Experimental observations on the sex ratio of adult *Schistosoma mansoni*, with comments on the natural male bias

J. BOISSIER *and* H. MONÉ*

Centre de Biologie et d'Écologie Tropicale et Méditerranéenne (*UMR 5555 du CNRS*), *Université de Perpignan, 66860 Perpignan Cedex, France*

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SUMMARY

The sex ratio of adult worms has been observed biased towards males in *Schistosoma mansoni* under natural conditions. The origin of this bias is unknown. This paper determines whether males are more infective than females under controlled experimental bisexual conditions, and hence if the sex ratio is male-biased as a consequence of this. The male and female cercarial infectivities in uni- and bisexual vertebrate host infections using a range of controlled cercarial sex ratios were studied. The results showed that, in experimental unisexual infections, male cercariae were more infective than females, and that in experimental bisexual infections, male cercarial infectivity was similar to that of female, irrespective of cercarial sex ratio. Furthermore, cumulative male and female cercarial infectivity was maximal when sex ratio was equilibrated. The unbiased sex ratios obtained in our experimental bisexual infections are discussed in terms of behavioural and/or biochemical male–female interaction. Alternative explanations of the natural biased sex ratio are proposed.

Key words: *Schistosoma mansoni*, sex ratio, cercariae, infectivity, transmission.

INTRODUCTION

Schistosoma mansoni is a dioecious trematode whose sex ratio is known to be male-biased in the definitive host. This bias has been observed under natural conditions: published values of male:female worm ratios, obtained from infected *Rattus rattus* in Guadeloupe (West Indies) include 1·1, 1·5 and 1·6 (Imbert-Establet, 1982; Théron *et al*. 1992; Barral *et al*. 1996). The origin of this bias may be found either in the steps preceding cercarial penetration, the process of penetration or during cercarial development into adult. In order to explain the bias, several authors have performed experimental controlled unisexual infections for each step of the life-cycle. Boissier, Morand & Moné (1999) have reviewed these studies and showed that only studies on cercarial infectivity could provide an explanation (Evans & Stirewalt, 1951; Stirewalt & Fregeau, 1968; Taylor & Andrews, 1973; Rowntree & James, 1977; Liberatos, 1987; Cheever, Lewis & Wynn, 1997): a better male cercarial infectivity may thus explain the bias.

However, experimental unisexual infections of the definitive host do not reflect the natural conditions because no possible interaction exists between sexes. The term 'sex ratio' cannot be used for such experimental unisexual infections: sex ratio cannot be calculated from the ratio of male/female worms when males and females are recovered from separate definitive hosts. Boissier *et al*. (1999) did not use the term male-biased sex ratio but expressed the higher male infectivity in terms of better male life-history performance. The term 'sex ratio' should be used only for mixed sex (bisexual) infections of the definitive host where a true operational sex ratio exists.

This paper investigates whether males are still more infective than females in controlled experimental bisexual conditions and thus if the sex ratio is male-biased as a consequence of this. It determines also if the infectivity of one sex is dependent on the presence of the other sex. Furthermore, it aims to study the proportionality between cercarial and adult sex ratios. In order to answer these questions, the cercarial infectivity of male and female cercariae were compared in both uni- and bisexual infections of the vertebrate host using a range of controlled sex ratios.

MATERIALS AND METHODS

Parasite and host strains

The host–parasite system used was an albino variety of *Biomphalaria glabrata* from Brazil and a strain of *S. mansoni*, also from Brazil, maintained in Swiss OF1 mice.

Snail infection and maintenance

The molluscs, measuring 4–5 mm in diameter, were exposed individually to a single miracidium in 5 ml of spring water. The next day, the snails were

* Corresponding author: Tel: +33 04 68 66 21 84. Fax: +33 04 68 66 22 81. E-mail: mone@univ-perp.fr

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Table 1. Comparison between male and female infectivities according to cercarial sex ratios

