SCHISTOSOMA JAPONICUM STRAINS: DIFFERENTIATION BY RAPD AND SSR-PCR

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Abstract. Eight geographical isolates of *Schistosoma japonicum* from Taiwan and mainland China and one isolate of *Schistosoma mansoni* were studied by RAPD analysis using six arbitrary primers and SSR-PCR analysis using a $(CA)_8RY$ primer. The genetic distance was determined by the percentage of unshared bands. The RAPD and SSR-PCR results showed that the genetic distance between *S. mansoni* and *S. japonicum* was more than 0.900 and 0.850 respectively; the genetic distance between the eight geographical isolates of *S. japonicum* was 0.000 to 0.232 and 0.066 to 0.368 respectively. These results demonstrated the usefulness of RAPD and SSR-PCR for showing the differences of inter- and intra-species of *Schistosoma*. The results also suggest that there is genetic diversity among the different geographical strains of *S. japonicum* in China.

INTRODUCTION

Schistosomiasis is a chronic and debilitating parasitic disease. It affects millions of people throughout the world and is responsible for many deaths every year. *Schistosoma japonicum* and *Schistosoma mansoni* are the major schistosome species that afflict humans. Parasitological evidence indicates that geographical strains of *S. japonicum* show considerable intraspecific variation. It is well known that the endemic areas of schistosomiasis japonica in the Chinese mainland are mainly confined to the regions south of the Yangtze River; these areas are not contiguous and are geographically and topographically isolated. Given this geographical isolation, it is likely that more than one strain of *S. japonicum* exists in the vast Chinese mainland (He *et al*, 1994). However, studies of the genetic variation of *Schistosoma* may have been limited by poor technique, such as flawed morphological, immunological, and biochemical analyses.

Recently, there have been important advances in the application of recombinant DNA techniques. A number of DNA-based methods have been developed to investigate genetic diversity, to differentiate strains and species, and to analyze the phylogenies of schistosomes. Dias-Neto *et al* (1993) demonstrated that RAPD (random amplified polymorphic DNA markers) allowed the identification of strains of *S. mansoni* and species of *Schitosoma*; Pillay and Pillay (1994) showed intraspecific DNA polymorphisms among the isolates of *S. mansoni* with Sman-24, a portion of a repetitive sequence of *S. mansoni*, used as a primer for PCR amplification.

Microsatellite DNA, also known as simple sequence repeats (SSR) or short tandem repeats, are genomic sequences that consist of a mono-, di-, tri- or tetra-nucleotide motif repeated in multiple tandem copies. Zietkiewicz *et al* (1994) used oligonucleotides complementary to them as single primers to amplify the inter-repeat segments and reveal the polymorphic banding patterns. They designated this technique as simple sequence repeat-anchored PCR amplification (SSR-PCR), and they obtained excellent results operating under stringent conditions using the primer $(CA)_8RY$ etc (R=puRine, Y=pYrimidine).

We investigated the RAPD and SSR-PCR

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profiles of *Schistosoma* isolates and found that they all have polymorphic banding patterns. The dendrograms, drawn up on the basis of genetic distances revealed by the RAPD and SSR-PCR bands, are similar.

MATERIALS AND METHODS

Parasite isolates

Seven natural geographical isolates of *S. japonicum* were obtained from different parts of China: Sj1 (Songzi County, Hubei Province); Sj2 (Zhongxiang County, Hubei Province); Sj3 (Yangxin County, Hubei Province); Sj4 (Wuhan City, Hubei Province); Sj5 (Tianquan County, Sichuan Province); Sj6 (Xinjian County, Jiangxi Province); Sj7 (Yueyang City, Hunan Province). In addition, a *S. japonicum* isolate from Taiwan (Sj8) was donated by Professor Li and a *S. mansoni* isolate (Sm) was donated by Professor Ruppel of University of Heidelberg, Germany.

Isolation of genomic DNA

For each isolate, 30 adult worms (mixed sexes) were ground with a glass homogenizer; DNA extraction buffer was then added; extraction was by, phenol:chloroform:isoamyl alcohol and precipitation was by 100% ethanol.

PCR amplification

RAPD: Six arbitrary primers were used as described by Barral *et al* (1993) (Table 1). The random amplification procedure was performed as described by Williams *et al* (1990). The PCR reaction mixture was subjected to electrophoresis in a 1% agar gel and photographed.

