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# Host-parasite life-histories of the diurnal vs. nocturnal chronotypes of *Schistosoma mansoni*: adaptive significance

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## Abstract

**OBJECTIVES** To optimise host-to-host transmission, digenean trematodes (parasites) synchronise their cercarial emission patterns with the aquatic activities of their vertebrate hosts. *Schistosoma mansoni* has two different circadian chronotypes: a diurnal shedding pattern with a mean peak occurring at 11:00 h, and a nocturnal shedding pattern with a mean peak occurring at 20:00 h. We analysed the life-history variations between these two chronotypes at the levels of the parasite and its hosts.

**METHODS** For each chronotype, we quantified three life-history traits related to the parasite (prepatent period, infection rate and cercarial production) and analysed the morphometry and the morphology of the parasite eggs; we also quantified three life-history traits related to the snail intermediate host (shell diameter, fecundity and survival rate) and one life-history trait related to the experimental definitive host (survival rate). A phylogeny based on the mitochondrial cytochrome-oxidase gene was made on samples of both chronotypes.

**RESULTS** Life-history analysis revealed significant variations between the two chronotypes. Life-history traits were optimal for both the parasite and the snail host for the diurnal chronotype compared to the nocturnal one. The new chronotype behaved like an allopatric population towards its snail host. Phylogenetic analysis supports the hypothesis of a lateral transfer of *S. mansoni* from humans to *Rattus rattus*. These results were interpreted in terms of an ongoing sympatric speciation.

**CONCLUSION** The nocturnal chronotype of *S. mansoni* showed non-adapted life-history traits in its relation with the snail intermediate host *Biomphalaria pfeifferi*. The emergence of this new phenotype is probably linked to divergent natural selection.

**keywords** *Schistosoma mansoni*, nocturnal chronotype, cercarial emergence pattern, circadian rhythm, sympatric speciation

## Introduction

In human parasitic diseases, the species *Schistosoma mansoni* (digenean trematode) is an important agent responsible for bilharziasis or intestinal schistosomiasis. It is a gonochoric parasite that nests in the venous circulatory system of the intestine, where it performs its sexual reproduction producing large quantities of embryonated eggs, many of which leave the circulatory system to reach the intestine and are expelled with stools. Contact of the eggs with freshwater causes hatching liberating the swimming larvae (miracidia). Each miracidium infects, by active penetration, the snail intermediate host of the genus *Biomphalaria* where asexual multiplication occurs. After the prepatent period, the infected snails shed, at a

specific time of the day, tens or even hundreds of swimming larvae (cercariae) that infect humans or reservoir hosts, such as rodents, by active penetration when the hosts come into contact with water.

Cercarial emission is an adaptive trait that has co-evolved with the definitive host behaviour [1]. The recent discovery of an exclusively nocturnal chronotype in *Schistosoma mansoni* [2], linked to its adaptation to a rodent host, showed that this species may have undergone significant genetic variation to switch from a photophase emission to exclusively emission during the scotophase. This switch in circadian behaviour raises questions since it should have been accompanied by some other traits, especially those responsible for the host–parasite life-histories. The present work aims to compare the life-history

traits of human-adapted diurnal and rodent-adapted nocturnal chronotypes of *S. mansoni* in order to understand how this behavioural diversity could be interpreted in terms of some biological concepts such as sympatry, allopatry and parasite speciation.

## Material and methods

### Biological material

We used two populations of *Schistosoma mansoni* originating from Dhofar region (Oman) characterised by a different cercarial shedding pattern. The diurnal chronotype was originally collected from human definitive hosts, whereas the nocturnal chronotype was originally collected from naturally infected *Rattus rattus* and *Biomphalaria pfeifferi* [2]. The biological material for the present study has been collected for both chronotypes according to each aim, phylogeny and life-history traits.

Regarding phylogeny, the adult worms were from the G0 to the G5 generations. Twenty-four adult male specimens of *S. mansoni* from Dhofar (Oman) were used: 10

worms of the diurnal chronotype and 14 worms of the nocturnal chronotype (Table 1). We also used two adult male worms of *Schistosoma rodhaini* from Burundi known for having nocturnal chronotype.

