Molecular Ecology (2002) **11**, 1231–1238

# Sex-specific genetic structure in *Schistosoma mansoni*: evolutionary and epidemiological implications

F. PRUGNOLLE,* T. DE MEEÛS,* P. DURAND,* C. SIRE† and A. THÉRON†
*Centre d'Etude du Polymorphisme des Micro-organismes, UMR 9926 CNRS-IRD, Institut de Recherche et Développement, 911 avenue Agropolis, BP 5045, 34032 Montpellier, France, †UMR 5555 CNRS-UP, CBETM, Université de Perpignan, 52 Avenue de Villeneuve, 66860 Perpignan, France*

**Abstract**

**We studied the population genetic structure of 360 and 1247 adult *Schistosoma mansoni* using seven microsatellite and seven random amplified polymorphic DNA (RAPD) markers, respectively. Parasites were collected from their natural definitive host *Rattus rattus* in Guadeloupe (West Indies). We found a sex-specific genetic structure, a pattern never before reported in a parasitic organism. Male genotypes were more randomly distributed among rats than female genotypes. This interpretation was consistent with a lower differentiation between hosts for males relative to females, the higher genetic similarity between females in the same host and the observed local (i.e. within-individual-host) differences in allele frequencies between the two sexes. We discuss our results using ecological and immunological perspectives on host–parasite relationships. These results change our view on the epidemiology of schistosomiasis, a serious disease affecting humans in African and American intertropical zones.**

*Keywords*: host–parasite interactions, microsatellites, RAPD, *Rattus rattus*, *Schistosoma mansoni*, sex-specific genetic structure

*Received 2 January 2002; revision received 12 March 2002; accepted 12 March 2002*

## Introduction

The study of population genetic structure of living organisms is central to the understanding of micro-evolutionary processes (Nevo 1978; Hudson *et al*. 1992; Ross & Keller 1995). For small animals, and in particular parasites, the analysis of genetic variation is the only method to investigate population parameters such as gene flow, size of reproductive units and breeding strategies (Nadler 1995). Molecular tools in particular allow sex-biased dispersal patterns to be investigated (e.g. Favre *et al*. 1997; Prugnolle & de Meeûs 2002).

Sex-biased dispersal is a pattern where the dispersal rate of one sex is higher than the dispersal rate of the other (Greenwood 1980). When sex-biased dispersal occurs in fragmented populations, one may expect genotypes of the more dispersing sex to be more randomly distributed among populations than genotypes of the other sex (Prout 1981; Favre *et al*. 1997). This phenomenon therefore generates a sex-specific genetic structure that may be detected from genetic markers (Favre *et al*. 1997; Mossman & Waser 1999). Most studies of sex-biased dispersal have concerned mammals (Favre *et al*. 1997; Seielstad *et al*. 1998; Mossman & Waser 1999) and birds (Clarke *et al*. 1997; Piertney *et al*. 1998; Gibbs *et al*. 2000) but little is known about sex-bias in other taxa. Notably, this pattern has never been investigated in gonochoric parasite species.

*Schistosoma mansoni* (trematode platyhelminth) is a gonochoric blood fluke parasitizing mainly humans. In this species, males and females noticeably differ in several aspects of their biology (see Boissier *et al*. 1999 for a review). The most discussed differences in life history traits concern the adult stage in the definitive vertebrate host where a male-biased sex ratio is observed (Morand *et al*. 1993). However, some differences have also been observed between sexes in the larval stages of the parasite and in the pathogenicity for the intermediate and the definitive hosts. For example, Boissier *et al*. (1999) showed that male cercariae were more infective than females. Conversely,

Correspondence: Franck Prugnolle. Fax: 33(0)4 67 41 62 99; E-mail: prugnoll@mpl.ird.fr

the production of female cercariae was greater, as was their lifespan. Such discrepancies between sexes may induce differences in the transmission patterns of males and females and thus influence the population genetic structure of each sex within and between definitive host individuals.

In the insular focus of Guadeloupe (French West Indies), *S. mansoni* has undergone a host shift from humans toward the murine host *Rattus rattus*, which is now its principal (if not only) host on this island (Théron & Pointier 1995). This situation provides an opportunity to collect adult parasites and to study accurately the adult population genetic structure of this parasite.