		Bisexual infections (cercarial sex ratios)							
Infectivity (Mean ± S.E.)	Unisexual infections	0·25	0·33	0·67	1·00	1·50	3·00	4·00	Pooled sex ratios
Experiment 1									
Male	0·22 ± 0·01	—	0·25 ± 0·03	—	0·31 ± 0·07	—	0·23 ± 0·06	—	0·26 ± 0·03
N	3	—	3	—	3	—	3	—	9
Female	0·18 ± 0·00	—	0·19 ± 0·02	—	0·28 ± 0·05	—	0·26 ± 0·07	—	0·24 ± 0·03
N	3	—	3	—	3	—	3	—	9
Significance	*	—	N.S.	—	N.S.	—	N.S.	—	N.S.
Experiment 2									
Male	0·26 ± 0·04	0·22 ± 0·03	—	0·20 ± 0·02	0·28 ± 0·04	0·24 ± 0·04	—	0·20 ± 0·03	0·23 ± 0·01
N	5	5	—	5	5	4	—	5	24
Female	0·14 ± 0·01	0·21 ± 0·02	—	0·19 ± 0·02	0·28 ± 0·04	0·22 ± 0·04	—	0·20 ± 0·03	0·22 ± 0·01
N	5	5	—	5	5	4	—	5	24
Significance	*	N.S.	—	N.S.	N.S.	N.S.	—	N.S.	N.S.

N.S., Not significant; $*P < 0·05$.

isolated in plastic glasses containing 150 ml of spring water. They were fed *ad libitum* with fresh lettuce, and water was changed weekly and maintained at a constant temperature of 26 °C and a photo-period of light : dark : 12 : 12. Five weeks after exposure, the cercarial sex from each mollusc was determined by infection and subsequent perfusion of mice.

Mice infection, cercarial infectivity and worm sex ratio determination

Mice were anaesthetized with a mixture of Rompun (20 mg/ml; Bayer) 0·50 ml and Imalgène (100 mg/ml; Rhône Mérieux) 1·00 ml in 8·5 ml of autoclaved NaCl 8·5 (‰) by injection of 0·1 ml/10 g of body weight. Mice abdomens were shaved and exposed to 200 cercariae for 45 min. Worms were recovered and worm sex ratio was determined 8 weeks post-infection for all mice. Mice were killed by a lethal intraperitoneal injection of sodium pentobarbital (1·1 ml in 10 ml of 10% ethanol). Adult worms were recovered by retrograde perfusion of the hepatic portal system with citrate (7·5‰) saline (8·5‰) solution administered through the left ventricle (Duwall & DeWitt, 1967). Worms trapped in the liver or mesenteric system were collected after excision of these organs. All worms were counted. Cercarial infectivity was calculated for each sex by dividing the number of worms which had developed into an adult by the number of cercariae given. Worm sex ratio was calculated for each mouse as the proportion of male to female worms.

Experimental design

Two experiments were performed. In both experiments, pairs of molluscs were constituted by 1 mollusc harbouring a male cercarial infection and 1 mollusc harbouring a female cercarial infection. In the first experiment, 3 different pairs were used at 100 days post-infection. For each pair, 3 cercarial sex ratios (defined as the number of male : female cercariae) were given to mice: 0·33 (50 : 150), 1 (100 : 100) and 3 (150 : 50). In the second experiment, 5 different pairs of molluscs were used at 110 days post-infection. For each pair, 5 cercarial sex ratios were given to mice: 0·25 (40 : 160), 0·67 (80 : 120), 1 (100 : 100), 1·5 (120 : 80) and 4 (160 : 40). Unisexual infections of mice were performed (control groups) using 200 cercariae shed from each infected mollusc used in both experiments.

Statistical tests

Statistical analyses were performed using StatView 4.5. Correlation tests were performed to assess possible relationships between cercarial sex ratios and worm sex ratios. The two-slope test (Zar, 1996) was used to compare 2 simple regression equations. The nonparametric Mann–Whitney U-test was used to compare infectivities.

