Heredity of specific host-finding behaviour in *Schistosoma mansoni* miracidia

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SUMMARY

Two strains of *Schistosoma mansoni* were used to investigate the hereditary basis of species-specific host recognition by analysing behavioural responses of miracidia to snail-conditioned water. An Egyptian strain of *S. mansoni*, capable of distinguishing its host snail *Biomphalaria alexandrina* from other snails was cycled repeatedly through *Biomphalaria glabrata*, the intermediate host of a Brazilian strain known to respond even to non-susceptible snails with high intensity. After 5 cycles in the non-natural host, miracidia of the Egyptian strain still retained their preference for the original host snail. In a second experiment, host-finding behaviour of hybrids between these two parasite strains was studied. In the F_1 generation, hybrids of both parental combinations showed the same low degree of specificity as the pure-bred Brazilian strain. Approximately one quarter of F_2 hybrids proved to be as discriminatory as the Egyptian strain, confirming dominant Mendelian inheritance of non-specificity in schistosome miracidial host-finding behaviour. Moreover, hybrids seem to have lost the ability to develop in *B. alexandrina*, possibly suggesting a link between host recognition and host compatibility. The heredity of this behavioural trait is of evolutionary and epidemiological significance, since a shift to low host-finding specificity might have been a prerequisite for *S. mansoni* to acquire new host snails after being introduced to South America by the slave trade.

Key words: *Schistosoma mansoni*, miracidia, host-finding behaviour, hybrids, species-specificity, adaptation.

INTRODUCTION

The specificity of parasite–host interactions has received great attention by parasitologists and evolutionary biologists. A high degree of host specificity can be considered as evidence for a long co-evolutionary history of a parasite–host system, but is also found as a consequence of locally dynamic co-evolution on the level of different genotypes within a host population. In trematode–snail interactions, which are generally regarded as highly specific (Wright, 1971), compatibility patterns of species or strains of parasites and hosts have been used for phylogenetic studies (Blair, Davis & Wu, 2001) as well as for investigations of parasite–host co-evolution on a local scale (Lively, 1999; Lively & Dybdahl, 2000). Especially in medically relevant blood flukes snail compatibility is also a topic of practical importance for epidemiological surveys and development of biological control methods. This is the reason why most work on snail compatibility and the snail's internal defence system against trematode infections was performed with *Schistosoma mansoni*

(Basch, 1976; Morgan *et al*. 2001), the cause of intestinal schistosomiasis in man. Host snails of the genus *Biomphalaria* respond with humoral and cellular mechanisms to *S. mansoni* sporocysts (reviewed e.g. by Bayne & Yoshino, 1989; Adema & Loker, 1997; Bayne, Hahn & Bender, 2001). It was recently demonstrated by selection experiments under laboratory conditions with different snail lines that, in the system *S. mansoni–B. glabrata*, compatibility characteristics seem to be inherited with resistance being dominant over susceptibility (Webster & Woolhouse, 1998; Webster, 2001).

However, specific interactions between schistosomes and their snail hosts start prior to penetration and development in the snail. Like first larvae of most digeneans, schistosome miracidia locate and penetrate their intermediate hosts actively. After hatching and dispersal, they approach their specific host snails' preferred microhabitat, guided by environmental stimuli (Saladin, 1979). Once miracidia have reached the area, where an encounter is likely, they search for a suitable host snail by chemo-orientation in response to glycoconjugates derived from the snail's mucous surface layer (Haberl *et al*. 1995). These 'miracidia attracting glycoconjugates' (MAGs) also stimulate the parasite's behaviour after contact with the snail host (Haberl & Haas, 1992). In nature, these parasite–host interactions preceding the actual penetration are essential for transmission