In this study, we examined the male and female population genetic structure of *S. mansoni* in the black rat (*R. rattus*) at a local scale in Guadeloupe using two kinds of genetic markers [microsatellites and random amplified polymorphic DNA; (RAPD)]. The descriptors we used ($F_{IS}$, $F_{ST}$, relatedness, and assignment indices) converged to the same conclusion that *S. mansoni* has a sex-specific genetic structure, a pattern never seen previously for a parasitic organism. We discuss our results using ecological and immunological perspectives on host–parasite relationships. The evolutionary and epidemiological implications of this sex-specific population structure are also considered.

## Materials and methods

### *Studied species*

*S. mansoni* is a gonochoric trematode. Adult parasitic worms (males and females) typically reside in the mesenteric venules of the vertebrate host. They sexually reproduce and the eggs break out of the venules into the intestinal lumen, to be discharged into the faeces of the host. Faecal contamination of water is followed by the hatching of these eggs into miracidia. The miracidia then infect the intermediate host (a gastropod mollusc) present in the water and transform into sporocysts. The sporocysts undergo several rounds of asexual reproduction that lead to the production of thousands of cercariae, a second free-living motile larval stage, infective for the definitive vertebrate host (Combes 2001).

### *Transmission site*

The study was performed at Dans Fond, one of numerous transmission sites located along the marshy forest focus of Grande Terre island, Guadeloupe (Fig. 1a–c). The study area is approximately 300 m long and 60 m wide and is subjected to seasonal flooding (from August to December) and drying (from April to July).

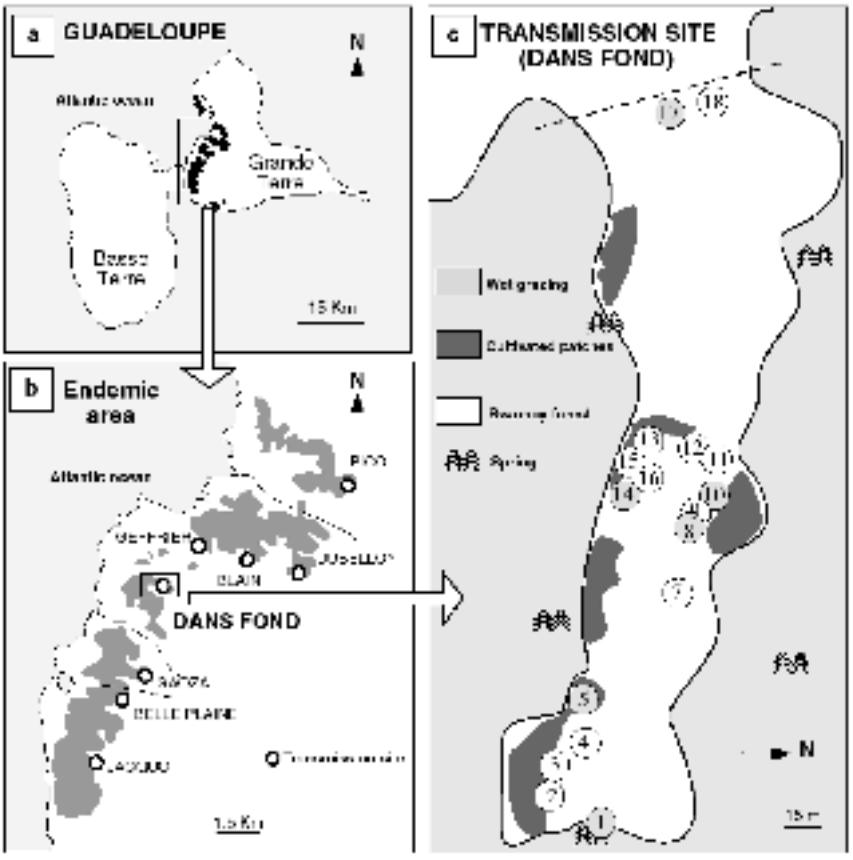


**Fig. 1** The murine focus of *S. mansoni* in Guadeloupe: (a) location of the marshy forest of Grande-Terre; (b) transmission sites within the endemic area and the studied locality of Dans Fond; (c) ecological feature of the transmission site of Dans Fond with the spatial distribution of the 18 trapped rats (modified from Sire *et al.* 2001). Rats whose numbers are marked in grey are those involved in the microsatellite study.

### *Sampling*

Eighteen rats (numbered from 1 to 18) were captured during the dry season in June 1997 using 162 traps baited with coconut and deployed during five consecutive nights at the transmission site. Adult schistosomes were recovered from each rat using a standard perfusion technique (Duvall & Dewitt 1967). They were carefully washed in physiological saline solution and stored in 70% ethanol after isolation of male and female paired worms. Rat 6 did not contain schistosomes and rat 9 contained only males.