RESULTS

Male versus female cercarial infectivity

The comparisons between male and female cercarial infectivities, according to the cercarial sex ratios, are presented in Table 1 for both experiments. In unisexual infections, male infectivities were significantly higher than female infectivities for both experiments. In bisexual infections, and for Experiment 1, there was no difference between male and female infectivities even if male values tend to be higher than female values for 0·33 and 1·00 sex ratios. In Experiment 2, which used a higher sampling than in Experiment 1, there was no difference between male and female infectivities. In bisexual pooled sex ratios, male infectivities were not significantly dif-

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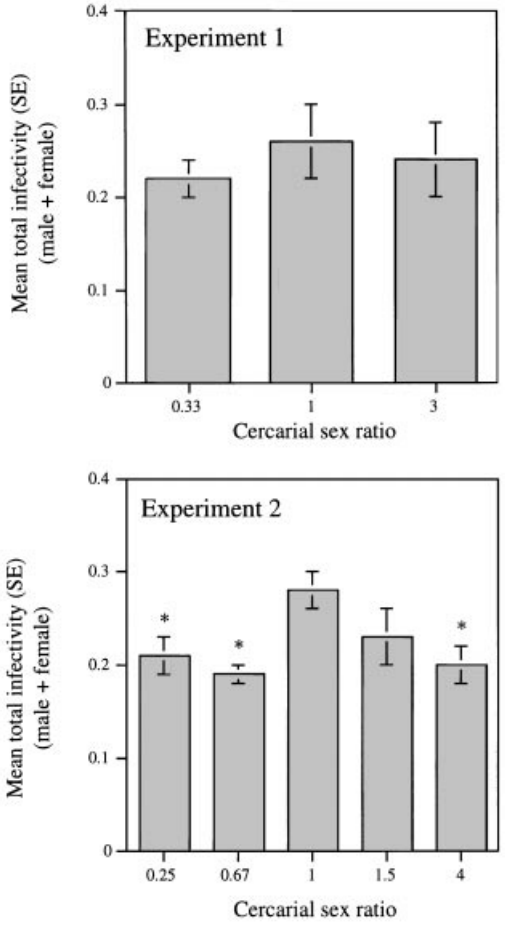

Fig. 1. Relationships between cercarial sex ratio and mean total infectivity (±S.E.) *Significant difference with the equilibrated sex ratio.

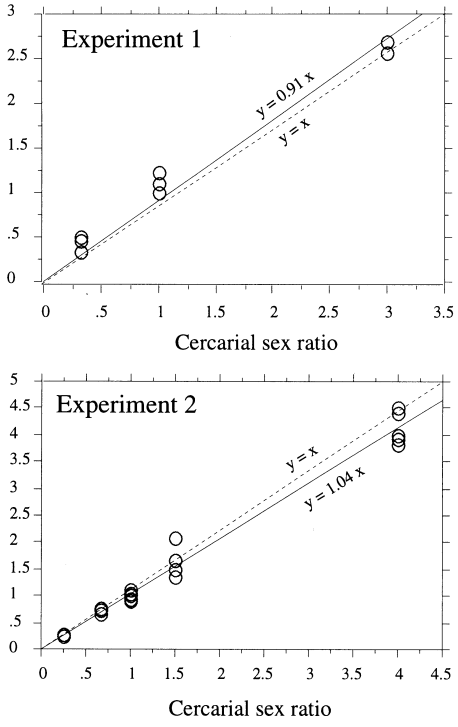

Fig. 2. Relationships between cercarial sex ratio and adult sex ratio.

ferent from male infectivities in unisexual infections (Exp. 1: $U = 8{\cdot}50$, $P = 0{\cdot}35$; Exp. 2: $U = 45{\cdot}50$, $P = 0{\cdot}40$). In bisexual pooled sex ratios, female infectivities were higher than female infectivities in unisexual infections; the difference was not significantly different in the first experiment ($U = 6{\cdot}00$, $P = 0{\cdot}16$) but significantly different in the second one ($U = 10{\cdot}00$, $P < 0{\cdot}05$).

The relationships between cercarial sex ratios and mean total (male + female) infectivities are presented in Fig. 1 for both experiments. Infectivities were maximal using equilibrated cercarial sex ratios. In the first experiment, there was no difference between the infectivity obtained with an equilibrated sex ratio and those obtained with non-equilibrated sex ratios ($P > 0{\cdot}05$). In the second experiment, infectivity obtained with an equilibrated sex ratio was significantly higher than with most non-equilibrated sex ratios: 0·25 ($U = 17$, $P < 0{\cdot}05$), 0·67 ($U = 17$, $P < 0{\cdot}05$) and 4 ($U = 24$, $P < 0{\cdot}05$).

