REVIEW

STRAIN COMPLEX OF *SCHISTOSOMA JAPONICUM* IN THE MAINLAND OF CHINA

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Abstract. The present paper deals with studies on the characteristics of *Schistosoma japonicum* isolated from five localities in the mainland of China. The following items were observed and compared including morphometric data, susceptibility of six mammalian hosts, prepatent period, compatibility between larvae and snail hosts, size of hepatic granuloma produced by eggs, immunoreactions in experimental animals, sensitivity to praziquantel, SDS-PAGE protein pattern and its antigenicity analysis, DNA hybridization and genetic variation and differentiation by analysis with multilocus enzyme electrophoresis. By means of these multidisciplinary methods, from morphological to molecular level, the following conclusions may be drawn from our results. The evidence indicates firstly that *S. japonicum* in the mainland of China comprises a strain complex with several components of geographically distributed strains. At least four distinct strains exist, *ie* Yunnan, Guangxi, Sichuan and Anhui-Hubei. Characteristics of each strain are distinct and the results of these studies lead to discussion on the problem of the intraspecific and inter-strain differentiation of *S. japonicum* in the mainland of China.

INTRODUCTION

Up to the present, *Schistosoma japonicum* endemic in the mainland of China has been considered to be only one strain, the Chinese mainland strain. It is well known that the endemic areas of schistosomiasis japonica in the Chinese mainland are mainly confined to regions south of Yangtze River, but they are discontinuous, showing conspicuous geographic and topographic isolation. It is very likely that due to geographic isolation, more than one strain of *S. japonicum* exists in the vast Chinese mainland. So far, little is known about the characteristics of schistosomes isolated from various topographic areas. We have tackled the problem by comparing the biological, biochemical, pathological, immunological and chemotherapeutic characteristics of *S. japonicum* isolated from different localities.

Fig 1—Southern part of the mainland of China showing historical distribution of schistosomiasis japonica (barred area) and source of schistosome isolates used in the present study.

DISTRIBUTION OF ISOLATES

Naturally infected *Oncomelania hupensis* were collected from the following five localities (Fig 1):

1. Guichi in Anhui (A), at the lower reaches of the Yangtze River in East China;
2. Jianli in Hubei (H), at the middle reaches of the Yangtze River in Mid China;
3. Guiping in Guangxi (G), a karst land in South China;
4. Tianquan in Sichuan (S), a mountainous region in West China;
5. Eryuan in Yunnan (Y), high plateau in Southwest China.

S. japonicum cercaria shedding was stimulated by fluorescent lights and these cercariae were coded isolates A, H, G, S and Y, respectively.

MORPHOMETRIC DATA

Size of cercariae

The size of cercariae collected from pooled naturally infected snails from the five different isolates is shown in Table 1. The body index of the Yunnan isolate, which is defined as cercaria body length × body width (mm) × 1000, was found to be smaller than those of the other 4 isolates, this difference being significant at $p<0.01$ (He *et al,* 1991a).

Size of adult worms and number of testes

Measurements of adult worms from the five different isolates were taken from five kinds of animal hosts infected with the same number of cercariae and for the same duration of infection. Results showed that the mean length of mature pair-worms from the Yunnan isolate was considerably smaller than that of the other 4 isolates from the permissive hosts such as mice, hamsters, jirds, rabbits and rhesus monkeys (Fig 2). This difference was significant at $p<0.05$ by analysis of variance.

The testes in male *S. japonicum* varied from 2 to 13, the majority being 7 in number. The percentages (45.5-63.9%) of specimens with 7 testes in the Yunnan isolate from the five permissive hosts were significantly less than those (71.1-90.5%) in the other 4 isolates, this difference being significant at $p<0.01$ (He *et al,* 1991b).

Size and shape of mature eggs

Comparison was made with mature eggs of *S. japonicum* from the feces of the five permissive hosts. It was found that the size and shape of the eggs varied not only in different host species infected with the same isolate of the parasite, but also among host individuals of the same species infected with the same isolate of parasite. Judging from the index defined as ratio of egg width/length × 100, the eggs of the Sichuan isolate were broad and short in shape, giving a high index (73.8 ± 5.6-77.3 ± 7.1). Those of Guangxi (66.8 ± 8.2-73.1 ± 8.0) and Hubei (69.8 ± 6.7-75.7 ± 5.2) isolates were oblong, giving the lowest index. The other two isolates from Yunnan (71.7 ± 6.9-75.5 ± 8.3) and Anhui (72.5 ± 6.3-74.9 ± 21.2), lay between those two extremes (He *et al,* 1991b).

