Compatibility of *Schistosoma mansoni* Cameroon and *Biomphalaria pfeifferi* Senegal

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SUMMARY

The vectorial capacity of *Biomphalaria pfeifferi* from Ndiangue, Senegal, was investigated with an allopatric isolate of *Schistosoma mansoni* from Nkolbisson, Cameroon. The snail infection rate after exposure to a single miracidium per snail (MD1) was 56·3 %, and 91·6 % for snails exposed to 5 miracidia per snail (MD5). The minimum pre-patent period was 21 days. The mean total cercarial production for the MD1 group was 18511 cercariae per snail, and 9757 cercariae for the MD5 group. The maximum production of cercariae for 1 day was 4892 observed in a snail from the MD1 group at day 43 post-infection. The mean longevity of snails was higher in group MD1 (88 days p.i.) than in group MD5 (65 days p.i.). The chronobiological emergence pattern revealed a circadian rhythm with one shedding peak at mid-day. Comparisons are made with the vectorial capacity of the sympatric combination of *B. pfeifferi* Senegal/*S. mansoni* Senegal.

Key words: *Schistosoma mansoni* Cameroon, *Biomphalaria pfeifferi* Senegal, chronobiology, snail–parasite compatibility .

INTRODUCTION

The vectorial capacity of *Biomphalaria pfeifferi* Senegal with *Schistosoma mansoni* Senegal was recently investigated (Tchuem Tchuenté *et al*. 1999). The study clearly demonstrated an unusually high compatibility between the intermediate snail host and the parasite, with a snail infection rate of 87 % after exposure to a single miracidium (MD1) and 100 % after exposure to 5 miracidia per snail (MD5), with a mean total production of 50456 cercariae per snail. The chronobiological cercarial production pattern showed a peak around mid-day, a typical 'human' pattern. The high degree of compatibility between intermediate host and parasite in Northern Senegal is probably an important factor in providing an explanation for the spread of the parasite in the Senegal river basin since its introduction about 1988, and the increase in levels of prevalence and intensity in the human population (Picquet *et al*. 1996). However, one of the questions arising from the Tchuem Tchuenté *et al*. (1999) study was whether the highly compatible intermediate host–parasite relationship was due to the snail, to the parasite or to both. In an attempt to shed light on aspects of this question, the vectorial capacity of *B. pfeifferi* Senegal with *S. mansoni* Cameroon has been investigated.

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MATERIALS AND METHODS

Schistosome and snail host

The isolate of *S. mansoni* was made in Cameroon in May 1998 by exposing laboratory bred *B. pfeifferi* Senegal to miracidia hatched from the faeces of 2 infected children who were from Nkolbisson, near Yaoundé. The snails were transported to the laboratory of the Biomedical Parasitology Division, The Natural History Museum, London, where the parasite isolate was established and maintained in mice and *B. pfeifferi* Senegal.

Snails were bred in the laboratory from wild caught *B. pfeifferi* collected in Ndiangue, near Richard-Toll, Northern Senegal, for experimental infections.

Snail infection experiments

In order to study the vectorial capacity of the Senegalese *B. pfeifferi* with the Cameroonian *S. mansoni*, 2 groups of 100 laboratory-bred snails, with a shell diameter of 5–7 mm and aged approximately 4 weeks, were selected. These were designated as group MD1 and group MD5 (MD = miracidial

dose). They were exposed individually to 1 miracidium or 5 miracidia of *S. mansoni*, respectively, in a well of Disposo-Tray™ containing 1 ml of snail conditioned water (bore hole water which is left standing for 3 days prior to being passed through Whatman Gamma 25 μm filters). They were left overnight before being transferred to polypropylene trays and fed *ad libitum* with lettuce. The water temperature was maintained at 26 °C for the duration of the experiment, and the snails were regularly examined for cercarial shedding from day 20 onwards.

Snail size was measured before the exposure to the parasite, at 4 weeks post-exposure and at snail death, using a calliper ruler. For each snail, the largest diameter of the shell was measured.

Cercarial production

Twenty-seven days after exposure, 20 snails were randomly selected from each of the 2 groups of infected snails. These 40 snails were then separated and maintained individually in small plastic pots containing 25 ml of snail conditioned water until they died. The water was changed daily, and the snails were fed daily with dry lettuce. The number of cercariae produced per individual snail was counted daily from day 30 post-infection (p.i.) to day 57 p.i. After this period, counting of cercariae was performed on a weekly basis until the death of the snail. For each individual snail, the number of cercariae contained in three 1 ml aliquots (stained with Lugol's iodine solution) of the thoroughly mixed parasite suspension was counted, and the cercarial production was calculated. Water was changed for each snail on a daily basis. For the total cercariae produced by each snail, the daily count (performed once per week) from day 64 onwards was multiplied by 7 to produce a weekly estimate of cercarial production.