SSR-PCR: PCR amplification was conducted as described by Zietkiewicz *et al* (1994) with minor modifications. Amplification was performed in 1x reacting buffer, 1.875 mM $MgCl_2$, 0.2 mM each dNTPs, 1 unit of Taq DNA polymerase, 0.82 μM primer $(CA)_8RY$ with 10~20 ng of genomic DNA per 20 μl reaction. The PCR reaction mixture was subjected to electrophoresis in a 1% agar gel and photographed.

Data analysis

The multiband profiles revealed by RAPD and SSR-PCR were scored by eye for a numerical taxonomy analysis based on the proportion of shared bands (S) in the total profiles between all possible pairs as described by Nei and Li (1979). The result was transformed to a dissimilarity coefficient (D = 1 —S) which was used to measure genetic distance. The dendrograms were constructed from the distance matrices by using the SAS (Statistical Analysis System) computer program.

RESULTS

RAPD

RAPD was performed with six different arbitrary primers (Table 1). In total, there were 135 amplified bands, varying from 100 to 3,000 bp. The banding patterns between *S. mansoni* and *S. japonicum* were very different and the banding patterns of the isolates from the Chinese mainland and Taiwan were polymorphic (Fig 1). The banding patterns of the eight *S. japonicum* isolates were polymorphic and showes marked similarity (Fig 2). Only five of the bands shared by *S. mansoni* and *S. japonicum* were of the same size: the genetic distance between them was more than 0.900 (data not shown). The different isolates of *S. japonicum* shared 48 to 69 bands: the genetic distance between them was 0.000 to 0.232. On the basis of genetic distance, we drew up dendrograms to show the relationship between

Table 1
Sequence of arbitrary primers.

Primer name	Sequence	G+C(%)
A7	5′-GAAACAAATG-3′	60
A8	5′-GTGACGTAGG-3′	60
A9	5′-GGGTAACGCC-3′	70
B5	5′-TGCGCCCTTC-3′	70
B6	5′-TGCTCTGCCC-3′	70
B10	5′-CTGCTGGGAC-3′	70

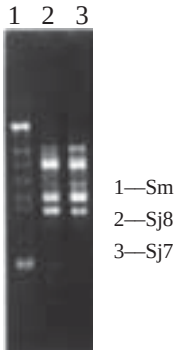

Fig 1–The interspecies multiband profiles amplified with primer A7.

the different isolates (Fig 4).

SSR-PCR

SSR-PCR was performed with a 5′ anchored primer-$(CA)_8RY$. The amplified bands varied in size from 100 to 1,500 bp. The banding patterns of *S. mansoni* and *S. japonicum* were markedly different; the banding patterns of the isolates from the Chinese mainland and Taiwan were polymorphic (Fig 3). The banding patterns among eight isolates are polymorphic with high similarity (Fig 4).

DISCUSSION

When applied to the study of schistosomes, the RAPD markers method has proved useful in analyzing the different problems associated with genetic diversity. Its main advantages over the other techniques are speed,

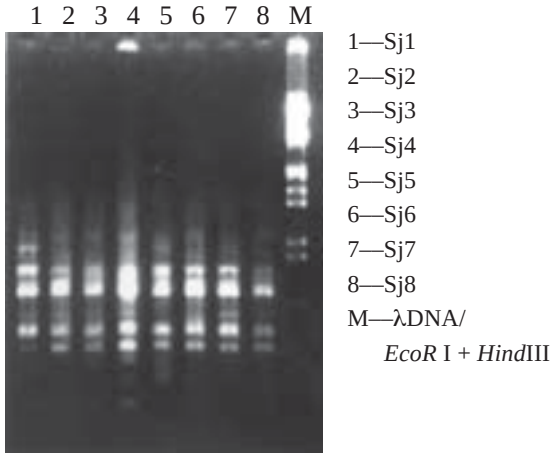

Fig 2–The multiband profiles of 8 isolates *S. japonicum* revealed by primer A8.

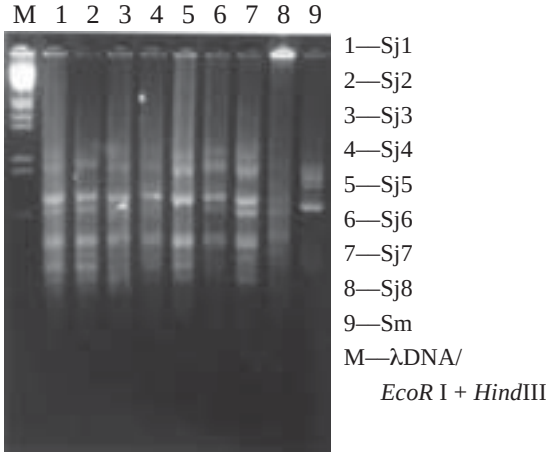

Fig 3–The interspecific and intraspecific banding of *S. japonicum* revealed by $(CA)_8RY$ primer.