Table 1

Size of cercariae from five different isolates of *S. japonicum* in the mainland of China.

Isolate	Body index $\bar{X} \pm SD$	Tail index $\bar{X} \pm SD$
Anhui	8.6 ± 0.9 (113)	3.3 ± 0.3 (111)
Hubei	8.7 ± 0.9 (105)	3.2 ± 0.5 (103)
Guangxi	8.8 ± 0.6 (100)	3.3 ± 0.4 (83)
Sichuan	8.5 ± 0.4 (105)	3.2 ± 0.3 (103)
Yunnan	8.0 ± 0.7 (110)	3.1 ± 0.4 (110)

Note: Number of cercariae measured in parentheses

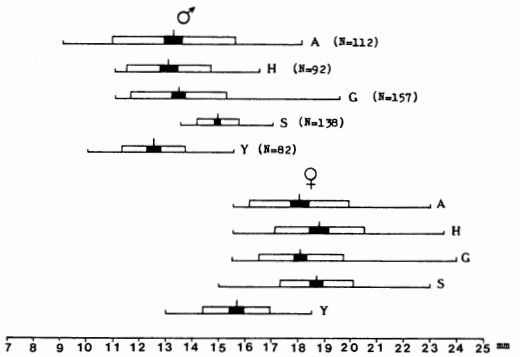

Fig 2—Body length of pair-worms in five different isolates of *S. japonicum* in rhesus monkeys from the mainland of China. N is the number of statistics in each sample. In each sample the horizontal line indicates the range of measurements; the crossbar, the mean; the hollow rectangle, 1 standard deviation on each side of the mean; the solid rectangle, 2 standard errors on each side of the mean.

SUSCEPTIBILITY OF MAMMALIAN HOSTS

Worm recovery

The worm recovery of the five different isolates of *S. japonicum* in 6 kinds of animal species including mouse, rat, hamster, jird, rabbit and rhesus monkey on 42 days post-infection has been made (He *et al,* 1990). The mean percentage of worm return in rats infected with five isolates was markedly lower than that of the other animals and worms recovered were immature, indicating that the rat is nonpermissive hosts for the five different isolates of *S. japonicum* in the mainland of China. With the exception of the rat, all the animals un-

der study were permissive hosts for five isolates of *S. japonicum,* though their worm recovery rates varied with different isolates.

Prepatent period

The prepatent period of the five isolates of *S. japonicum* has been determined in mice, hamsters, jirds, rabbits and rhesus monkeys (He *et al,* 1991b). The mean prepatent period in different host species were 35.0 ± 0.8 to 36.4 ± 1.0 days for the Anhui isolate; 34.5 ± 1.2 to 36.4 ± 1.2 days for the Hubei isolate; 34.5 ± 1.3 to 35.8 ± 0.6 days for the Sichuan isolate; 35.1 ± 1.0 to 37.3 ± 1.9 days for the Guangxi isolate and 36.1 ± 1.9 to 37.8 ± 0.8 days for the Yunnan isolate. In general, the prepatent periods were longer in the mice, hamsters and rhesus monkeys infected with Yunnan and Guangxi isolates, than those with Sichuan isolate.

Fuzzy cluster analysis

Computerized numerical systematics was used to analyse the five different isolates of *S. japonicum* by means of the fuzzy clustering method with 32 quantitative data of each isolate about the morphometric and biological characters mentioned above (He *et al,* 1991d). A dendrogram of the five isolates of *S. japonicum* was constructed on the basis of similarity coefficients. The phylogenetic relationships revealed by the present study showed that the two isolates of Anhui and Hubei came together in one group, then the isolates of Yunnan and Guangxi gathered in another group, while the Sichuan isolate was alone and closely related to the group of Anhui-Hubei isolates.

COMPATIBILITY BETWEEN LARVAE AND *ONCOMELANIA* SNAILS

Snails from Hubei and Anhui were readily infected with the local isolate of *S. japonicum* and cross infection also took place readily between the snails and the parasites from these two places, the infection rates of Hubei snails infected with Hubei or Anhui parasites were 57.7% and 43.8%, as well as those of Anhui snails infected with Anhui or Hubei parasites being 52.2% and 44.6%, respectively. This suggested that the schistosomes and their snail hosts from both Hubei and Anhui, the middle and lower reaches of the Yantze River, were compatible (He *et al,* 1991c).