Chronobiology of cercarial emergence

The rhythm of emergence of *S. mansoni* cercariae was studied according to the methods described by Théron (1982): water kept at a constant temperature (26 °C), balanced photo-period (light/dark: 12 h/12 h) and photo-phase 06.00 h to 18.00 h (2000 lux), with light intensity gradually increasing at the beginning and decreasing at the end of the photophase. Quantitative determination of cercarial emergence was ascertained by using a cercariometric apparatus, allowing the automatic hourly deposition of emitted cercariae into collecting vessels. The contents of the vessels were concentrated by filtration through a polyamide filter (25 μm pore size), and stained with Lugol's iodine solution. Cercariae trapped on the filter were counted under a stereoscopic microscope. The emergence rhythms were studied for 10 snails in both MD1 and MD5 groups, on 2 consecutive days. The chronobiological data were transformed into circular variables (Chassé & Théron, 1988), after which the mean vector was calculated.

Statistical analysis

Cercarial production was analysed for individual snails using linear regression of log transformed values, and tests for specific effects were obtained as *t*-tests or Mann–Whitney *U*-test. Results were considered statistically significant at $P < 0{\cdot}05$. The 28-day production (i.e. from days 30 to 57) was analysed to assess the difference between the 2 groups MD1 and MD5. The rate of change of cercarial production (i.e. the slope of a regression of log of cercarial production on day for the period 30 to 57 days) was analysed allowing for initial size and 28-day production. The mortality rate of snails (log transformed) was regressed allowing for 28-day production and initial size index to determine the differences between the 2 groups. The survival times of the 2 exposure groups were plotted as life-tables.

RESULTS

Snail–parasite compatibility

The results of the snail infection experiments revealed infection rates of 56·3 % for the *B. pfeifferi* exposed to a single miracidium, and 91·6 % for the snails exposed to 5 miracidia. The minimum prepatent period was 21 days. At day 27 post-infection, 4 of the 100 snails of the MD1 group and 17 of the MD5 group had died, giving mortality rates of 4 % and 17 %, respectively.

Cercarial production

The results of the cercarial production counts are summarized in Table 1. The total production of cercariae per infected snail during its life-span ranged from 150 to 46484 cercariae for snails in the MD1 group, and from 192 to 28683 cercariae for those in the MD5 group, with a mean of 18511 cercariae for MD1 group, and 9757 cercariae for MD5 group ($t = -2{\cdot}39$, D.F. = 38, $P < 0{\cdot}05$).

The pattern of daily cercarial production per snail is shown in Fig. 1. The mean daily cercarial production per snail was high between day 30 and day 71 post-infection. Thereafter, the cercarial production decreased gradually until the death of the snail. The mean daily production of cercariae per snail (average from day 30 to day of death) was greater, but not significantly, in the MD1 group (386 cercariae) compared to the MD5 group (371 cercariae) ($t = -0{\cdot}2$, D.F. = 38, $P > 0{\cdot}05$).

The maximum production of cercariae by 1 snail for 1 day was 4892 cercariae, observed in a snail from

Table 1. Cercarial production and longevity of *Biomphalaria pfeifferi* from Ndiangue, Northern Senegal, infected with allopatric *Schistosoma mansoni* from Nkolbisson, Cameroon

No. of miracidia per snail	No. of snails per group	Mean total cercariae per infected snail (minimum–maximum)	Mean daily cercariae per infected snail (maximum)	Mean longevity of infected snails* (minimum–maximum)
1	20	18511 (150–46484)	386 (4892)	88 (31–202)
5	20	9757 (192–28683)	371 (3125)	65 (30–178)

* Number of days post-infection.