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success of miracidia within their life-span of only a few hours. Furthermore, Combes & Moné (1987) pointed out that previous contact of miracidia with non-host organisms may reduce their ability to further develop in a suitable snail species. To avoid such detrimental failures trematodes should, therefore, have evolved precise species-specific mechanisms of host finding and host recognition. In fact, we found that an Egyptian (laboratory) strain of *S. mansoni* discriminated between its host snail *B. alexandrina* and other snail species and strains, already during approach and after contact (Kalbe, Haberl & Haas, 1996; Haberl *et al.* 2000; Hassan *et al.* 2003). Also miracidia of the liver fluke *Fasciola hepatica*, and of the bird schistosomes *Trichobilharzia ocellata* and *T. franki*, respond exclusively to MAGs from their respective hosts and not to those from even closely related snail species (Kalbe, Haberl & Haas, 1997, 2000; Kock, 2001). Several infection experiments, where miracidia had the choice between different snail species, support our assumption that species-specific host-finding is the normal case rather than an exception in digenean miracidia (Christensen, Nansen & Frandsen, 1976; Chipev, 1993; Toledo *et al.* 1999). Although high specificity in miracidial host finding is obviously advantageous in endemic areas, where the parasite's specific host snails occur permanently within a diverse mollusc fauna, it is clear that, on the other hand, this trait hinders the acquisition of new host species. Especially long-persisting digeneans of migratory, long-living definitive hosts may be faced with a situation where the required snail species is not available and a host switch is the only chance for further reproduction. Under these circumstances, parasites should adjust their host-finding behaviour and respond to a broad range of potential new host organisms. In fact, contrary to *S. mansoni* from Egypt, miracidia of two Brazilian strains showed no preference for their host *B. glabrata* over several other snails (Kalbe *et al.* 1996). This result concurs with observations that various aquatic organisms interfere with successful infection of *B. glabrata* snails by miracidia of Carribean or Latin American schistosome strains (Barbosa & Carneiro, 1965; Chernin & Perlstein, 1971; Upatham, 1972; Moné & Combes, 1986). This lack of specificity might be a consequence of the fact that *S. mansoni* was introduced to America by the slave trade and only recently acquired *B. glabrata* as a new intermediate host (Files & Cram, 1949; Després, Imbert-Establet & Monnerot, 1993; Morgan *et al.* 2001).

We used our model system of an Egyptian *S. mansoni* strain whose miracidia respond highly specifically to snail host cues, and a Brazilian strain with less distinctive host-finding behaviour, to investigate the hereditary background of a shift in the degree of specificity. In order to determine whether a sudden switch from high to low specificity occurs, we analysed miracidial behaviour of the Egyptian strain after repeated cycling through the non-host snail *B. glabrata*. To obtain information about the genetic basis of this species-specific recognition, we produced hybrids of these two *S. mansoni* strains and compared their capability to discriminate between different snail species.

MATERIALS AND METHODS

Parasites and hosts

The Brazilian *S. mansoni* strain (BR) was isolated in Belo Horizonte (Brazil) together with its host snail *B. glabrata* in 1967. Since 1996, this parasite was cycled through an albino strain of *B. glabrata*. The Egyptian *S. mansoni* strain (ET) originated from Egypt and was maintained at the University of Massachusetts, Lowell, USA. In 1990, it was re-imported to Egypt and maintained together with its intermediate host *B. alexandrina* at the Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Cairo. *Lymnaea stagnalis* as experimental non-host snails were originally collected from ponds near Erlangen, Germany, and reared in the laboratory for several years.

Snails were kept under a day–night rhythm of 12:12 h at 27 °C (*L. stagnalis* at 18 °C) in aerated tap water and fed on lettuce and fish food. Parasites were cycled through female CD-1 mice infected by the paddling method (Webbe & James, 1971) and eggs were obtained from mouse faeces, which were washed with cold saline and transferred to tap water. The hatched miracidia were concentrated in side-arm flasks by illumination and used for the experiments within 1–3 h after hatching.

In this study, two different methods were used to infect snails: for repeated cycling in different species of intermediate hosts, batches of 20–35 young snails (diameter 3–6 mm) were incubated overnight in glass beakers containing 300 ml of tap water and 5 *S. mansoni* miracidia per snail. For crossing experiments, single miracidia were pipetted into 5 ml glass vials. Before a snail was added, it was confirmed under a dissecting microscope that the vials contained not more than 1 miracidium. In the case of these mono-miracidial infections, snails were left in the vials for 24 h to make sure that non-penetrated miracidia were dead before the snails were transferred to aquaria in groups. After 6–8 weeks, surviving snails were checked for cercarial shedding by illuminating them individually in 40 ml glass beakers.

Sex determination of cercariae and cross-infections

Schistosomes are dioecious, therefore offering the possibility to produce hybrids of different strains of the parasite. Freshly isolated cercariae of individual snails were washed in deionized water, and single larvae were transferred into PCR tubes and

lyophilized. Cercariae were incubated in 25% ammonia solution for 10 min at 95 °C according to the method of Grevelding, Kampkötter & Kunz (1997). Determination of the gender of cercariae was performed by polymerase chain reaction (PCR) of the sex specific W1 sequence (Webster, Mansour & Bieber, 1989) employing the protocol and primers described by Gasser, Moharan & Mitchel (1991). Amplification products were analysed on Sybr Green-stained 1% TBE agarose gels.