Relationship between cercarial and adult sex ratios

The relationships between cercarial sex ratios and adult sex ratios are presented in Fig. 2 for both experiments. There were highly significant positive correlations between the sex ratios of cercariae administered and sex ratios of the adult worms recovered from mice (Exp. 1: $n = 9$, $r = 0{\cdot}99$, $P < 0{\cdot}001$; Exp. 2: $n = 25$, $r = 0{\cdot}99$, $P < 0{\cdot}001$). Regression equations were not significantly different from the straight line with a slope of 1 (Exp. 1: $t = 0{\cdot}04$, D.F. = 3, $P > 0{\cdot}05$; Exp. 2: $t = 0{\cdot}02$, D.F. = 7, $P > 0{\cdot}05$).

DISCUSSION

The results showed that, in experimental bisexual infections, female cercarial infectivity is similar to that of males, irrespective of cercarial sex ratio. Furthermore, cumulative male and female cercarial infectivity is maximum when the sex ratio is equilibrated.

Similar infectivities between both sexes in experimental bisexual infections show that simultaneous presence of both sexes do not give the same results as in unisexual infections. In experimental unisexual infections, our results showed that cercarial male infectivity, and thus life-history performance, was higher than that of the female. This result agrees with that of Boissier *et al.* (1999) using a statistical method (meta-analysis) on the pooled experimental results from the literature. In our bisexual infections, no male-bias emerged from the adult sex ratios, which remained similar to the cercarial sex ratios.

The unbiased sex ratios obtained in our experimental bisexual infections could be explained by a male–female interaction. The presence of the male

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may have stimulated the female infectivity, making it comparable to that of males. At the same time, the presence of female cercariae apparently does not stimulate male cercarial infectivity.

In *S. mansoni*, many experimental biochemical and behavioural studies have been published concerning female stimulation mediated by males. For example, the presence of the male is necessary for the acquisition and the maintenance of female sexual maturity (Popiel, 1986), the synthesis of female DNA (Den Hollander & Erasmus, 1985), or molecules which are known in other organisms to be involved in mitogenic processes (Schussler, Grevelding & Kunz, 1997). Female attraction towards the male is caused, in the absence of worm-tactile behaviour, by males emitting pheromones that attract females (Imperia & Fried, 1980; Eveland, Fried & Cohen, 1982; Haseeb & Eveland, 1991).

Maximum cercarial infectivity occurring for an equilibrated sex ratio could be a direct consequence of behavioural and/or biochemical interactions between the sexes. On the one hand, 1 male can only protect 1 female in its gynecophoral canal. On the other hand, *in vitro*, 1 female, but not 2, attracts 1 male and, 1 male, but not 2, attracts 1 female (Eveland, Fried & Cohen, 1983). These last results can be interpreted from the 'window' and/or 'shut down' mechanisms described by Kemp & Devine (1982) in digeneans where the behavioural responses are density dependent and thus pheromonal concentration dependent. From these mechanical protection and/or pheromonal control mechanisms, a maximum infrapopulation developmental success will be achieved for an equilibrated sex ratio. In natural populations of *S. mansoni* in *Rattus rattus*, Morand *et al.* (1993) observed such a tendency in sex ratio equilibrium when infection intensity increases.

Both the stimulation of the female by the male as well as a maximal infectivity when sex ratio is equilibrated maximize the number of pairings and thus the number of eggs produced and hence favour schistosome transmission.