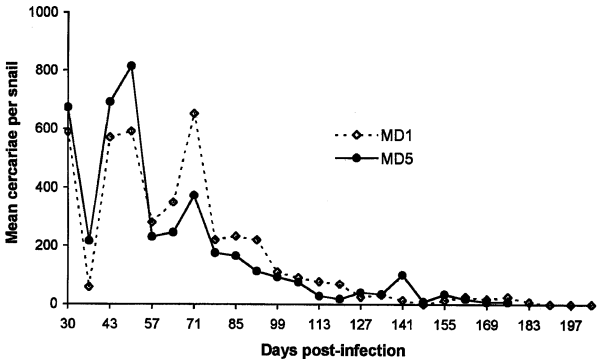

Fig. 1. Mean daily number of cercariae of *Schistosoma mansoni* from Nkolbisson, Cameroon produced per individual *Biomphalaria pfeifferi* Richard-Toll, Northern Senegal exposed either to 1 miracidium (MD1) or 5 miracidia (MD5). The starting number of snails was 20 individuals per group.

group MD1 at day 43 post-infection. A frequency distribution of daily production of cercariae was evaluated and the results are summarized in Table 2. From the total of 1000 individual daily counts made, the median was 233 cercariae shed per snail per day, with 73·2% lower than 500 cercariae and only 8·6% exceeding 1000 cercariae.

Snail growth

Prior to infection, the mean large diameter of snails per group was 4·68 mm for both MD1 and MD5 groups; it was 7·43 mm and 7·41 mm at 4 weeks post-infection ($U = 181$, $P > 0{\cdot}05$) and 8·25 mm and 7·76 mm at snail death ($U = 194{\cdot}5$, $P > 0{\cdot}05$) for groups MD1 and MD5, respectively.

Snail mortality

Figure 2 shows the mortality of infected *B. pfeifferi* in the 2 groups of snails selected and followed up as a life-table. The maximum life-span of an individual infected snail was 202 days p.i. for group MD1, and 178 days p.i. for group MD5. The mean longevity of snails was significantly higher in group MD1 (88 days p.i.) than in group MD5 (65 days p.i.) ($t = -1{\cdot}5$, D.F. = 38, $P < 0{\cdot}05$) (Table 1).

Table 2. Frequency distribution of daily levels of *Schistosoma mansoni* (Cameroon) cercarial production per individual *Biomphalaria pfeifferi* (Senegal)

	Group MD1*		Group MD5*		MD1+MD5	
	N†	Frequency (%)	*N*	Frequency (%)	*N*	Frequency (%)
Levels of cercariae output						
< 100	177	30·4	121	28·9	298	29·8
100–199	93	16	59	14·1	152	15·2
200–299	67	11·5	64	15·3	131	13·1
300–399	49	8·4	41	9·8	90	9
400–499	29	5	32	7·7	61	6·1
500–999	109	18·7	73	17·5	182	18·2
1000–1499	40	6·9	22	5·3	62	6·2
1500–1999	6	1	3	0·7	9	0·9
2000–2499	6	1	2	0·5	8	0·8
⩾ 2500‡	6	1	1	0·2	7	0·7
Total no. of counts made	582	100	418	100	1000	100
Median of cercariae output/snail	221		250		233	

* Group MD1, group of snails exposed individually to 1 miracidium; Group MD5, group of snails exposed individually to 5 miracidia.
† *N*, Number of counts made.
‡ The highest daily production obtained was 4892 cercariae.

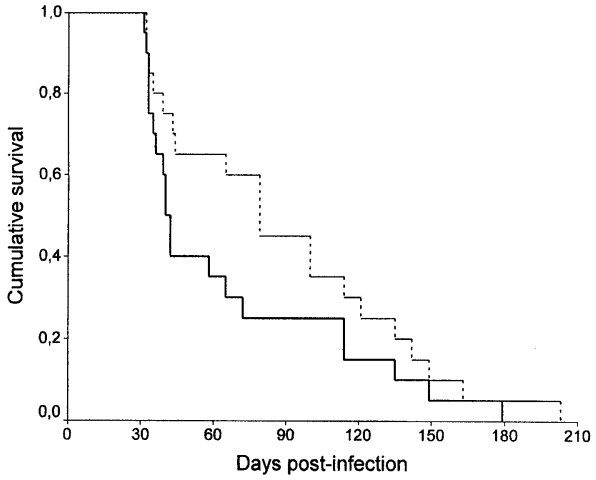

Fig. 2. Comparative survival curves of *Biomphalaria pfeifferi* from Ndiangue, Northern Senegal exposed either to 1 miracidium (-----) or 5 miracidia (——) of allopatric strain of *Schistosoma mansoni* from Nkolbisson, Cameroon. The starting number of snails was 20 individuals per group.