Since all cercariae from a monomiracidial-infected snail are genetically a clone, the sex of all larvae from a given snail was known when it was determined for one of them. Mice were infected with 60 male and 60 female cercariae originating from a single snail respectively. The possible hybrid combinations ET♂ × BR♀ and BR♂ × ET♀, as well as pure-bred controls (ET♂ × ET♀ and BR♂ × BR♀) were used to produce F_1 miracidia. F_2 generations were raised from both pure-bred strains and from one of the hybrids (BR♂ × ET♀)♂ × (BR♂ × ET♀)♀. The whole crossing experiment was performed twice.

Test substrates

Snail-conditioned water (SCW) was prepared by incubating 1 *Biomphalaria* snail per ml of tap water (1 *L. stagnalis* per 3 ml) for 2 h at 27 °C (*L. stagnalis* at 18 °C). After molecular filtration in a stirred cell (Filtron, Northborough, Massachusetts, USA) the carbohydrate content of the fraction > 30 kDa was determined colorimetrically by the sulphuric acid method (Dubois *et al.* 1956) with mannose serving as the standard. For experiments in which the effect of SCW from different snail species on the miracidial host-finding behaviour was compared, all of these substrates were adjusted to a uniform sugar content of 0·1 µg/ml corresponding to dilutions of 1:10 in both *Biomphalaria* species and 1:30 in the case of *L. stagnalis*. In a second experiment SCW of *L. stagnalis* was offered undiluted and in dilutions up to 1:100 equivalent with a carbohydrate content of 3 to 0·03 µg/ml.

Miracidial behaviour and experimental conditions

Chemo-orientation of miracidia was investigated in a one-arm chamber as described previously (Haas *et al.* 1995; Haberl *et al.* 1995). Test substrates were applied to the end of a straight chamber, and the behaviour of miracidia entering or leaving the substrate-containing section was recorded. In case of effective SCW, the normally straight-swimming miracidia show an increase in the rate of change of direction (RCD) when entering the point of inoculation and a turnback swimming response when leaving it. The responses of 10–50 individual miracidia per replicate were recorded and the percentages of these two chemokinetic responses were calculated independently. Experiments were performed at 27 °C by placing the chamber on a precision heating plate and keeping all substrates in a water bath. The test substrates, the water containing the miracidia, and the tap water for filling the chamber, were adjusted to pH 7·5 with Tris–HCl (9 mM). Two or three of the *S. mansoni* strains/ crossings were compared on each experimental day and, for all investigations, aliquots of identical SCW samples were offered. For all experiments a blind protocol was used. The observer never knew which type of substrate and which strain or hybrid was under study.

Data analysis

The arcsin-square root transformed data from behavioural experiments and monomiracidial infections were analysed with the help of the statistical analysis software SPSS. Standard errors ('SE') were retransformed after computation. Means were compared using the TUKEY multiple *t*-test procedure with a significance level of 0·05 (indicated by *).

Data from behavioural experiments with miracidia of the hybrid F_2 (BR♂ × ET♀)♂ × (BR♂ × ET♀)♀ were used to test if host-finding specificity follows a Mendelian segregation pattern. Hypothetical values for a 3:1 ratio were calculated for miracidial responses to non-host SCW of *L. stagnalis* in that particular dilution, where the greatest differences between the *S. mansoni* ET strain and the BR strain occurred. Within this range, hypothetical percentages for a 3:1 ratio in the miracidial responses, 'Increase of RCD' and 'Turnback swimming', were calculated according to the formula

$$(\%\mathrm{responses}_{BR} - \%\mathrm{responses}_{ET}) \times 0\cdot75 + \%\mathrm{responses}_{ET}.$$

On the basis of these values and the total number of observations, the theoretical number of responding miracidia for a 3:1 were determined. Subsequently, a chi-square analysis (χ^2) was used to compare the actually observed and the predicted numbers of responses.