Regarding the life-history traits we studied the survival of the vertebrate experimental host (the mouse) towards the two chronotypes using data collected from the G2 to the G27 generations. The two chronotypes were then maintained in the laboratory using laboratory bred pigmented *Biomphalaria pfeifferi* as intermediate hosts, and Swiss OF1 mouse as definitive host. Life cycles were maintained as follows: eggs of *S. mansoni* were collected from the liver and small intestine of Swiss OF1 mice previously infected with 120 or 80 cercariae. The liver was ground and mixed in a saline solution (8.5‰ NaCl) and filtered through a series of four sieves with pore sizes of 425, 180, 106 and 45 µm. The eggs on top of the 45 µm sieve were placed in a glass beaker that contained well water (at 25 °C) to allow the egg hatching. After egg hatching, miracidia were collected under a binocular microscope using a Pasteur pipette and placed one by one in 24 well plates. Each well received a healthy pigmented *B. pfeifferi* in the

**Table 1** *Schistosoma mansoni* adult specimens used for the molecular analysis. Three haplotypes (\*) were found for the diurnal chronotype. Only one haplotype, identical to the diurnal haplotype *Sm-DC1/Hs*, was observed for the nocturnal chronotype

Specimen	COI accession number	Generation	Country, region, locality	Latitude (East)	Longitude (North)
<i>Sm-DC1/Hs</i> *	MK172830	G <sub>1</sub>	Oman, Dhofar region, Sheer locality	54°11'10.41"	17°11'41.21"
<i>Sm-DC2/Hs</i> *	MK172831				
<i>Sm-DC3/Hs</i> *	MK172832				
<i>Sm-DC4/Hs</i>	MK172830	G <sub>2</sub>			
<i>Sm-DC5/Hs</i>	MK172830	G <sub>3</sub>			
<i>Sm-DC6/Hs</i>	MK172832				
<i>Sm-DC7/Hs</i>	MK172830				
<i>Sm-DC8/Hs</i>					
<i>Sm-DC9/Hs</i>					
<i>Sm-DC10/Hs</i>					
<i>Sm-NC1/Rr</i>	MK172833	G <sub>0</sub>	Oman, Dhofar region, Tibraq locality	54°19'35.09"	17°05'58.11"
<i>Sm-NC2/Rr</i>					
<i>Sm-NC3/Rr</i>					
<i>Sm-NC4/Rr</i>					
<i>Sm-NC5/Rr</i>		G <sub>1</sub>			
<i>Sm-NC6/Rr</i>					
<i>Sm-NC7/Rr</i>					
<i>Sm-NC8/Rr</i>					
<i>Sm-NC9/Rr</i>		G <sub>3</sub>			
<i>Sm-NC10/Rr</i>		G <sub>5</sub>			
<i>Sm-NC1/Bp</i>	MK172834	G <sub>0</sub>			
<i>Sm-NC2/Bp</i>					
<i>Sm-NC3/Bp</i>					
<i>Sm-NC4/Bp</i>					

*Sm*, *Schistosoma mansoni*; DC, diurnal chronotype; NC, nocturnal chronotype; *Hs*, *Homo sapiens*; *Rr*, *Rattus rattus*; *Bp*, *Biomphalaria pfeifferi*; G, generation.

presence of well water at 25 °C. The 24 well plates were then placed in a room at a constant temperature (25 °C), balanced photoperiod (light/dark: 12 h/12 h) and balanced photophase (06:00 am to 06:00 pm) for 24 h before grouping them in an aquarium with 2 liters of well water and fed *ad libitum* with fresh lettuce.

The life-history traits of each member of the host-parasite combination *S. mansoni*/*B. pfeifferi* were studied using the G14 generation. For each chronotype, the study was conducted twice, using two populations of *B. pfeifferi* originating from two waterbodies, Siginitti 54°28'08.99" (E); 17°08'36.44" (N) (Experiment 1) and Tibraq 54°19'35.09" (E); 17°05'58.11" (N) (Experiment 2). We considered these experiments as equivalent since the snails were originating from the same drainage basin (Wustah mountains, Dhofar, Oman) and since they were shown to segregate in PCA analysis based on microsatellites genetic comparison [3]. The snail mean diameters at the beginning of the experiments are presented in Table 2; they were not significantly different whatever the chronotype and whatever the experiment.

#### Life-history traits parameters

The interaction between the rodent experimental definitive host and *S. mansoni* was studied by analysing the survival data of female Swiss OF1 mice, commonly used in the laboratory for the maintenance of life cycles. The study was conducted with two cercarial doses per mouse: 120 and 80 cercariae. For the 120 cercarial dose, we collected 144 data from G2 to G6 generation (2003–2010) for the diurnal chronotype and 96 data from G4 to G13 (2004–2010) for the nocturnal chronotype. For the 80 cercarial dose, we collected 66 data from G11 to G21 (2013–2017) for the diurnal chronotype and 90 data from G15 to G27 (2012–2017) for the nocturnal chronotype.

The interaction between *B. pfeifferi* and *S. mansoni* was studied by analysing six life-history traits, three were related to the parasite and three to the snail host.

