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The interaction of *Schistosoma haematobium* and *S. guineensis* in Cameroon

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Abstract

Interactions between schistosomes are complex with some different species being able to mate and hybridize. The epidemiology of schistosomiasis in specific areas of South West Cameroon has evolved remarkably over 30 years as a result of hybridization between *Schistosoma guineensis* and *S. haematobium*. Morphological and biological data suggest that *S. haematobium* replaced *S. guineensis* in areas of Cameroon through introgressive hybridization. Data are reported on the use of single stranded conformational polymorphism (SSCP) analysis of the nuclear ribosomal second internal transcribed spacer (ITS2) of individual schistosomes from hybrid zones of Cameroon. The data show that since 1990 *S. haematobium* has completely replaced *S. guineensis* in Loum, with *S. haematobium* and the recombinants still present in 2000. This study illustrates the complexities of the dynamics between *S. haematobium* and *S. guineensis* in South West Cameroon.

Introduction

One of the most intriguing features about schistosomes is that they are dioecious and male and female worms have to come together in the definitive host to mate thus enabling the female worm to produce eggs so that the life-cycle may continue. Heterospecific interactions sometimes occur between male and female schistosomes if they infect the same definitive host. The outcome of such heterospecific interactions depends upon the phylogenetic distance of the two species involved, usually resulting in either parthenogenesis or hybridization. These interspecific interactions may potentially have important consequences on parasite epidemiology, and in some cases may influence the progressive exclusion of a particular species from a given area (Southgate *et al.*, 1982, 1998; Tchuem Tchuenté *et al.*, 1993, 1996, 1997; Southgate, 1997).

Until recently there were three known species of schistosome infecting man in Africa, *S. mansoni*, *S. haematobium* and *S. intercalatum*. For about the last 30 years two distinct strains of *S. intercalatum* have been recognized, one from the Democratic Republic of Congo and the other from the Lower Guinea region of West Africa (e.g. Cameroon, Gabon, Equatorial Guinea and São Tomé). The two strains differed from each other in a number of features, including pre-patent periods in the intermediate and definitive hosts, egg morphology, intermediate host–parasite relationships and isoenzymes (Wright *et al.*, 1972). Recent studies on hybrid breakdown between the two strains of *S. intercalatum* and phylogenetic relationships based on three mitochondrial genes (cytochrome oxidase subunit 1, NADH dehydrogenases subunit 6 and the small ribosomal RNA gene) supported

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the division of *S. intercalatum* into two separate species (Pagès *et al.*, 2002; Kane *et al.*, 2003) The parasite from Lower Guinea has been re-described as *Schistosoma guineensis* (Pagès *et al.*, 2003). Ecological changes in parts of South West Cameroon, West Africa have created areas of sympatry between *S. haematobium* and *S. guineensis* enabling these two species of schistosome to interact and hybridize in Nature and this has had a dramatic effect of the epidemiology of these two species in these areas (Southgate *et al.*, 1976).

For example, the epidemiology of schistosomiasis in Loum, Cameroon evolved in a remarkable manner over about 30 years (fig. 1). In 1968, only *S. guineensis* was known to occur in Loum transmitted through its intermediate snail host *Bulinus forskalii* and the parasite eggs were passed in the faeces of the infected definitive hosts (van Wijk, 1969). However, by 1972, a number of children were found to be passing schistosome eggs in their urine, and these eggs ranged in size and shape from forms characteristic of *S. haematobium* to those of *S. guineensis*. Thus, it seemed evident that between the late 1960s and early 1970s *S. haematobium* became established in Loum, and laboratory snail infection experiments subsequently demonstrated that cases of natural hybridization were occurring between *S. guineensis* and *S. haematobium* (Wright *et al.*, 1974; Southgate *et al.*, 1976).

The hybridization of these two species in Loum had a dramatic effect on the epidemiology of schistosomiasis in the area, and observations in 1978 confirmed the earlier predictions of a swing from intestinal schistosomiasis to urinary schistosomiasis (Rollinson & Southgate, 1985). The data from Ratard *et al.* (1990) also supported the same swing with only 5% of children passing eggs in their faeces and 33% passing eggs in their urine. In 1996, a survey examining 426 schoolchildren in Loum, aged between 4 and 17 years, demonstrated a prevalence of 52% with urinary schistosomiasis and none with intestinal schistosomiasis (Tchuem Tchuenté *et al.*, 1997). This survey indicated that in a period of about 25 years *S. haematobium* had completely replaced *S. guineensis* through introgressive hybridization, possibly associated with an interspecific competitive exclusion mechanism (Southgate *et al.*, 1982; Tchuem Tchuenté *et al.*, 1996).

