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Interactions between *Schistosoma intercalatum* (Zaire strain) and *S. mansoni*

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Abstract

Schistosoma mansoni and *S. intercalatum*, two schistosomes from different evolutionary lineages, are parasitic in humans and therefore able to co-infect the same host where they occur sympatrically in Africa. Previous studies of mating interactions between these species in mice, using the Lower Guinea strain of *S. intercalatum*, have demonstrated the competitive dominance of *S. mansoni* over *S. intercalatum* in terms of pairing ability, which is potentially an important mechanism restricting the distribution of *S. intercalatum* in Africa. The study presented here examines the mating interactions in mice between *S. mansoni* and the Zaire (Democratic Republic of Congo) strain of *S. intercalatum*, which differs from the Lower Guinea strain in many biological characteristics. Analysis of the data showed a preponderance of intraspecific pairs over interspecific, demonstrating a specific mate preference system for both species. Mating competition between these species and the ability of males of both species to effect a change of mate by pulling paired females away from their partners was indicated. Comparisons are made between the competitive mating abilities of both strains of *S. intercalatum* relative to those of *S. mansoni*, with the data suggesting that *S. mansoni* is competitively dominant to *S. intercalatum* (Zaire) in sequential infections but to a lesser extent than for *S. intercalatum* (Lower Guinea). Additional factors which may contribute to the confinement of *S. intercalatum* (Zaire) to the Democratic Republic of Congo are discussed.

Introduction

Two distinct strains of *Schistosoma intercalatum* are currently recognized: the Lower Guinea and Zaire (Democratic Republic of Congo) strains, which differ in their geographical distribution and a number of biological characteristics such as intermediate host specificity, prepatent periods, isoenzyme profiles and chronobiological cercariae shedding patterns (Wright *et al.*, 1972, 1979; Pagès & Théron, 1990). In addition, recent data have shown considerable molecular divergence between the strains (Pagès *et al.*, 2001a,b). There is some uncertainty as to the origin of the two strains. The first reports of mesenteric terminal-spined schistosomiasis were in 1923, by Chesterman from Yakusu, near Stanleyville (Kisangani) in the Democratic Republic of Congo (Chesterman, 1923), and by Clapier from Libreville in Gabon (Clapier, 1923), but it was Fisher (1934) who officially described *S. intercalatum* as a new species from the Stanleyville region. A study by Deschiens & Delas (1969) suggested that the parasite was spreading northwards into Cameroon with immigrant manual workers. These authors assumed that the Stanleyville focus was the true origin of the *S. intercalatum* parasite, and that its outward spread led to its divergence into two different strains through geographical isolation. However, Browne (1969) noted that there was no evidence that the Stanleyville focus was older than the Libreville one, and Wright *et al.* (1972) interpreted the numerous biological differences between the strains as indicative that they had

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been separated for some time. Indeed, more recent studies incorporating the molecular divergence between the strains confirm that their differentiation from one another is ancient, and probably took place at the same time as speciation of all the other *S. haematobium*-group schistosomes (Wright *et al.*, 1972; Desprès *et al.*, 1992; Pagès *et al.*, 2001a,b). Wright *et al.* (1972) postulated that, as the two strains are unable to utilize each other's snail host (*Bulinus africanus* group snails for the Zaire strain, and *B. forskalii* group snails for the Lower Guinea strain), this parasite originated in an ancestral bulinid snail of forest regions and the two strains diverged with separate lines of snails derived from the common stock.

Of the two, it is the Lower Guinea strain which has the wider distribution, occuring in Gabon, Cameroon, Nigeria, Equatorial Guinea and Sao Tomé, whereas the Zaire strain appears confined to the Democratic Republic of Congo (Tchuem Tchuenté *et al.*, 1997a). However, in comparison to the extremely wide distribution of their definitive (human) and intermediate hosts across Africa, the distribution of both strains appears to be highly restricted (Tchuem Tchuenté *et al.*, 1996, 1997a).

One biological reason postulated for this concerns the tendency of *S. intercalatum* cercariae, in contrast to other African schistosomes, to form non-infective aggregates in response to small temperature changes. The aggregates are formed by the release of the adhesive post-acetabular gland secretion which causes the cercariae to stick together. It has been suggested that *S. intercalatum* originated in streams within tropical rainforest areas, and only comparatively recently spread into water bodies in more open areas of savannah. Here cercariae would experience greater diurnal temperature changes, and thereby aggregate more readily, so impeding invasion of the definitive host and limiting its spread (Southgate, 1978).

Other factors possibly restricting the distribution of *S. intercalatum* concern mating interactions of *S. intercalatum* with other schistosome species with which it is sympatric. For example, the introduction of *S. haematobium* by immigrants in 1968 into the town of Loum, Cameroon where *S. intercalatum* (Lower Guinea) was indigenous led to the large-scale replacement of *S. intercalatum* by *S. haematobium* through hybridization (Southgate, 1978; Tchuem Tchuenté *et al.*, 1997b). *Schistosoma haematobium* and *S. intercalatum* are closely related (both belonging to the *S. haematobium* group) and will hybridize both in nature and in the laboratory when they co-parasitize the same individuals (Wright *et al.*, 1974; Southgate *et al.*, 1976; Wright & Southgate, 1976; Tchuem Tchuenté *et al.*, 1997a,b). Hybrid offspring of *S. haematobium* ♂ × *S. intercalatum* ♀ crosses are viable (Southgate *et al.*, 1976), and several generations of backcrossing with parental species (introgressive hybridization) may cause the emergence of a new strain of *S. haematobium*, as at Loum. Key to the replacement of *S. intercalatum* was the competitive dominance of *S. haematobium* over *S. intercalatum* (Lower Guinea) with regard to pairing in mixed infections, as demonstrated by Southgate *et al.* (1982).

