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Isoenzyme analysis of *Schistosoma haematobium*, *S. intercalatum* and their hybrids and occurrences of natural hybridization in Cameroon

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Abstract

Isoelectric focusing of glucose-6-phosphate dehydrogenase (G6PD) produced clearly identifiable profiles for *S. haematobium* and *S. intercalatum* and their hybrids. To provide a more detailed analysis of the interactions of *S. haematobium* and *S. intercalatum* in South West Cameroon over the last 12 years, G6PD analyses were carried out on individual schistosomes collected in Kumba in 1990, Loum in 1990, 1999 and 2000 and Barombi Mbo and Barombi Kotto in 1999. Studies were also carried out on the two parental species *S. haematobium* Barombi Mbo, *S. intercalatum* Edea and subsequent generations of hybrids resulting from laboratory crosses of the two parental species. The isoenzyme analysis demonstrated that the 1990 isolate from Kumba, was a recombinant of *S. intercalatum* × *S. haematobium*, and that 30% of individual schistosomes collected in 1990 in Loum were also recombinants. The remainder gave data indicative of *S. haematobium*. In 1999, 12.5% of individuals from Loum showed recombination and 10% in 2000. Results from the most recent parasitological survey in October 2000 showed the persistence of the recombinant population in addition to that of *S. haematobium*. There was also evidence of recombination having taken place in Barombi Kotto but not Barombi Mbo. This study demonstrates how the situation has changed over the last 12 years, and emphasizes the importance of assessing morphological, biological and molecular data together to gain a true picture of the rapidly evolving situation.

Introduction

The epidemiology of schistosomiasis in Loum has evolved in a remarkable manner over the last 30 years. In 1968 only *Schistosoma intercalatum* was known to occur in Loum and this species was transmitted by *Bulinus forskalii*. In a survey of 500 schoolchildren aged between 4 and 15 years, utilizing rectal scraping techniques, van Wijk (1969) reported a prevalence of 54.2%. However, by 1972 *S. haematobium* had become well established and 15% of 225 children 4–15 years of age had terminal-spined eggs in their urine which ranged in size and shape from forms characteristic of *S. haematobium* to those of *S. intercalatum*. *Schistosoma intercalatum* infections were apparently declining, with only 25% of those same children passing eggs in their stools. Thus, it seemed

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evident that between the late 1960s and early 1970s *S. haematobium* became established in Loum, and laboratory experiments subsequently demonstrated that cases of natural hybridization were occurring between *S. intercalatum* and *S. haematobium*. It became apparent that the hybrid parasite was much more viable from the *S. haematobium* ♂ × *S. intercalatum* ♀ cross than the reverse cross (Wright *et al.*, 1974; Southgate *et al.*, 1976). Furthermore, it was demonstrated that the hybrid parasite was able to utilize both *B. forskalii* and *B. truncatus* as intermediate hosts, unlike the parental parasites. A clear correlation between the distribution of the snail hosts in the river Mbette and its tributaries and the distribution of schistosomes in the human populations was demonstrated (Southgate *et al.*, 1976). These same authors postulated that *B. truncatus*, the host of *S. haematobium*, probably became established in Loum as a result of forest clearance. Once the snails became established in Loum and environs it is relatively easy to envisage *S. haematobium* being introduced to and becoming established with the movements of people to Loum from areas of high endemicity for *S. haematobium*. Observations in 1978 confirmed the earlier predictions of a swing to urinary schistosomiasis (38.7%, n = 93) from intestinal schistosomiasis (6.5%, n = 93) (Rollinson & Southgate, 1985). The data from Ratard *et al.* (1990) also showed a further decline among schoolchildren passing eggs in the stools to 5% and an increase to 33% of those passing eggs in their urine. Examination of 426 schoolchildren in Loum, aged between 4 and 17 years, in 1996 demonstrated a prevalence of 52% (221 children) with urinary schistosomiasis and none with intestinal schistosomiasis (Tchuem Tchuenté *et al.*, 1997). In a period of about 25 years *S. haematobium* completely replaced *S. intercalatum* through introgressive hybridization, possibly associated with an interspecific competitive exclusion mechanism (Southgate *et al.*, 1982; Tchuem Tchuenté *et al.*, 1996). Morand *et al.* (2002) showed, using a mathematical model, that the unequal sex ratios in schistosome populations in favour of the male sex, and the greater ability of male *S. haematobium* worms to pair with female worms than male *S. intercalatum*, are critical factors in driving the outcome of this interaction, that is, the replacement of *S. intercalatum* by *S. haematobium* through introgression.

