unite in the anterior part of the body and the uterus contains no more than 4 eggs. The egg is spindle shape and has a prominent lateral spine. This species is prevalent in Africa, Near East, South America, and West Indies. Its intermediate hosts

are snails of *Biomphalaria* (*B. glabrata*, *B. alexandrina*, *B. sudanica*, *B. ruppellii,* and *B. pfeifferi*). Gerbils, opossums rodents and monkeys are the reservoir hosts of *S. mansoni* (Beaver *et al*, 1984). Moreover, special characteristics in the ultra-structure of the tegumental surface in the F1 hybrid schistosome between *S. mekongi* in man and *S. japonicum*-like (Malaysian) in rodents have also been reported (Kruatrachue *et al*, 1987).

Although *S. mansoni* and *S. japonicum* are distinctive species, their experimental cross has been accomplished in mice and hamsters, but relatively few of the resulting ova reach the miracidial stage (Vogel, 1942). Miracidia from the crossing of male *S. mansoni* and female *S. japonicum* can infect the normal snail hosts of both species (*Biomphalaria* and *Oncomelania*), but those from the reciprocal crossing can infect only *Biomphalaria* snails. However, the fact that the resulting cercariae and adults resemble in morphology and behavior the maternal species may suggest that they have derived from parthenogenetic development.

Vogel (1941) infected mammalian hosts with heterologous schistosome species in his experiments as follows: *S. mansoni* (female) x *S. japonicum* (male), *S. japonicum* (female)x *S. mansoni* (male), *S. mansoni* (female) x *S. haematobium* (male), *S. haematobium* (female)x *S. mansoni* (male), *S. haematobium* (female)x *S. japonicum* (male), and *S. japonicum* (female)x *S. haematobium* (male). In each case, the males copulated with the females of different species and stimulated female's development to sexual maturity. The growth of the females was also stimulated but they did not attain the full size from the homologous mating. In some instances, the growth of the male worms was stimulated and in others it seemed to be retarded. Short (1948) performed similar crossing experiments using males of *Schistosomtium douthitti* and females of *Schistosoma mansoni*. Although the females remained small, many of them attained sexual maturity. Thus it seems that the influence of the male schistosome on the growth and sexual development of the female is not species specific (Vogel, 1941), nor is it genus specific (Short, 1948).

In the crosses between *S. mansoni*, *S. haematobium*, and *S. japonicum*, the F1 eggs, which produced, were of low viability and infectivity to the snail hosts (Vogel, 1941, 1942). The results of the present study confirm the findings of Vogel (1941, 1942). Relatively few hybrid eggs became mature, either in the tissues or in the feces of infected mice. A low percentage of miracidial hatching, as well as a relatively low infectivity of F1 miracidia were observed. Moreover, we found the life span of *B. grabrata* was shortened to less than 30 days after the first emergence of with hybrid cercariae resulting from the cross of female *S. mansoni* and male *S. japonicum*. In addition, the location of the ovary in the hybrid females from *S. japonicum* x *S. mansoni* was mostly in the posterior part (89%) of the worms. However, in the reciprocal crossing (*S. mansoni* x *S. japonicum*), all of the ovaries were located in the anterior part of worms.

In the present study, the hybrid eggs (*S. japonicum* x *S. mansoni*) could be recovered from the liver and small intestine at 33 days and in the stool at day 46 after infection. However, the corresponding figures for the normal eggs of *S. japonicum* were days 26 and 42, both appearing at earlier dates. It is interesting to note that there was no significant difference in the morphology of the normal and hybrid eggs. These results agree with the findings of Vogel (1942) that the characteristics of worms and eggs of the hybrids are determined by the maternal species. Moreover, similar findings were also obtained in a previous hybridization study (Lo, 1989). Although Vogel (1942) suggested that this phenomenon is probably due to parthenogenesis, it is difficult to ascertain the actual occurrence of parthenogenesis in natural populations of schistosomes (Jourdane *et al*, 1995) and further studies on this topic at the molecular level are necessary (LoVerde and Chen, 1991).

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