Schistosoma intercalatum is also found sympatrically in Africa with *S. mansoni* (Tchuem Tchuenté *et al.*, 1993) and, like *S. haematobium*, *S. mansoni* is thought to be limiting the distribution of *S. intercalatum* for several reasons.

Firstly, as demonstrated by Tchuem Tchuenté *et al.* (1996) using mixed infections of *S. mansoni* and *S. intercalatum* (Lower Guinea) in rodents, infection with *S. mansoni* induces a significant degree of cross-protection against subsequent infection with *S. intercalatum*, but infection with *S. intercalatum* does not cross-protect against reinfection with *S. mansoni*. In nature, *S. mansoni*-induced immunity against *S. intercalatum* in infected individuals will make it difficult for *S. intercalatum* to become established in areas where *S. mansoni* is already present.

Secondly, similar to the situation with *S. haematobium*, the competitive dominance of *S. mansoni* over *S. intercalatum* in mixed infections may limit the distribution of *S. intercalatum* (Tchuem Tchuenté *et al.*, 1996). In the experiments of Tchuem Tchuenté *et al.* (1993, 1994, 1996) using *S. intercalatum* (Lower Guinea) in mixed infections with *S. mansoni*, not only was *S. mansoni* shown to be better at forming pairs than *S. intercalatum*, but also maintained a stronger homospecific mate preference. As *S. mansoni* and *S. intercalatum* cannot hybridize, being from different evolutionary groups, offspring of heterospecific pairings would be parthenogenetic and of very low viability, and so homospecific pairing is preferable. It is likely that as *S. intercalatum* has only recently become sympatric with other schistosome species, pre-zygotic reproductive isolating traits such as competitive pairing and strong homospecific mate preference have not been strongly selected for (Tchuem Tchuenté *et al.*, 1995; Southgate *et al.*, 1998; Pagès *et al.*, 2001b). Using data from the Tchuem Tchuenté *et al.* (1993) investigation into mating interactions between *S. intercalatum* (Lower Guinea) and *S. mansoni*, Tchuem Tchuente *et al.* (1996) used a mathematical model of mating probabilities in these mixed infections to suggest that the basic transmission rate of *S. intercalatum* could be reduced by a factor of 5 in areas of sympatry with *S. mansoni*, independent of the effect of *S. mansoni*-induced cross-protection against *S. intercalatum*.

A third reason for *S. mansoni* outcompeting and so limiting the distribution of *S. intercalatum* concerns the fact that *S. mansoni* populations will always be greater in nature than those of *S. intercalatum*, by virtue of its greater cercarial productivity, its greater worm burden and shorter patency period in definitive hosts (Loker, 1983).

Given the wide distribution of both *S. haematobium* and especially *S. mansoni* across much of Africa, all of the above immunological and competitive mating factors make it very difficult for *S. intercalatum* to spread. Indeed the relatively new foci of *S. intercalatum* reported in Sao Tomé and Equatorial Guinea corrrespond to situations where both *S. haematobium* and *S. mansoni* are absent (Tchuem Tchuenté *et al.*, 1996; Jourdane *et al.*, 2001).

So far, all work on mating interactions of *S. intercalatum* with *S. mansoni* and *S. haematobium* has used the Lower Guinea strain of *S. intercalatum*. Given the clear biological differences between the two strains outlined above, together with the fact that they cannot utilize each other's intermediate snail host, there is currently much debate over whether or not the two strains are in fact separate species (Jourdane *et al.*, 2001; Pagès *et al.*, 2001a). Therefore, it cannot necessarily be assumed that

the Zaire strain will interact in the same way as the Lower Guinea strain with other species of schistosome.

The aims of the present study were to elucidate the mating interactions of *S. intercalatum* (Zaire) with *S. mansoni*, to establish which (if any) species emerged as competitively dominant, and more specifically if *S. mansoni* could limit the distribution of *S. intercalatum* (Zaire) through competitive dominance as appears to be the case for *S. intercalatum* (Lower Guinea). It should also provide further insight into the differences between the two strains of *S. intercalatum*.

Materials and methods

Origin of species

The isolate of *S. intercalatum* (Zaire strain) used in these experiments originates from Kinshasa, Democratic Republic of Congo, where an autochthonous focus of *S. intercalatum* was described in 1987 by De Clercq. Tchuem Tchuenté *et al.* (1997a) noted a possible PGM isoenzyme difference between this isolate and the original one from Kisangani described by Fisher (1934), but it utilizes the same snail host (*B. globosus*) and remains distinct from the Lower Guinea strain. It was isolated in 1994 from faeces of infected primary school children in Kinshasa by L.A. Tchuem Tchuenté and maintained in the laboratory at the University of Perpignan in *B. globosus* from Zambia and Swiss OF1 mice for several years. It was introduced into the laboratories of The Natural History Museum in 2001 courtesy of L.A. Tchuem Tchuenté where it was maintained in laboratory-bred *B. wrighti* from Oman, and albino TO mice for two cycles prior to its use in this study.

The isolate of *S. mansoni* originated from Richard Toll, Senegal and was isolated from wild-caught *Biomphalaria pfeifferi* in 1993. It has been maintained in laboratory-bred *B. pfeifferi*, *B. glabrata* and albino TO mice to date.