The focus at Kumba in 1990 appears to be of recent origin: schistosomiasis probably became established there sometime during 1965 and 1990. This represents another example of new urban foci of *S. haematobium*. The town of Kumba, in the Department of Meme, South-West Province of Cameroon, lies at 4°38′N, 9°26′E, on the banks of the Kumba River and is an administrative and commercial centre with a population of about 90,000 of mixed background and socio-economic status. Sama & Ratard (1994) reported that the area of Kumba was a new and intense focus of schistosomiasis and it is now known that urinary schistosomiasis occurs in this area. Although fountains provide clean water, the population of Kumba still uses the river and streams, which cross the town, for most of its bathing, laundry and swimming, providing transmission sites for schistosomiasis.

The village of Barombi Mbo lies at 4°40′N, 9°23′E, and is situated 1 km from Kumba, about 400 m back from the north-west shore of the crater lake to which it gives its name. The transmission of *S. haematobium* in the village of Barombi Mbo has been recognized for some considerable time (Duke & Moore, 1976). Only one species of bulinid snail is found in Lake Barombi Mbo, *B. truncatus*. However, in the streams in the environs of Kumba, *B. forskalii* has been found. Parasite material isolated by Drs D. Rollinson and G. Greer in 1990 (personal communication) from people based in Kumba has been included in the study. In 1990 a survey by Rollinson and Greer (personal communication) reported that there was evidence of hybridization occurring in Kumba on the basis of polymorphic eggs being passed by children in their urine samples, indicating that possible hybridization between *S. haematobium* and *S. intercalatum* had been taking place.

Barombi Kotto is also an area of interest as it has been predicted that hybridization between *S. intercalatum* and *S. haematobium* may have taken place in the area, due to the fact that both the snail intermediate hosts *B. camerunensis* and *B. truncatus* are found, facilitating the possible establishment of both *S. haematobium* and *S. intercalatum* and hence hybridization of these two species in the area (Wright & Southgate, 1976).

The aim of the present study was to analyse parasite material collected in Kumba in 1990, Loum in 1990, 1999 and 2000 and Barombi Mbo and Barombi Kotto in 1999, using isoenzymes with a view to a more precise characterization of variation within the populations of isolates collected from areas around Cameroon to identify recombination taking place and to compare these individuals with laboratory-bred parental species and hybrids of the early generations.

Three enzyme systems were tested and one, glucose-6-phosphate dehydrogenase (G6PDH), which had been particularly valuable in previous studies (Southgate *et al.*, 1982), proved to be the most effective. Therefore this enzyme system was utilized for this preliminary study of the evolution of hybridization between *S. intercalatum* and *S. haematobium* in Cameroon.

Materials and methods

Schistosoma haematobium

Schistosoma haematobium was isolated from the urine of five infected schoolchildren in the village of Barombi Mbo in 1999, and was maintained in the laboratory in *Bulinus wrighti* and the golden hamster, *Mesocricetus auratus*.

Schistosoma intercalatum

Schistosoma intercalatum was isolated in 1998 from a batch of 26 naturally infected *Bulinus forskalii* collected in Edea, Cameroon and was routinely maintained in Swiss mice and *B. wrighti*.