RESULTS

Repeated cycling through a non-natural host snail

Infection rates of *S. mansoni* ET cycled through the Brazilian snail *B. glabrata* albino ranged between 12·3 and 18·7%. No obvious change in infection rates was observed with repeated cycling through the non-natural host. Even after 5 cycles, the ET miracidia retained their specificity in host-finding behaviour: the responses to *B. alexandrina* SCW was not altered, and they responded to SCW of the experimental intermediate host with the same low intensity as to the non-host snail *L. stagnalis* or the control. The
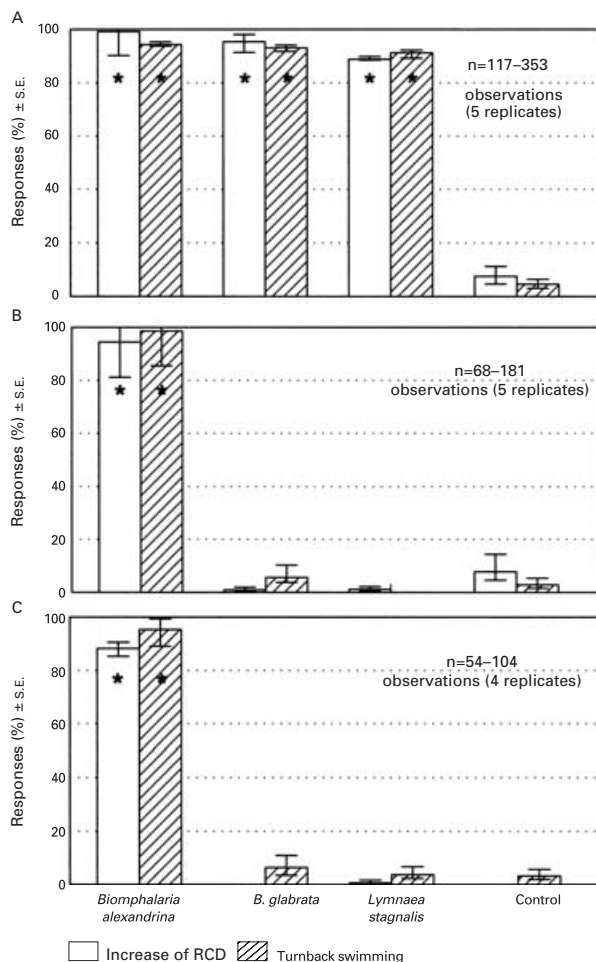
Fig. 1. Comparison of miracidial responses (%) to snail conditioned water from specific intermediate hosts, experimental hosts and the non-host *Lymnaea stagnalis*. (A) Brazilian *Schistosoma mansoni* cycled through its host snail *Biomphalaria glabrata*; (B) Egyptian *S. mansoni* cycled through its host snail *B. alexandrina*; (C) Egyptian *S. mansoni* cycled experimentally through *B. glabrata* (5 cycles). *$P < 0.05$ *vs* control (*t*-test).

BR strain showed similarly high rates of responses towards SCW of all 3 snails (Fig. 1).

Hybrids of two S. mansoni *strains*

In both *S. mansoni* strains, high amounts of the sex-specific repetitive W1 sequence could be amplified by PCR only in female cercariae. Some males produced a very faint band at approximately 0·5 kb indicating a low number of W1 copies (not shown).

Crossing of the parasite strains produced viable offspring in both combinations of the parental sexes. Hybrids showed comparable low specificity in their host-finding behaviour as the pure-bred BR strain, with the hybrid ET♂ × BR♀ apparently responding to a lower extent to SCW of *B. glabrata* and *L. stagnalis* than BR♂ × ET♀. Only the ET♂ × ET♀ miracidia retained their specificity for their host snail *B. alexandrina* (Fig. 2). However, experiments with diluted SCW of the non-host *L. stagnalis* revealed

that both hybrids (Fig. 3C, D) responded with a similar sensitivity as the pure-bred BR strain (Fig. 3A). The pure-bred ET strain responded only weakly to undiluted *L. stagnalis* SCW (a concentration 30 times higher than in the previous experiment), whereas the following dilutions elicited no behavioural changes at all (Fig. 3B). Comparable activity/viability in all 4 strains was indicated by the fact that all miracidia responded to SCW of *B. alexandrina* in a dilution of 1 : 10 with similar high intensity (Figs 2 and 3).

The specificity of chemo-orientation in the F_2 hybrid (BR♂ × ET♀)♂ × (BR♂ × ET♀)♀ was studied with a similar experimental procedure. At a dilution of 1 : 5, the highest differences between both pure bred parental strains in responses to *L. stagnalis* SCW were found. Within this range, 76·3% of the F_2 hybrid miracidia would be expected to show an 'increase of RCD' in case of a 3 : 1 ratio. This does not differ significantly ($\chi^2 = 2 \cdot 00$, s.f. = 1, $P = 0 \cdot 65472$) from the observed value of 73·3% (s.e. $+3 \cdot 17/-3 \cdot 47$; 120 observation, 4 replicates). Likewise, the observed 71·3% F_2 hybrid miracidia performing 'turnback swimming' (s.e. $+5 \cdot 34/-6 \cdot 06$; 244 observations, 4 replicates) were approximately matched to the expected value for a 3 : 1 ratio of 76·1% ($\chi^2 = 1 \cdot 281$, d.f. = 1, $P = 0 \cdot 25763$) at this particular concentration (Fig. 4). These proportions indicate that non-specific host-finding behaviour is genetically dominant following simple Mendelian inheritance.

