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Hybrids between *Schistosoma mansoni* and *S. rodhaini*: characterization by isoelectric focusing of six enzymes

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Abstract. Results are reported of enzyme analyses by isoelectric focusing in polyacrylamide gels of individual extracts from *Schistosoma mansoni* (Guadeloupe), *S. rodhaini* (Burundi), and their experimental hybrids (first and second generation). The distinctive patterns of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PD), acid phosphatase (AcP), phosphoglucomutase (PGM) and glucose phosphate isomerase (GPI) enable the characterization of the two parental strains and the hybrids. Particular observations, such as the existence of a polymorphism at both MDH-1 and MDH-2 loci and a sex-linked heredity for GPI, are discussed. A genetic interpretation is proposed to explain the patterns observed for MDH and GPI (with a dimeric structure) and for PGM (monomeric structure); a comparison is made with electrophoretic data available for *S. mansoni* and *S. rodhaini*.

In the complex of African species of the *Schistosoma* genus, schistosomes belonging to the same group (lateral spined egg or terminal spined egg) can hybridize experimentally and produce viable and fertile offspring (Le Roux 1954; Taylor 1970). Within some foci of schistosomiasis, where several species coexist, changes in transmission conditions can result in the breaking down of prezygotic isolating processes and lead to a natural hybridization between schistosomes from different species (Southgate et al. 1976; Wright and Ross 1980; Rollinson and Southgate 1985). The detection of possible natural hybrids may be a problem, since classical identification criteria (egg morphology, adult morphoanatomy, host-parasite specificity) are sometimes difficult to apply without a good knowledge of the intraspecific variability of the species involved (Wright and Southgate 1976).

Recent improvements in electrophoretic techniques have widened the range of isoenzymatic markers, which allow the rapid and easy identification of hybrids. Isoelectric focusing in polyacrylamide gels, successfully used by Wright and Ross (1980) to characterize hybrids between *S. haematobium* and *S. matthei*, seems to be well adapted to this type of endeavor: this technique has high resolution and enables the individual analysis of schistosomes of both sexes. In this article we present the results obtained by this technique for the characterization of experimental hybrids between two species of schistosomes from the lateral-spined-egg group: *S. mansoni* and *S. rodhaini*.

Material and methods

Experimental hybridizations. The following strains were used: (a) a strain of *S. mansoni* from Guadeloupe (French West Indies), maintained in *Biomphalaria glabrata* (Guadeloupe) and SWISS OF1 mice; and (b) a strain of *S. rodhaini* from Burundi, maintained in *B. glabrata* (Brazil, albino variety) and SWISS OF1 mice. Crossbreeding was carried out by infecting mice with 100 cercariae from each parental (P) schistosome species. These cercariae were obtained from snails harbouring a single parasite genotype either after infection with a single miracidium of unknown sex (in the case of *S. rodhaini*), or after clones of identified sex were established (in the case of *S. mansoni*) using the technique of microsurgical sporocyst transplantation (Jourdane and Théron 1980).

F1 miracidia, produced by genetically identical couples for a given crossbreed, were used to infect

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B. glabrata (Guadeloupe and Brazil), whose cercariae were used to infect mice, which gave F1 adults. Second-generation schistosomes (G2) were obtained by a similar process from miracidia bred by several couples of F1 individuals (genetically different). In every case (P, F1, G2), adult schistosomes recovered by perfusing mice 8 or 9 weeks after infection were rinsed in saline solution (9 g NaCl) and stored in liquid nitrogen after males and females were dissociated.

Sample preparation. Individual extracts were prepared by crushing worms on a glass slide in 2–3 µl distilled water at 1 °C. The resulting homogenates were immediately absorbed on 2 × 8 mm (male) or 1 × 10 mm (female) tabs of Whatman number 3 chromatography paper, which were stored in liquid nitrogen prior to enzyme analysis.