Snails from Sichuan and Yunnan were refractory to infection with schistosome isolates from Hubei and Anhui, the infection rates were 0, 0, 1.9 and 0% respectively. In contrast to these results, the isolates from Sichuan and Yunnan were able to develop in *Oncomelania hupensis* snails from Hubei and Anhui, resulting in infection rates of 10.2% and 7.0% or 44.5% and 13.4% respectively.

Although the Guangxi isolate of *S. japonicum* developed readily in both Anhui snails (30.7%) and Guangxi snails (9.4%), the average precercarial period was 100.9 ± 13.3 days in the former which was significantly longer than 76.9 ± 2.6 days in the latter ($p < 0.01$). None of the other snails from Sichuan and Yunnan became infected. On the other hand, snails from Guangxi infected with Anhui parasites also had a longer precercarial period of 92.7 ± 14.5 days, than that of 81.6 ± 13.7 days in Anhui snails. The difference between the precercarial period was statistically significant ($p < 0.05$).

PATHOGENECITY

Schistosome eggs deposit in host tissues to bring about granulomatous lesions are the main factor of pathogenesis. Thus the size of hepatic granuloma produced by eggs from the five different isolates of *S. japonicum* was compared. The productive stage of egg granulomas containing a single mature egg were measured in the liver sections of C57BL mice and rhesus monkeys infected with each of isolates. The volume of each granuloma was calculated by the formula $V = \pi AB^2/6$, in which A and B stand for the two perpendicular axes.

It was notes that the mean volume of egg granulomas from each isolate differed with host species, and the average size of the egg granuloma was larger in rhesus monkey than in the mouse. In the host of C57BL inbred mouse, the mean volume of egg granulomas for Yunnan and Guangxi isolates was significantly larger than that detected in isolates of Sichuan, Anhui and Hubei (Fig 3). In the case of rhesus monkey, the volume of egg granulomas was also larger in both Yunnan and Guangxi isolates than in those of Sichuan, Anhui and Hubei isolates. These differences were statistically significant ($p < 0.05$). Our results suggested that the different isolates of *S. japonicum* in the mainland of China seem to be different in their pathogenicity or virulence (He *et al,* 1992c).

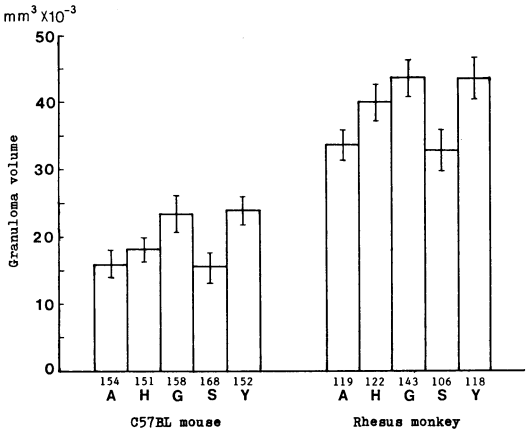

Fig 3—Mean volume of single egg granulomas of the productive stage in liver of animals infected with five different isolates of *S. japonicum* from the mainland of China. Numbers in each isolate are granulomas measured.

IMMUNOREACTIONS IN EXPERIMENTAL ANIMALS

Circumoval precipitin test (COPT)

Observations were made on the immunoreactivities toward egg antigens of different experimental animals including mouse, rat, hamster, rabbit and rhesus monkey infected with *S. japonicum* of the five different isolates. Ten animals of each kind of rodent and 4-5 animals of both rabbits and rhesus monkeys were used for each isolate (Xue *et al*, 1992a). Sera from the same sorts of animals were separately pooled according to the different sources of infection mentioned above. Each serum sample was tested with ova from the same or different isolates. Thus the level of antibodies detected by homologous or heterologous antigens was compared. The results varied with host species and sources of different isolates (Table 2), but no significant difference was found by

Table 2

COPT results of sera from various animals infected with five different isolates of *S. japonicum* to schistosome eggs of different sources.