### *Genotyping*

DNA extraction and protocol for DNA amplification are presented in Durand *et al*. (2000) for microsatellites and Barral *et al*. (1996) for RAPD.

For microsatellites, of the 18 trapped rats, three males (rat 1, 5 and 14) and three females (rats 8, 10 and 17) were randomly chosen and 60 worms/host (30 males and 30 females) were sampled. Worms were genotyped for seven microsatellite loci (GenBank accession numbers: AF202965, AF202966, AF202967, AF202968, R95529, L46951 and M85305) (Durand *et al*. 2000). Electrophoretic gels were read by two different researchers (yielding identical results). Individuals that raised reading problems were genotyped at least twice.

RAPD data were obtained from five primers (OP-A9, 5′-GGGTAACGCC-3′; OP-A10, 5′-GTGATCGCAG-3′; OP-A13, 5′-CAGCACCCAC-3′; OP-G13, 5′-CTCTCCGCCA-3′; OP-B6, TGCTCTGCCC-3′; corresponding to seven independent characters) for a total of 731 males and 516 females from 17 rats (nine males and eight females). For each primer used, a tube containing all the components except for the template DNA was included as a control to detect potential contamination. Controls were always negative. Controls with host DNA gave a completely different pattern and RAPD marker differences detected between individual worms were not correlated to a potential host DNA contamination. The major criteria for taking a fragment into account were reproducibility and sharpness of the fragments. We scored each variable band in each individual, assuming that it represented an allele at a unique biallelic locus.

### *Statistical analyses for microsatellite data*

Genetic variability was measured by Nei's (1987) unbiased mean heterozygosity (*Hs*) using FSTAT version 2.9 (Goudet 1995).

Population genetic structure was investigated using Wright's *F*-statistics (Wright 1965). $F_{IS}$ measures the within-sample magnitude of departures from Hardy–Weinberg equilibrium expectations while $F_{ST}$ measures the genetic differentiation between different samples. These parameters were estimated by Weir & Cockerham's (1984) unbiased estimators $f$ (for $F_{IS}$) and $\theta$ (for $F_{ST}$) and computed with FSTAT version 2.9.

An assignment index (Paetkau *et al*. 1995) was computed for each individual. The assignment index (*AI*) corresponds to the expected frequency of a genotype across all loci in the population in which it was found. Since we were not interested in population effects (which may arise from differences in genetic diversity), *AI* values were corrected (*AIc*) by subtracting population means after log-transformation (to avoid rounding errors with very small numbers) (Favre *et al*. 1997). It follows that *AIc* values averaged zero for each population, and that negative values characterized individuals with a lower probability than average. *AIc* were computed using the program BIASDISP, which is freely downloadable from http://www.unil.ch/izea/softwares/biasdisp.html.

A measure of Hamilton's (1971) relatedness (*R*) was computed using Queller & Goodnight's (1989) estimator (FSTAT version 2.9). This measure corresponds to the average relatedness of individuals within samples when compared to the whole.

The significance of departure of $F_{IS}$ from 0 was tested by randomizing alleles between individuals in each sample (15 000 permutations). The probability of obtaining a value as extreme as or more extreme than the observed $F_{IS}$ was then computed. The significance of genetic differentiation was tested with the allelic *G*-based test (Goudet *et al*. 1996) after 15 000 permutations of genotypes between samples. These randomization tests were performed using FSTAT version 2.9.

To test whether $F_{IS}$, $F_{ST}$ and mean *AIc* differed significantly between the two sexes, we used a randomization approach using the program BIASDISP. If males and females present the same genetic structure, the statistics used do not depend on the variable 'sex'. Let $X_m$ and $X_f$ be the statistic of interest for males and females, respectively. The statistic for each sex over all populations and the absolute difference $\Delta X_{obs} = |X_m - X_f|$ are computed. Sex is then randomly assigned 10 000 times to each individual (keeping the genotypes in their original sample and the sex ratio in each sample constant). The probability of the data under the null hypothesis that genetic structure is independent of sex (*P-value*) is then computed by the proportion of randomly obtained values of $\Delta X$ which are equal to or more extreme than the observed value (see Goudet *et al*. (2002) and Prugnolle & de Meeûs (2002) for a discussion on the power of the different tests based on these different statistics).