Electrophoretic methods. Enzyme analyses were conducted by isoelectric focusing in polyacrylamide gels. The gel solution was prepared according to the Desaga method, using 2.81 ml acrylamide (30% in water), 2.48 ml *N,N'*-methylenediacrylamide (1% in water), 1.16 ml ampholytes (pharmalytes), 9.56 ml distilled water, and 0.5 ml ammonium peroxidisulfate (1.5% in water). Polyacrylamide gels (0.5 mm thick) were prepared according to the LKB capillary casting method by pouring the gel solution on a Gel Bond PAG film between two glass plates with 0.5-mm longitudinal spacers. Isoelectric focusing was carried out at 4 °C using a Desaphor HF focusing chamber, a Pharmacia ECPS 3000/150 power supply, a Pharmacia VH1 volt-hour (Vh) integrator and a Haake D8-G cryostat.

Enzyme systems used in the study included lactate dehydrogenase (LDH; E.C. 1.1.1.27), malate dehydrogenase (MDH; E.C. 1.1.1.37), glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49), hexokinase (HK; E.C. 2.7.1.1), phosphoglucomutase (PGM; E.C. 2.7.5.1), acid phosphatase (AcP; E.C. 3.1.3.2), and glucose phosphate isomerase (GPI; E.C. 5.3.1.9).

Gels with pH range 3–10 were used for LDH, MDH, G6PD and AcP; those with pH range 4–9 (pharmalytes, 4–6.5 and 6.5–9), for AcP and GPI; gels with pH range 4–6.5, for HK and PGM. Samples were applied 3 cm from the anodal edge of the gel for LDH, MDH, AcP and GPI and 3 cm from the cathodal edge of the gel for G6PD, HK and PGM.

Without prefocusing of the gel isozyme separation was begun under 250 V and was conducted

Table 1. Staining solutions used for the seven enzymatic systems included in the study

		Enzymes						
		LDH	MDH	G6PD	HK	PGM	AcP	GPI
Buffers:	Sodium acetate 0.15 *M* (pH 5.0)						50 ml	
	TRIS HCl 0.5 *M* (pH 7.1)	16 ml	8 ml					
	TRIS HCl 0.2 *M* (pH 8.0)			50 ml	50 ml	50 ml		50 ml
Substrates:	Lithium lactate	350 mg						
	Malic acid[a]		5 ml					
	Glucose-6-phosphate			40 mg				
	D-glucose				80 mg			
	Glucose-1-phosphate					100 mg		
	Glucose-1,6-diphosphate					0.2 mg		
	Naphthyl phosphate						60 mg	
	Fructose-6-phosphate							10 mg
Other constituents:	Distilled water	24 ml	38 ml					
	Magnesium chloride 0.5 *M*			1 ml	3 ml	1 ml		2 ml
	Manganese chloride 10%						1 ml	
	NAD	20 mg	25 mg					10 mg
	NADP			10 mg	15 mg	10 mg		10 mg
	ATP				80 mg			
	G6PD[b]				15 units	10 units		20 units
	Fast blue RR salt						60 mg	
	NBT	7 mg	15 mg	5 mg	8 mg	5 mg		5 mg
	MTT	7 mg		5 mg	8 mg	5 mg		5 mg
	PMS[b]	3 mg	3 mg	5 mg	8 mg	5 mg		5 mg

[a] 10.6 g sodium carbonate and 1.34 g D-L-malic acid in water for 100 ml solution
[b] Added to staining solution just before pouring

with a constant power of 3–5 W. Current decreased by approximately 20 mA during the course of the run, and isoelectric focusing was complete when 2400 Vh was attained.

Enzyme staining. After their removal from the cooling plate, gels were rinsed and stained using a 0.5% agar overlay for all systems except MDH and AcP. Recipes used for staining solutions, modified from Wright et al. (1979) and Fletcher et al. (1981 b), are given in Table 1. Gels were stained at 37 °C in a dark environment until enzyme patterns developed; they were then rinsed, fixed in a 7% acetic acid solution and photographed immediately. Isoelectric points were determined by using Pharmacia isoelectric focusing calibration kits.

Results

HK

Identical HK patterns were observed for *S. mansoni* and *S. rodhaini.* Hence, this system could not be used for the characterization of hybrids between these two species.

LDH

LDH showed no variation within each parental strain used for the crossbreedings and gave clearly different patterns for *S. mansoni* and *S. rodhaini.* The pattern observed for the F2 hybrid seems to be purely additive, being the combination of the patterns of the two parents (Fig. 1). Nevertheless, the major activity at pH 7.8 could also be the consequence of an addition band, analogous to that observed in the case of a dimeric enzyme. For the G2 schistosomes, patterns for the males (eight worms analysed) were identical with those obtained for *S. rodhaini* and for the F1 hybrid. The results obtained from females were disregarded because of poor resolution.