Experimental infections

The experimental design was aimed at determining how male and female worms of both species interact in mixed infections in the vertebrate host, and whether a specific mate recognition system and mating competition exists between the two species. Mice were exposed individually by the paddling technique to a fixed number of male and female cercariae of both species in three experiments. These were of two types, the first of which (experiment 1) involved the simultaneous infection of mice with 150 cercariae of *S. intercalatum* (Zaire) and 10 days later with 150 cercariae of *S. mansoni*, to ensure that both species reached patency simultaneously and therefore had total choice of mate from either species. The second type of experiment (experiments 2 and 3) involved the sequential infection of mice, with the time interval between infections adjusted according to the differences in patency of the two species in mice (approximately 45 days for *S. intercalatum* and approximately 35 days for *S. mansoni* (Loker, 1983)) so that the first species would reach patency 2–3 weeks before the second. Mice in experiment 2 were infected firstly with 150 cercariae of *S. mansoni* and then with 150 cercariae of *S. intercalatum* (Zaire) one week later, whilst mice from experiment 3 were infected first with 150 cercariae of *S. intercalatum* (Zaire) and 4 weeks later with 150 cercariae of *S. mansoni*. In sequential infections it is assumed that the first species will form homospecific pairs, and the usual male-bias of schistosome infections (Liberatos, 1987; Mitchell *et al.*, 1990) should result in an excess of 'species 1 males' which may compete with 'species 2 males' for 'species 2 females'.

Mice were killed and worms collected by perfusion and dissection of the hepatic portal vein and mesenteric venous systems of each infected mouse 6–8 weeks post-reinfection. Each pair and any unpaired worms recovered from infected mice were segregated into individual containers and the pairs were separated. Individual females were taxonomically identified by microscopic examination of the morphometry and number of the intrauterine eggs: *S. mansoni* females have single lateral-spined eggs *in utero*, whereas *S. intercalatum* females produce several eggs which have a conical posterior end tapering to a terminal posterior spine. Since the taxonomic identity of the male worms could not be determined by morphometry, individual male worms were examined for glucose-6-phosphate-dehydrogenase (G6PD) activity using the isoelectric focusing technique (Wright *et al.*, 1979; Fletcher *et al.*, 1981; Wright & Ross, 1983). Male worms of the isolates of both species used in this study are monomorphic for G6PD but differ in their pI values, producing an identifiable profile which enabled distinction to be made between the two species of schistosome. Each male and female worm within each pair and any unpaired worms were identified and recorded for each mouse. Data were analysed using the Mantel-Haenszel test to evaluate the significance of observed proportions (Mantel & Haenszel, 1959; Southgate *et al.*, 1982; Webster *et al.*, 1999).

Results

Experiment 1: infections with S. intercalatum *(Zaire) and 10 days later with* S. mansoni

Table 1 summarizes the worms recovered from each mouse and how they paired. Two homospecific and two heterospecific types of pairing were obtained, and there was a surplus of unpaired males of both species. Three multiple worm pairs were obtained in total, one from mouse 4, mouse 5 and mouse 6. In all cases these pairings consisted of one male paired with two females: in mice 4 and 5 all worms involved were *S. mansoni*, and in mouse 6, they were all *S. intercalatum* worms.

Overall, 196 homospecific pairs and 94 heterospecific pairs were obtained. To test whether this difference was indicative of any species preference, the Mantel-Haenszel test was carried out on the proportions of males of both species paired homospecifically and heterospecifically. The result was highly significant ($\chi^2 = 51.101$, $P < 0.001$), showing that both species have a strong preference for homospecific mating.

Of paired *S. intercalatum* males, 85.6% formed homospecific pairs, and 14.4% heterospecific pairs, whereas 55.2% of paired *S. mansoni* males formed homospecific pairs, and 44.8% heterospecific pairs. This indicates that *S. intercalatum* has the stronger homospecific mate

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Table 1. Data from mice infected first with 150 cercariae of the Zaire strain of *Schistosoma intercalatum* (Int), and 10 days later with 150 cercariae of *S. mansoni* (Ms) so that both species reach patency simultaneously.

Mouse	Ms♂	Int♂	Ms♂ × Ms♀	Int♂ × Int♀	Ms♂ × Int♀	Int♂ × Ms♀	Multiple pairings (♂:♀)
1	5	1	7	5	8	0	
2	4	0	13	17	14	0	
3	4	0	21	8	6	0	
4	10	0	7	7	3	0	1 Ms:2 Ms
5	2	0	10	12	2	0	1 Ms:2 Ms
6	0	0	11	15	0	1	1 Int:2 Int
7	2	0	7	12	20	7	
8	8	2	7	6	10	2	
9	0	0	8	4	4	2	
10	6	3	4	15	10	5	
Total	41	6	95	101	77	17	

preference. However, it should be noted that the sex ratio of males: females for *S. intercalatum* in this experiment was 0.8:1, and this female bias may have influenced this result (see later discussion).

The proportion of all *S. intercalatum* males which were paired was 95.2%, whereas that of *S. mansoni* males was 80.8%. This difference was statistically significant according to Mantel-Haenszel analysis ($\chi^2 = 11.394$, $P < 0.005$), with *S. intercalatum* males being more successful than *S. mansoni* males at pairing. Again, it should be noted that this result may have been influenced by the female bias of the *S. intercalatum* sex ratio in this experiment.

Experiment 2: infections with S. mansoni *and 1 week later with* S. intercalatum *(Zaire)*

Two homospecific and two heterospecific types of pairing were obtained in this experiment (table 2) together with one multiple-worm pair from mouse 1, which consisted of one *S. intercalatum* male paired with one *S. intercalatum* female and one *S. mansoni* female. The proportion of males which formed pairs was 87.4% for *S. mansoni* males and 74.2% for *S. intercalatum* males. Mantel-Haenszel analysis showed this difference to be highly significant ($\chi^2 = 4.744$, $P < 0.05$), with *S. mansoni* males being better able to form pairs than *S. intercalatum* males.