Thus, what could be the explanation for the biased-sex ratio towards males in natural bisexual infections (Imbert-Establet, 1982; Théron *et al.* 1992; Barral *et al.* 1996) compared to our unbiased experimental sex ratio? Three explanations can be proposed for the natural bias. The first possibility could be that the bias exists before cercarial penetration. Since males and females have similar infectivities in our bisexual experimental infections, the bias could take place at any step from egg-hatching to cercarial penetration. Boissier *et al.* (1999) showed that most of those performances were similar between the sexes and that, when different, they were higher in females than in males. Some other characteristics have yet to be studied, such as the host-finding efficiency of male and female cercariae, and this may explain the bias. The second possibility could be that the bias comes from the vertebrate host and is due to repeated exposures. In natural conditions, the infection of the definitive host by both sexes may be accomplished either by simultaneous or by trickle repeated exposures. Our experiments used simultaneous infections by male and female cercariae. In natural conditions, such simultaneous mixed sex exposures are highly likely. Molluscs harbouring mixed sex infections are more numerous than expected (Woolhouse, Chandiwana & Bradley, 1990), and most infected snails are observed to be spatially aggregated (Sire *et al.* 1999). However, some other authors suppose that the usual pattern of exposure is by trickle repeated infections (Sturrock, Cottrell & Kimani, 1984; Mitchell *et al.* 1990). Would our results be the same if infections by male and female cercariae were not performed simultaneously? Such experiments would be possible by the fact that mice infected with cercariae of only one sex are not resistant to reinfection (Roberts, Boot & Wilson, 1988), resistance being correlated with the presence of eggs resulting from a bisexual infection (Harrison, Bickle & Doenhoff, 1982). Finally, the third possibility could be that the bias is due to a competition between genetically different adults. A genetic diversity among the individuals of the same sex would create competition for access to the opposite sex. This competition would be higher between females and would induce the male-biased sex ratio. This hypothesis is supported by both natural and experimental results. A high genetic diversity among adults has been found in naturally-infected *Rattus rattus* (Barral *et al.* 1996). In our experimental protocol, we have eliminated such a genetic diversity; mice were infected by genetically identical male cercariae and genetically identical female cercariae. The adults coming from these cercariae had to choose between sexual partners that were all genetically identical. By eliminating the genetic diversity, we would have suppressed the competition and thus the bias. Finally, the capacity of schistosomes to choose a partner has been shown in heterospecific mating experiments (Tchuem Tchuenté *et al.* 1995) and may support the existence of a competition for the access to the other sex.

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REFERENCES

BARRAL, V., MORAND, S., POINTIER, J. P. & THERON, A. (1996). Distribution of schistosome genetic diversity within naturally infected *Rattus rattus* detected by RAPD markers. *Parasitology* **113**, 511–517.

BOISSIER, J., MORAND, S. & MONÉ, H. (1999). A review of performance and pathogenicity of male and female *Schistosoma mansoni* during the life cycle. *Parasitology* **119**, 447–454.

CHEEVER, A. W., LEWIS, F. A. & WYNN, T. A. (1997). *Schistosoma mansoni*: unisexual infections sensitize mice for granula formation around intravenously injected eggs. *Parasitology Research* **83**, 57–59.

DEN HOLLANDER, J. E. & ERASMUS, D. A. (1985). *Schistosoma mansoni*: male stimulation and DNA synthesis by the female. *Parasitology* **91**, 449–457.

DUWALL, R. H. & DEWITT, W. B. (1967). An improved perfusion technique for recovering adult schistosomes from laboratory animals. *American Journal of Tropical Medicine and Hygiene* **16**, 483–486.

EVANS, A. S. & STIREWALT, M. A. (1951). Variations in infectivity of cercariae of *Schistosoma mansoni*. *Experimental Parasitology* **1**, 19–33.

EVELAND, L. K., FRIED, B. & COHEN, L. M. (1982). *Schistosoma mansoni*: adult worm chemoattraction with and without barriers. *Experimental Parasitology* **54**, 271–276.

EVELAND, L. K., FRIED, B. & COHEN, L. M. (1983). *Schistosoma mansoni*: adult worm chemoattraction with barriers of specific molecular weight exclusions. *Experimental Parasitology* **56**, 255–258.

HARRISON, R. A., BICKLE, Q. & DOENHOFF, M. J. (1982). Factors affecting the acquisition of resistance against *Schistosoma mansoni* in the mouse. Evidence that the mechanisms which mediate resistance during early patent infections may lack immunological specificity. *Parasitology* **84**, 93–110.

HASEEB, M. A. & EVELAND, L. K. (1991). *Schistosoma mansoni*: a chemoattractive factor released by males and its receptor in females. *Experientia* **47**, 970–974.