Crosses and production of hybrid generations

Schistosoma haematobium and *S. intercalatum* miracidia were obtained from laboratory animals by the technique described by Taylor (1970). A total of 100 *B. wrighti* (about 3–5 mm height) were exposed individually to one miracidium, 50 snails for each parasite species, so that

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each infected snail would produce single sex cercariae. Snails were maintained in trays with 'snail-conditioned water' at about 25°C. They were carefully monitored and fed dry lettuce *ad libitum*. Twenty five days post-exposure snails were placed into individual pots containing clean snail-conditioned water and exposed to a light source to stimulate them to liberate cercariae. After 2–3 h the pots were examined under a binocular microscope for the presence of cercariae, and any snail found to be infected was isolated and given an identity number. Uninfected snails were examined every other day for the next 10 days. All infected snails were kept individually in a pot and the water changed every other day. Control animals were infected with cercariae from each individual snail to facilitate identification of the sex of the cercariae by examining the adult worms at a later date. Hamsters were used for *S. haematobium* and mice were used for *S. intercalatum*. To produce hybrids, hamsters were infected with cercariae shed from two known *B. wrighti*, one shedding *S. intercalatum* cercariae and the other *S. haematobium* cercariae. Each cross was blind, that is, at the time of the double infection the sexes of the cercariae were unknown. Approximately 100 cercariae of each species were used in each infection. After the patency date of the control infections, 60–70 days for *S. haematobium* and 45–50 days for *S. intercalatum*, the animals were culled, perfused and the worms obtained were examined under a binocular microscope to determine their sex. Thus, the sex of the cercariae being produced by each individual snail was determined, allowing identification of animals that were infected with schistosomes of different species and different sexes. For experimental animals that had been infected with *S. haematobium* and *S. intercalatum* of different sexes, an appropriate time was selected some weeks after the patency date of both *S. haematobium* and *S. intercalatum* to allow adult worms to pair, mature and produce eggs in quantity. The hybrid parasites were passaged to produce F_1 and F_2 generations of all stages of the life cycle, as described by Taylor (1970). The hybrid parasites were passaged through hamsters and the intermediate molluscan hosts *B. truncatus* and *B. forskalii*. Schistosomes that were subsequently perfused or dissected from the laboratory animals were stored in liquid nitrogen.

Natural schistosome isolates

A total of eight different natural schistosome isolates from Cameroon were analysed by isoelectric focusing:

Isolate 1 was made in Kumba, Cameroon in 1990 by exposing laboratory bred *B. wrighti* to miracidia hatched from polymorphic shaped eggs found in the urine of a 14-year-old schoolchild.
Isolate 2 was made in Loum, Cameroon in 1990 by exposing laboratory bred *B. wrighti* to miracidia hatched from eggs passed in the urine of a schoolchild.
Isolate 3 was made in Loum, Cameroon 1990 from cercariae shed from naturally infected *B. truncatus* found in the area.
Isolate 4 was made in Loum, Cameroon 1999 by exposing laboratory bred *B. wrighti* to miracidia hatched from eggs passed in the urine of five heavily infected schoolchildren (Loum 1).
Isolate 5 was made in Loum, Cameroon 1999 by exposing laboratory bred *B. wrighti* to miracidia hatched from eggs passed in the urine of one very heavily infected schoolchild (Loum 2).
Isolate 6 was made in Barombi Mbo, Cameroon 1999 by exposing laboratory bred *B. wrighti* to miracidia hatched from eggs passed in the urine of three heavily infected schoolchildren.
Isolate 7 was made in Barombi Kotto, Cameroon 1999 from cercariae shed from naturally infected *B. camerunensis* found in the area.
Isolate 8 was made in Loum, Cameroon in 2000 by exposing laboratory bred *B. wrighti* to miracidia hatched from eggs passed in the urine of thirteen infected schoolchildren.

Each isolate was maintained in the laboratory in *B. wrighti* and the golden hamster, *Mesocricetus auratus*. The schistosomes were perfused from the hamsters and stored in liquid nitrogen until 20 individual male and 20 individual female schistosomes from each isolate were analysed.

Laboratory schistosomes

Ten individual males and ten individual female schistosomes of the following were also analysed:

1. Parental pure *S. haematobium* from Barombi Mbo.
2. Parental pure *S. intercalatum* from Edea.
3. F_1 of *S. haematobium* ♂ × *S. intercalatum* ♀ hybrid cross.
4. F_2 of *S. haematobium* ♂ × *S. intercalatum* ♀ hybrid cross.
5. F_1 of *S. haematobium* ♀ × *S. intercalatum* ♂ hybrid cross.
6. F_2 of *S. haematobium* ♀ × *S. intercalatum* ♂ hybrid cross.