Monomiracidial infection rates in two Biomphalaria *species*

The monomiracidial infection series with both *S. mansoni* strains and their hybrids in the two *Biomphalaria* species revealed that cercarial shedding of the BR strain occurred only in its host snail *B. glabrata* (Table 1). The ET strain developed in both snail species, although with less success in *B. glabrata*. Interestingly, the infection rates of both hybrids in *B. alexandrina* were extremely low, similar to the BR strain. However, survival rates of these snails were lower than of *B. glabrata*, possibly indicating a higher mortality of *B. alexandrina* with pre-patent infections. Nevertheless, these results could be a first hint on a correlation between the ability to discriminate *B. alexandrina* from other snails during approach and the capability to develop within this snail.

DISCUSSION

In this study we demonstrated that unspecific miracidial host-finding behaviour of a Brazilian *S. mansoni* strain is genetically dominant over the ability to distinguish suitable hosts from non-host organisms in an Egyptian strain. This low host-searching specificity was obviously advantageous for *S. mansoni* after it had been transferred from Africa
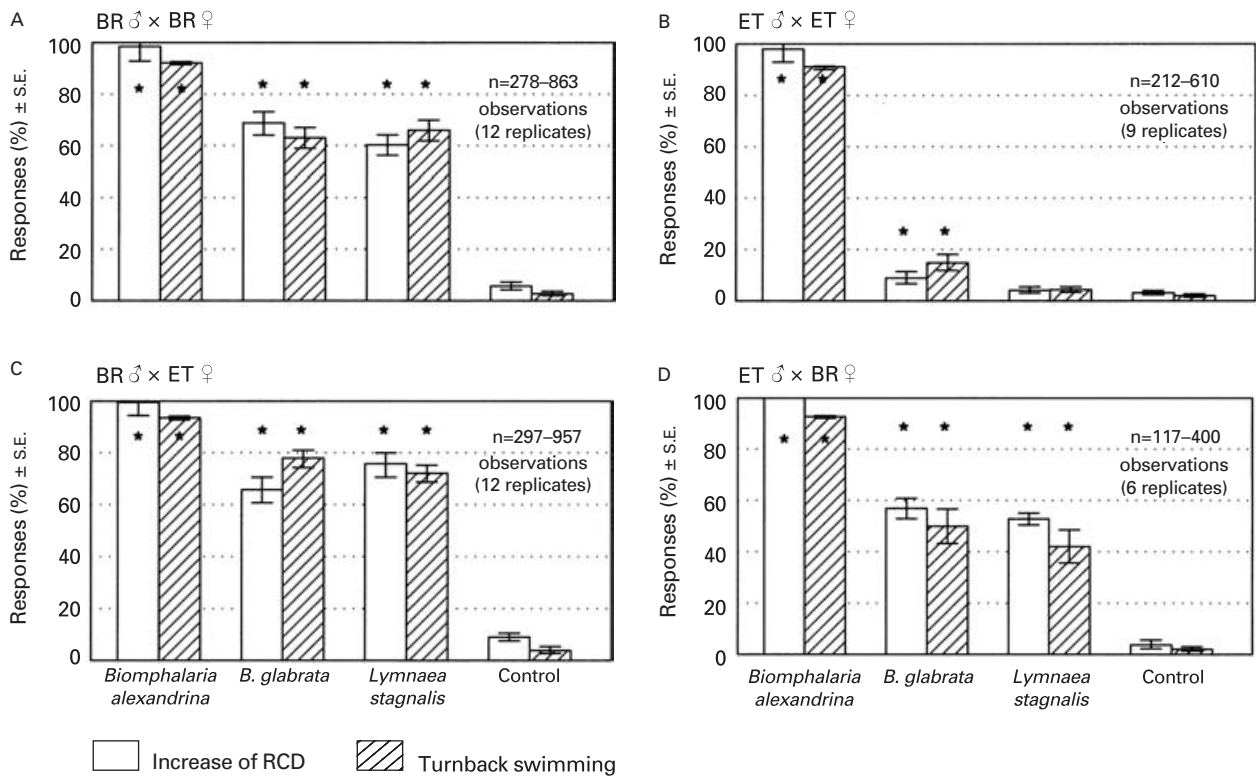
Fig. 2. Miracidial responses (%) to snail conditioned water from two *Biomphalaria* species and the non-host *Lymnaea stagnalis*. (A) Pure-bred Brazilian *Schistosoma mansoni* strain; (B) pure-bred Egyptian *S. mansoni* strain; (C) F_1 hybrid of male Brazilian and female Egyptian *S. mansoni* strain; (D) F_1 hybrid of male Egyptian and female Brazilian *S. mansoni* strain. *$P < 0.05$ *vs* control (*t*-test).
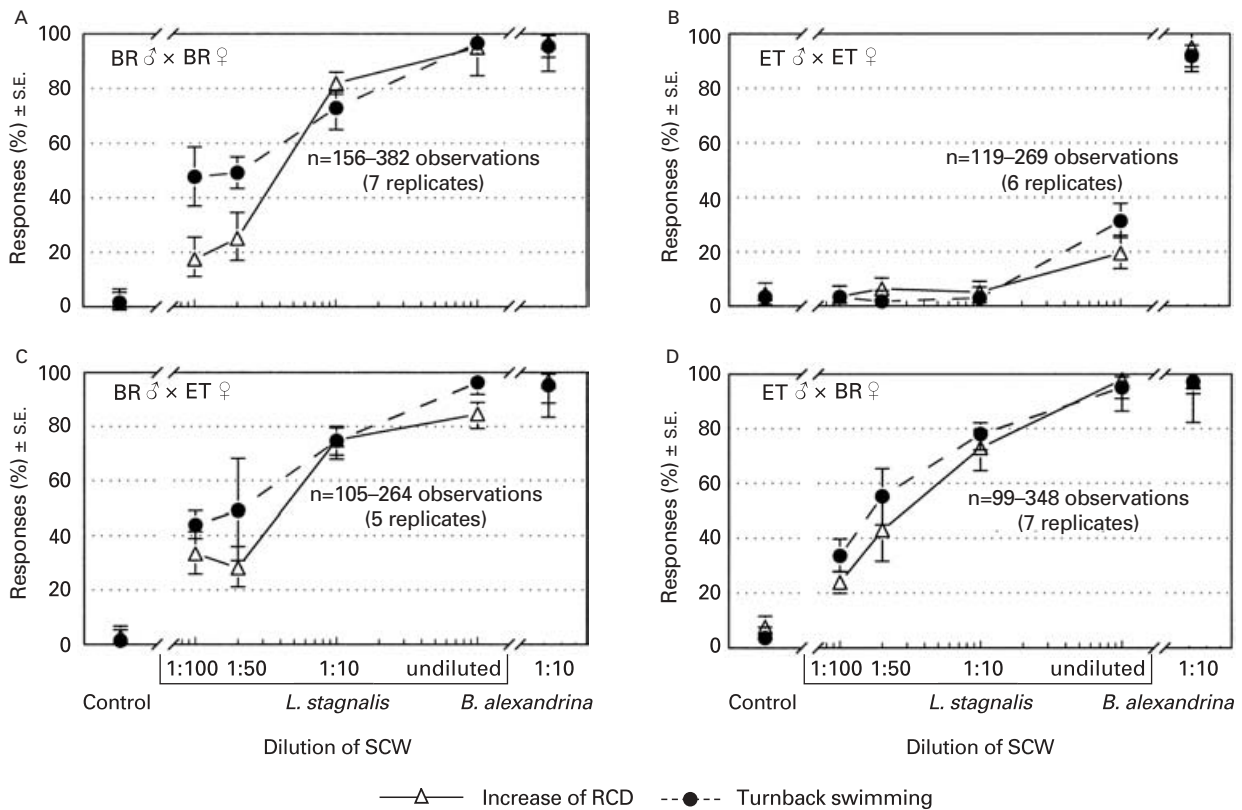