G6PD

This system, for which activity was observed between pH 5.8 and 6.1, was beset with resolution problems because of the diffusion of bands of activity when gels with pH gradient 4–6.5 were used. Only gels with pH gradient 3–10 gave results that could be interpreted. G6PD was monomorphic for each parental strain and gave distinct patterns for *S. mansoni* and *S. rodhaini.* A four-banded pattern was observed for the F1 hybrid (Fig. 2), with the two external bands at the same level as those observed for the parents (pH 5.9 and 6.1). On the other hand, the two central bands had slightly different pHi values compared with that of the fraction at pHi 6.0 for both parents. The G2 schistosomes gave patterns identical with those obtained from the F1 hybrid and the parents.

PGM

Two loci have previously been identified for PGM in both *S. mansoni* and *S. rodhaini* (Fletcher et al. 1981 a, b). PGM showed no variation within each parental strain, and both PGM-1 and PGM-2 loci gave distinct patterns for the two species. In both species PGM-1 showed a pattern with two major fractions that behaved as a single unit. The F1 hybrid pattern appeared to be a combination of the two parental ones, with no additional fraction, suggesting a monomeric structure for this enzyme (Fig. 3). The G2 shistosomes gave patterns similar to those of the parents and the F1 hybrid.

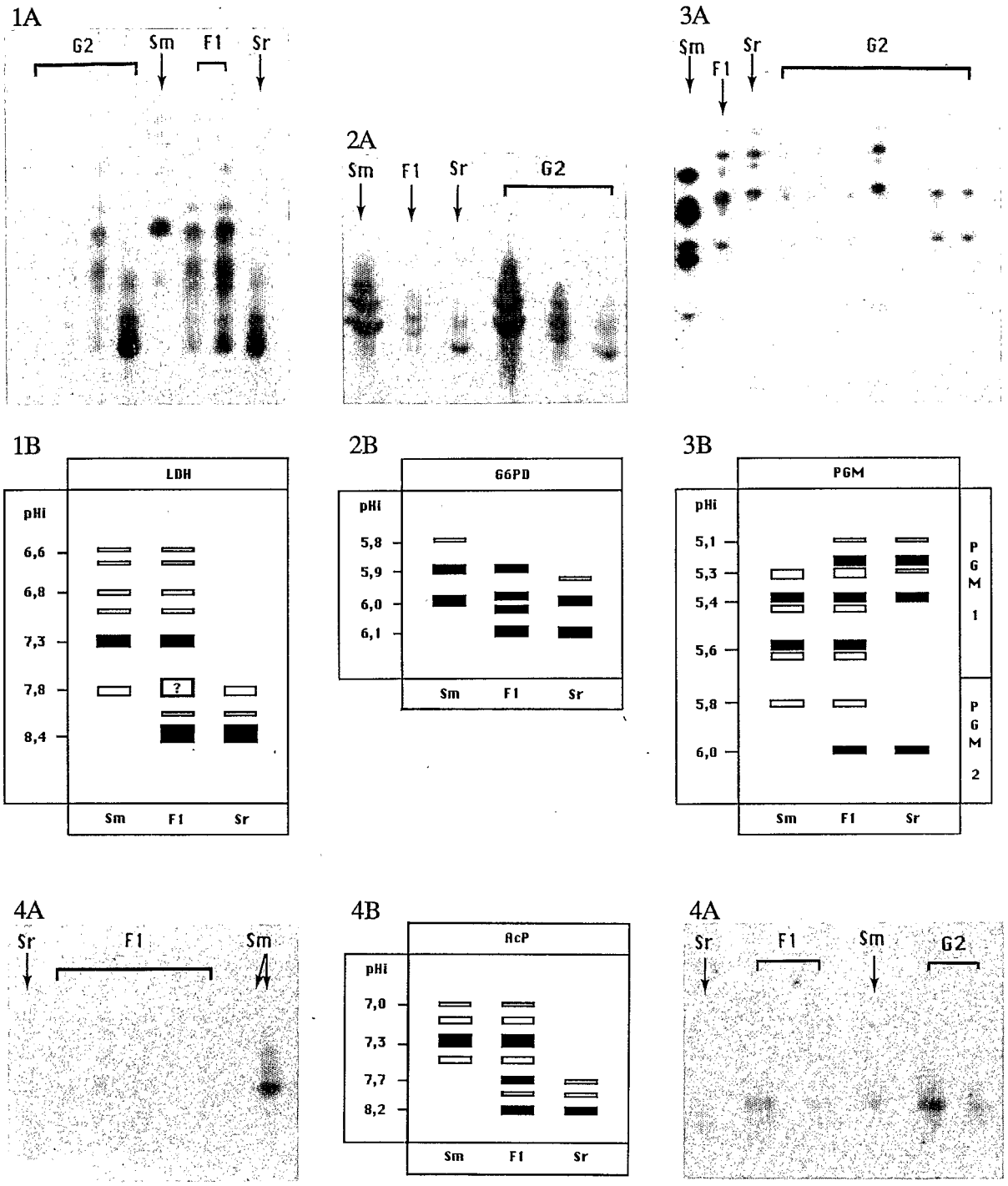
GPM-2 was characterized by a single band of activity, weaker for *S. rodhaini* than for *S. mansoni.* The F1 hybrid possesses both bands (the one from *S. mansoni* being stronger than that from *S. rodhaini*), indicative of a monomeric structure for PGM-2 (Fig. 3). The G2 male schistosomes gave patterns identical to those observed for the parents and the F1 hybrid. The patterns obtained for the G2 females could not be interpreted because of weak activity at this locus.