Animal species	Source of egg	Mean COPT rate (%)				
		Anhui	Hubei	Sichuan	Yunnan	Guangxi
Mouse	Anhui	54	55	30	53	31
	Hubei	27	33	28	23	31
	Sichuan	31	36	30	29	33
	Yunnan	52	34	35	31	34
Rat	Anhui	1	7	10	8	4
	Hubei	0	8	8	4	7
	Sichuan	1	4	6	8	6
	Yunnan	2	7	6	16	9
Hamster	Anhui	50	47	34	28	26
	Hubei	23	27	30	22	28
	Sichuan	35	28	30	37	27
	Yunnan	53	40	26	25	26
Rabbit	Anhui	46	59	23	43	26
	Hubei	37	39	16	39	25
	Sichuan	34	31	15	37	15
	Yunnan	40	39	26	38	24
Rhesus monkey	Anhui	36	43	25	36	34
	Hubei	26	25	35	26	34
	Sichuan	23	29	29	23	37
	Yunnan	24	33	38	18	42

analysis of variance in various hosts or in rhesus monkey alone ($F=1.35$, $p=0.2889$).

Latex agglutination test (LAT)

Soluble egg antigen (SEA) prepared from an Anhui isolate was used, and the level of antibodies was measured. Results showed no significant differences among the five different isolates ($F=95$, $p=0.467$) in their four permissive hosts (Table 3), while in the rat, a non-permissive host, all were negative.

Enzyme linked immunosorbent assay (ELISA)

The soluble egg antigen merely prepared from Anhui isolate was used and the results are shown in Table 4. Though the level of antibodies differed among the five different isolates ($F=3.03$, $p=0.0487$) in their permissive hosts, the mean OD values of antisera induced by the other 4 different heterologous isolates were not lower than that induced by the Anhui homologous isolate, suggesting that the positive antibody detection rate might not be influenced by antigen prepared from *S. japonicum* eggs of different isolates in the mainland of China.

SENSITIVITY TO PRAZIQUANTEL

Altogether 549 of C57BL inbred mice infected with each of the Anhui, Hubei, Sichuan and Yunnan isolates of *S. japonicum* were treated with praziquantel (PZQ) and the parasiticidal effects were compared (He *et al*, 1992b). Worm reduction rate was recorded to assess systematically the sensitivity of 4 different isolates to PZQ in the mouse. Three dosage-levels representing ED40, ED50 and ED60 of PZQ, *ie* 150, 230 and 310 mg/kg body weight in single doses were used.

Table 3

LAT results of sera from various animals infected with five different isolates of *S. japonicum* to Anhui schistosome SEA.

Animal species	Mean titer				
	Anhui	Hubei	Sichuan	Yunnan	Guangxi
Mouse	11.3	32.0	22.6	16.0	42.3
Rat	0	0	0	0	0
Hamster	16.0	32.0	16.0	8.0	8.0
Rabbit	16.0	16.0	32.0	32.0	16.0
Rhesus monkey	11.3	16.0	32.0	11.3	11.3

Table 4

ELISA results of sera from various animals infected with five different isolates of *S. japonicum* to Anhui schistosome SEA.

Animal species	Mean OD value				
	Anhui	Hubei	Sichuan	Yunnan	Guangxi
Mouse	1.00	1.26	1.02	1.33	1.95
Rat	0.50	0.65	0.64	0.64	0.59
Hamster	0.50	0.64	0.73	0.51	0.63
Rabbit	0.70	1.97	1.51	2.00	1.91
Rhesus monkey	0.70	0.91	0.99	0.72	1.51

The worm development rates of control groups infected with schistosomes from Anhui, Hubei, Sichuan and Yunnan were 75.5, 81.8, 81.5 and 83.0%, respectively. At the dosage level of 150 mg/kg, the worm reduction rates for the 4 different isolates were 36.0, 33.9, 25.5 and 35.6%, respectively. At the dosage level of 230 mg/kg, the rates were 47.1, 46.0, 38.1 and 47.7%, while at the dosage level of 310 mg/kg, they were 59.3, 58.6, 50.8 and 61.7%, respectively. The results indicated that the worm reduction rate of the Sichuan isolate was lower than that of the other three isolates, however, the differences were not statistically significant, suggesting that schistosomes of Anhui, Hubei, Sichuan and Yunnan isolates bear resemblance in drug response.

PROTEIN PATTERN AND ANTIGENICITY ANALYSIS

SDS-PAGE analysis

Homogenate prepared from *S. japonicum* adult worms of the five different isolates were analyzed by SDS-PAGE (Xue *et al,* 1992b). Results indicated that with Coomassie blue staining male worms of *S. japonicum* basically had similar patterns of 17-20 bands, no obvious difference could be observed among these five isolates. With silver staining both male and female worms of Guangxi isolate showed some definite differences in their protein profile, namely, absence of one band between 50-75 kDa in male worms and marked reduction in quantity of >110 and 30 kDa bands in female worms (Figs 4-5). There were no obvious differences among other isolates in either male or female worms. It suggested that the protein components of Guangxi isolate must have some differential specificities.

