Mating interactions between *Schistosoma haematobium* and *S. mansoni*

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Abstract

Schistosoma haematobium and *S. mansoni* are two medically important schistosomes, commonly occurring sympatrically in Africa and so potentially able to infect the same human host. Experiments were designed to study the mating behaviour of these two species in mixed infections in hamsters. Analysis of the data obtained showed that both heterospecific and homospecific pairs readily form. No significant difference was seen between the two species in their ability in forming pairs, however, *S. mansoni* showed a greater homospecific mate preference. Analysis of the data using the Mantel-Haenszel test suggests that mating competition does occur between *S. haematobium* and *S. mansoni*, the former being the more dominant species. Both species appeared to be able to change mate, with *S. haematobium* showing a greater ability in taking *S. mansoni* females away from *S. mansoni* males when introduced into a pre-established *S. mansoni* infection highlighting the competitiveness of *S. haematobium*. The significance of the results is discussed in relation to the epidemiological consequences occurring in Senegal, and other areas where both species are sympatric.

Introduction

Schistosomes have achieved a wide geographical distribution causing the most significant helminth disease of mankind. One of the most intriguing features about schistosomes is that they are dioecious not hermaphrodite, like most other trematodes. Mate finding and pairing occurs in the hepatic portal system before migration to the egg-laying site (Armstrong, 1965; Wilson *et al*., 1978; Miller & Wilson, 1980). Even when male and female worms belong to different species, successful heterospecific pairing may occur resulting in parthenogenesis or hybridization depending on the phylogeny of the two species involved (Armstrong, 1965; Tchuem Tchuenté *et al*., 1994; Jourdane *et al*., 1995; Southgate *et al*., 1998).

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It was originally thought that pairing of adult schistosomes, of the same or different species in the definitive host was random and occurred by trial and error (Armstrong, 1965). More detailed recent studies, however, have indicated that specific mate preference systems, mating competition and change of mate can occur (Tchuem Tchuenté *et al*., 1993, 1995, 1996b; Southgate *et al*., 1995).

The three species of human schistosomes existing in Africa, i.e. *S. haematobium, S. mansoni* and *S. intercalatum*, sometimes overlap in their distribution, giving areas of sympatry between species. It is known that interspecific interactions occur in such areas potentially having important consequences on parasite epidemiology. For example, it may influence the progressive exclusion of a particular species from a given area (Tchuem Tchuenté *et al*., 1993, 1996a, 1997; Southgate *et al*., 1995, 1998; Southgate, 1997).

Mating behaviour between *S. haematobium* and *S. intercalatum*, and between *S. mansoni* and *S. intercalatum*

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and their consequences have already been studied (Southgate *et al.*, 1982; Tchuem Tchuenté *et al.*, 1993, 1994, 1995, 1996b), whereas interactions between *S. haematobium* and *S. mansoni* have not yet been investigated.

Field studies in Cameroon (Ratard *et al.*, 1991), and in the Delta of the Senegal River (Ernould, 1996), where *S. haematobium* and *S. mansoni* are both established, showed that *S. mansoni* eggs were being excreted in urine samples of infected patients. This suggests that the two species are pairing heterospecifically, with a possible dominance of *S. haematobium* males to mate and migrate with *S. mansoni* females to the urinary oviposition site, resulting in the production of lateral spined eggs in the urine. This phenomenon readily occurred if the host was more heavily infected with *S. haematobium* than *S. mansoni.* If the *S. mansoni* infection was more abundant, however, relatively more *S. mansoni* eggs were excreted in faecal samples, suggesting that homospecific pairing was more readily occurring between the *S. mansoni* males and females (Ernould, 1996). These observations were based entirely on field data and as yet the actual pairing and competition of these two species of schistosome have not been studied in the laboratory. Therefore, the purpose of this study was to provide an insight into the mating behaviour between *S. mansoni* and *S. haematobium* in mixed infections in the definitive host.