AcP

AcP, which was monomorphic for each parental strain, showed distinct patterns for *S. mansoni* (major activity at pH 7.3) and *S. rodhaini* (major activity at pH 8.2). The F1 hybrid was characterized by a pattern with three major bands of activity (pH 7.3, 7.7 and 8.2), suggesting a dimeric structure for this enzyme (Fig. 4). The band corresponding to *S. rodhaini* showed less activity than that for *S. mansoni*, the additional fraction at pH 7.7 being of intermediate intensity between both bands. The G2 schistosomes gave patterns identical to those obtained from the parents and the F1 hybrid.

GPI

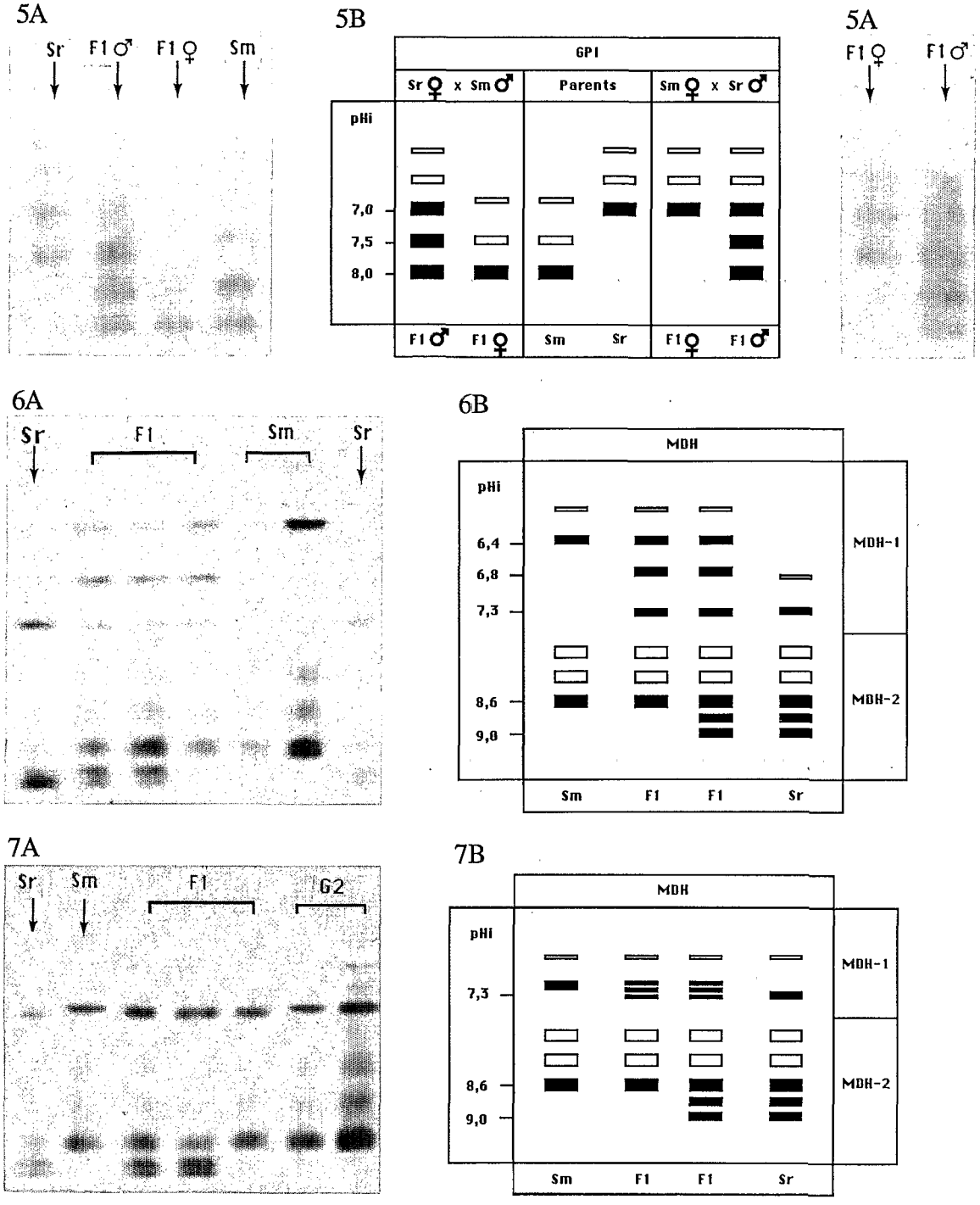
GPI was monomorphic for each parental strain and showed distinct patterns for *S. mansoni* (major activity at pH 8.0) and *S. rodhaini* (major activity at pH 7.0). The results obtained differed according to the particular cross.



Figs. 1–4. A Isozyme patterns observed after isoelectric focusing of individual extracts from *S. mansoni* (*Sm*), *S. rodhaini* (*Sr*), and the hybrids [first (*F1*) and second (*G2*) generation] and **B** their genetic interpretation: *1*, LDH; *2*, G6PD; *3*, PGM; *4*, AcP (*left*, pH gradient 4–9; *right*, pH gradient 3–10)

S. rodhaini female × *S. mansoni male*. The F1 hybrids showed a three-banded pattern for the males (activity at pH 7.0, 7.5 and 8.0), indicative of a dimer (Fig. 5), whereas the females gave a pattern identical to that obtained from the male parent (*S. mansoni*). The G2 schistosomes were not obtained for this cross.