Extracts of each worm were subjected to electrophoretic analysis for glucose-6-phosphate dehydrogenase activity using the technique described by Wright *et al.* (1979).

Results

The parental species *S. intercalatum* and *S. haematobium* used in the hybridization experiments are monomorphic for G6PD and the patterns are distinctive, allowing easy identification of the two parental species (fig. 1). Each individual male and female *S. haematobium* schistosome gave a distinctive profile at pH 5–6, and each individual male and female *S. intercalatum* gave a profile ranging from about pH 6–7. A pattern containing all the bands found in the profiles of both the parental species with the two external bands at the same level as those seen for the parents was observed in the F_1 and F_2 hybrid generations produced from both crosses in the laboratory hybridization experiments. This profile was very distinguishable from the parental species allowing identification of the hybrid offspring from the two parental species (figs 1 and 2). This enzyme system facilitated the screening of large numbers individual worms for isolates collected from Cameroon, to establish if there were any hybrids among the populations.

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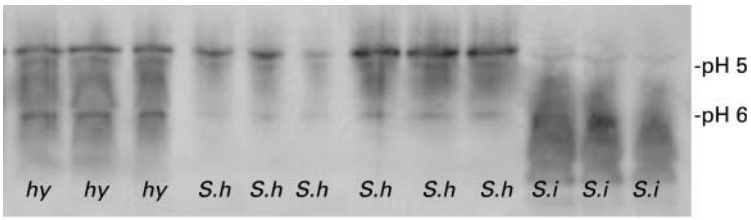

Fig. 1. Glucose-6-phosphate dehydrogenase profiles of the individual parental schistosomes (*S. h*, *Schistosoma haematobium* and *S. i*, *S. intercalatum*) and the laboratory hybrids of these two species (*hy*).

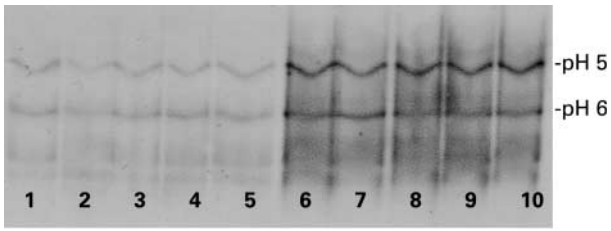

Fig. 2. Glucose-6-phosphate dehydrogenase profiles of laboratory *Schistosoma haematobium* ♂ × *S. intercalatum* ♀ F_1 hybrids (lanes 1–5 ♀, 6–10 ♂).

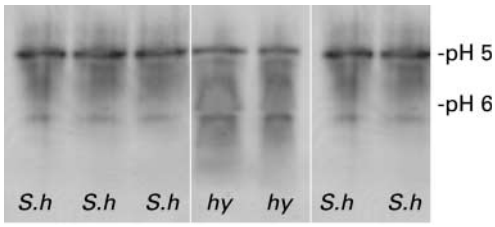

Fig. 4. Glucose-6-phosphate dehydrogenase profiles representing the individual hybrid (*hy*) and *Schistosoma haematobium* (*S. h*) schistosomes from the 1990, 1999 and 2000 isolates from Loum.

Isolate 1. All individuals produced a hybrid profile (100% recombinants) (fig. 3).
Isolate 2. Six males produced a hybrid profile (30% recombinants), 14 males produced an *S. haematobium* profile, six females produced a hybrid profile (30% recombinants) and 14 females produced an *S. haematobium* profile (fig. 4). The overall % of recombinants was 30%.
Isolate 3. All individuals produced an *S. haematobium* profile (fig. 4).
Isolate 4. Four males produced a hybrid profile (20% recombinants), 16 males produced an *S. haematobium* profile, three females produced a hybrid profile (15% recombinants) and 17 females produced an *S. haematobium* profile (fig. 4).
Isolate 5. Two males produced a hybrid profile (10% recombinants), 18 males produced an *S. haematobium* profile, one female produced a hybrid profile (5% recombinants) and 19 females produced an *S. haematobium* profile (fig. 4). The overall percentage of recombinants from the two isolates was 12.5%.