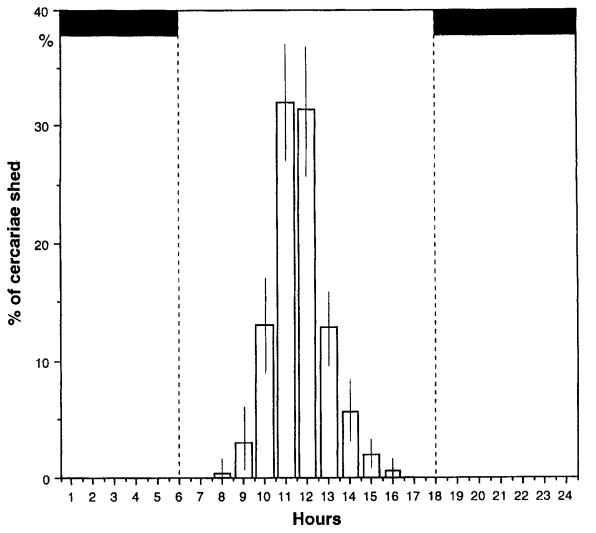

Fig. 3. Histogram of the daily emergence pattern of cercariae of *Schistosoma mansoni* from Nkolbisson, Cameroon. Vertical bars represent S.D.

Chronobiology of cercarial emergence

The results of the chronobiological cercarial emergence pattern are represented in Fig. 3, showing a circadian rhythm. There was only 1 shedding peak at 11h58, with an angular deviation of 1h02, representing 32 % of the daily production of cercariae.

DISCUSSION

The snail infection data of 56·3 % for MD1, and 91·6 % for MD5 is indicative of a less compatible host–parasite relationship between *S. mansoni* Cameroon/*B. pfeifferi* Senegal than between *S. mansoni* Senegal/*B. pfeifferi* Senegal where the corresponding infection rates were 87·3 % and 100 % (Tchuem Tchuenté *et al.* 1999). Thus, it is apparent that the snail infection rate varies according to the origin of the parasite and the intermediate host, and reflects the snail–host compatibility. Also, the cercarial production differs significantly between the 2 combinations: the allopatric *B. pfeifferi* Senegal/*S. mansoni* Cameroon MD1 and MD5 groups produced approximately only one third and one fifth, respectively, of the sympatric *B. pfeifferi*/*S. mansoni* Senegal MD1 and MD5 groups. There was a significant difference in the cercarial output between the group of snails exposed to 1 miracidium and that exposed to 5 miracidia, unlike the *S. mansoni* Senegal/*B. pfeifferi* Senegal combination. The snails exposed to 1 miracidium produced approximately twice as many cercariae as those snails which were exposed to 5 miracidia. Apparently, the relation between cercarial output and miracidial dose is highly variable, for cercarial production may be either higher for plurimiracidial infections compared with monomiracidial (Théron, 1985; Théron, Pagès & Rognon, 1997) or inversely smaller for plurimiracidial infections (Mouahid & Combes, 1987).

There was a correlation between MD1 and MD5 and the mortality of snails in the *S. mansoni* Cameroon/*B. pfeifferi* Senegal combination: those snails which had been exposed to 5 miracidia each died sooner than those exposed to 1 miracidium. Moreover, the MD5 snails produced fewer cercariae than the MD1 snails.

The mean longevity was significantly longer for MD1 (88 days) than for MD5 (65 days), and the maximum life-span of MD1 (202 days p.i.) was longer than that for MD5 (178 days p.i.) for *S. mansoni* Cameroon, which were shorter than the equivalent recordings for *S. mansoni* Senegal, that is, 100 days p.i. for MD1 and 143 days p.i. for MD5, with maximum life-spans of 239 days p.i. and 231 days p.i. for MD1 and MD5, respectively.

The analysis of the frequency of cercarial counts reflects the differences in vectorial capacity between the isolate of *S. mansoni* from Cameroon and that of *S. mansoni* from Senegal in the same intermediate host. Indeed, the combined MD1 and MD5 production shows that 55 % of the daily counts were over 200 cercariae per snail, and 8·6 % over 1000 cercariae per snail for *S. mansoni* Cameroon/*B. pfeifferi* Senegal, which contrasts with 81 % and 15 %, respectively, for *S. mansoni* Senegal/*B. pfeifferi* Senegal. Once again these figures reflect differences in vectorial capacity between the isolate of *S. mansoni* from Cameroon and that of *S. mansoni* from Senegal in the same intermediate host. Frandsen (1979) showed for *S. mansoni*/*Biomphalaria* spp. that only 20–30 % of the daily counts were greater than 200 cercariae, and only 1 % of counts over 1000 cercariae per snail per day. The results for the *S. mansoni* Cameroon/*B. pfeifferi* Senegal regarding infection rates, mean longevity of infected