S. mansoni female × *S. rodhaini male*. Male F1 hybrids showed a three-banded hetetozygote pattern comparable with that observed for the reverse cross (Fig. 5), whereas the females gave a pattern identical to that obtained from the parental male (*S. rodhaini*). The G2 schistosomes showed either the F1 hybrid pattern for the males (12 worms



Figs. 5–7. **A** Isozyme patterns observed after isoelectric focusing of individual extracts from *S. mansoni* (*Sm*), *S. rodhaini* (*Sr*), and the hybrids (*F1*) and **B** their genetic interpretation: **Fig. 5**, GPI (*left*, Sr female × Sm male; *right*, reverse crossing); **Fig. 6**, MDH (*Sm*, mdh-1 a/mdh-1 a); **Fig. 7**, MDH (*Sm*, mdh-1 b/mdh-1 b)

analysed) or the two homozygote patterns for the females (8 worms analysed).

MDH

Two loci have previously been identified for MDH in both *S. mansoni* and *S. rodhaini* (Fletcher et al. 1981 a, b; Imbert-Establet et al. 1984; Rollinson et al. 1986). MDH-1 is polymorphic in *S. mansoni* and possesses two alleles, MDH-1 a and MDH-1 b. The patterns observed in our different crossbreeding experiments corresponded to the AA (major activity at pH 6.4) and BB (major activity at pH 7.2) homozygote phenotypes. MDH-1 is

monomorphic in *S. rodhaini*, whose homozygote pattern CC (major activity at pH 7.3) resolved in a position close to that of the BB type observed for *S. mansoni*. The results obtained with MDH-1 differed according to the parental phenotype of *S. mansoni*.