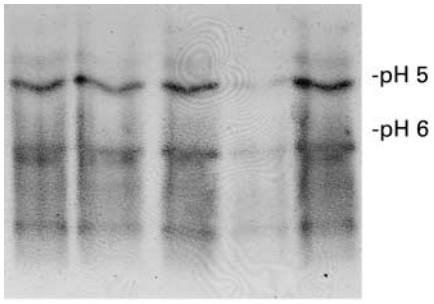

Fig. 3. Glucose-6-phosphate dehydrogenase hybrid profiles from individual schistosomes of the 1990 isolate from Kumba.

Isolate 6. All individuals produced an *S. haematobium* profile (fig. 5).
Isolate 7. All individuals gave *S. haematobium* profiles, apart from two out of 20 individual males 10% gave an unusual profile (? recombinants) (fig. 6).
Isolate 8. Three males produced a hybrid profile (15% recombinants), 17 males produced an *S. haematobium* profile, one female produced a hybrid profile (5% recombinants) and 19 females produced an *S. haematobium* profile (fig. 4). The overall % of recombinants was 10%.

Discussion

Isoelectric focusing of isoenzymes has been used effectively for the identification and genetic analysis of schistosome species and populations. In this study glucose-6-phosphate dehydrogenase was shown to produce an identifiable profile for the two parental species *S. haematobium* and *S. intercalatum* and then also

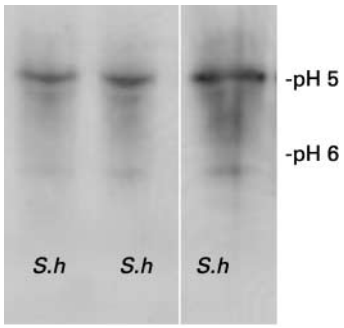

Fig. 5. Glucose-6-phosphate dehydrogenase profile of individual schistosomes (*S. h*, *Schistosoma haematobium*) from the 1999 isolate from Barombi Mbo 1999.

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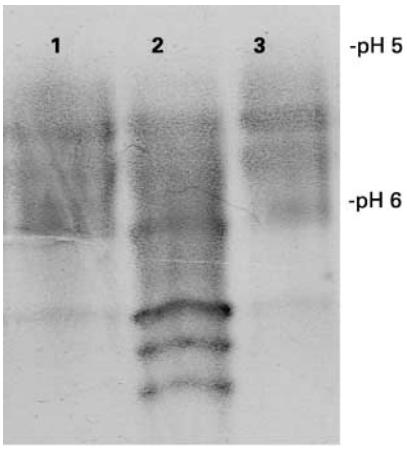

Fig. 6. Glucose-6-phosphate dehydrogenase profiles from individual schistosomes from the 1999 isolate from Barombi Kotto. The unusual profile is represented in track 2.

for the hybrid of the two species and therefore was used to analyse the natural isolates from Cameroon.

There are not only problems in defining the limits of the population but also in estimating the adequacy of the sample size relative to the population as a whole. Many hundreds of miracidia may be hatched from the eggs in a single urine sample from one infected person. Whether these larvae are the progeny of all the paired female worms present, or of only some of them, is unknown. Also, maintenance of the schistosome isolates in the laboratory results in each isolate passing through a number of bottlenecks thereby reducing the genetic variability of the population. Thus, from the initial sample there is a progressive diminution in the representation status of the material throughout the process leading to analysis. Also, only a certain number of individuals from each isolate can actually be analysed and it is predicted that these selected individuals can offer some information about the population as a whole.

The enzyme data showed clear genetic inheritance from both the parental species as the hybrid generations contained both the *S. intercalatum* and *S. haematobium* profile in their G6PD profile. This hybrid profile was seen for all the generations analysed, for both male and female individuals and for both types of crosses *S. haematobium* ♂ × *S. intercalatum* ♀ and the reverse cross, the latter of which was previously considered to be non-viable (Southgate *et al.*, 1982).