The sex ratio of males:females in this experiment was 1.3:1 for *S. mansoni*, and therefore the assumption that all *S. mansoni* females would have initially been paired with *S. mansoni* males, leaving an excess of *S. mansoni* males which may compete with *S. intercalatum* males for *S. intercalatum* females, is a reasonable one.

However, despite this assumption a total of nine heterospecific *S. intercalatum* ♂ × *S. mansoni* ♀ pairs were obtained from mice 1, 3, 5, 7 and 9 (table 2). In mice 1, 3, 5 and 7, there was at least one unpaired *S. mansoni* male for each *S. intercalatum* ♂ × *S. mansoni* ♀ pairing, which the *S. intercalatum* males had outcompeted (table 2). In mouse 9, which yielded two *S. intercalatum* ♂ × *S. mansoni* ♀ pairs but only one unpaired *S. mansoni* male, one of these two pairs may have resulted from there being one fewer *S. mansoni* males than *S. mansoni* females: thus an *S. intercalatum* male paired with the extra *S. mansoni* female. A Mantel-Haenszel test carried out on the proportions of males of both species paired with *S. mansoni* females returned a highly significant result ($\chi^2 = 30.069$, $P < 0.001$), indicating that *S. mansoni* males were better overall at pairing with *S. mansoni* females than were *S. intercalatum* males. Therefore, *S. intercalatum* males are unable to outcompete *S. mansoni* males for *S. mansoni* females to any significant extent.

Data from mice 2, 3, 5, 7 and 8 indicate that 21 of the 32 heterospecific *S. mansoni* ♂ × *S. intercalatum* ♀ pairs obtained in total arose from there being a deficit of *S. intercalatum* males for the *S. intercalatum* females to pair with. Four of the 32 pairings may have involved *S. mansoni* males that had been actively displaced from their original homospecific *S. mansoni* ♀ partners by competitor *S. intercalatum* males, as indicated by the presence of a

Table 2. Data from mice infected first with 150 cercariae of *Schistosoma mansoni* (Ms) and 1 week later with 150 cercariae of *S. intercalatum* (Int), Zaire so that *S. mansoni* reaches patency 2 weeks before *S. intercalatum*.

Mouse	Ms♂	Int♂	Ms♂ × Ms♀	Int♂ × Int♀	Ms♂ × Int♀	Int♂ × Ms♀	Multiple pairings (♂:♀)
1	5	0	10	9	0	1	1 Int:1 Int + 1 Ms
2	0	1	9	7	7	0	
3	2	2	14	5	10	2	
4	1	1	14	6	0	0	
5	4	13	17	13	1	3	
6	1	0	11	8	0	0	
7	4	0	16	8	10	1	
8	4	7	14	0	4	0	
9	1	1	15	7	0	2	
Total	22	25	120	63	32	9	

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corresponding *S. intercalatum* ♂ × *S. mansoni* ♀ pair for each of the four *S. mansoni* ♂ × *S. intercalatum* ♀ pairings. These displaced males may have managed to re-pair with a heterospecific female. For each of the remaining seven heterospecific *S. mansoni* ♂ × *S. intercalatum* ♀ pairs at least one unpaired *S. intercalatum* male was also obtained which is assumed to have been outcompeted by the *S. mansoni* males for the *S. intercalatum* females. The difference in the proportions of males of each species paired with *S. intercalatum* females was significant according to Mantel-Haenszel analysis ($\chi^2 = 5.041$, $P < 0.05$) indicating that overall, *S. intercalatum* males are better than *S. mansoni* males at pairing with *S. intercalatum* females.

Experiment 3: infections with S. intercalatum *(Zaire) and 4 weeks later with* S. mansoni

Table 3 summarizes the worms obtained from each mouse and how they paired. Four main types of pairing were found: two homospecific and two heterospecific. There were no multiple-worm pairs. The proportion of males which formed pairs was 98.6% for *S. intercalatum*, and 82.6% for *S. mansoni*. The results of a Mantel-Haenszel test carried out on these proportions was not significant, and therefore males of each species do not significantly differ in their ability to form pairs.

The sex ratio of males:females was 1.7:1 for *S. intercalatum* in this experiment, and therefore the assumption that all *S. intercalatum* females would have initially been paired with *S. intercalatum* males, leaving an excess of *S. intercalatum* males which may compete with *S. intercalatum* males for *S. intercalatum* females, is a reasonable one.

Nevertheless, 12 heterospecific *S. mansoni* ♂ × *S. intercalatum* ♀ pairs were obtained in total from mice 2, 3, 4, 6, 8 and 9. Three of the *S. mansoni* ♂ × *S. intercalatum* ♀ pairs resulted from a deficit of *S. intercalatum* males for the females to pair with in mouse 2. There was at least one unpaired or non-homospecifically paired *S. intercalatum* male for each of the nine remaining pairs, indicating that these *S. intercalatum* males had been outcompeted for *S. intercalatum* females by the *S. mansoni* males. However, Mantel-Haenszel analysis revealed a highly significant difference in the proportions of males of each species paired with *S. intercalatum* females, ($\chi^2 = 20.487$, $P < 0.001$), with *S. intercalatum* males being better able to do so than *S. mansoni* males. Overall, *S. mansoni* males are unable to outcompete *S. intercalatum* males for *S. intercalatum* females to any significant extent.