S. mansoni AA × *S. rodhaini CC*. The F1 hybrid showed a three-banded pattern (activity at pH 6.4, 6.8 and 7.3) characteristic of a dimer (Fig. 6), with a lower intensity for the fraction corresponding to *S. rodhaini* (7.3) than for that corresponding to *S. mansoni* (6.4). The G2 schistosomes were not obtained for this cross.

S. mansoni BB × *S. rodhaini CC*. The F1 hybrid showed only one broad band of activity at pH 7.2–7.3 (Fig. 7), which genetically corresponds to an heterozygote with three bands that are indistinguishable because of their proximity. The G2 schistosomes gave patterns identical to those of the parents and the F1 hybrid.

MDH-2 is polymorphic for *S. rodhaini* and possesses two alleles, MDH-2a and MDH-2b. The *S. rodhaini* used in our crossbreeding experiments all showed the three-banded heterozygote phenotype AB (major activity at pH 8.6, 8.8 and 9.0). The dissymmetry of the pattern can be explained by the compression of the fraction at pH 9.0 against the basic strip, due to the pH gradient (3–10) established in the gel. MDH-2 is monomorphic for *S. mansoni*, with an homozygote pattern A′A′ identical to the type AA observed for *S. rodhaini*.

S. mansoni A′A′ × *S. rodhaini AB*. The F1 hybrid showed either one major band of activity at pH 8.6 (corresponding to the genotype A′A) or a three-banded pattern with major activity at pH 8.6, 8.8 and 9.0 (corresponding to the genotype A′B), indicative of a dimer (Figs. 6, 7). The G2 schistosomes gave patterns similar to those observed for the F1 hybrids: activity at pH 8.6 (corresponding to genotypes AA, A′A or A′A′) and activity at pH 8.6, 8.8 and 9.0 (corresponding to genotypes AB or A′B). The BB homozygote was not observed among the 40 G2 schistosomes analysed for MDH-2.

Discussion

All the enzymatic systems tested (LDH, G6PD, AcP, PGM, GPI and MDH), with the exception of HK, enabled *S. mansoni* (Guadeloupe) and *S. rodhaini* (Burundi) to be distinguished by their phenotypes. Fletcher et al. (1981a) could distinguish *S. mansoni* (Puerto Rico and South Africa) from *S. rodhaini* (Kenya) by starch gel electrophoresis using MDH, PGM and GPI, although G6PD, HK and AcP showed identical patterns for both species. Boissezon and Jelnes (1982) obtained slightly different results (positive for PGM and GPI, negative for MDH, G6PD and HK) by polyacrylamide gel electrophoresis. The fact that it was possible to use G6PD and AcP to distinguish the two parental species can be explained by either the different geographic origin of the schistosomes analysed or the higher resolution of the isoelectric focusing (IEF) technique. Indeed, our results agree with those obtained by Rollinson et al. (1986) using IEF in polyacrylamide gel for *S. mansoni* and *S. rodhaini* with the same geographical origins as used in this study. The crosses carried out between *S. mansoni* and *S. rodhaini* led to viable and fertile offspring that were characterized by their enzyme profiles for six of the seven systems tested. These experimental hybridizations also help to answer questions relating to the genetic interpretation of the sometimes complex patterns observed with the IEF technique.

Our results concerning MDH, PGM and GPI validate the existence of a dimeric structure for MDH and GPI and a monomeric structure for PGM, as suggested by Fletcher et al. (1981a, b) and Rollinson et al. (1986); they also confirm the existence of two loci for MDH and PGM. Analyses for GPI were characterized by the lack of heterozygote patterns in the female, as has previously been noted for other species of schistosomes such as *S. bovis* (Southgate et al. 1980) and *S. intercalatum* (Brown et al. 1984). This phenomenon can be explained by the absence of the GPI gene on the W chromosome, only present in the female (heterogametic on schistosomes: Grossman et al. 1981; Short 1983). Thus, in the case of two a and b alleles located on the Z chromosome, a female may have the homozygote genotypes Za/W or Zb/W, whereas the male possesses the homozygote genotypes Za/Za or Zb/Zb and the heterozygote genotype Za/Zb. These results show the existence of a sex-linked heredity for GPI in *S. rodhaini*. This phenomenon has been previously demonstrated for *S. mansoni* (Jelnes 1983).