Enzyme analysis resulted in the finding of an isoenzyme profile that can be used as a genetic marker to identify recombination events occurring in populations of natural isolates. Isolates from different areas in Cameroon where hybridization has been suggested to have taken place were used in this enzyme study ranging back to 1990 so as to throw light on the dynamics of the interactions between *S. haematobium* and *S. intercalatum*. The data show the complexity of the interactions between *S. intercalatum* and *S. haematobium* and their hybrids over time, and strongly indicate that the evolution of the spread and dynamics of schistosomiasis in Loum should not be based solely on morphological and biological data. The enzyme data presented here clearly show that *S. haematobium* and recombinants, resulting from the *S. haematobium* ♂ × *S. intercalatum* ♀ cross and subsequent crosses and backcrosses, were present in Loum in the year 2000, thus lending support to the mathematical model of Morand *et al.* (2002). These data indicate the dynamics and complexities of the hybridization process between *S. intercalatum* and *S. haematobium* and demonstrate how the situation has changed over the last 12 years, and emphasizes the importance of evaluating morphological, biological and molecular data together to gain a true picture of the rapidly evolving situation. The data show that the isolates from Barombi Mbo were all *S. haematobium* as expected, because the transmission focus is isolated and only *B. truncatus* snails were found in Lake Barombi Mbo, and these are able to transmit *S. haematobium* but not *S. intercalatum*. However, there was some variation in the schistosome population in Barombi Kotto. Most of the schistosomes analysed were identified as *S. haematobium*, but 10% of the individuals, presented somewhat unusual profiles. Wright (1973) suggested that hybridization had occurred in this area. *Bulinus truncatus* and *B. camerunensis* are found in Lake Barombi Kotto, which would allow *S. haematobium* and *S. intercalatum* to become established, and hence interactions to take place between the two species. There is no other evidence to support the interpretation that these individual schistosomes, that presented unusual profiles, are from a hybrid line or are recombinants, but the data do show that there is variation within the population of schistosomes collected from Barombi Kotto, unlike that from Barombi Mbo.

Numerous hypotheses have been postulated to explain the rapidly changing situation in Loum, Cameroon as a result of the interaction between *S. haematobium* and *S. intercalatum* (Wright & Southgate, 1976). These enzyme data have generated some new ideas relating to the dynamics of the situation and the current schistosome populations in Loum, Cameroon. The enzyme data clearly suggest a shift from *S. intercalatum* to *S. haematobium* over the last 30 years with *S. haematobium* and recombinants still present today.

The enzyme studies confirmed that the progeny from every individual female worm exhibiting polymorphic intrauterine eggs isolated from Kumba were recombinants, and 30% of the next generation of schistosomes originating from isolates from Loum 1990, exhibiting intra-uterine eggs typical of *S. haematobium*, were also recombinants. This figure dwindled to 12.5% and 10% of the progeny from intrauterine eggs typical of *S. haematobium*, from Loum in 1999 and 2000, respectively, appearing as recombinants. However, there was no evidence for *S. intercalatum* being present in any of the samples, despite the continued presence of *B. forskalii* in Loum. The results clearly indicate that *S. haematobium* is replacing *S. intercalatum* in an area where 30 years ago only *S. intercalatum* was found. Suggestions to find an explanation for this dramatic change in prevalence of the two species have come from both environmental and behavioural standpoints, in addition to genetic dynamics within the populations (Southgate *et al.*, 1976, 1982; Southgate, 1978). The parental species and the hybrids have been studied in the laboratory, and environmental

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changes in Loum have been reported over the last 30 years (Wright & Southgate, 1976; Tchuem Tchuenté *et al.*, 1997). There are several traits that give *S. haematobium* reproductive, behavioural and genetic advantages over *S. intercalatum* which could afford some explanation, together with environmental factors, as to why *S. haematobium* is replacing *S. intercalatum*, and explaining why only *S. haematobium* and the recombinants are found in Loum, Cameroon today (Morand *et al.*, 2002).

The enzyme data support the fact that *S. haematobium* and the hybrid have replaced *S. intercalatum* in Loum. The use of this isoenzyme study has allowed analysis of populations of individual schistosomes, although additional studies using molecular techniques are needed to unravel further the intricacies of the interaction of *S. haematobium* and *S. intercalatum*.