Enzyme linked immunoelectrotransfer blot (EITB) analysis

The antigenically active components among numerous SDS-PAGE resolved worm extracts prepared from Anhui, Hubei, Yunnan, Sichuan and Guangxi isolates were tested with antisera against snails collected from the same or different areas (Qiu *et al,* 1992). Figs 6 and 7 show the EITB results of worm extracts from the five different isolates versus 4 antisera against snail hosts from Anhui, Hubei, Yunnan and Sichuan, respectively. Female worm antigens of Anhui, Hubei, Yunnan and Sichuan isolates reacting with their

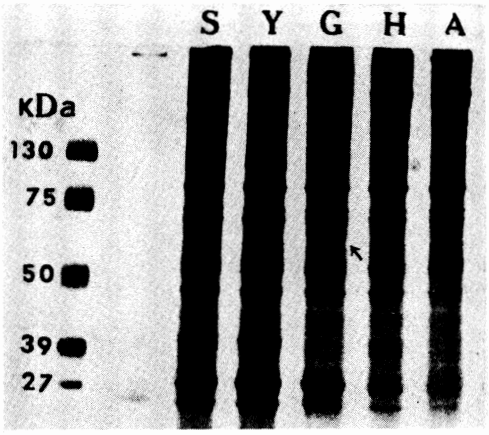

Fig 4—SDS-PAGE protein patterns of five different isolates of male *S. japonicum* from the mainland of China.

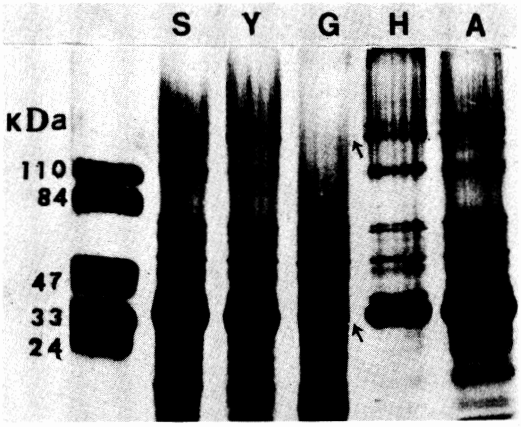

Fig 5—SDS-PAGE protein patterns of five different isolates of female *S. japonicum* from the mainland of China.

natural snail hosts' antisera revealed 7-10 bands with molecular weights ranging from 17 to >110 kDa, the MW of all the main bands being >33 kDa, while the five worm antigens mentioned above which reacted with antisera against snails from other places showed 6-9 bands. It suggested that *S. japonicum* from different areas of the mainland of China has common antigens with snails collected from various localities.

Fig 6 revealed that extracts from Anhui and Hubei female worms had similar EITB patterns, and extracts from Yunnan female worms showed a distinct band of 84 kDa when tested with antisera against snails from Anhui, Hubei and Yunnan,

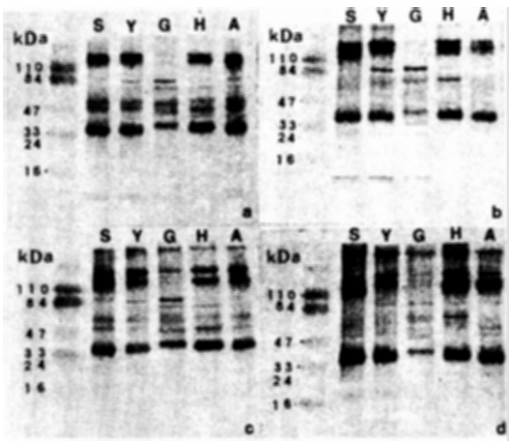

Fig 6—EITB patterns of anti-*Oncomelania* sera from four localities against antigens of five different isolates of female *S. japonicum* in the mainland of China.
a: R anti-Anhui snail serum
b: R anti-Hubei snail serum
c: R anti-Yunnan snail serum
d: R anti-Sichuan snail serum