Materials and methods

Parasites and hosts

Schistosoma haematobium was isolated from wild caught *Bulinus globosus* at Mbodiene, Senegal (August 1997), and maintained in laboratory *B. wrighti, B. globosus* and golden hamster *Mesocricetus auratus.*

Schistosoma mansoni was isolated from wild caught *Biomphalaria pfeifferi* at Richard Toll, Senegal (November 1993), and maintained in laboratory *B. pfeifferi, B. glabrata* and albino mice.

Experimental infections

The experimental design was aimed at determining whether there is a mate recognition system or any competition between the two species of schistosome, and if so, how male and female worms of either species interact in mixed infections. Hamsters were exposed individually, by the paddling technique, to cercariae of *S. haematobium* and *S. mansoni* in three different experiments:

1. Ten hamsters were exposed simultaneously to 100 cercariae of *S. haematobium* and 100 cercariae of *S. mansoni*.
2. Seventeen hamsters were exposed first to 100 cercariae of *S. haematobium* and then 70 days later to 100 cercariae of *S. mansoni*.
3. Ten hamsters were exposed first to 100 cercariae of *S. mansoni* and then 30 days later to 100 cercariae of *S. haematobium.*

Hamsters were killed and worms collected by perfusion and dissection of the hepatic portal vein and mesenteric venous systems of each infected hamster; 60–70 days post-infection; 25–30 days after re-infection and 60–70 days after re-infection for experiments 1, 2 and 3, respectively. Each pair and any unpaired worms were segregated into individual containers and pairs were then separated. The taxonomic identity of individual females was determined by examination of the morphology of the intrauterine egg. Female worms of *S. mansoni* produced only single lateral spined eggs, whereas *S. haematobium* females produced several terminal spined eggs *in utero.* In the case of immature females, where no egg was present, the taxonomic identity of the female worm was distinguished by the position of the ovary.

Since the taxonomic identity of the male worms could not be determined by morphometry, individual male worms were examined for glucose-6-phosphate dehydrogenase (1.1.1.49), (G6PD), using the isoelectric focusing technique (IEF) (Wright & Ross, 1979, 1983; Fletcher *et al.*, 1980, 1981; De Boissezon & Jelnes, 1982). Male worms of *S. haematobium* and *S. mansoni* are both monomorphic for G6PD but differ in their pI values, producing an identifiable profile which enabled distinction between the two species of schistosome (fig. 1). Each male and female worm within each pair and any unpaired worms were identified and recorded for each hamster. The data were analysed using the Mantel-Haenszel test to evaluate the significance of the observed proportions (Mantel & Haenszel, 1959; Southgate *et al.*, 1982).

Results

Four types of pairings were obtained from hamsters, two being homospecific (*S. haematobium* male × *S. haematobium* female and *S. mansoni* male × *S. mansoni* female) and two heterospecific (*S. haematobium* male × *S. mansoni* female and *S. mansoni* male × *S. haematobium* female). Single worms of both species and of both sexes were also

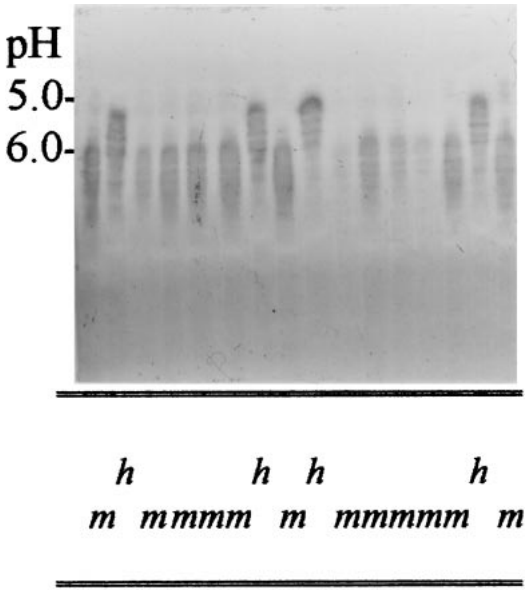

Fig. 1. Isoenzyme patterns of individual worm extracts of *Schistosoma haematobium* (*h*), and *S. mansoni* (*m*), separated by isoelectric focusing of G6PD in a polyacrylamide gel.