**Table 2** Diameter of *Biomphalaria pfeifferi* (mean in mm ± SE) at their exposure to diurnal and nocturnal chronotypes for experiments 1 and 2

Experiment	Diurnal	Nocturnal
1	4.26 ± 0.13 (N = 32)	4.53 ± 0.17 (N = 19) NS
2	4.53 ± 0.17 (N = 45) NS	4.30 ± 0.15 (N = 31) NS

N, Number of snails used; NS, non significant (Mann–Whitney U-test).

The parameters measured for *S. mansoni* were prepatent period (number of days from miracidial penetration to the first cercarial emission), infection rate (number of snails successfully infected divided by the number of surviving exposed snails), and cercarial production (number of cercariae produced per infected snail per day). Cercarial production was measured from the first day of cercarial emission until the 91st day post-infection. The cercariae produced daily by each snail were filtered through a Nitrel filter ( $\phi = 25 \mu\text{m}$ ), stained with 5% Lugol solution, and counted under a binocular microscope. Six *B. pfeifferi* among the positive snails were used to measure cercarial production. When one of them died, it was immediately replaced by another positive snail of the same combination of similar size, until stock exhaustion. For each chronotype, we also studied the morphometry of 50 eggs recovered from mice faeces. We retained only the eggs containing a living miracidium. Morphometry was done under a microscope.

Three parameters were measured weekly for the snail intermediate host: growth (shell diameter), fecundity and survival, from the time of infection (week 0) until the end of the experiment which lasted 13 weeks. Shell diameter was measured with a digital caliper. Fecundity was determined by measuring the mean weekly number of eggs per snail.

#### DNA extraction, PCR amplification and sequencing

The adult male worms were preserved in 95% ethanol and stored for DNA extraction. DNA from each worm was extracted using QIAmp® DNA Mini Kit (Qiagen) as described in the manufacturer's protocol. The polymerase chain reaction (PCR) was employed to amplify the cytochrome C oxidase gene (Cox1) using: two primers Cox1-schist F5'-TCTTTTRGATCATAAGCG-3' and Cox1-schist R 5'-TAATGCATMGGAAAAAACA-3' [4] and two internal primers Cox-man-F1 5'-AATTTTAAATAGGTG-GATTTGG-3' and Cox-man-R1 5'-AATCCATAATAAC-CAAACGAC-3' [2]. Each PCR reaction was carried out in a total volume of 25  $\mu\text{l}$ : DNA 2  $\mu\text{l}$ , 5 $\times$  Green GoTaq™ Buffer (Promega) 5  $\mu\text{l}$ ; MgCl<sub>2</sub> 25 mM, 1  $\mu\text{l}$ ; dNTPs 2 mM, 2.5  $\mu\text{l}$ ; primers 10  $\mu\text{M}$ , each 1  $\mu\text{l}$ ; Go Taq™ DNA Polymerase (Promega) 0.3  $\mu\text{l}$  and H<sub>2</sub>O milliQ, 12.2  $\mu\text{l}$ . This mix was placed in an Eppendorf Mastercycler. PCR cycling conditions were as follows: initial denaturation step at 94 °C for 120 s; denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and elongation at 72 °C for 120 s, for 40 cycles; extension step at 72 °C for 7 min. In order to assess the validity of results obtained from each PCR assay, PCR negative assay (H<sub>2</sub>O milliQ added as template) was done. PCR products of

the expected size (1100 bp) were obtained. DNA bands were isolated from a 1% agarose gel and purified using Wizard® SV Gel and PCR Clean-Up System (Promega). PCR products were sequenced using a dideoxy-dye-terminator method (CEQ™ DTCS-Quick Start kit, Beckman Coulter) and a CEQ™ 8000 apparatus (Beckman Coulter). Sequences were obtained using the CEQ™ 8000 sequence analysis software.

### Phylogenetic analysis

The different steps of the phylogenetic analysis were conducted on MEGA 7.0.9 beta software [5]. DNA multiple sequence alignments were performed according to codons using MUSCLE program [6]. Poorly aligned and highly variable region alignments were automatically removed using GBLOCKS 0.91b [7–9]; [http://molevol.cmima.csic.es/castresana/Gblocks\\_server.html](http://molevol.cmima.csic.es/castresana/Gblocks_server.html)) with the following three options: smaller final blocks, gap positions within the final blocks and less strict flanking positions. Phylogenetic tree was obtained via maximum-likelihood (ML) method on the MEGA software. Different models of DNA evolution for coding sequences and associated parameters were estimated using MEGA software [10] and the best parameters were identified based on the Bayesian Information Criterion (BIC). An ML tree was then computed with MEGA software using the best nucleotide substitution model Hasegawa-Kishino-Yano (HKY+G) and validated via bootstrap procedure with 1000 replicates. The tree was built using only haplotypes according to each host from which each chronotype was originated (Humans, *Rattus rattus* and *Biomphalaria pfeifferi*). We also used *S. mansoni* Cox1 sequence from Puerto Rico (accession number AF216698), *S. mekongi* (accession number AF217449) and *S. japonicum* (accession number NC\_002544). The accession numbers of the sequences used in this study are MK172830, MK172831 and MK172832 for the diurnal chronotype and

MK172833 and MK172834 for the nocturnal chronotype (Table 1).