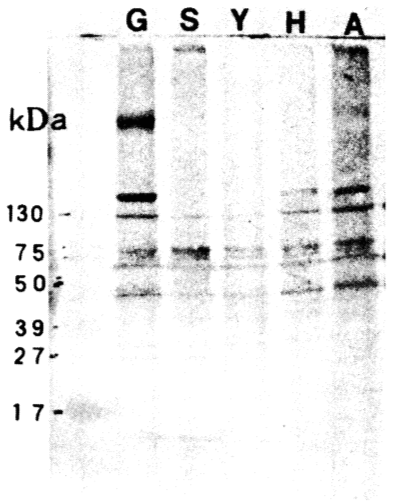

Fig 7—EITB patterns of anti-Anhui *Oncomelania* serum against antigens of five different isolates of male *S. japonicum* in the mainland of China.

but very weak reactions could be seen when tested with antisera against Sichuan snails. However, when Sichuan female worm antigen were tested with antisera against Anhui, Hubei and Yunnan snails, such a band could never be seen. As compared to the extracts of female worms from the other four isolates, there were marked differences in EITB of Guangxi isolate. The 2 dark and dense bands with MW slightly greater than 110 kDa did not appear (versus antisera against snails from Anhui, Hubei and Sichuan) or appeared very vaguely (versus antisera against snails from Yunnan), moreover, the 84 kDa band was observed when any of the 4 anti-snail antisera was used. The EITB pattern of male worms from Guangxi isolate showed 2 main bands of MW> 130 kDa against anti-Anhui snail antiserum which corresponded with the result of male worms of Anhui isolate (Fig 7). But these bands could not be seen with male worms of isolates from Yunnan and Sichuan.

DNA HYBRIDIZATION

DNA hybridization to identify the polymorphic structural variants of the five different isolates of *S. japonicum* was carried out (Xie *et al,* 1993). The probe pSM889 sent by Drs Simpson and McManus was one of three fragments of a ribosome RNA gene digested by BamHI restriction endonuclease. The genomic DNA of male worms from the five isolates extracted with phenol/chloroform was digested by restriction enzymes EcoRI and BamHI, respectively. The resulting fragments were separated by 0.8% agarose gel electrophoresis and Southern blot transferred to nylon membrane. After preparing and purifying, the probe was labeled and hybridized to the membrane according to the method of Boehringer Mannheim's Nonradioactive DNA Labeling and Detection Kit.

The hybridization of DNA digested by EcoRI showed that the five isolates have the same four major bands in which the molecular weights of two bands are about 2.3 and 4.4 Kb, and those of the other two bands are between 4.4-6.6 Kb (Fig 8). However, the minor bands of hybridization of the five isolates are different, not only in number but also in molecular weight. The number of minor bands observed in the five isolates was as follows: Sichuan 2, Yunnan 2, Guangxi 2, Hubei 2, Anhui 4, with molecular weights between 2.3-4.4 Kb, and Sichuan 3, Yunnan 5, Guangxi 3, Hubei 3, Anhui 3, with molecular weights between 4.4-9.6 Kb. Our results revealed that the major DNA fragments containing the ribosomal gene unit were the same among the five isolates but that minor fragments containing the gene varied.

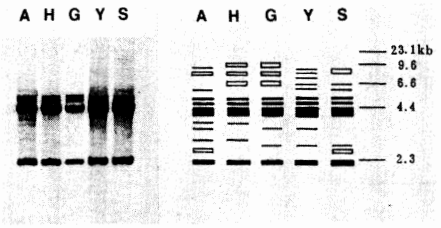

Fig 8—Southern blots of EcoR I-digested *S. japonicum* DNA were hybridized with pSM889 probe, showing major bands and some differences on the minor bands of hybridization were observed among the five isolates.

GENETIC CRITERIA

The genetic variation and differentiation of the five different isolates of *S. japonicum* were compared by multilocus enzyme electrophoresis in polyacrylamide gels of individual male worm extracts (He *et al,* 1991e). The enzyme systems used in this study included GDH, G6PD, LDH, MDH, PGI, PGM and SOD (Fig 9). Of 9 loci examined in the 7 isozyme systems, 4 were found to be polymorphic, the proportion of polymorphic loci being 44.4%. These were LDH-1, LDH-2, MDH and PGM respectively, the remainder GDH, G6PD-1, G6PD-2, PGI and SOD being monomorphic. The allele frequencies at polymorphic loci coding for enzyme in the five different isolates of *S. japonicum* are shown in Table 5. The average heterozygosity varied between 0.223 and 0.425, with a mean of 0.332 (He *et al,* 1992a). Nei's genetic distance (D) among the populations of the five different isolates in the mainland of China gave values between 0.001 and 0.039 with an average of 0.023 (Table 6), indicating that these isolates are closely related.