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Table 1. Data from hamsters (Ham) simultaneously infected with 100 cercariae of *Schistosoma haematobium* (Sh) and 100 cercariae of *S. mansoni* (Sm).

Ham	Sm♂ × Sm♀	Sh♂ × Sh♀	Sm♂ × Sh♀	Sh♂ × m♀	Sm♂	Sh♂	Sm♀	Sh♀
1	8	3	1	8	3	0	0	0
2	6	8	1	10	0	1	0	0
3	2	9	1	16	11	3	0	0
4	15	0	0	2	3	1	0	0
5	9	0	4	0	0	0	1	7
6	12	0	0	1	0	0	0	0
7	17	1	3	2	0	0	0	0
8	15	0	1	3	0	0	0	0
9	14	2	0	4	0	1	0	0
10	27	4	0	1	0	3	0	0
Total	125	27	11	47	17	9	1	7

found in the hamsters. The data in each experiment were analysed to identify if there was any competition occurring between the two species.

Experiment 1: simultaneous infections

Mating interactions between the two species of schistosome extracted from each individual hamster are shown in table 1. All female worms obtained were paired, apart from those in hamster 5 which were due to the absence of *S. haematobium* males and very low numbers of *S. mansoni* males. There was no significant difference between the proportions of paired *S. mansoni* males (88.9%) and paired *S. haematobium* males (89.1%). Ninety two percent of the *S. mansoni* paired male worms formed homospecific pairs compared to 8% that formed heterospecific pairs. Thirty six percent of the *S. haematobium* male worms that paired formed homospecific pairs compared to 64% that paired heterospecifically.

For hamsters 1, 3 and 4, *S. haematobium* males were better than *S. mansoni* males at pairing with *S. mansoni* females, resulting in an excess of unpaired *S. mansoni* males. The reverse, however, was seen in hamster 2, where one *S. mansoni* male was paired with a *S. haematobium* female. To test whether this observed mating competition is due to some species preference, the proportions of males, from both species that paired heterospecifically were compared. The difference was statistically significant ($\chi^2 = 22.0$, $P < 0.001$) with *S. haematobium* males being more successful than *S. mansoni* males at pairing with *S. mansoni* females.

Experiment 2: infections with S. haematobium *and 60 days later with* S. mansoni

Data summarized in table 2 show that 70% of all *S. haematobium* male worms formed pairs and 76% of the *S. mansoni* male worms formed pairs indicating that there is no significant difference in the ability between the two different species in forming pairs in the hamster. All the female worms obtained were paired and there was an excess of male worms of both species in each case.

Table 2. Data from hamsters (Ham) infected first with 100 cercariae of *Schistosoma haematobium* (Sh) and 70 days later with 100 cercariae of *S. mansoni* (Sm).

Ham	Sm♂ × Sm♀	Sh♂ × Sh♀	Sm♂ × Sh♀	Sh♂ × Sm♀	Sm♂	Sh♂
1	4	2	2	5	3	1
2	2	3	0	1	2	0
3	14	0	1	1	8	1
4	15	0	0	2	9	1
5	21	0	1	1	2	0
6	15	0	2	1	11	0
7	12	0	3	0	2	1
8	14	3	0	0	4	1
9	18	0	2	0	8	1
10	11	0	5	0	1	1
11	20	0	0	0	1	0
12	25	2	1	2	10	3
13	5	1	0	7	8	2
14	16	1	4	1	12	3
15	13	4	0	0	3	1
16	16	1	3	1	1	1
17	20	0	1	1	0	0
Total	241	17	25	23	85	17

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Table 3. Data from hamsters (Ham) infected first with 100 cercariae of *Schistosoma mansoni* (Sm) and 30 days later with 100 cercariae of *S. haematobium* (Sh).