The data generated for AcP, LDH and G6PD raise problems, some of which remained unsolved: 1. For the F1 hybrid, AcP showed at three-banded pattern indicative of a dimer. However, variation occurred in the intensity of the staining of the bands corresponding to the parental parasites, a phenomenon also observed to a lesser degree for MDH-1 and PGM-2. The lower intensity of the AcP fraction from *S. rodhaini* in the hybrid may

show a weaker enzyme production for the part of the genome corresponding to *S. rodhaini* than for that of *S. mansoni*.

2. LDH, which has a tetrameric structure in most living organisms, did not show the five-banded pattern expected for the hybrid (expected in the case of an heterozygote with a locus possessing two different alleles for each parental species); this may be the consequence of the expression of a regulating gene controlling the proportions of the different LDH allozymes. Imbert-Establet et al. (1984) observed LDH polymorphism in a strain of *S. mansoni* and could select parasites with distinct LDH types by single miracidial infections and sporocyst transplantation. Studies based on intraspecific crossbreeds might elucidate the genetic nature of the patterns obtained in the case of *S. mansoni* (Fletcher et al. 1981a, b; Imbert-Establet et al. 1984; Rollinson et al. 1986), for which it has not been possible to propose a genetic interpretation.

3. G6PD gave results that raise questions similar to those raised by the data put forward by Wright and Ross (1980, 1983): these authors observed a pattern characteristic of a dimer for the F1 hybrids between *S. haematobium* and *S. matthei,* although the supposed heterozygote between two types of *S. haematobium* did not show any central additional fraction, suggesting a monomeric structure for G6PD. Our results shown a pattern for the F1 hybrids that does not correspond to either that expected in the case of a dimer (no additional central band) or that expected in the case of a monomer (the two central bands are offset in relation to those observed at pH 6.0 for both parents); they do not lead to precise genetic interpretation of the patterns obtained with G6PD.

More detailed research is therefore required for LDH and G6PD and could be complemented by the study of other enzymatic markers such as AK (adenylate kinase; E.C. 2.7.4.3), LGG (leucylglycylglycine aminopeptidase; E.C. 3.4.1.3), and MPI (mannose-phosphate isomerase; E.C. 5.3.1.8), which have been successfully used to distinguish *S. mansoni* and *S. rodhaini* (Fletcher et al. 1981a; Boissezon and Jelnes 1982).

The characterization of schistosome hybrids between *S. mansoni* and *S. rodhaini* has been the object of detailed studies, starting with morphological markers (eggs, number of testicles: Taylor 1970), behavioural markers (cercarial rhythms of emergence: Théron 1989) and compatibility with vector snails (Taylor 1970). Our work complements these analyses by the use of biochemical markers and confirms the existence of close phyletic relationships between *S. mansoni* and *S. rodhaini*. Although the existence of natural hybridization between these two species has not yet been demonstrated, some records point to such a possibility: *S. mansoni* and *S. rodhaini* are sympatric in several African foci; *S. rodhaini*, a rodent parasite, has been found in man (Gillet 1960; D'Haenens and Santele 1955); and, finally, *S. mansoni* var *rodentorum,* described in African rodents by Schwetz (1954), possessed eggs with a morphology very similar to that of eggs from experimental hybrid schistosomes. The use of electrophoretic techniques should provide partial answers to some of the questions raised by the population biology of these schistosomes and, more generally, to the problem of the existence of genetic flow in the complex of species of the *Schistosoma* genus.