Male and female average relatedness coefficients were compared using their 95% confidence intervals obtained by a randomization procedure (bootstrapping over loci) with FSTAT version 2.9. If confidence intervals do not overlap between the two sexes, one can conclude that a significant difference exists.

**Table 1** Comparison between *S. mansoni* male and female genetic estimators ($f$, θ, *AIc* and *R*) computed from microsatellites and RAPD

| | Microsatellites | | | RAPD | | |
|---|---|---|---|---|---|---|
| | Females | Males | *P*-value | Females | Males | *P*-value |
| *N* | 180 | 180 | | 516 | 731 | |
| $F_{IS}$ ($f$) | −0.103*** | −0.045* | 0.061 | | | |
| $F_{ST}$ (θ) | 0.070*** | 0.035*** | 0.002 | 0.143*** | 0.072*** | 0.0001 |
| *AIc* | 0.271 | −0.271 | 0.052 | | | |
| *R* | 0.143 | 0.071 | | | | |
| CI | [0.107; 0.184] | [0.044; 0.093] | | | | |

For RAPD, only $F_{ST}$ estimators (θ) were compared. For $F_{IS}$ ($f$) and $F_{ST}$ (θ), the significance of departure from zero is given by $*P < 0.05$, $***P < 0.001$. Male and female average relatedness coefficients (*R*) were compared using their 95% confidence intervals (CI; obtained by bootstrapping over loci).
*N*, total number of sampled individuals.
*P*-value, probability of equal values between females and males.

In the case of multiple testing we used an exact binomial test to assess when the proportion of significant *P*-values was significantly higher than expected ($\alpha = 0.05$) under the null hypothesis (unilateral test performed by S-PLUS 2000, Professional release 1, MathSoft, Inc.).

### *Statistical analyses for RAPD data*

RAPD patterns were scored at each 'locus' using (1) for presence or (0) for absence of a band. With dominant markers, such as RAPDs, heterozygous individuals cannot be distinguished from homozygous ones (Hadrys *et al*. 1992), limiting the use of the classical indices of population genetics (*Hs*, $F_{IS}$). Therefore, we have only computed θ (Weir & Cockerham 1984) from the RAPD data (after their diploidization) by using FSTAT version 2.9 (see the FSTAT version 2.9 Helpfile, updated from Goudet 1995). Comparison between male and female $F_{ST}$ has been realized as detailed for microsatellite data.

## Results

### *General microsatellite polymorphism*

The unbiased mean heterozygosity was moderate for microsatellite markers and equal to 0.531. The number of alleles per locus varied between two and 11 alleles (mean 4.6). In schistosomes, the female is the heterogametic sex and the male is the homogametic one. The patterns displayed by our seven microsatellite loci appear incompatible with a linkage to a sexual chromosome of any of them. Overall differentiation between parasite infrapopulations (between hosts) is highly significant ($\theta = 0.039$; $P < 10^{-4}$). Thus, different hosts harbour genetically differentiated schistosome infrapopulations.

### *Sex-specific genetic structure*

Even if some of the parameter estimates showed only marginally significant deviations from the null expectation of no difference between sexes ($F_{IS}$, *AIc*), all the measures used (for both microsatellites and RAPD) agreed with the general conclusion that male genotypes were more randomly distributed than female genotypes among hosts. Some of these parameters gave highly significant values (e.g. for microsatellites $F_{ST}$, $P = 0.002$). This interpretation was consistent with the lower differentiation between hosts displayed by males relative to females (Table 1). The higher genetic similarity between females (Table 1) of the same host and the observed local (i.e. within-individual-host) differences in allele frequencies between the two sexes were consistent with this conclusion (Table 2). Neither *S. mansoni* males' $F_{ST}$ nor *S. mansoni* females' $F_{ST}$ were affected by host sex (Fig. 2a,b). However, even if the signal stays in the same direction, host sex seems to affect the intensity and the variance of the response (Fig. 2a,b).

There was a significant heterozygote excess and a significant between-host differentiation for both female and male schistosomes (Table 1).