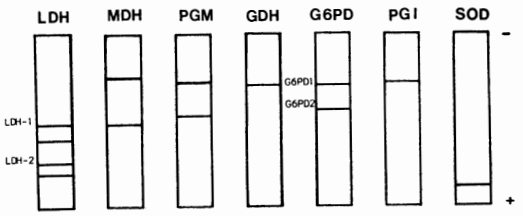

Fig 9—Diagrammatic representation showing 9 loci in the 7 isoenzyme systems examined of *S. japonicum* in the mainland of China.

DISCUSSION

In one species all organisms in a particular area might constitute a strain if they are sufficiently different from populations in other areas. Thompson and Lymbery (1990) propose that parasite strains should be described by a combination of genetic and biological characteristics, for instance, a strain can be considered as being not only genetically differentiated from another population but also differing in one or more epidemiologic features. Based on the results of our studies above, it is reasonable to consider that *S. japonicum* in the mainland of China is not a single strain, it is actually a strain complex with several components of geographically distributed strains. Characteristics of worms isolated from Hubei and Anhui Provinces are nearly the same, one may therefore conclude that schistosomes in the middle and lower reaches of the Yangtze River are of one strain. Endemic areas of schistosomiasis in Yunnan Province are on a highland approximately 2,000 m above sea level, while those of Sichuan Province are in the Ming River valley and of Guangxi Autonomous Region are in the karst land. These diverse habitats obviously strongly affect snail ecology, hence the schistosome strains. Thus, taking an integral consideration of our results presented above, the epidemiological features and the results obtained by using molecular techniques (Jin *et al,* 1989; Tan *et al,* 1990; Luo *et al,* 1992; Zeng *et al,* 1993), it has been well established that *S. japonicum* from the five different isolates in the mainland of China possesses distinct characteristics and that at least four geographic strains exist. The designation and characteristics of them are as follows:

Chinese Yunnan strain: Distributed over the high plateau of altitude 1,350-2,450 m in Yunnan Province, Southwest China. Local smooth shell of *O. hupensis* serves as intermediate host, while the larvae can also develop to mature cercariae in *O. hupensis* of both smooth and ribbed shell from any other regions in the mainland of China.

Chinese Guangxi strain: Distributed over the karst land of altitude 200-400 m in Guangxi Zhang Autonomous Region, South China. Local *O. hupensis* of smooth shell serves as intermediate host, though the larvae can develop to mature cercariae in *O. hupensis* of ribbed shell, the average precercarial period takes significantly longer times.

Table 5

Allele frequencies at polymorphic loci coding for enzyme in five different isolates of *S. japonicum* from the mainland of China.

Locus	Allele	Anhui	Hubei	Guangxi	Sichuan	Yunnan
LDH-1	N	52	47	39	44	20
	0.55	0.654	0.660	1.000	0.943	0.775
	0.63	0.346	0.340	0	0.057	0.225
	H	0.453	0.449	0	0.107	0.349
LDH-2	N	52	47	39	44	20
	0.47	0.625	0.638	1.000	0.943	0.725
	0.80	0.375	0.362	0	0.057	0.275
	H	0.469	0.462	0	0.107	0.399
MDH	N	50	40	18	58	34
	0.24	0.360	0.325	0.500	0.500	0.382
	0.55	0.640	0.675	0.500	0.500	0.618
	H	0.461	0.439	0.500	0.500	0.480
PGM	N	48	46	15	50	16
	0.27	0.198	0.152	0.267	0.450	0
	0.46	0.802	0.848	0.733	0.550	1.000
	H	0.318	0.258	0.391	0.495	0
	$\bar{H}$	0.425	0.402	0.223	0.302	0.307

N: Sample number; H: Heterozygosity

Table 6

Nie's genetic identity, above diagonal, and genetic distance, below diagonal, between five different isolates of *S. japonicum* from the mainland of China based on available electrophoretic data for nine loci.

	Anhui	Hubei	Guangxi	Sichuan	Yunnan
Anhui	-	0.999	0.964	0.965	0.992
Hubei	0.001	-	0.963	0.962	0.994
Guangxi	0.036	0.037	-	0.995	0.973
Sichuan	0.036	0.039	0.005	-	0.962
Yunnan	0.008	0.006	0.027	0.038	-

Chinese Sichuan strain: Distributed over the northwest and southwest parts of mountainous region of altitude 400-1,600 m in Sichuan Basin, West China. Local *O. hupensis* of smooth shell serves as intermediate host, the larvae can develop to mature cercariae in *O. hupensis* of ribbed shell, but the infection rate is quite low.