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References

Boissezon B, Jelnes JE (1982) Isoenzyme studies on cercariae from monoinfections and adult worms of *Schistosoma mansoni* (10 isolates) and *S. rodhaini* (one isolate) by horizontal polyacrylamide gel electrophoresis and staining of eight enzymes. Z Parasitenkd 67:185–196

Brown DS, Sarfati C, Southgate VR, Ross GC, Knowles RJ (1984) Observations on *Schistosoma intercalatum* in southeast Gabon. Z Parasitenkd 70:243–253

D'Haenens G, Santele A (1955) Sur un cas humain de *Schistosoma rodhaini* trouvé aux environs d'Elisabethville. Ann Soc Belg Med Trop 35:497

Fletcher M, Loverde PT, Kuntz RE (1981a) Electrophoretic differences between *Schistosoma mansoni* and *S. rodhaini.* J Parasitol 67:593–595

Fletcher M, Loverde PT, Woodruff DS (1981b) Genetic variation in *Schistosoma mansoni:* enzyme polymorphisms in populations from Africa, Southwest Asia, South America and the West Indies. Am J Trop Med Hyg 30:406–421

Gillet J (1960) Note sur un foyer de bilharziose murine à *Schistosoma rodhaini* Brumpt au Kivu. Ann Soc Belg Med Trop 40:639–641

Grossman AI, Short RB, Cain GD (1981) Karyotype evolution and sex chromosome differentiation in schistosomes (Trematoda, Schistosomatidae). Chromosoma 84:413–430

Imbert-Establet D, Rollinson D, Ross GC (1984) Sélection de génotypes de *Schistosoma mansoni* et leur maintenance par transplantations sporocystiques. CR Acad Sci Paris Ser III 11:459–462

Jelnes JE (1983) Phosphoglucose isomerase: a sex-linked character in *Schistosoma mansoni.* J Parasitol 69:780–781

Jourdane J, Théron A (1980) *Schistosoma mansoni;* cloning by microsurgical transplatation of sporocysts. Exp Parasitol 50:349–357

Le Roux PL (1954) Hybridisation of *Schistosoma mansoni* and *Schistosoma rodhaini.* Trans R Soc Trop Med Hyg 48:3–4

Rollinson D, Southgate VR (1985) Schistosome and snail populations: genetic variability and parasite transmission. In:

Rollinson D, Anderson RM (ed) Ecology and genetics of host-parasite interactions. Academic, London, pp 91–109

Rollinson D, Imbert-Establet D, Ross GC (1986) *Schistosoma mansoni* from naturally infected *Rattus rattus* in Guadeloupe: identification, prevalence and enzyme polymorphism. Parasitology 93:39–53

Schwetz J (1954) On two schistosomes of wild rodents of the Belgian Congo: *Schistosoma rodhaini* Brumpt, 1931 and *Schistosoma mansoni* var. *rodentorum* Schwetz, 1953 and their relationship to *S. mansoni* of man. Trans R Soc Trop Med Hyg 48:89–100

Short RB (1983) Presidential address. J Parasitol 69:3–22

Southgate VR, Van Wijk HB, Wright CA (1976) Schistosomiasis at Loum, Cameroun; *Schistosoma haematobium, S. intercalatum* and their natural hybrid. Z Parasitenkd 49:145–159

Southgate VR, Rollinson D, Ross GC, Knowles RJ (1980) Observations on an isolate of *Schistosoma bovis* from Tanzania. Z Parasitenkd 63:241–249

Taylor MG (1970) Hybridization experiments on five species of African schistosomes. J Helminthol 49:253–314

Théron A (1989) Hybrids between *Schistosoma mansoni* and *S. rodhaini;* characterization by cercarial emergence rhythms. Parasitology (in press)

Wright CA, Ross GC (1980) Hybrids between *Schistosoma haematobium* and *S. matthei* and their identification by isoelectric focusing of enzymes. Trans R Soc Trop Med Hyg 74:326–332

Wright CA, Ross GC (1983) Enzyme analysis of *Schistosoma haematobium*. Bull WHO 61:307–316

Wright CA, Southgate VR (1976) Hybridization of schistosomes and some of its implications. In: Taylor A, Muller R (eds) Genetic aspects of host-parasite interactions. Blackwell Scientific, Oxford, pp 55–86

Wright CA, Southgate VR, Ross GC (1979) Enzymes in *Schistosoma intercalatum* and the relative status of the lower Guinea and Zaire strains of the parasite. Int J Parasitol 9:523–528

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