Heterospecific *S. intercalatum* ♂ × *S. mansoni* ♀ pairs were obtained from all mice in this experiment except mouse 6. Seventeen out of the 37 pairs recovered in total arose from there being a deficit of *S. mansoni* males compared with *S. mansoni* females. Eight out of the 37 were obtained along with a *S. mansoni* ♂ × *S. intercalatum* ♀ pair. Therefore, the eight heterospecifically paired *S. intercalatum* males may have been those actively displaced from their original *S. intercalatum* female partners by competitor *S. mansoni* males, and which subsequently managed to re-pair with heterospecific females. For each of the remaining 12 heterospecific *S. intercalatum* ♂ × *S. mansoni* ♀ pairs at least one unpaired *S. mansoni* male was also obtained which is assumed to have been outcompeted by the *S. intercalatum* males for the *S. mansoni* females. The difference in the proportion of males of each species paired with *S. mansoni* females was highly significant according to Mantel-Haenszel analysis ($\chi^2 = 15.503$, $P < 0.001$), indicating that *S. intercalatum* males are unable to outcompete *S. mansoni* males for *S. mansoni* females to any significant extent.

Discussion

In all three experiments both homospecific and heterospecific pairs were obtained, and all females recovered were paired and contained eggs, thereby confirming that pairing and reproductive stimulation of females is possible between different species of schistosome, even where they belong to different evolutionary groups (Tchuem Tchuenté *et al.*, 1994; Khalil & Mansour, 1995; Southgate *et al.*, 1998).

Mice infected simultaneously with *S. intercalatum* (Zaire) and *S. mansoni* (experiment 1) yielded a significant preponderance of homospecific pairs over heterospecific, indicating the presence of a specific mate preference system, as demonstrated for the *S. intercalatum* (Lower Guinea)/*S. mansoni*; *S. haematobium*/*S. mansoni*; and *S. margrebowiei*/*S. mansoni* models of mating between

Table 3. Data from mice infected first with 150 cercariae of *Schistosoma intercalatum* (Int), and 4 weeks later with 150 cercariae of *S. mansoni* (Ms) so that *S. intercalatum* reaches patency 3 weeks before *S. mansoni*.

Mouse	Ms♂	Int♂	Ms♂ × Ms♀	Int♂ × Int♀	Ms♂ × Int♀	Int♂ × Ms♀
1	1	11	2	5	0	7
2	1	0	9	1	4	1
3	0	10	6	27	3	2
4	1	0	8	11	2	2
5	6	0	5	20	0	0
6	3	3	7	6	1	1
7	4	3	15	14	0	6
8	1	8	9	8	1	1
9	5	17	9	8	1	10
10	2	1	8	5	0	7
Total	24	53	78	105	12	37

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schistosomes from different evolutionary groups (Tchuem Tchuenté *et al.*, 1993; Webster *et al.*, 1999; Cosgrove & Southgate, 2002).

In this model, *S. intercalatum* (Zaire) exhibited a significantly greater homospecific mate preference than *S. mansoni*, in contrast to *S. intercalatum* (Lower Guinea) which had a lesser homospecific mate preference than *S. mansoni* (Tchuem Tchuenté *et al.*, 1993). However, it should be borne in mind that the *S. mansoni* isolate used by Tchuem Tchuenté *et al.* (1993) originated from Brazil, whereas the isolate used in these experiments came from Senegal, so it is conceivable that some of the differences in the data between Tchuem Tchuenté *et al.* (1993) and those presented here may possibly be correlated with the different isolates of *S. mansoni*. On the other hand, the work of Fletcher *et al.* (1981) mitigates against this idea, as they demonstrated, using isoenzymes, a relative lack of genetic divergence between Old and New World isolates of *S. mansoni*. The reproductive disadvantage of heterospecific pairing in yielding offspring of reduced viability has been mentioned earlier, but parthenogenetic offspring are particularly undesirable for *S. intercalatum* males in mixed infections with *S. mansoni*. This is because the *S. mansoni* ♂ × *S. intercalatum* ♀ cross yields sterile eggs, whilst the *S. intercalatum* ♂ × *S. mansoni* ♀ cross yields some parthenogenetic, matroclonal eggs of low viability, which, being matroclonal, may produce a small number of *S. mansoni* worms (Wright & Southgate, 1976). Therefore, the (albeit small) advantage of parthenogenesis in mixed infections (in allowing the propagation of some germ plasm) is enjoyed only by (female) *S. mansoni*, and therefore provides a further incentive for *S. intercalatum* to avoid heterospecific pairing.

However, the sex ratio of *S. intercalatum* (Zaire) was female-biased in this experiment (0.8:1 males:females), which may have had a significant influence on the results by increasing the chances of *S. intercalatum* (Zaire) being able to pair homospecifically over those of *S. mansoni* whose sex-ratio was male-biased (as sex ratios of schistosomes in nature usually are). The excess single *S. intercalatum* females would then be available for the excess single *S. mansoni* males to pair with, so increasing the proportion of heterospecifically paired *S. mansoni* males.

Similarly, the female-bias of the *S. intercalatum* (Zaire) infection may have influenced the finding that *S. intercalatum* (Zaire) males were better at pairing than *S. mansoni* males in experiment 1, which again differs from the *S. intercalatum* (Lower Guinea)/*S. mansoni* mating model where *S. mansoni* males were equally good at pairing as *S. intercalatum* (Lower Guinea) males (Tchuem Tchuenté *et al.*, 1993). In the simultaneous *S. intercalatum* (Lower Guinea)/*S. mansoni* experiments of Tchuem Tchuenté *et al.* (1993), equal numbers of males and females of each species were used.

Although there are indications from pairing ability and the strength of homospecific mate preference in experiment 1 that *S. intercalatum* (Zaire) has the competitive advantage over *S. mansoni* in simultaneous infections, which is the reverse of the situation with the Lower Guinea strain of *S. intercalatum*, the possible influence of the female-biased sex ratio of *S. intercalatum* (Zaire) must be taken into account.