### Statistical analyses

Fisher's exact test (infection rate), the Mann–Whitney test (U) and autocorrelation were used as statistical analysis tools for the various comparisons. Survival rates were examined by Kaplan–Meier survival analysis and survival curves were compared using the log rank test. All data were analysed using StatView v.5 (SAS Institute Inc), SPSS (Version 10.05, Chicago, IL, USA), and GraphPad InStat (Version 3.05, GraphPad Software, La Jolla, CA, USA). Means and standard-errors (SEs) for all values were given. A *P*-value less than 0.05 was considered significant.

The French “Direction Départementale de la Protection des Populations” provided (i) authorisation to our laboratory (UMR 5244) for experiments on animals (living vertebrates) N°: C 66-136-01 with prefectural order N°: 2012-201-0008 and (ii) certificate for animal experimentation for Dr Hélène Moné, authorisation N°: C 66.11.01; articles N°: R 214-87, R 214-122 and R 215-10. Housing, breeding and animal care adhered to national ethical requirements.

## Results

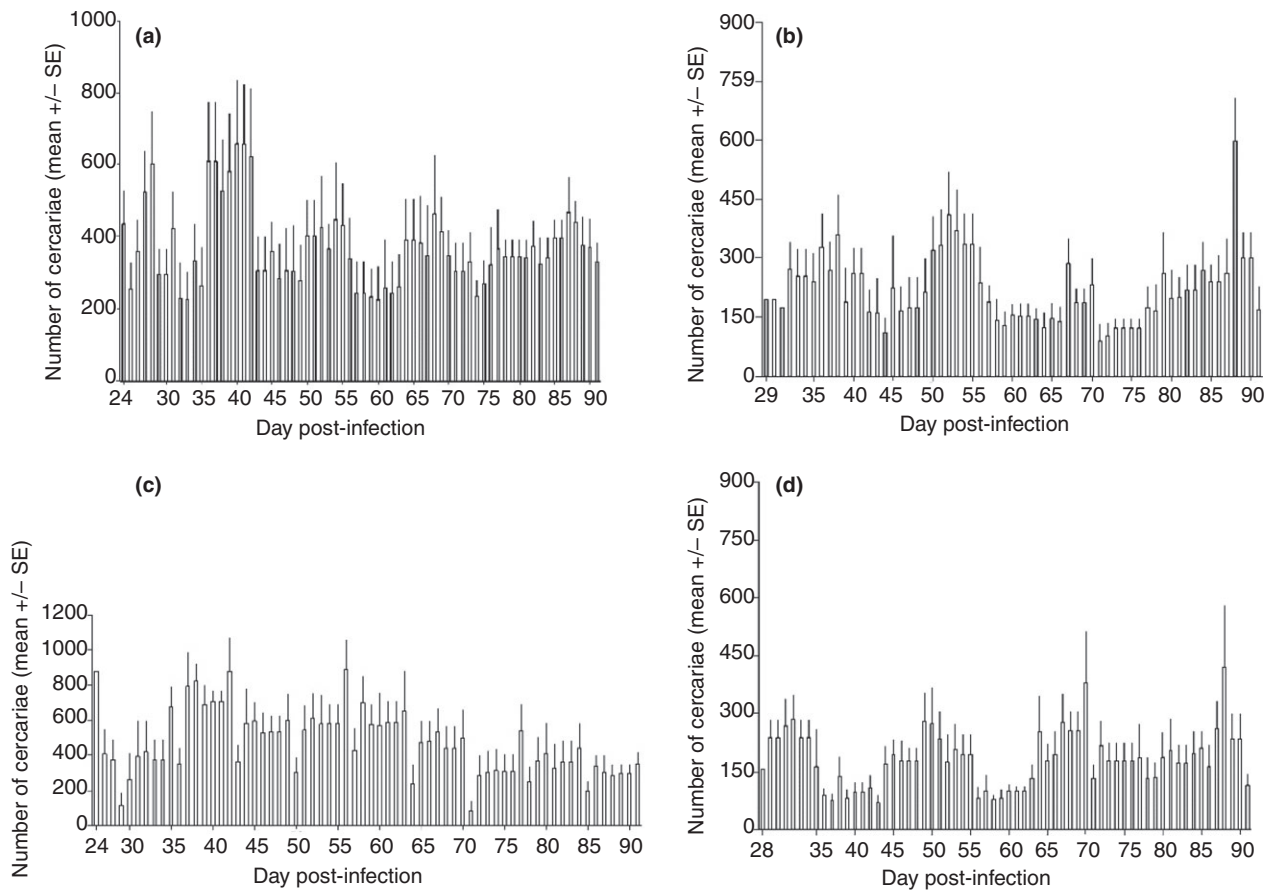
### Parasite life-history traits

In either experiment (1 or 2, see Table 3), mean pre-patent period was significantly shorter in the diurnal than the nocturnal chronotype ( $P < 0.05$ ); infection rates did not significantly differ between the two chronotypes; cercarial production of the diurnal chronotype was significantly higher than that of the nocturnal chronotype ( $P < 0.05$ ). Cercarial production followed a rhythmic pattern (Figure 1a, b and d) with alternating periods of high and low production, except for the diurnal

**Table 3** Diurnal vs. nocturnal *Schistosoma mansoni* life-history traits on *Biomphalaria pfeifferi* from Siginitti (experiment 1) and from Tibraq (Experiment 2)

	Experiment 1		Experiment 2	
	Diurnal (N = 32)	Nocturnal (N = 19)	Diurnal (N = 45)	Nocturnal (N = 31)