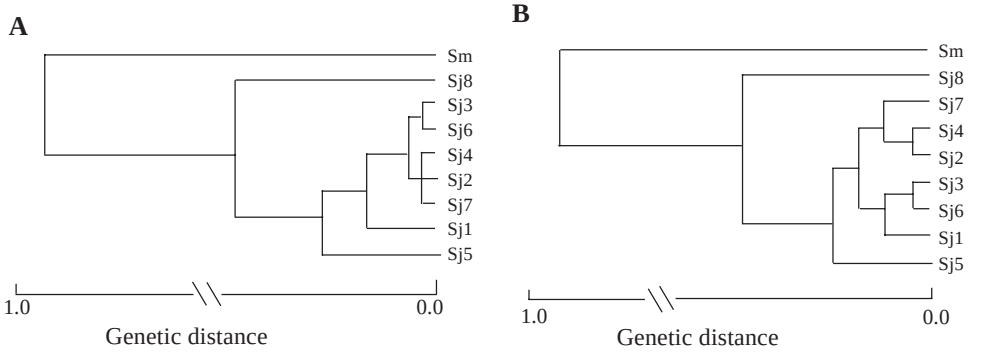

Fig 4–Dendrograms showing the relationships of different schistosome isolates. A: according to RAPD; B: according to SSR-PCR.

reproducibility, technical simplicity, high resolution, and a considerable reduction of the amount of DNA required for routine analysis. Using this technique, markers that can discriminate between species or strains were revealed quickly and without the need for sequence information or radiolabeling.

SSR-PCR is another useful DNA marker. It has many of the advantages associated with RAPD; it is quick, simple, specific, and requires less DNA; it can also detect the whole genome through the SSR primer. SSR-PCR offers a flexible approach to the study of DNA variation in most eukaryotic genomes, and may be of use in population genetics, molecular taxonomy, genome mapping, and in screening for somatic mutations (Thibodean *et al*, 1993).

In our study, the RAPD and SSR-PCR markers showed that *S. mansoni* and *S. japonicum* can be viewed as two different species because of the great genetic distance between them. These results reflect the fact that these two species of *Schistosoma* have been geographically isolated for a long time; these results also match those obtained by other biochemical and DNA analyses, such as multilocus enzyme electrophoresis (Wang *et al*, 1999), and the sequencing of the gene coding for rRNA (Simpson *et al*, 1984).

In our study, the genetic distance between the Chinese mainland isolate and the Taiwan isolate was more than 0.200: greater than that between the Chinese mainland isolates themselves. The Chinese mainland and Taiwan have been seperated by the straits for a long time: it is easy to understand that the *S. japonicum* endemic to these two regions may have developed genetic diversity because of geographical isolation and different ecological conditions. The *S. japonicum* endemic in Taiwan does not infect humans, it is a zoophilic strain (Feng and Niu, 2001); our RAPD and SSR-PCR studies have confirmed this.

The endemic areas for schistosomiasis japonica in the Chinese mainland are mainly confined to the regions south of the Yangtze River: these areas are not contiguous and are isolated. He *et al* (1994) studied the characteristics of *S. japonicum* isolated from five localities in the Chinese mainland and concluded that *S. japonicum* in the Chinese mainland comprises a strain complex of several components of geographically distributed strains and at least four distinct strains (Yunan, Guangxi, Sichuan and Anhui-Hubei). Our results showed that the RAPD and SSR-PCR amplified bands of seven isolates from the Chinese mainland are not identical. The genetic distance between Sj5 (Sichuan Province) and the other six isolates was more than that between the other six isolates. Sichuan Province is southwest of the Yangtze River with high latitudes. The intermediate host - *Oncomelania hupensis* - thrives in mountainous or hilly regions and is therefore less affected by the annual inundation of the Yangtze. These factors may be added to the geographical isolation and the different ecological conditions. The other six isolates Sj1, Sj2, Sj3, Sj4, Sj6 and Sj7, are usually found in the middle or lower Yangtze River areas. The intermediate host of these isolates lives in marshland and lakes. Some of these regions may be separated to some degree by natural barriers and therefore some of the isolates are different and genetic diversity may be shown (Fig 4).

In conclusion, RAPD and SSR-PCR markers provide powerful resolution and are a helpful tool for the investigation of genetic variation within the genus *Schistosoma* and the species *S. japonicum*. These techniques provide a method for the rapid differentiation of species and strains that may in combination with biochemistry and molecular biology, help us to understand schistosomasis.

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