One possible reason for there being more females than males of *S. intercalatum* (Zaire) in experiment 1 and not in experiments 2 or 3 is that the *S. intercalatum* (Zaire) infections for experiment 1 were carried out very soon after the infected snails (laboratory *Bulinus wrighti*) reached patency (45 days after miracidial infection). Not all snails will start to shed at the same time, and it may have been possible that some of the early shedders were shedding only female cercariae. Frandsen (1977) reported that snails shedding only female *S. intercalatum* (Zaire) cercariae shed for only 32 days, whereas snails shedding only male cercariae shed for up to 119 days. Therefore, by the time the *S. intercalatum* (Zaire) infections for experiments 2 and 3 were carried out (over one month later), some infected snails may produce fewer female cercariae, hence restoring the usual sex balance of more males to females. However, it should be noted that there is some debate on whether male cercariae have a shorter prepatent period in the snail host than females: male cercariae of an Iranian and a Mauritian strain of *S. haematobium* matured faster in *Bulinus* than female cercariae (Wright & Bennett, 1967; Wright & Knowles, 1972), whereas no difference was found between the maturation time of male and female cercariae of *S. intercalatum* (both strains) (Frandsen, 1977) and of *S. mansoni* (Liberatos, 1987).

In this model of mating interactions, several multiple-worm pairs were recovered. In experiment 1, the three multiple worm pairs recovered in total all consisted of one male paired with two homospecific females. It would appear that in pairing with two *S. mansoni* females, one *S. mansoni* male outcompeted ten and two unpaired *S. mansoni* males in mice 4 and 5 respectively. In mouse 6, one *S. intercalatum* (Zaire) male outcompeted one other *S. intercalatum* (Zaire) male in pairing with two *S. intercalatum* (Zaire) females. It is likely that the outcompeted *S. intercalatum* (Zaire) male is the male partner of the single heterospecific *S. intercalatum* (Zaire) ♂ × *S. mansoni* ♀ pair recovered from mouse 6: i.e. it had managed to re-pair with a surplus *S. mansoni* female. All three multiple-worm pairs from experiment 1 demonstrate intraspecific male-male competition for females, and it is apparent that both *S. mansoni* and *S. intercalatum* (Zaire) males are able to pair with multiple females.

In experiment 2, where *S. mansoni* reached patency in mice 2 weeks before *S. intercalatum* (Zaire), one multiple worm pair was recovered from mouse 1, consisting of an *S. intercalatum* (Zaire) male paired with one female of each species. This pairing may have arisen by a homospecifically paired *S. intercalatum* (Zaire) male pairing with an additional *S. mansoni* female, outcompeting five unpaired *S. mansoni* males in the process and demonstrating the involvement of interspecific male-male competition in multiple pairings.

Tchuem Tchuenté *et al.* (1993) obtained *S. intercalatum* (Lower Guinea) males paired with two to three females of *S. intercalatum* (Lower Guinea) or *S. mansoni* in experiments where *S. mansoni* males were absent. Therefore it appears that both strains of *S. intercalatum* are able to pair homospecifically or heterospecifically with more than one female.

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That multiple pairings are not reported more frequently from models of mating interactions between different species of schistosome may in part be due to the fact that multiple pairs could easily become separated when recovering worms from rodents by perfusion so that one or more of the participant worms is classified as unpaired.

Statistical analysis of data from experiment 2 shows that *S. mansoni* males are significantly better at pairing than *S. intercalatum* (Zaire) males. This might be expected, given that homospecific *S. mansoni* pairing would have taken place initially in the absence of competition from *S. intercalatum* males. Also, overall, *S. mansoni* males were better than *S. intercalatum* males at pairing with *S. mansoni* females. Again, this is as expected since the pairing of species 2 (*S. intercalatum*) males with species 1 (*S. mansoni*) females is a much more active process involving the males pulling away homospecifically paired species 1 females from their partners (except for where there is an excess of *S. mansoni* females over *S. mansoni* males as for one *S. intercalatum* ♂ × *S. mansoni* ♀ pair in mouse 9). That several *S. intercalatum* ♂ × *S. mansoni* ♀ pairs were recovered, however, shows that *S. intercalatum* males had the capability to 'actively compete' for females in this manner. It also indicates that *S. intercalatum* (Zaire) males may be more competitive than *S. intercalatum* (Lower Guinea) males, as in experiment 7 of the Tchuem Tchuenté *et al.* (1993) study, mice infected with male and female *S. mansoni* and male *S. intercalatum* (Lower Guinea) yielded only homospecific *S. mansoni* ♂ × *S. mansoni* ♀ pairs.

It should be noted that in sequential infections (experiments 2 and 3), the number of males of the second infection paired with females of the first infection may have been higher had the worms been perfused from animals more than 8 weeks post-reinfection, since Tchuem Tchuenté *et al.* (1995) described the phenomenon of change of mate 'as a progressive process, requiring up to 8 weeks' to happen. However, in the present experiments, it was not possible to delay the culling of mice any longer due to the pathogenicity of the worms.

Because the *S. mansoni* infection in experiment 2 was male-biased (1.3:1 males:females), it can be assumed that the excess of unpaired *S. mansoni* males competed with unpaired *S. intercalatum* (Zaire) males for *S. intercalatum* (Zaire) females. Neither types of pairing involve 'active competition' of the kind described above, instead involving 'passive' heterospecific pairing by *S. mansoni* males, and 'passive' homospecific pairing by *S. intercalatum* (Zaire) males. Overall, *S. intercalatum* (Zaire) males were significantly better at pairing with *S. intercalatum* (Zaire) females.