Pre-patent period (mean day ± SE)	26.56 ± 1.33	32.38 ± 0.73*	28.30 ± 0.26	30.29 ± 0.35*
Infection rate (%)	64	42 (NS)	44	68 (NS)
Cercarial production/day/snail (mean number of cercariae ± SE)	372 ± 68 (N = 6)	221 ± 35* (N = 6)	464 ± 74 (N = 6)	183 ± 16* (N = 6)

NS, non-significant; SE, Standard-Error; \* $P < 0.05$  (Mann–Whitney *U*-test for pre-patent period and cercarial production; Fisher exact test for infection rate).



**Figure 1** Mean daily cercarial productions of diurnal (a and c) and nocturnal (b and d) *Schistosoma mansoni* chronotype from *Biomphalaria pfeifferi* from Siginitti (a and b (Experiment 1)) and from Tibraq (c and d (Experiment 2)). Bars represent standard errors.

chronotype in experiment 2 (Figure 1c). Between two periods of high or low cercarial production, the autocorrelation test indicated a periodicity of 14 days for the diurnal chronotype and of 15–20 days for the nocturnal chronotype.

There was no significant difference in the measurements of the eggs of diurnal and nocturnal chronotypes. However, whatever the chronotype, the morphological study of the eggs collected in the stools revealed the presence of a polymorphism based on the shape of the egg shell with the presence of two morphotypes A and B (Figure 2): eggs with A morphotype had the classical form of *S. mansoni*, whereas eggs with B morphotype showed a beginning of curvature at the opposite end of the lateral spine but not as pronounced as in *S. rodhaini* eggs, and oriented at the opposite side of the lateral spine. The diurnal chronotype contained 92% of the A morphotype whereas the nocturnal chronotype only contained 64%. The rest of the eggs presented the B morphotype; thus

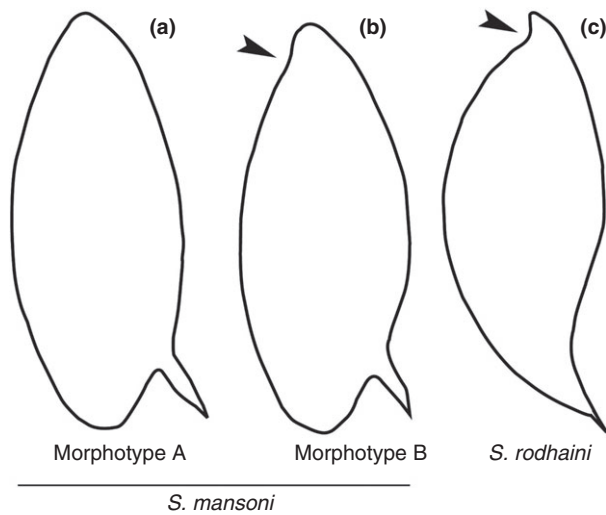
the proportion of the B morphotype was significantly higher in the nocturnal chronotype (Chi-square = 9.18;  $P = 0.0034$ ).