IMBERT-ESTABLET, D. (1982). Infestation naturelle des rats sauvages par *Schistosoma mansoni* en Guadeloupe: Données quantitatives sur le développement et la fertilité du parasite. *Annales de Parasitologie Humaine et Comparée* **6**, 573–585.

IMPERIA, P. S. & FRIED, B. (1980). Pheromonal attraction of *Schistosoma mansoni* females toward males in the absence of worm-tactile behavior. *Journal of Parasitology* **66**, 682–684.

KEMP, W. M. & DEVINE, D. D. (1982). Behavioral cues in trematodes life cycles. In *Cues that Influence Behavior of Internal Parasites* (ed. Bailey, W. S.), pp. 67–84. Agricultural Research Service, US Department of Agriculture: Washington, DC, USA.

LIBERATOS, J. D. (1987). *Schistosoma mansoni*: Male-biased sex ratios in snails and mice. *Experimental Parasitology* **64**, 165–177.

MITCHELL, G. F., GARCIA, E. G., WOOD, S. M., DIASANTA, R., ALMONTE, R., CALICA, E., DAVERN, K. M. & TIU, W. U. (1990). Studies on the sex ratio of worms in schistosome infections. *Parasitology* **101**, 27–34.

MORAND, S., POINTIER, J. P., BOREL, G. & THERON, A. (1993). Pairing probability of schistosomes related to their distribution among the host population. *Ecology* **74**, 2444–2449.

POPIEL, I. (1986). Male-stimulated female maturation in *Schistosoma*: a review. *Journal of Chemical Ecology* **12**, 1745–1754.

ROBERTS, S. M., BOOT, C. & WILSON, R. A. (1988). Antibody responses of rodents to a tegument membrane preparation from adult *Schistosoma mansoni*. *Parasitology* **97**, 425–435.

ROWNTREE, S. & JAMES, C. (1977). Single sex cercariae of *Schistosoma mansoni*, a comparison of male and female infectivity. *Journal of Helminthology* **51**, 69–70.

SCHUSSLER, P., GREVELDING, C. G. & KUNZ, W. (1997). Identification of Ras, MAP kinases, and a GAP protein in *Schistosoma mansoni* by immunoblotting and their putative involvement in male–female interaction. *Parasitology* **115**, 629–634.

SIRE, C., DURAND, P., POINTIER, J. P. & THERON, A. (1999). Genetic diversity and recruitment pattern of *Schistosoma mansoni* in a *Biomphalaria glabrata* snail population: a field study using random-amplified polymorphic DNA markers. *Journal of Parasitology* **83**, 436–441.

STIREWALT, M. A. & FREGEAU, W. A. (1968). Effect of selected experimental conditions on penetration and maturation of cercariae of *Schistosoma mansoni* in mice. *Experimental Parasitology* **22**, 73–95.

STURROCK, R. F., COTTRELL, B. J. & KIMANI, R. (1984). Observations on the ability of repeated, light exposures to *Schistosoma mansoni* cercariae to induce resistance to reinfection in Kenyan baboons (*Papio anubis*). *Parasitology* **88**, 505–514.

TAYLOR, M. G. & ANDREWS, B. J. (1973). Comparison of the infectivity and pathogenicity of six species of African schistosomes and their hybrids. 1. Mice and hamsters. *Journal of Helminthology* **4**, 439–453.

TCHUEM TCHUENTE, L. A., SOUTHGATE, V. R., IMBERT-ESTABLET, D. & JOURDANE, J. (1995). Change of mate and mating competition between males of *Schistosoma intercalatum* and *S. mansoni*. *Parasitology* **110**, 45–52.

THERON, A., POINTIER, J. P., MORAND, S., IMBERT-ESTABLET, D. & BOREL, G. (1992). Long-term dynamics of natural populations of *Schistosoma mansoni* among *Rattus rattus* in patchy environment. *Parasitology* **104**, 291–298.

WOOLHOUSE, M. E., CHANDIWANA, S. K. & BRADLEY, M. (1990). On the distribution of schistosome infections among host snails. *International Journal for Parasitology* **20**, 325–327.

ZAR, J. H. (1996). *Biostatistical Analysis*. 3rd Edn. Prentice-Hall International, London.