## Discussion

A sex-specific genetic structure of the gonochoric parasite *S. mansoni* was demonstrated at a local scale in Guadeloupe. Male genotypes were more randomly distributed among hosts than female genotypes. To our knowledge, the present study is the first to show a sex-related population structure in a natural population of parasites. Moreover, some results could suggest an interaction of this pattern with host sex. Unfortunately, our sampling does not allow further discussion of this interesting issue. Below we discuss in detail several of the possible reasons that

**Table 2** Genetic differentiation between *S. mansoni* females and males within each infrapopulation with (θ +) and without (θ –) repeated genotypes

| | Microsatellites | | |
|---|---|---|---|
| Rat no. | θ + | θ – | RAPD θ |
| 1 | 0.043*** | 0.019 | −0.001 |
| 2 | — | | 0.007 |
| 3 | — | | 0.436*** |
| 4 | — | | 0.0452 |
| 5 | 0.003 | −0.001 | 0.014* |
| 7 | — | | 0.097*** |
| 8 | 0.023* | 0.023* | 0.025** |
| 10 | 0.011 | 0.007 | 0.047*** |
| 11 | — | | 0 |
| 12 | — | | 0.139*** |
| 13 | — | | 0.234*** |
| 14 | 0.017* | −0.003 | 0.143*** |
| 15 | — | | 0.590*** |
| 16 | — | | 0.065*** |
| 17 | 0.055*** | 0.037*** | 0.242*** |
| 18 | — | | 0.042 |

The binomial probability of randomly observing a more extreme number of *P*-values inferior or equal to 0.05 is $P < 10^{-4}$ for microsatellites and RAPD with repeated genotypes and equal to 0.033 for microsatellites without repeated genotypes. RAPD were not polymorphic enough to be studied without repeated genotypes. No results are shown for Rat 9 because it contained only male schistosomes. Levels of significance of $F_{ST}$ (θ) are given by $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

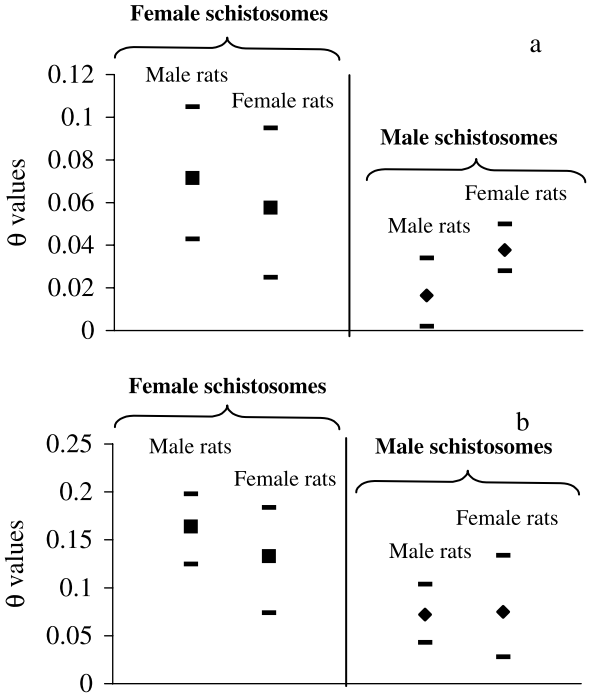


**Fig. 2** Comparison between mean differentiation values (θ) (±95% confidence interval) observed for male and female *S. mansoni* in male and female rats. (a) Using microsatellites (three male and three female rats); (b) using RAPD (nine male and eight female rats).

could explain the sex-specific genetic structure observed globally.

Sex-specific genetic structure is not a stable state since it requires that the factors leading to it occurs at each generation (see Goudet *et al*. (2002) and Prugnolle & de Meeûs (2002) for a more extensive discussion on this point).

Sex-specific genetic structure may reflect differences in the patterns of dispersal between sexes (Favre *et al*. 1997; Mossman & Waser 1999; Prugnolle & de Meeûs 2002). In our case, the results obtained suggest that among-host dispersal of male *S. mansoni* is higher than female dispersal. Mating behaviour studies of this parasite have demonstrated that males are very active in the mating process (Amstrong 1965). Moreover, the sex ratio is biased towards males in definitive hosts (Morand *et al*. 1993). Therefore, *S. mansoni* males are in competition for access to females (Morand & Muller-Graff 2000). According to the hypothesis of Dobson (1982), males should disperse more than females to avoid local mate competition among kin. This pattern of dispersal also limits the risk of breeding with close kin individuals of the opposite sex (Perrin & Mazalov 2000). Therefore, the avoidance of locale mate competition and of inbreeding could have played a role in the evolution of a sex-specific dispersal pattern.

