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RESEARCH COMMUNICATION

FURTHER OBSERVATIONS ON THE ELECTROPHORETIC CHARACTERIZATION OF SOUTH AFRICAN *SCHISTOSOMA MATTHEEI* AND *S. HAEMATOBIUM*

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ABSTRACT

KRUGER, F. J., 1988. Further observations on the electrophoretic characterization of South African *Schistosoma mattheei* and *S. haematobium*. *Onderstepoort Journal of Veterinary Research*, 55, 67–68 (1988)

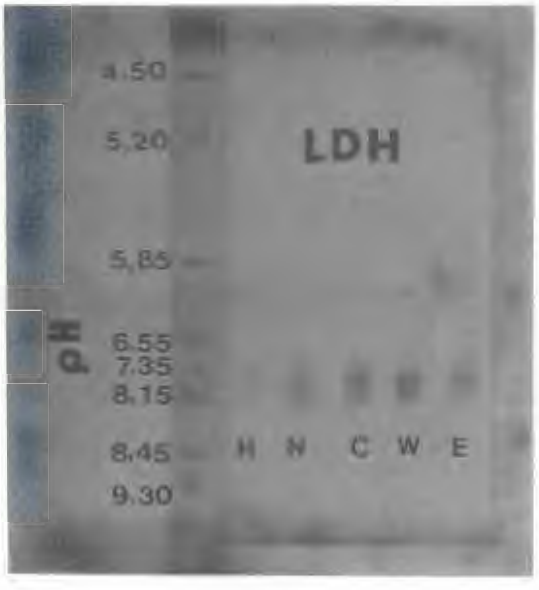
Eleven enzymes, which were used to compare South African *S. mattheei* and *S. haematobium* in a former study, were employed to study intraspecific variation within *S. mattheei*, using starch gel electrophoresis and iso-electric focusing where resolution in starch gel was poor.

Acid phosphatase varied intraspecifically within *S. mattheei* in that the most southern population differed from the northern populations. Malate dehydrogenase also varied intraspecifically. Three populations which occur sympatric with *S. haematobium* had a MDH-1 allele in common with the human schistosome while an allopatric population did not.

Kruger (1987) studied the electrophoretic patterns of acid phosphatase (ACP), aldolase (ALD), glucose-6-phosphate dehydrogenase (G6PD), glutamate oxaloacetate transaminase (GOT), hexokinase (HK), lactate dehydrogenase (LDH), leucylglycylglycine aminopeptidase (LGG), malate dehydrogenase (MDH), 1-naphthyl acetate esterase (EST), octanol dehydrogenase (ODH) and phosphoglucomutase (PGM) from South African *Schistosoma mattheei* and *S. haematobium* on starch gel. It was concluded that these 2 species are closely related when compared to the interspecies relationships within other species groups. This paper reports on additional biochemical-systematic observations on the 2 spesies.

Because ACP, LDH and ALD produced diffuse patterns on starch gel (Kruger, 1987), these enzymes were investigated by isoelectric focusing (IEF) to obtain improved resolution. Focusing was performed in 5 % Ampholine Pagplates (LKB, Bromma) at a pH of 3,5–9,5. The instructions supplied by the manufacturer were closely followed. In order to determine the pH gradient profile of the gels, a broad pH calibration kit (Pharmacia Fine Chemicals) was used.

An electrophoretic study was conducted on the *S. mattheei* populations from the Eastern Transvaal, Western Transvaal, Northern Natal and Eastern Cape previously studied by Kruger, Schutte, Visser & Evans (1986) and by Kruger, Hamilton-Attwell & Schutte (1986). Except for the 3 enzymes mentioned above, which were analysed by IEF, electrophoresis was performed on 7,5 % starch gels using the enzymes and method described by Kruger (1987).



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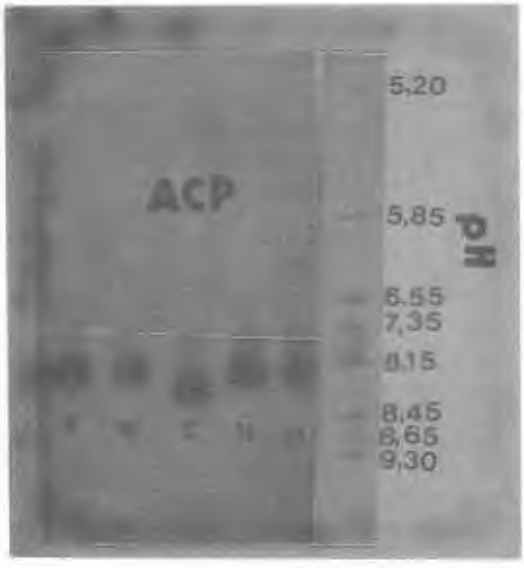

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FIG. 1, 2 & 3 Iso-electric focusing of lactate dehydrogenase (LDH), aldolase (ALD) and acid phosphatase (ACP) from 4 populations of *S. mattheei* and South African *S. haematobium* on 5 % polyacrylamide gels, pH 3,5–9,5. (E,W,C,N = *S. mattheei* from the Eastern Transvaal, Western Transvaal, Eastern Cape and Northern Natal: H = *S. haematobium*)