Ham	Sm♂ × Sm♀	Sh♂ × Sh♀	Sm♂ × Sh♀	Sh♂ × Sm♀	Sm♂	Sh♂
1	13	1	1	4	1	0
2	8	2	0	2	7	2
3	0	0	1	1	0	0
4	6	0	0	0	2	1
5	7	0	0	1	1	0
6	23	0	0	1	3	3
7	11	0	0	2	7	5
8	12	0	0	1	2	1
9	8	0	0	0	4	6
10	13	0	0	1	0	0
Total	101	3	2	13	27	18

Since *S. haematobium* was introduced first, it was assumed that all the possible *S. haematobium* male × *S. haematobium* female homospecific pairings occurred prior to the introduction of *S. mansoni*. Data from hamsters 1, 7, 9, 10, 12, 14 and 16, however, show that out of the 25 *S. mansoni* male × *S. haematobium* female heterospecific pairs that formed, 40% occurred leaving an excess of unpaired *S. haematobium* males. Data from hamsters 1–6, 12, 13, 14 and 16 show that 87% of the *S. haematobium* male × *S. mansoni* female heterospecific pairs formed, leaving an excess of single *S. mansoni* males. These data were found to be statistically significant ($\chi^2 = 10.6$, $P < 0.001$) with *S. haematobium* males being more successful than *S. mansoni* males in pairing with *S. mansoni* females.

Experiment 3: infections with S. mansoni *and 35 days later with* S. haematobium

In this experiment there was a very low worm return especially that of *S. haematobium* females which has to be taken into consideration when interpreting the data (table 3). Only five *S. haematobium* females were obtained from ten hamsters. All the female worms were paired and there was an excess of single male worms of both species. Forty seven percent of the *S. haematobium* male worms and 79.2% of the *S. mansoni* male worms formed pairs.

Since *S. mansoni* was introduced first, it was assumed that all the *S. mansoni* males will have paired homospecifically prior to the introduction of *S. haematobium*. Data from hamsters 1, 2, 5, 6, 7, and 8, however, show that 62% of the *S. haematobium* male × *S. mansoni* female heterospecific pairings were formed leaving an excess of single *S. mansoni* males. No *S. mansoni* male was seen to out-compete a *S. haematobium* male in forming pairs with a *S. haematobium* female. These data proved to be statistically significant ($\chi^2 = 8.1$, $P < 0.001$) with *S. haematobium* males being more dominant than *S. mansoni* males in pairing with *S. mansoni* females.

Discussion

Schistosoma haematobium and *S. mansoni* are known to occur sympatrically in many areas of Africa, and so able to infect the same human host. In mixed infections in hamsters interspecific interactions occurred between these two species, indicating that there are no obvious factors preventing heterospecific pairing in the same definitive host. Each heterospecifically paired female was mature, which confirms previous observations that full maturation and reproductive stimulation is not species dependent (Khalil & Mansour, 1995; Southgate *et al.*, 1998).

Data obtained from hamsters that were simultaneously infected with both *S. haematobium* and *S. mansoni*, suggested that when given the choice, *S. mansoni* exhibits greater specific mate recognition than *S. haematobium*, preferentially mating with partners of the same species. This observation, however, does not take into account the high number of *S. mansoni* females compared with *S. haematobium* females in each infection, enabling *S. mansoni* females to be involved in a higher proportion of the pairings that took place, making interpretation of the data difficult.

Previous studies have shown that schistosomes are not always faithful and change of mate can take place (Tchuem Tchuenté *et al.*, 1995, 1996b). The data from experiments 2 and 3 suggest that this phenomenon could be occurring between *S. haematobium* and *S. mansoni*, with males of both species being able to take away females from already established homospecific pairs. There seems, however, to be a significant difference, between the species, in their ability to do this: *S. haematobium* males are better at removing *S. mansoni* females from *S. mansoni* males, compared with *S. mansoni* males taking away *S. haematobium* females from *S. haematobium* males. This dominance of *S. haematobium* is also highlighted by the statistical analysis of data from all three experiments, showing that there is mating competition occurring between the two species. Males of *S. haematobium* compete with, and are more successful than, *S. mansoni* males in pairing with *S. mansoni* females, leaving a significant number of unpaired *S. mansoni* males.

This preliminary investigation does not take into account the ratios of the schistosomes in each infection, which may influence the dynamics of the interactions observed. However, it provides insights into the mating interactions between *S. haematobium* and *S. mansoni*, allowing us to explain possibly further events involving transmission and epidemiology of these two species in nature.