Chinese Anhui-Hubei strain: Distributed over the territories of altitude below 200 m along the Yangtze Valley within Hubei and Anhui Provinces. Local ribbed shell of *O. hupensis* serves as intermediate host, while the larvae are refractory to development in smooth shell of *O. hupensis* from Yunnan and Sichuan Provinces.

Although each of the four geographic strains of *S. japonicum* shows its distinct characteristics, we believe that they were descended from a common ancestor. The mainland of China was formed

under the combined influences of the Indian, Pacific and Siberian Plates, beginning in the mid-Cenozoic history. The collision, rotation and dispersion of these plates gave rise to the present-day structure of the mainland of China. Among these influences was the collision of the Indian Plate and the mainland of China in the late Tertiary. The point of contact ran from the west to the east, resulting in the formation of the Himalaya Uplift and the transformation of the western China terrain. Because only *Oncomelania* can serve as the intermediate host of *S. japonicum,* and so the distribution of *S. japonicum* followed the distribution of the susceptible oncomelanid snails. If we wish to speculate the intraspecific differentiation of *S. japonicum,* we must also consider the origin and distribution of the oncomelanid snail. Davis (1980) reported the line tracing *Oncomelania* and its precursors from South African Gondwanaland through India to northwestern Burma through the mainland of China to Taiwan Province, the Philippines, and Sulawesi. He therefore suggests that the introduction of the Pomatiopsidae snails to the mainland of Asia was via the Indian Plate. It is worth noting that so far in the mainland of China, *Oncomelania*-like snail fossils have only been discovered in Guangxi and Yunnan. The snail fossils from Guangxi date to the Tertiary Period, while those from Yunnan have yet to be dated (Nanjing Institute of Geology and Palaeontology, Academia Sinica, personal communication). Furthermore, according to the reports of the Institute of Geology, Academia Sinica (1959), the formation of the Guangxi territory occurred in the late Cretaceous and early Tertiary and the Guangxi riverine system originated in Yunnan during the mid-Cenozoic era, while the alluvial plain of the middle and lower Yangtze River Valley occurred in the Quaternary, later in geological time.

Based on the outline of geological events and zoographical data given above, we believe that *S. japonicum* and its snail hosts originated in the Old World tropics in the vicinity of Yunnan Province and then radiated north along the Ming River valley into Sichuan Province; east along the Yangtze River Valley to Shanghai at the mouth of the Yangtze River; and south along the Mekong River into Laos, Cambodia, Thailand, Malaysia and Vietnam, although it is no longer found there. Snails may have reached Guangdong Province by radiating south from Hunan Province. The origin of schistosomes in Fujian Province may have been with the arrival of the snails from Zhejiang Province. It is almost certain that both schistosomes and snails spread from Fujian Province to Taiwan Province, and from there into Japan, the Philippines and Sulawesi. The dispersion should have been completed before the islands were separated from the Asian continent. During the process of evolution, this parasite was constantly multiplying in some places, spreading into other places, receding and becoming extinct in others, and thus forming the present apparently discontinuous pattern of geographic distribution. Because the ecological conditions and general adaptability were different in separate geographical localities, the schistosomes in different areas in Asia became distinct geographic strains, *ie* the Chinese mainland, Taiwan Province, Japanese, Philippine and Indonesian (Hsü and Hsü, 1958; Cross, 1976), while in the Chinese mainland this strain further evolved at least into four distinct strains, including Yunnan, Guangxi, Sichuan and Anhui-Hubei. These four newly evolved strains still have their main characteristics in common. Their Nei's genetic distance (D) gave an average value of 0.023 (He *et al,* 1992a), and this is markedly less than that of 0.3 among the four areas of Japan, Philippines, the mainland of China and Taiwan Province (Fletcher *et al,* 1980; Woodruff *et al,* 1987; Merenlender *et al,* 1987), indicating that the degree of genetic differentiation in the populations of *S. japonicum* in the mainland of China is significantly lower than those of other Asian areas.

ACKNOWLEDGEMENTS

This project was supported by the National Natural Science Foundation of China. We are grateful to Liu Hou-Xiang, Zhang Yong-Hong, Chen Wei, Xia Feng and Zhu Chen-Wen for their participating in a part of the work.

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