#### Host life-history traits

In either experiment (1 or 2) and for either chronotype used, *B. pfeifferi*'s growth curves were close, with rapid growth during the first 3 weeks followed by a slowdown until week 13 (Table 4; Figure 3). In experiment 1, mean weekly shell diameter showed no significant difference between the two chronotypes, whereas in experiment 2, mean weekly shell diameters were significantly smaller with nocturnal chronotype since week 2 post-infection. Snails infected with diurnal *S. mansoni* chronotype continued to spawn even though the values (Table 4) were far below the values of healthy snails ( $145.00 \pm 23.24$  to  $159.80 \pm 10.43$  for a period of 13 weeks). Snails infected with the nocturnal *S. mansoni* chronotype produced a

tiny amount of eggs or did not lay eggs at all. Egg-laying began in the 3rd week post-infection and was not continuous. In the 4 host-parasite combinations studied, the proportion of egg-laying snails was very small and laying could take place until the 13th week. Survival of *B. pfeifferi* differed between experiment 1 and 2 (Table 4). In experiment 1 no significant difference was found between the chronotypes while in experiment 2, the survival of the snails infected with the nocturnal chronotype was significantly shorter.

Mice survival (in days) according to the cercarial dose and to the chronotype was as follows: (i) For the cercarial dose of 120 it was 127 days  $\pm$  5 ( $N = 144$ ) for the



**Figure 2** Whatever the chronotype, diurnal *vs.* nocturnal, *Schistosoma mansoni* eggs showed two morphotypes: (a) eggs with a typical morphology of *S. mansoni* ( $L = 143 \mu\text{m}$ ) and (b) eggs with, at the opposite end of the lateral spine, a beginning of curvature but not as pronounced as in *Schistosoma rodhaini* ( $L = 130 \mu\text{m}$ ). (c) *S. rodhaini* typical egg ( $L = 139 \mu\text{m}$ ). L: egg length.

diurnal chronotype and 149 days  $\pm$  10 ( $N = 96$ ) for the nocturnal chronotype. (ii) For the cercarial dose of 80 it was 145 days  $\pm$  8 ( $N = 66$ ) for the diurnal chronotype and 172 days  $\pm$  13 ( $N = 90$ ) for the nocturnal chronotype. For both cercarial doses, the Mann–Whitney *U*-test showed no significant difference between the two chronotypes ( $P = 0.5$  and  $P = 0.8$  for each cercarial dose, respectively). However, mice survival increased with decreasing cercarial dose with a significant difference only for the diurnal chronotype ( $P = 0.01$  and  $U = 3748$ ).

### Phylogeny

The phylogenetic results based on the 1054 bp Cox1 analysis revealed the presence of 3 haplotypes in the diurnal chronotype whose accession numbers are MK172830 for 7 individuals represented by *Sm-DC1/Hs*, MK172831 represented by *Sm-DC2/Hs* and MK172832 for 2 individuals, represented by *Sm-DC3/Hs*. Regarding the nocturnal chronotype, two accession numbers were given, MK172833 for the 10 adult worms (*Sm-NC1/Rr* to *Sm-NC10/Rr*) collected from naturally infected host *Rattus rattus* and MK172834 for the 4 adult worms (*Sm-NC1/Bp* to *Sm-NC4/Bp*) collected from naturally infected *Biomphalaria pfeifferi* and after mouse experimental infection. Whatever the host, only one haplotype, identical to the diurnal haplotype *Sm-DC1/Hs*, was observed in the nocturnal chronotype (Table 1 and Figure 4).

### Discussion

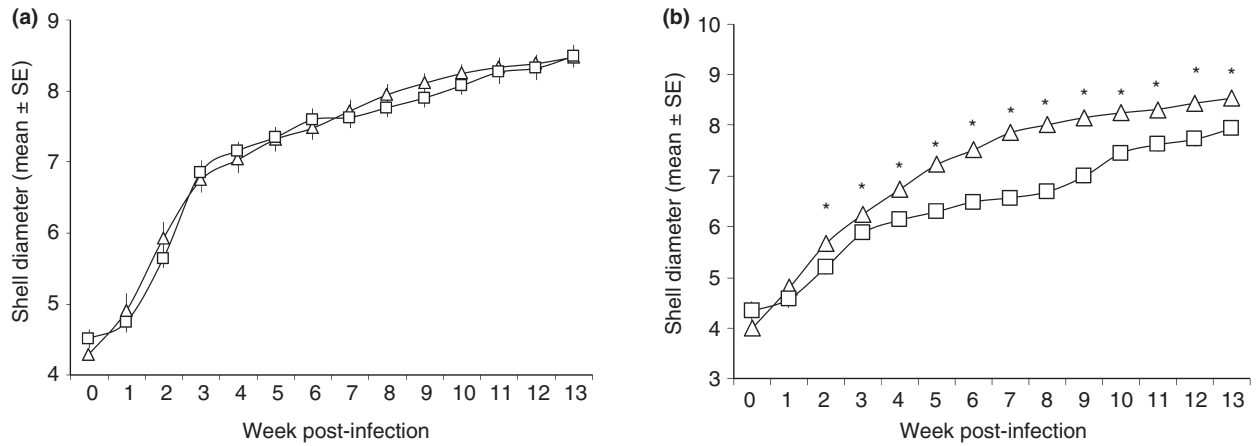
We explored the host-parasite life-history traits thanks to the dual and remarkable presence of two *S. mansoni* chronotypes (diurnal *vs.* nocturnal) in the same region and found that the diurnal and the nocturnal chronotypes had different life-history traits. The diurnal chronotype had a significantly shorter prepatent period, the same infection rates in the snails and a significantly higher