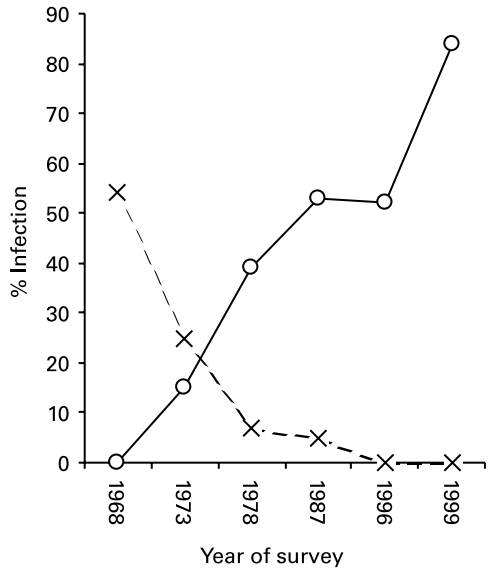

Fig. 1. Changes in prevalence of urinary —o— (*Schistosoma haematobium*) and intestinal —×— (*S. guineensis*) schistosomiasis in Loum from 1968 to 1999.

Reports of hybridization between *S. haematobium* and *S. guineensis* in Loum and other areas of Cameroon were based primarily upon egg morphology and intermediate host compatibility (Duke & Moore, 1976; Wright & Southgate, 1976). A molecular technique known as single-stranded conformational polymorphism (SSCP) proved to be useful in schistosome identification (Kane *et al.*, 2002). In this paper data are reported on the use of SSCP analysis of the nuclear ribosomal second internal transcribed spacer (ITS2) of individual schistosomes from isolates collected from areas of Cameroon where hybridization between *S. haematobium* and *S. guineensis* has thought to have occurred, and also on laboratory-bred parental and hybrid schistosomes of these two species. This study was aimed at providing a more detailed analysis of the interactions and dynamics of *S. haematobium* and *S. guineensis* in South West Cameroon between 1989 and 2001.

Materials and methods

Origin of parasite material

Schistosoma haematobium

The *S. haematobium* isolate originated from the village of Barombi Mbo, Cameroon. *S chistosoma haematobium* eggs were collected and amalgamated from five urine samples collected from local schoolchildren. The eggs were hatched and *B. wrighti* were infected. The isolate was maintained in the laboratory as described in Webster *et al.* (2003).

Schistosoma guineensis

Twenty-six naturally infected *B. forskalii* were collected from Edea, Cameroon in 1998. *Schistosoma guineensis* cercariae shed from these snails were used to establish the infection in the laboratory as described in Webster *et al.* (2003).

Laboratory S. haematobium, S. guineensis *crosses and production of hybrid schistosomes*

Laboratory hybridization experiments were carried out using single miracidium snail infections to produce generations of *S. haematobium* ♂ × *S. guineensis* ♀ and *S. guineensis* ♂ × *S. haematobium* ♀ hybrids for molecular analysis as described in Webster *et al.* (2003).

Natural isolates

Isolate 1 originated from polymorphic shaped eggs passed in the urine of a young boy from Kumba in 1990, isolate 2 from *S. haematobium* shaped eggs passed in the urine samples of young children from Loum in 1990, isolate 3 from cercariae from naturally-infected

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B. truncatus snails collected from Loum in 1990, isolate 4 from *S. haematobium*-shaped eggs passed in the urine samples of young children from Loum in 1999 and isolate 5 from *S. haematobium*-shaped eggs passed in the urine samples of young children from Loum in 2000. Each isolate was established and maintained in the laboratory and schistosomes recovered for molecular analysis as described in Webster *et al.* (2003).

SSCP ITS2 analysis

Twenty male and 20 female worms were analysed from each of the following samples: (i) *S. haematobium*; (ii) *S. guineensis*; (iii) *S. haematobium* ♂ × *S. guineensis* ♀F_1 laboratory hybrid; (iv) *S. guineensis* ♂ × *S. haematobium* ♀F_1 laboratory hybrid; (v) isolate 1; (vi) isolate 2; (vii) isolate 3; (viii) isolate 4; (ix) isolate 5.

DNA extraction, fluorescent ITS2 amplification and SSCP analysis

Samples were recovered from liquid nitrogen and thawed slowly on ice. DNA from each individual worm was extracted by the method described in Webster *et al.* (2003).

Amplifications from each individual worm were performed in a total reaction volume of 25 μl containing a Ready-to-go polymerase chain reaction (PCR) bead (components are: 1.5 units DNA Taq Polymerase, 10 mM Tris-HCl pH 9, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP + stabilizers), Primers: ITS2 SSCP1 2 μl (31.6 pmol), ITS2 SSCP2 2 μl (29.05 pmol), DNA O.5 μl ~25 ng and 20.5 μl sterile distilled PCR water to make the reaction up to 25 μl. Fluorescently labelled ITS2 PCR products were generated using the forward primer ITS2 SSCP1 (5′GCATATCAACGCGGG 3′) (Kane *et al.*, 2002) and was labelled at the 5′ end with FAM while the sequence of the reverse primer, ITS2 SSCP2, was (5′ ACAAACCGTAGACCGAACC 3′) (Kane *et al.*, 2002) and was labelled at the 5′ end with HEX. Thermal cycling was performed in a Perkin Elmer 9600 Thermal Cycler programmed for an initial denaturing step of 10 min at 94 °C, and then 30 cycles of 1 min at 94 °C (denaturing), 1 min at 58 °C (annealing), 1 min at 72 °C (elongation), and there was a final extension period of 10 min at 72 °C. Samples were kept at 4 °C until they were visualized on an ethidium bromide agarose gel. All PCR products were purified using Qiagen PCR Purification Kits (Qiagen).