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References

Duke, B.O.L. & Moore, P.J. (1976) The use of a molluscicide, in conjunction with chemotherapy, to control *Schistosoma haematobium* at the Barombi Lake foci in Cameroon. III. Conclusions and costs. *Tropical Medicine and Parasitology* **27**, 505–508.

Morand, S., Southgate, V.R. & Jourdane, J. (2002) A model to explain the replacement of *Schistosoma intercalatum* by *Schistosoma haematobium* and the hybrid *S. intercalatum / S. haematobium* in areas of sympatry. *Parasitology* **124**, 401–408.

Ratard, R.C., Kouemeni, L.E., Ekani Bessala, M.L., Ndamkou, C.N., Greer, G.J., Spilsbury, J. & Cline, B.L. (1990) Human schistosomiasis in Cameroon. I. Distribution of schistosomiasis. *American Journal of Tropical Medicine and Hygiene* **42**, 561–572.

Rollinson, D. & Southgate, V.R. (1985) Schistosome and snail populations: genetic variability and parasite transmission. pp. 99–109 *in* Rollinson, D. & Anderson, R.M. (*Eds*) *Ecology and genetics in host–parasite interactions*. London, Academic Press.

Sama, M.T. & Ratard, R.C. (1994) Water contact and schistosomiasis in Kumba, south-western Cameroon. *Annals of Tropical Medicine and Parasitology* **88**, 629–634.

Southgate, V.R. (1978) On factors possibly restricting the distribution of *Schistosoma intercalatum* Fisher, 1934. *Zeitschrift für Parasitenkunde* **56**, 183–193.

Southgate, V.R., Van Wijk, H.B. & Wright, C.A. (1976) Schistosomiasis at Loum, Cameroun. *Schistosoma haematobium* and *S. intercalatum* and their natural hybrid. *Zeitschrift für Parasitenkunde* **49**, 145–159.

Southgate, V.R., Rollinson, D., Ross, G.C. & Knowles, R.J. (1982) Mating behaviour in mixed infections of *Schistosoma haematobium* and *S. intercalatum*. *Journal of Natural History* **16**, 491–496.

Taylor, M.G. (1970) Hybridization experiments on five species of African schistosomes. *Journal of Helminthology* **44**, 253–314.

Tchuem Tchuenté, L.A., Morand, S., Imbert-Establet, D., Delay, B. & Jourdane, J. (1996) Competitive exclusion in human schistosomes: the puzzling restricted distribution of *Schistosoma intercalatum*. *Parasitology* **113**, 129–136.

Tchuem Tchuenté, L.A., Southgate, V.R., Nijiokou, F., Nijiné, T., Kouemeni, L.E. & Jourdane, J. (1997) The evolution of schistosomiasis at Loum, Cameroon: replacement of *Schistosoma intercalatum* by *S. haematobium* through introgressive hybridization. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 664–665.

van Wijk, H.B. (1969) *Schistosoma intercalatum* infection in school-children of Loum, Cameroun. *Tropical and Geographical Medicine* **21**, 375–382.

Wright, C.A. & Southgate, V.R. (1976) Hybridization of schistosomes and some of its implications. pp. 55–86 *in* Taylor, A.E.R. & Muller, R. (*Eds*) *Genetic aspects of host–parasite relationships*. Oxford, Blackwell Scientific.

Wright, C.A., Southgate, V.R., Wijk, H.B. & Moore, P.J. (1974) Hybrids between *Schistosoma haematobium* and *S. intercalatum* in Cameroon. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **68**, 413–414.

Wright, C.A., Southgate, V.R. & Ross, G.C. (1979) Enzymes in *Schistosoma intercalatum* and the relative status of the Lower Guinea and Zaire strains of the parasite. *International Journal for Parasitology* **9**, 523–528.

Wright, W.H. (1973) Geographic distribution of schistosomes and their intermediate hosts. pp. 32–247 *in* Ansari, N. (*Ed.*) *Epidemiology and control of schistosomiasis (bilharziasis)*. Baltimore, University Park Press.

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