**Table 4** *Biomphalaria pfeifferi* from Siginitti (Experiment 1) and from Tibraq (Experiment 2) life-history traits, infected either with the diurnal or the nocturnal *Schistosoma mansoni* chronotype

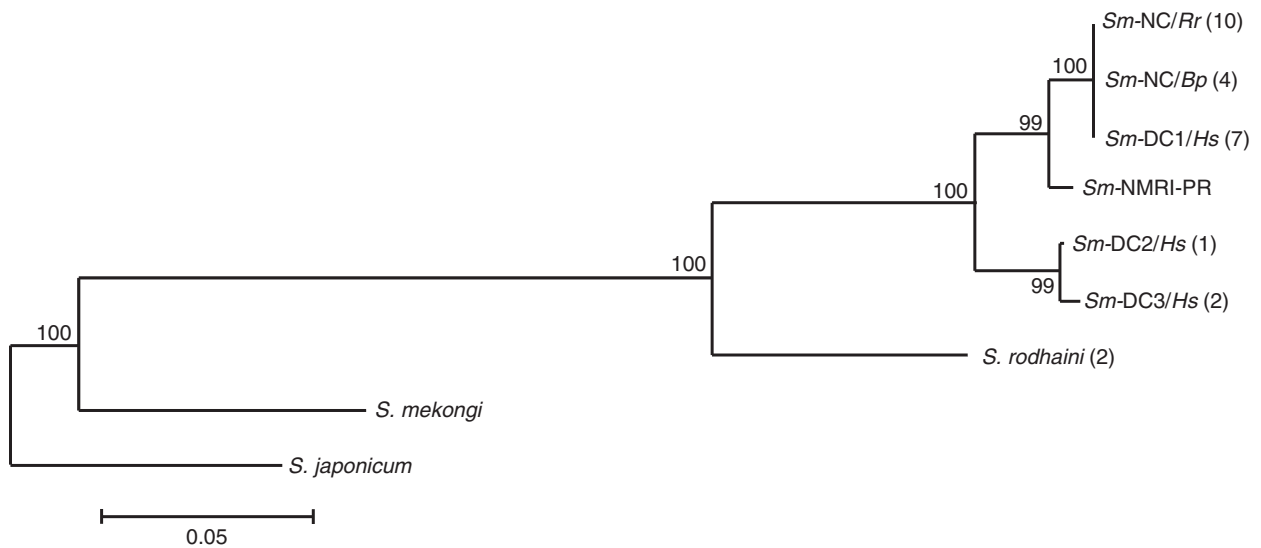
	Experiment 1		Experiment 2	
	Diurnal ( $N = 18$ )	Nocturnal ( $N = 8$ )	Diurnal ( $N = 20$ )	Nocturnal ( $N = 17$ )
Shell diameter (mm)	8.48 $\pm$ 0.12	8.49 $\pm$ 0.16 NS	8.52 $\pm$ 0.12	7.92 $\pm$ 0.11*
Fecundity	7.44 $\pm$ 5.64	0.75 $\pm$ 0.80 NS	9.88 $\pm$ 4.06	0.00
Survival (%)	13/18: 0 egg	7/8: 0 egg	10/20: 0 egg	17/17: 0 egg
	90	100 NS	85	47*

Snail growth is expressed by the shell diameter and Fecundity is expressed by the mean number of eggs/week/snail. NS, non significant; \* $P < 0.05$  (Mann–Whitney *U*-test). Survival is determined at the 13th week post-infection.





**Figure 3** Mean weekly diameter ( $\pm$  SE) of *Biomphalaria pfeifferi* (a: Experiment 1 and b: Experiment 2) infected with the diurnal (triangle) and the nocturnal (square) *Schistosoma mansoni* chronotypes during 13 weeks post infection. \*Significant difference (Mann-Whitney *U*-test;  $P < 0.05$ ).