snails and cercarial output are indicative of a less compatible host–parasite relationship than the *S. mansoni* Senegal/*B. pfeifferi* Senegal combination. These observations indicate a fast, local adaptation of the *S. mansoni* population to the *B. pfeifferi* in the Senegal river basin and agree with different studies showing that parasites are more adapted to sympatric hosts than to allopatric hosts of the same species (see Ebert (1994) for review). Theoretical studies predict that parasites are more likely to be adapted to their local host population than an allopatric host population. The data presented here and those of Tchuem Tchuenté *et al*. (1999) concerning reciprocal cross-infection studies using the same population of *B. pfeifferi* and 2 geographically distant populations of *S. mansoni* are consistent with the prediction of the model (Gandon *et al*. 1996).

The data from the chronobiology experiments showed that there was 1 peak of emergence, around mid-day, which is typical of the 'human' pattern. This is considered to be an adaptive behaviour of the parasite, under genetic control, shaped under the selective pressures exerted by the behaviour of the definitive hosts (Théron & Combes, 1998; Théron, 1989). *S. mansoni* Senegal possessed a similar chronobiology with just the 1 peak around mid-day.

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REFERENCES

CHASSÉ, J. L. & THÉRON, A. (1988). An example of circular statistics in chronobiological studies: Analysis of polymorphism in the emergence rhythms of *Schistosoma mansoni* cercariae. *Chronobiology International* **5**, 433–439.

EBERT, D. (1994). Virulence and local adaptation of horizontally transmitted parasite. *Science* **265**, 1084–1086.

FRANDSEN, F. (1979). Studies of the relationship between *Schistosoma* and their intermediate hosts. III. The genus *Biomphalaria* and *Schistosoma mansoni* from Egypt, Kenya, Sudan, Uganda, West Indies (St. Lucia) and Zaire (two different strains: Katanga and Kinshasa). *Journal of Helminthology* **53**, 321–348.

GANDON, S., CAPOWIEZ, Y., DUBOIS, Y., MICHALAKIS, Y. & OLIVIERI, I. (1996). Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proceedings of the Royal Society London*, *B* **263**, 1003–1009.

MOUAHID, A. & COMBES, C. (1987). Genetic variability of *Schistosoma bovis* cercarial production according to miracidial dose. *Journal of Helminthology* **61**, 89–94.

PICQUET, M., ERNOULD, J. C., VERCRUYSSE, J., SOUTHGATE, V. R., MBAYE, A., SAMBOU, B., NIANG, M. & ROLLINSON, D. (1996). The epidemiology of human schistosomiasis in the Senegal River Basin. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 340–346.

TCHUEM TCHUENTÉ, L. A., SOUTHGATE, V. R., THÉRON, A., JOURDANE, J., LY, A. & GRYSEELS, B. (1999). Compatibility of *Schistosoma mansoni* and *Biomphalaria pfeifferi* in Northern Senegal. *Parasitology* **118**, 595–603.

THÉRON, A. (1982). Le compartiment cercaire dans le cycle de *Schistosoma mansoni* Sambon, 1907. Ecologie de la transmission bilharzienne en Guadeloupe. Doctoral thesis, University of Perpignan, France.

THÉRON, A. (1985). Dynamics of the cercarial production of *Schistosoma mansoni* in relation with the miracidial dose exposure to the snail host *Biomphalaria glabrata*. *Annales de Parasitologie Humaine et Comparée* **60**, 665–674.

THÉRON, A. & COMBES, C. (1988). Genetic analysis of cercarial emergence rhythms of *Schistosoma mansoni*. *Behavior Genetics* **18**, 201–209.

THÉRON, A. (1989). Hybrids between *Schistosoma mansoni* and *S. rodhaini*: characterization by cercarial emergence rhythms. *Parasitology* **99**, 225–228.

THÉRON, A., PAGÈS, J. R. & ROGNON, A. (1977). *Schistosoma mansoni*: distribution patterns of miracidia among *Biomphalaria* snail as related to host susceptibility and sporocyst regulatory processes. *Experimental Parasitology* **85**, 1–9.