Experiment 6 in the Tchuem Tchuenté *et al.* (1993) study, using mice infected with males of both species but with only *S. intercalatum* (Lower Guinea) females, provides a directly comparable test of the relative abilities of males to pair with with *S. intercalatum* (Lower Guinea) females. No significant difference in this ability was found, again indicating that the Zaire strain of *S. intercalatum* is slightly more competitive in its interactions with *S. mansoni* than the Lower Guinea strain: *S. mansoni* males are outcompeted for *S. intercalatum* (Zaire) females by *S. intercalatum* (Zaire) males, but are equally good as *S. intercalatum* (Lower Guinea) males at pairing with *S. intercalatum* (Lower Guinea) females.

Statistical analysis of data from experiment 3, where *S. intercalatum* (Zaire) was the first infection and commenced pairing 3 weeks before *S. mansoni* was ready to do so, showed that there was no significant difference in the ability of males of both species to form pairs. In other words, when *S. intercalatum* (Zaire) has the advantage of being the first species to enter the host, it is only as good as *S. mansoni* at forming pairs, whereas when *S. mansoni* was the initial infection (experiment 2) it was significantly better at pairing than *S. intercalatum* (Zaire) males. Therefore, in sequential infections, *S. mansoni* males appear to have the competitive edge over *S. intercalatum* (Zaire) males with regard to their ability to form pairs.

Despite the recovery in experiment 3 of 12 *S. mansoni* ♂ × *S. intercalatum* (Zaire) ♀ pairs, nine of which were due to *S. mansoni* males actively pulling away paired *S. intercalatum* (Zaire) females from their homospecific partners, overall *S. intercalatum* (Zaire) males were significantly better at pairing with *S. intercalatum* (Zaire) females than *S. mansoni* males. A comparable test for the relative ability of *S. mansoni* and *S. intercalatum* (Lower Guinea) males to pair with *S. intercalatum* (Lower Guinea) females, involving active competition on the part of the *S. mansoni* males and 'passive' competition from the *S. intercalatum* (Lower Guinea) males was carried out by Tchuem Tchuenté *et al.* (1995) using mice infected first with males and females of *S. intercalatum* (Lower Guinea) and later reinfected with *S. mansoni* males. Surprisingly, the experiment revealed *S. mansoni* males as significantly better able to pair with female *S. intercalatum* (Lower Guinea) than *S. intercalatum* (Lower Guinea) males: an as yet unique example of 'active' competition winning over more 'passive' competition to a statistically significant extent.

The sex ratio of *S. intercalatum* (Zaire) was male-biased in experiment 3 (1.7:1 males:females), so that excess unpaired *S. intercalatum* (Zaire) males would have been available for 'passive' competition with *S. mansoni* males for *S. mansoni* females. Statistical analysis of the data revealed that, overall, *S. mansoni* males were better at pairing with *S. mansoni* females than were *S. intercalatum* (Zaire) males. Tchuem Tchuenté *et al.* (1993) carried out a comparable test for the relative ability of males to pair with *S. mansoni* females using mice infected with males of *S. intercalatum* (Lower Guinea) and *S. mansoni* but with only *S. mansoni* females. A similar result was obtained: male *S. mansoni* were better at pairing with female *S. mansoni* than male *S. intercalatum* (Lower Guinea).

As seen with the sequential infections in the *S. margrebowiei*/*S. mansoni* model (Cosgrove & Southgate, 2002), the worm return of each species in sequential infections can be used to determine whether the initial infection induces immunological cross-protection against reinfection by the second species. In experiment 2, with *S. mansoni* as the initial infection, the worm return of *S. mansoni* is 22.4% whilst that of *S. intercalatum* (Zaire) is 14.4%, which is 64.3% of the *S. mansoni* return. In experiment 3, with *S. intercalatum* (Zaire) as the initial infection, the worm return of *S. intercalatum* (Zaire) is 20.8% whilst that of *S. mansoni* is 15.3%, which is 73.6% of

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the *S. intercalatum* (Zaire) return. Therefore there does appear to be a similar degree of heterologous cross-protection induced by the initial infection against the second infection, in both situations. This differs from the results of Tchuem Tchuenté *et al.* (1996) which found that the cross-protection induced by *S. mansoni* against *S. intercalatum* (Lower Guinea) was not reciprocal, thus highlighting a possibly important immunological difference between the Zaire and Lower Guinea strains of *S. intercalatum*. However, when comparing degrees of cross-protection in experiments 2 and 3, it should be noted that the time interval between the initial and challenge infections differed (being 1 week and 4 weeks, respectively), which may have resulted in differing levels of immunity. As with the heterologous immunity observed in the Tchuem Tchuenté *et al.* (1996) study and the *S. margrebowiei*/*S. mansoni* model of Cosgrove & Southgate (2002), the cross-protection observed in experiments 2 and 3 is likely to result from the immunization process itself rather than to egg-induced immunity, because the first infection would not have reached patency by the time of reinfection with the second species in either of the experiments.