SSCP analysis of each purified PCR product was carried out on an ABI Prism 377^{TM} automated sequencer, using a Perkin Elmer Applied Biosystems protocol but with specific modifications and run conditions as described by Kane *et al.* (2002).

Results

Single-stranded conformational polymorphism analysis of the ITS2 fragments produced electronic images clearly showing unique SSCP profiles for *S. haematobium* and *S. guineensis* (fig. 2). Characteristic 4-banded SSCP profiles were produced for the laboratory *S. haematobium* × *S. guineensis* hybrids incorporating all bands from both the parental species (fig. 2).

ITS2 SSCP analysis of the adult worms originating from the natural isolates demonstrated that hybridization between *S. haematobium* and *S. guineensis* had occurred with some individual worms presenting SSCP ITS2 profiles identical to that of the laboratory bred hybrids (fig. 3). All schistosomes from the Kumba 1990 samples presented a hybrid profile, and 33% of individual schistosomes originating from eggs collected from Loum in 1990, and 5% of individuals from Loum collected in 1999 and 2000 produced hybrid profiles. All other individuals examined presented profiles characteristic of *S. haematobium* and no individual male or female worm analysed in this study produced a *S. guineensis* profile.

Discussion

Single stranded conformational polymorphism ITS2 analysis enabled the identification of *S. haematobium*/*S. guineensis* hybrids facilitating the characterization of hybrid populations in nature. The SSCP data clearly suggest a major shift from *S. guineensis* to *S. haematobium* over a 30-year time period in Cameroon. All of the individuals examined originating from polymorphic eggs collected from Kumba in 1990 were recombinants and 33% of schistosomes examined originating from isolates from Loum in 1990 exhibiting intra-uterine eggs typical of *S. haematobium* were found to be recombinants, and this figure dwindled to a mere 5% of individuals collected in 1999 and 2000 from Loum. There was no evidence of *S. guineensis* transmission taking place in these areas despite the presence of its intermediate snail host *Bulinus forskalii*. SSCP analysis showed that some individuals from isolates that were considered to be *S. haematobium* based solely on egg morphology were actually hybrids and this detail would have been lost without this sort of analysis. This indicates that whilst morphological data are very important they cannot be relied on too heavily in this type of study.

Molecular data presented here clearly show that *S. haematobium* has completely replaced *S. guineensis* in Loum, and that *S. haematobium* and the recombinants were present in Loum in the year 2000. There are several traits that place *S. haematobium* with reproductive, behavioural

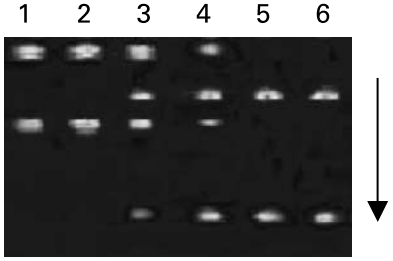

Fig. 2. Electronic image of the SSCP ITS2 profiles from the laboratory parental and hybrid schistosomes. Lanes 1–2, *Schistosoma guineensis*; lane 3, (S. h ♂ × S. g ♀) F_1 hybrid; lane 4, (S. h ♀ × S. g ♂) F_1 hybrid; lanes 5-6, *S. haematobium*.

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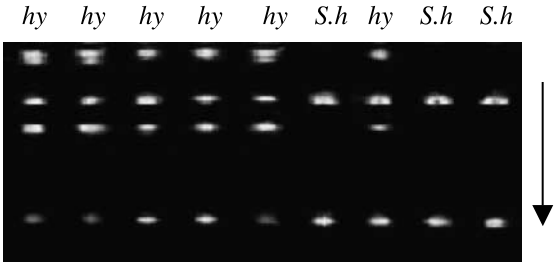

Fig. 3. Electronic image of the SSCP ITS2 profiles from natural isolates from Cameroon. *hy,* hybrid; *S.h, Schistosoma haematobium*.

and genetic advantages over *S. guineensis*, which could afford some explanation, together with environmental factors, as to why *S. haematobium* has replaced *S. guineensis* through introgressive hybridization (Morand *et al.*, 2002).

The data show the complexity of the interactions between *S. guineensis* and *S. haematobium* and their hybrids in Cameroon over time, and demonstrate how the epidemiology of schistosomiasis changed between 1990 and 2000, strongly indicating that the dynamics of the interactions of these parasites at any particular time should not be based solely on morphological and biological data.

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