Fig. 3. Miracidial responses (%) to snail conditioned water from the non-host *Lymnaea stagnalis* in decreasing dilutions and from *Biomphalaria alexandrina*. (A) Pure-bred Brazilian *Schistosoma mansoni* strain; (B) pure-bred Egyptian *S. mansoni* strain; (C) F_1 hybrid of male Brazilian and female Egyptian *S. mansoni* strain; (D) F_1 hybrid of male Egyptian and female Brazilian *S. mansoni* strain.

Table 1. Infection rates in *Biomphalaria glabrata* and *B. alexandrina* after exposure to single miracidia of a Brazilian and an Egyptian *Schistosoma mansoni* strain and their F_1 hybrids in both parental combinations

| *S. mansoni* strain/crossing	Host snail	Infection rate of surviving snails (% ± S.E.)		Survival rate of snails (% ± S.E.)		Number of snails (replicates)
BR♂ × BR♀	*B. glabrata*	30·8	+10·0/−8·9	78·4	+4·4/−11·1	610 (6)
	B. alexandrina	0	—	56·9	+2·9/−2·9	211 (4)
ET♂ × ET♀	*B. glabrata*	6·5	+3·4/−2·5	95·6	+0·5/−0·5	227 (3)
	B. alexandrina	26·3	+12·9/−10·6	57·6	+12·3/−13·0	646 (7)
BR♂ × ET♀	*B. glabrata*	46·7	+7·3/−7·1	84·9	+0·1/−5·2	265 (4)
	B. alexandrina	1·3	+0·8/−0·4	69·3	+6·4/−7·2	231 (4)
ET♂ × BR♀	*B. glabrata*	29·4	+21·5/−16·5	89·9	+2·3/−5·0	189 (3)
	B. alexandrina	1·6	+1·6/−0·6	74·7	+8·2/−17·5	166 (2)
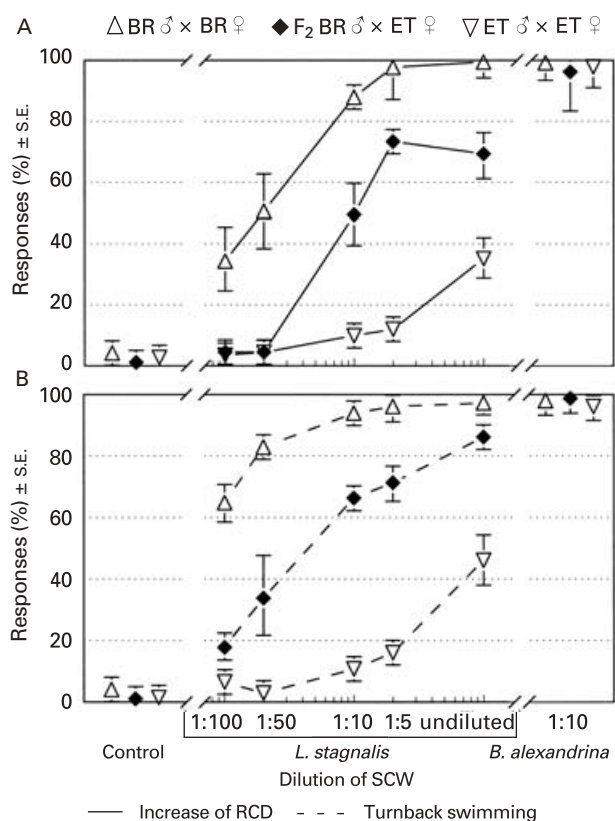
Fig. 4. Miracidial responses (%) of a F_2 hybrid of Brazilian and Egyptian *Schistosoma mansoni* to snail conditioned water from the non-host *Lymnaea stagnalis* in decreasing dilutions and from *B. alexandrina* as compared with the responses of the pure-bred parental strains. For clarity reasons, the behavioural patterns 'Increase of RCD' (A) and 'Turnback swimming' (B) are shown in separate graphs. Pure-bred BR strain, 89–696 observations (6–15 replicates); F_2 hybrid BR♂ × ET♀, 67–393 observations (4–9 replicates); pure-bred ET strain, 114–401 observations (6–15 replicates).

to America by the slave trade (Files & Cram, 1949; Després *et al*. 1993), where the parasite had to capture a new host snail species (Morgan *et al*. 2001).

There is one example showing that, in a population of *S. mansoni* introduced less than 400 years ago, heritable behaviour related to transmission has been

adapted to a new host: in different isolates of the parasite from the Carribean island of Guadeloupe, Chassé & Théron (1988) discovered differences in the circadian emergence rhythms of the cercariae released daily from snail hosts. Cercarial shedding maximum of *S. mansoni* infecting humans took place at noon, whereas cercarial emergence of the same species obtained from rodents with nocturnal activity exhibited a peak later in the afternoon. Reciprocal crosses of both chronobiological races resulted in F_1 offspring with a shedding maximum between the peaks of both parental lineages (Théron & Combes, 1988), indicating no clear genetical dominance of one of the two shedding patterns. Although limited genetic exchange is still possible, this sympatric alloxenic speciation (Théron & Combes, 1995) probably represents a continuous and ongoing process leading to distinct strains.