**Figure 4** ML tree based on mitochondrial cytochrome oxidase I sequences of *Schistosoma mansoni* individual adults according to each chronotype, diurnal vs. nocturnal. This analysis was computed using a 1054 bp-long multiple alignment of 24 *S. mansoni* Omani samples, one sample of *S. mansoni* from Puerto Rico (NMRI), two samples of *S. rodhaini* from Burundi, one sample of *S. japonicum* and one sample of *S. mekongi* for the outgroups. Node supports are indicated by the ML bootstrap values.

cerarial production than the nocturnal chronotype. Regarding the host life-history traits, shell diameter, fecundity and survival of the snails infected with the diurnal chronotype were either the same or significantly higher than those of the snails infected with the nocturnal chronotype and mouse survival did not differ between the two chronotypes. From the parasite's point of view, reducing its pre-patent period, enhancing cercarial

production, inducing higher growth, fecundity and survival in its snail host are factors that will enhance its transmission to the vertebrate host. Thus diurnal chronotype transmission is higher than nocturnal chronotype transmission. This allows us to say that the diurnal chronotype of *S. mansoni* seems to be better adapted to its sympatric snail intermediate host than the nocturnal chronotype *S. mansoni*. In fact, the diurnal parasite

chronotype behaves normally, with its snail host coming from a sympatric population whereas the nocturnal chronotype behaves as if the snail host is from an allopatric population. For example, Ibikounlé *et al.* showed that the prepatent period was significantly shorter for *S. mansoni* and for *S. haematobium* from Toho-Todougba (Benin) on their sympatric snail hosts *B. pfeifferi* and *Bulinus globosus*, respectively, compared to the pre-patent periods obtained for different allopatric populations of the snail hosts [11, 12]. The “allopatric” behaviour of the nocturnal chronotype supports the hypothesis of its recent appearance.

The morphology of the parasite eggs also differed between the chronotypes: the percentage of morphotype B (presence of a curvature) was significantly higher in the eggs of the nocturnal chronotype. This trait has been observed in a Guadeloupean transmission site, where *R. rattus* was involved in the life cycle of a diurnal *S. mansoni* population [13]. However, no explanation can be given at present on the possible (or not) implication these differences may have on schistosome transmission.

Whatever the chronotype, the cercarial production showed generally high and low cercarial production periods with a rhythmic pattern where periods of high production alternated with periods of low production. Such rhythmic variation in the cercarial production was shown in *S. mansoni* from Brazil on *Biomphalaria glabrata* from Brazil by [14] and in *S. bovis* from Spain on *Planorbium metidjensis* from Morocco [15]. These variations were closely related to the pattern of cercariogenesis and sporocystogenesis processes [16].

Molecular analysis revealed a greater diversity within the diurnal than the nocturnal chronotype and argues in favour of an emergence of the nocturnal phenotype from the diurnal phenotype. This is supported by a previous molecular study of various strains of *S. mansoni*, which showed that the lateral transfer from humans to rats is likely to have been recent and localised [17]. The scenario of the nocturnal chronotype emergence is probably linked to a diversifying natural selection that leads to its selection. The definitive host *Rattus rattus*, which constitutes a contrasting environment with a nocturnal behaviour compared to humans, allows this new trait to be selected. The diurnal chronotype widened its host spectrum by passing over the rat; it is not a host shift. We do not know which mechanism was at the origin of the chronobiological divergence of *S. mansoni* from Dhofar. Mutation and/or genetic drift are two main mechanisms. Many authors agree that divergent natural selection is a fundamental step in the process of speciation among sexually reproducing eukaryotes. The ethological divergence

in the profiles of the cercarial emission (diurnal or nocturnal) constitutes good conditions for a phenomenon of sympatric speciation (in the sense of Coyne and Orr [18]) to be triggered. Mating might not occur because of differences in cercarial shedding time. This behavioural divergence could act as a prezygotic reproductive barrier, reducing the possibility of males and females to meet in the same definitive host (Human or rodent) and consequently to contribute to a barrier to gene flow between the two chronotypes. We hypothesise that this prezygotic isolation is likely to create, in the long term, post-zygotic isolation as it currently occurs between species of schistosomes of the same group (terminal or lateral spine egg group).

Indeed, we are aware that the concept of species in the genus *Schistosoma* is to be taken with care. Since the 1970s, species of the same group are known to hybridise with a postzygotic isolation from the 1st to 3rd generation (depending on the crossed species and on the reciprocal crossing) due to high decrease in the miracidial viability and infectivity [19]. In schistosomes, no complete cessation of sexually mediated gene exchange exists and natural hybridisation between different species of schistosome in Africa explains the growing number of cases of introgressed species, such as *S. haematobium* introgressed by *S. bovis* [20]. If the gene flow is not interrupted between the two chronotypes, which is perfectly conceivable since we are within the same species, we could imagine the emergence of natural viable hybrid progeny with an ultradian chronotype (diurnal and nocturnal) adapted to both humans and rats.

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