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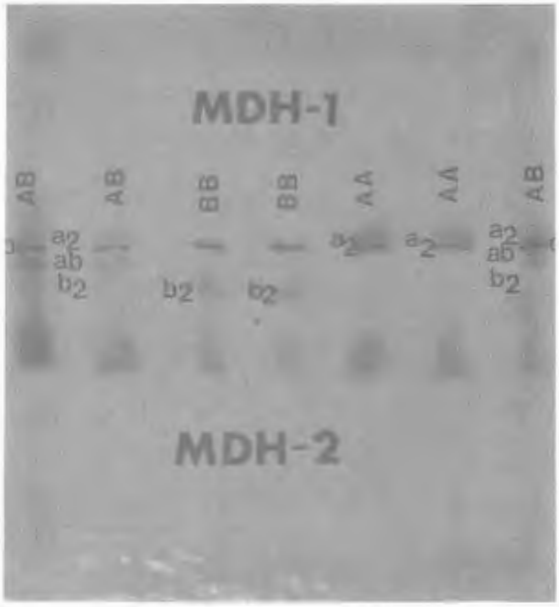

FIG. 4 Electrophoresis of malate dehydrogenase isoenzymes (MDH-1 & MDH-2) in 7,5 % strach gels. See text for the frequencies at which the alleles occurred in the different populations (AA = MDH-1AA homozygote: AB =MDH-1AB heterozygote: BB = MDH-1BB homozygote: a & b = subunits of dimeric MDH-1: O = origin)

It was found that in all the populations of *S. mattheei*, as well as in *S. haematobium*, LDH focused as 2 closely associated bands between pH 7,35 and pH 8,15 (Fig. 1). ALD focused as a single band between pH 6,55 and pH 7,35 in all the samples (Fig. 2). ACP focused as a single band slightly acidic to pI marker 8,15 (Fig. 3). However, the enzyme from the Eastern Cape *S. mattheei* population had a more alkaline pI than the other isolates of *S. mattheei* and *S. haematobium*. The Eastern Cape *S. mattheei* population is situated at the southernmost tip of the distribution range of *S. mattheei* (Gear, Pitchford & Van Eeden, 1980) and this observation may be an indication of a divergence between this population and the rest of the species further north.

The pI's recorded for LDH and ACP in the present study corresponded with the pH regions where the most intensive staining were recorded for LDH and ACP in *S. mattheei* and *S. haematobium* by Ross (1976), for LDH and ACP in *S. mattheei* by Ross, Southgate & Knowles (1978) and for LDH in *S. haematobium* by Wright & Ross (1983). As the above authors used gels with a narrower pH range, they have also recorded a number of additional, lighter, bands.

Of the 8 enzymes studied on starch gel, only MDH-1 exhibited intraspecific variation (Fig 4). Two allozymes (alleles) were recorded. Western Transvaal *S. mattheei* was monomorphic for MDH-1BB and *S. haematobium* was monomorphic for MDH-1AA. Northern Natal and Eastern Cape *S. mattheei* exhibited 2 MDH-1 patterns: firstly a single band with a similar Rf value to MDH-1AA, and secondly a triple-banded pattern. The Northern Natal isolate exhibited the 2 patterns in approximately a 2:1 ratio from the first intra-murine generation onwards. The Eastern Cape isolate exhibited the patterns in approximately a 9:1 ratio during the first 2 generations, after which the triple-banded pattern disappeared. The Eastern Transvaal population contained the MDH-1AA, the MDH-1BB and the triple-banded pattern during the first and second generations, after which the MDH-1BB pattern disappeared in the subsequent generations. A cross-breeding experiment conducted during the course of this study has shown that the triple-banded pattern is the MDH-1AB heterozygote. The 3 bands are to be attributed to the dimeric structure of the enzyme.

Rotmans (1978) has shown that the molecular mass of MDH from *S. mansoni* falls within the range of dimeric vertebrate MDH and has also recorded dimerization during gel filtration. MDH is known to be subject to post-translational modifications possibly as a result of epigenetic influences (Ferguson, 1980). This may explain the presence of the MDH-1AB heterozygote in the absence of the MDH-1BB homozygote since their founding in the Northern Natal and Eastern Cape populations and since the 3rd generation in the Eastern Transvaal population. Coles (1971) recorded similar structural changes in *S. mansoni* MDH-1 during passage through rodents.

Nevertheless the results recorded correspond with the morphological observations of Kruger *et al.* (1986) in that the Western Transvaal *S. mattheei* population, which occurs allopatric to *S. haematobium*, has the least in common with the human parasite. There are indications that MDH may serve as an indicator of micro-evolutionary changes in schistosomes as shown by the marked differences in the frequency of MDH-1 alleles in *S. mansoni* from man and *Rattus rattus* in Guadeloupe by Rollinson, Imbert-Establet & Ross (1986).

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