This dispersal bias must involve parasites at their larval stage before entering the definitive host. However, it is difficult to suppose that significant differences in dispersal of free-living larval stages could generate this pattern. Indeed, miracidial and cercarial lifespan (only some hours, Théron, personal communication) or motility seem too low (Combes 2001) to induce such marked differences between sexes. On the other hand, in Guadeloupe, the infection of the snail host is generally monomiracidial (Sire *et al*. 1999). A higher mobility and/or lifespan of molluscs infected by male miracidia would be a more likely explanation of this sex-biased dispersal. Comparing the lifespan and/or the mobility of molluscs when infected by a male or a female would test this hypothesis.

For *S. mansoni*, the life cycle includes an asexual multiplicative phase within the intermediate snail host which leads to the production of genetically identical infective cercariae. Very few studies have analysed the impact of clonal amplification on the genetic structure of parasites with a complex life cycle. However, Mulvey *et al*. (1991) suggested that $F_{ST}$ values could be increased between *Fascioloides magna* infrapopulations due to the replication of certain genotypes within each infrapopulation. In our case, it is clear that deleting repeated genotypes (determined by microsatellites + RAPD) has a substantial effect on $F_{IS}$ values corresponding to a significant role for clonally

**Table 3** Comparison between male and female schistosomes of genetic estimator values ($f$, θ, *AIc*, *R*) computed from microsatellite dataset without repeated genotypes

| | Microsatellites | | |
|---|---|---|---|
| | Females | Males | *P*-value |
| *N* | 143 | 162 | |
| $F_{IS}$ ($f$) | −0.017 | −0.048* | 0.305 |
| $F_{ST}$ (θ) | 0.045*** | 0.024*** | 0.031 |
| *AIc* | 0.018 | −0.016 | 0.905 |
| *R* | 0.087 | 0.049 | |
| CI | [0.062; 0.119] | [0.023; 0.070] | |

For $F_{IS}$ ($f$) and $F_{ST}$ (θ), the significance of departure from zero is given; *$P < 0.05$, ***$P < 0.001$. Male and female average relatedness coefficients (*R*) were compared using their 95% confidence intervals (CI; obtained by a bootstrapping over loci). RAPD were not sufficiently polymorphic to be studied here. Abbreviations are as in Tables 1 and 2.

identical individuals in shaping the within-infrapopulation genetic variability. However, $F_{ST}$ [and relatedness (*R*)] still converge to the conclusion of a sex-specific genetic structure (Table 3). Furthermore, the differentiation between males and females within hosts remains significant (Table 2). Therefore, clonality cannot be responsible for the observed pattern of sex-specific genetic structure.

Host immune selection against particular parasite genotypes could also result in the clustering of certain genotypes within hosts (Anderson *et al*. 1998). A complex interaction between the rat immunological system and the genetic variability of male and female schistosomes could explain the differences. Using evolutionary simulation models, Brown & Grenfell (2001) explore the hypothesis that established schistosome adults may adaptively manipulate their host's immune system to enhance the exclusion of larval competitors, resulting in concomitant immunity. Here we add that concomitant immunity may operate in a genotype-specific manner, and so contribute to the observed patterns of genetic distribution. In this respect, a higher concomitant immunity against males in the black rat could select for more genetic heterogeneity within males than within females. This hypothesis however, supposes that the antigenic variability differs between genders and correlates with the global genetic variation (i.e. microsatellites and RAPD).

The last hypothesis is related to a potential kin-selection process. Many theoretical models rely on a positive correlation between virulence and transmissibility (e.g. Frank 1994). They assume that high parasite replication rates are associated with a high probability of transmission (and, hence, increased parasite fitness), but also with high levels of damage to the host (high virulence). Therefore, parasites face a trade-off between damaging their hosts and the benefits of rapid growth and transmission. Furthermore, Bremermann & Pickering (1983) and Knolle (1989) demonstrated theoretically how competition among parasites within a host may favour the evolution of increased virulence. Conversely, Frank (1994) has demonstrated that increased relatedness within hosts tends to decrease competition and virulence and thus to increase the success of the local group of parasites by kin selection.

In *S. mansoni*, females are responsible for virulence which is directly associated with egg production (e.g. Davies *et al*. 2001). Indeed, some of the eggs are not released into the intestinal lumen but remain in the intestinal mucosa or even within the blood circulation. These eggs may become fixed in different organs, in particular the liver, where their accumulation may cause a marked pathology. In schistosomes, high virulence in the definitive host is associated with high egg production (Davies *et al*. 2001). Therefore, in order to reduce competition and virulence we may expect settled females to disfavour infection by unrelated females. This situation would effectively produce a sex-specific pattern of genetic distribution since only related females would be recruited by the definitive host.