When comparing the results of this present study (using the Zaire strain of *S. intercalatum*) with those of Tchuem Tchuenté *et al.* (1993, 1995) (using the Lower Guinea strain) it appears that *S. mansoni* is not as competitively dominant to the Zaire strain of *S. intercalatum* as it is to the Lower Guinea strain, as evinced by observations that the homospecific mate preference of *S. mansoni* is stronger than that of *S. intercalatum* (Lower Guinea) but weaker than that of *S. intercalatum* (Zaire); that *S. mansoni* males are equally as good as *S. intercalatum* (Lower Guinea) males but worse than *S. intercalatum* (Zaire) males both at pairing in simultaneous infections and pairing with *S. intercalatum* females in sequential experiments with *S. mansoni* as the initial infection; and that *S. mansoni* males are better than *S. intercalatum* (Lower Guinea) males but worse than *S. intercalatum* (Zaire) males at pairing with *S. intercalatum* females in simultaneous infections with *S. intercalatum* as the initial infection. Indeed, in the present study, *S. intercalatum* (Zaire) appears to be competitively dominant to *S. mansoni* in simultaneous infections (experiment 1) by having a stronger homospecific mate preference and greater pairing ability, although the aforementioned female-bias of the *S. intercalatum* (Zaire) infection in experiment 1 casts doubt on the validity of these results.

However, it is experiment 2, with *S. mansoni* as the initial infection, which most closely resembles the situation in nature, that of *S. intercalatum* encountering pre-established infections of *S. mansoni* in its attempt to spread. The competitive dominance of *S. mansoni* in this situation was demonstrated by its greater ability to form pairs and its ability to retain homospecific pairing by outcompeting *S. intercalatum* males for *S. mansoni* females. In the light of this, then, it can be suggested that the competitive dominance of *S. mansoni* might be a contributory factor to the restricted distribution of the Zaire strain of *S. intercalatum*.

Given the above, it might be predicted that *S. intercalatum* (Zaire) would be competitively dominant to *S. intercalatum* (Lower Guinea) in mixed infections of the two. Interestingly, a recent study by Pagès *et al.* (2001b) found mating between the two strains to be random, with no specific mate preference system in mice infected simultaneously with both strains. In contrast to many species within the *S. haematobium* group which have assortative mating as a pre-zygotic mating barrier but lack the post-zygotic mating barrier of hybrid infertility, there appears to be no assortative mating barrier between the strains of *S. intercalatum* but hybrids between the two strains are of reduced viability in the F_1 generation, and are sterile beyond the F_2 generation (Frandsen, 1978; Pagès *et al.*, 2001a). Pagès *et al.* (2001b) note that strong assortative mating tends to evolve when there are strong contact zones between different species, and therefore the geographical isolation of the Zaire and Lower Guinea strains of *S. intercalatum*, not usually so marked for other species of the *S. haematobium* group, can explain why the post-zygotic barrier of hybrid infertility is not reinforced by strong homospecific mate preference.

However, this lack of assortative mating does not exclude the possibility of one of the strains being more competitive at pairing than the other, since in the *S. haematobium*/*S. intercalatum* (Lower Guinea) mating model (Southgate *et al.*, 1982), mating was similarly random with no specific mate preference system, yet *S. haematobium* was competitively dominant to *S. intercalatum* (Lower Guinea) in terms of pairing ability.

If the Zaire strain is indeed more competitive than the Lower Guinea strain, this then begs the question as to why the Zaire strain is restricted to the Democratic Republic of Congo whilst the Lower Guinea strain is more widespread. One important reason suggested by Tchuem Tchuenté *et al.* (1997a) concerns the poor compatibility of *S. intercalatum* (Zaire) with many potential intermediate hosts (Wright *et al.*,1972; Frandsen, 1979c) in contrast to *S. intercalatum* (Lower Guinea). Apart from *B. wrighti*, which is a universal host for all schistosomes within the *S. haematobium* group (Wright & Knowles, 1972; Wright *et al.*, 1972; Southgate & Knowles, 1975; Frandsen 1979a,b,c) *S. intercalatum* (Zaire) is only compatible with a very few isolates of *B. globosus*: for example, those from Zaire and Zambia, but not those from Cameroon, Togo, and the Ivory Coast (Tchuem Tchuenté *et al.*, 1997a; Frandsen *et al.*, 1978). In addition, factors responsible for the reduction in prevalence (from 30% to less than 4% as reported by Tchuem Tchuenté *et al.*, 1997a) of *S. intercalatum* (Zaire) in the Kinshasa focus between 1987 and 1997 may play a role in restricting the overall distribution of the Zaire strain. For example, these authors found evidence of natural hybridization between *S. intercalatum* (Zaire) and *S. haematobium* in the area causing a decline in the transmission of pure *S. intercalatum* (Zaire). They also suggest that the intermediate host of the Zaire strain, *B. globosus*, may have been disappearing from the area, perhaps in the manner of *Biomphalaria camerunensis* in the early 1980s which led to the decline of *S. mansoni* transmission in the region in spite of the replacement of *B. camerunensis* by *B. pfeifferi*, another potential snail host for *S. mansoni* (De Clercq, 1987). For this reason, the reduced prevalence of *S. intercalatum* (Zaire) in Kinshasa cannot be blamed on its interactions with *S. mansoni*. Other factors implicated

in the regional decline of the Zaire strain include the increasing socio-economic status of the human population and sanitation improvements, such as the provision of piped water, reducing the extent of water contact by humans (Tchuem Tchuenté *et al.*, 1997a).

In summary, the mating model presented here describes further differences between the Zaire and Lower Guinea strains of *S. intercalatum*, and allows the prediction that the Zaire strain may be competitively dominant to the Lower Guinea strain to be made. There is evidence to suggest that *S. mansoni* may hinder the spread of *S. intercalatum* (Zaire) to areas where *S. mansoni* is already present both through its competitiveness at mating and by the induction of immunological cross-protection against *S. intercalatum* (Zaire), but to a lesser extent than for *S. intercalatum* (Lower Guinea). Nevertheless, *S. intercalatum* (Zaire) is more restricted in its distribution than *S. intercalatum* (Lower Guinea), which highlights the great importance of setting observations from laboratory models of mating interactions in a wider context incorporating other factors and influences on the transmission of schistosomes in the field.

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