Several studies have shown that, in mixed infections, there are no physiological barriers preventing encounters and mating of different species of schistosomes (Southgate *et al.*, 1982; Tchuem Tchuenté *et al.*, 1995, 1996b). It is generally assumed that the male worm carries the female worm to the definitive egg-laying site after pairing in the hepatic portal vein. According to the phylogeny of the two species, the interspecific interactions will lead either to hybridization or parthenogenesis. Pairings involving two species which belong to different groups, such as *S. haematobium* and *S. mansoni*, results in parthenogenesis and the production of non-viable egg(s) (Jourdane *et al.*, 1995; Khalil & Mansour, 1995; Southgate & Rollinson, 1987; Southgate *et al.*, 1982, 1995; Tchuem Tchuenté *et al.*, 1994). Where *S. haematobium* and *S. mansoni* are sympatric, such as in the Senegal River Basin, it has been observed that *S. mansoni* eggs are excreted in the urine of patients (Ernould, 1996). Conclusions drawn from our data possibly explain this phenomenon. The assumed dominance of *S. haematobium* males enable them to pair with and carry *S. mansoni* females to the urinary oviposition site, resulting in the parthenogenetic production of *S. mansoni* lateral spined eggs, that are excreted in the urine.

When competition exists between two species of schistosome that are able to infect the same host, there exists a risk of exclusion, either partially or totally, of one species by the other. Previous studies have shown that in simultaneous and sequential mixed infections of *S. mansoni* and *S. intercalatum*, *S. mansoni* males are dominant over *S. intercalatum* males in forming pairs with *S. intercalatum* females (Tchuem Tchuenté *et al.*, 1994). It was also observed that in the absence of *S. mansoni* females, unpaired *S. mansoni* males pull away *S. intercalatum* females from *S. intercalatum* males. It has been suggested that in situations of sympatry, this mating competition was probably an important factor limiting the distribution of *S. intercalatum* in Africa, with the dominant species causing the competitive exclusion of the less dominant species (Southgate *et al.*, 1982, 1998; Tchuem Tchuenté *et al.*, 1993, 1994, 1995, 1996a, 1997).

In this paper, the reverse situation appears to occur between *S. haematobium* and *S. mansoni.* In Egypt for example, in areas such as Fayoum where both species are sympatric, *S. mansoni* is observed to be replacing *S. haematobium* (Abdel-Wahab *et al.*, 1993). This phenomenon could be explained by numerous factors that influence the transmission of these two species. For example, *S. mansoni* has a shorter life cycle (hence a potentially faster reproduction rate), compared to *S. haematobium* and there is also, a natural large difference in the sex ratios, between the two species (Southgate *et al.*, 1998; Mitchell *et al.*, 1990). In a mixed infection, the *S. mansoni* worm burden is greater than *S. haematobium* and the number of *S. mansoni* females per male, in relation to *S. haematobium* females per male, is greater. The competitive pairing of *S. haematobium* males with *S. mansoni* females will result in non-viable reproduction until through subsequent re-infections of the host, conspecific females become available and a change of mate can take place (Tchuem Tchuenté *et al.*, 1994). This, together with the greater worm burden and shorter life cycle of *S. mansoni*, will result in more homospecific and hence viable *S. mansoni* pairs forming, in a given time, compared with that of *S. haematobium*. Also, due to major ecological changes, the snail host, *B. pfeifferi*, has become more abundant in the area, possibly increasing the transmission foci for *S. mansoni* (Abdel-Wahab *et al.*, 1993; Southgate, 1997). The combination of this change in the distribution of snail hosts, in favour of *S. mansoni*, together with the competitive mating of *S. haematobium* and differences in epidemiology between the species, could affect the replacement of *S. haematobium* by *S. mansoni.* Therefore environmental and *in vivo* interactions should be taken into account when relating observations found in the laboratory to events occurring in the field.

The intensity and prevalence of both urinary and intestinal schistosomiasis are increasing in many areas of Africa (Picquet *et al.*, 1996). It is, therefore, important to have a greater awareness of behavioural interactions between schistosome species, as they could have important consequences on the epidemiology of schistosomiasis.

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