Controlled infestations of experimental definitive hosts with more or less genetically related male and female *S. mansoni* should provide arguments for or against the immunological and the kin selection hypotheses.

Host–parasite interactions are often presented in the framework of the 'Red Queen hypothesis' and some authors have suggested that sex and polymorphism (Bremermann 1980; Mopper *et al*. 1991; Lively & Dybdahl 2000) are fundamental for both hosts and parasites to stay in the 'race'. Genetic differentiation between males and females necessarily leads to increased heterozygosity compared to Hardy–Weinberg expectation, in particular in offspring (Prout 1981; Aars & Ims 2000). This may thus provide a parasite with an advantage in the arms race against its hosts and particularly against the mollusc. The negative and significant $F_{IS}$ observed in male and female schistosomes (Table 1), even after removal of multilocus repeated genotypes (Table 3), are consistent with this argument.

This study necessarily modifies the perception we have of the epidemiology of this parasite that severely affects humans in tropical Africa and the neotropical zone (Combes 2001). It highlights the need to consider each sex separately in future studies of this parasite. This sex-specific genetic distribution could indeed determine differences in the parasite interactions with the host's immunological system as it may reflect antigenic differences between sexes. Moreover, this unexpected pattern could be encountered in other gonochoric invertebrates and in particular parasites, and will have to be checked in further studies on such organisms.

## Acknowledgements

We thank F. Renaud, S. Brown, F. Balloux, J. Goudet, Y. Michalakis, J. Boissier, M. Tibayrenc, N. Perrin, P. Jarne, J.P. Pointier and P. David for very useful discussions and suggestions. We also thank three anonymous referees. This work received financial support from the CNRS (PNDBE) and the MENRT (PRFMMIP *n*°95).

## References

Aars J, Ims RA (2000) Population dynamics and genetic consequences of spatial density-dependent dispersal in patchy populations. *American Naturalist*, **155**, 252–265.

Amstrong JC (1965) Mating behaviour and development of schistosomes in the mouse. *Journal of Parasitology*, **51**, 605–616.

Anderson TJC, Blouin MS, Beech R (1998) Population biology of parasitic nematodes: applications of genetic markers. *Advances in Parasitology*, **41**, 219–283.

Barral V, Morand S, Pointier JP, 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.

Bremermann HJ (1980) Sex and polymorphism as strategies in host–pathogen interactions. *Journal of Theoretical Biology*, **87**, 671–702.

Bremermann HJ, Pickering J (1983) A game theoretical model of parasite virulence. *Journal of Theoretical Biology*, **100**, 411–426.

Brown SP, Grenfell BT (2001) An unlikely partnership: parasites, concomitant immunity and host defence. *Proceedings of the Royal Society of London B*, **268**, 2543–2549.

Clarke AL, Saether BE, Roskaft E (1997) Sex biases in avian dispersal: a reappraisal. *Oikos*, **79**, 429–438.

Combes C (2001) *Parasitism. The Ecology and Evolution of Intimate Interactions*. Chicago Press, Chicago.

Davies CM, Webster JP, Woolhouse ME (2001) Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite. *Proceedings of the Royal Society of London B*, **268**, 251–257.

Dobson FS (1982) Competition for mates and predominant juvenile male dispersal in mammals. *Animal Behaviour*, **30**, 1183–1192.

Durand P, Sire C, Théron A (2000) Isolation of microsatellite markers in the digenetic trematode *Schistosoma mansoni* from Guadeloupe island. *Molecular Ecology*, **9**, 997–998.

Duvall RH, Dewitt WB (1967) An improved perfusion technique for recovering adult schistosomes from laboratory animals. *American Journal of Tropical Medicine and Hygiene*, **16**, 483–486.

Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal Crocidura russula: evidence from field data and microsatellites patterns. *Proceedings of the Royal Society of London B*, **264**, 127–132.

Frank SA (1994) Kin selection and virulence in the evolution of protocells and parasites. *Proceedings of the Royal Society of London B*, **258**, 153–161.

Gibbs HL, Dawson RJ, Hobson KA (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Molecular Ecology*, **9**, 2137–2147.

Goudet J (1995) Fstat (vers. 1.2). A computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.

Goudet J, Raymond T, De Meeûs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Goudet J, Perrin N, Waser P (2002) Test for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology*, in press.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Hadrys H, Balick M, Schierwater B (1992) Application of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology*, **1**, 55–63.

Hamilton WD (1971) Selection of selfish and altruistic behaviour in some extreme models. In: *Man and Beast: Comparative Social Behavior* (eds Eisenberg JF, Dillon WS), pp. 57–91. Smithsonian Institute Press, Washington DC.

Hudson RR, Boos DD, Kaplan NL (1992) A statistical test for detecting geographic subdivision. *Molecular Biology and Evolution*, **9**, 138–151.

Knolle H (1989) Host density and the evolution of parasite virulence. *Journal of Theoretical Biology*, **136**, 199–207.

Lively CM, Dybdahl MF (2000) Parasite adaptation to locally common host genotypes. *Nature*, **405**, 679–681.

Mopper S, Mitton JB, Witham TG, Cobb NS, Christensen KM (1991) Genetic differentiation and heterozygosity in pinyon pine associated with resistance to herbivory and environmental stress. *Evolution*, **45**, 989–999.

Morand S, Pointier JP, Borel G, Théron A (1993) Pairing probability of schistosomes related to their distribution among the host population. *Ecology*, **74**, 2444–2449.

Morand S, Muller-Graf CD (2000) Muscles or testes? Comparative evidence for sexual competition among dioecious blood parasites (Schistosomatidae) of vertebrates. *Parasitology*, **120**, 45–56.

Mossman CA, Waser PM (1999) Genetic detection of sex-biased dispersal. *Molecular Ecology*, **8**, 1063–1067.

Mulvey M, Aho JM, Lydeard C, Leberg PL, Smith MH (1991) Comparative population genetic structure of a parasite (*Fascioloides magna*) and its definitive host. *Evolution*, **45**, 1628–1640.

Nadler SA (1995) Microevolution and the genetic structure of parasite populations. *Journal of Parasitology*, **81**, 395–403.

Nei M (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nevo E (1978) Genetic variation in natural populations: patterns and theory. *Theoretical Population Biology*, **13**, 121–177.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Perrin N, Mazalov V (2000) Local competition, inbreeding, and the evolution of sex-biased dispersal. *American Naturalist*, **155**, 116–127.

Piertney SB, MacColl AD, Bacon PJ, Dallas JF (1998) Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology*, **7**, 1645–1654.

Prout T (1981) A note on the island model with sex dependent migration. *Theoretical and Applied Genetics*, **59**, 327–332.

Prugnolle F, de Meeûs T (2002) Inferring sex-biased dispersal from population genetic tools: a review. *Heredity*, **88**, 161–165.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Ross KG, Keller L (1995) Joint influence of gene flow and selection on a reproductively important genetic polymorphism in the fire ant *Solenopsis invicta*. *American Naturalist*, **146**, 325–348.

Seielstad MT, Minch E, Cavalli-Sforza LL (1998) Genetic evidence for a higher female migration rate in humans. *Nature Genetics*, **20**, 278–280.

Sire C, Durand P, Pointier JP, Théron A (1999) Genetic diversity and recruitment of *Schistosoma mansoni* in a *Biomphalaria glabrata* snail population: a field study using Random Amplified Polymorphic DNA markers. *Journal of Parasitology*, **85**, 436–441.

Sire C, Durand P, Pointier JP, Théron A (2001) Genetic diversity of *Schistosoma mansoni* within and among individual hosts (*Rattus rattus*): differentiation at microspatial scale. *International Journal for Parasitology*, **31**, 1609–1616.

Théron A, Pointier JP (1995) Ecology, dynamics, genetics and divergence of trematode populations in heterogeneous environments: the model of *Schistosoma mansoni* in the insular focus of Guadeloupe. *Research and Reviews in Parasitology*, **55**, 49–64.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution*, **19**, 395–420.

---

Franck Prugnolle is a PhD student working on the co-evolution between *Schistosoma mansoni* and its intermediate (*Biomphalaria glabrata*) and definitive host (*Rattus rattus*) in Guadeloupe. Thierry De Meeûs is a researcher in population genetics of host–parasite systems. Patrick Durand is a research technician in molecular biology. Christelle Sire is a PhD graduate and has worked on *S. mansoni* ecology. André Théron is a director of research working on the ecology and evolution of schistosomes and other parasites.

---