The behavioural adaptation investigated here is quite different: miracidia of the Brazilian *S. mansoni* strain did not shift their host-searching preference from an African snail to the American *B. glabrata*. Instead they seem to have abolished species-specificity in host-finding completely, as shown by the strong responses towards the allopatric non-host snail *L. stagnalis*. Although *B. alexandrina* appears to be almost insusceptible to the Brazilian strain, this snail was even more attractive to BR miracidia than their specific host *B. glabrata*. The natural distribution of *B. alexandrina* is restricted to the river Nile (Brown, 1994) and it does not occur in West and Central Africa, the geographical origin of American *S. mansoni* (Després *et al*. 1993). Therefore, the Egyptian parasites used here probably do not represent the ancestral population of the Brazilian strain in this study, but should be regarded as an example of an indigenous African *S. mansoni*. Hence, it would be very interesting to examine miracidial ability of West and Central African *S. mansoni* isolates to distinguish their intermediate hosts *B. pfeifferi* and *B. sudanica* from American host snails. Recent phylogenetic studies reveal that *B. glabrata* is more closely related to African *Biomphalaria* species than to other neotropical species of this genus (Woodruff & Mulvey,

1997; Campbell *et al.* 2000; DeJong *et al.* 2001). This close relationship might have contributed to the successful immunological adaptation of *S. mansoni* to *B. glabrata*. Nevertheless, it remains puzzling why the parasite did not also adjust its potentially highly specific host recognition mechanisms to this new host species. An explanation could be that changes in host-finding specificity take much more time than the indispensable ability to cope with the snail's internal defence system or that a reduction in specificity of the miracidial receptor(s) is an evolutionary one-way track. However, it is still not clear whether non-specifically responding miracidia have modified or lost receptors, or if they use additional receptors, recognizing more general characteristics of snail mucus. The latter scenario is likely since the Brazilian *S. mansoni* responds to a different fraction of *B. alexandrina* SCW than the Egyptian strain does (Haberl *et al.* unpublished observations) and to another fraction of *L. stagnalis* SCW than *Trichobilharzia ocellata* uses for specific host recognition (Kalbe *et al.* 2000). Additionally, the selection pressure for specificity in host finding, mainly determined by the abundance of host and non-host snails, could be different in the habitats in Africa and South America.

The extremely low infection rates of F_1 hybrids between Egyptian and Brazilian *S. mansoni* strains for *B. alexandrina* in this study disagree with many observations in other hybrids of *Schistosoma* strains or species, where the offspring usually were compatible with both parental snail hosts (Wright & Southgate, 1976; Paraense & Corrêa, 1981; Mutani, Christensen & Frandsen, 1985; Imbert-Establet & Combes, 1986; Tchuem Tchuenté *et al.* 1997; Webster & Southgate, 2003). This loss of specificity for *B. alexandrina* in their host-finding behaviour together with their ability to develop successfully in this snail might be a hint for a genetical linkage between these two aspects of parasite transmission. This link could also explain why the high degree of specificity has been maintained by the Egyptian *S. mansoni* strain even after several years of cycling in the laboratory. As miracidia are exposed to snails in a few ml of water they do not need effective host-finding and host-recognition mechanisms for transmission, but larvae unable to overcome the snail's internal defence system would be outcompeted, even under these artificial conditions.

A striking conclusion from the simple dominant Mendelian inheritance of non-specificity in snail recognition is that this complicated behavioural process seems to be controlled by a single gene or gene complex. It is possible that the dominant allele for non-host-specificity was always present at low frequencies in natural African *S. mansoni* populations. Even a small number of individuals with this genotype might have been sufficient to become the founder generation after introduction to America, since a reduced precision in discriminatory host finding increases the likelihood of encounters with potential new host species. However, in their natural habitat, maintenance of this genotype requires a high abundance of susceptible host snails in the sympatric mollusc fauna to ensure sufficient transmission rates by random contacts with suitable snails. Numerous reports from epidemiological surveys in Africa show that even in areas with a high prevalence of schistosomes in humans, infection rates in snails are astonishingly low. This seems to indicate that infecting a host snail is a very critical phase, probably under high selection pressure. Therefore, it appears uncertain whether miracidia with the disadvantage of carrying the non-specific host-finding allele (homozygous or heterozygous) are competitive enough to persist within a population capable of precise host-finding behaviour.

Alternatively, if the loss of specific host-finding behaviour appeared *de novo* in American *S. mansoni* strains, possible explanations could be either a random mutation or that digeneans might have evolved a kind of genetic switch mechanism. Although we could not demonstrate such an effect within only five generations of a laboratory strain in a non-natural host snail, it is conceivable that occasionally (or triggered by physiological parameters of the definitive host) a certain proportion of the parasite's offspring with the characteristic 'low host-finding specificity' appears. In case of a breakdown of the sympatric snail host population, or migration of the final host, these miracidia may become the founder generation of a non-specific strain. This new physiological strain would easily spread and replace the original genotype because of the genetical dominance of this trait. On the other hand, if the specific host species is available, these larvae would be readily outcompeted by their specifically responding siblings, which gain advantage from their reduced likelihood of detrimental encounters with non-host organisms (Combes, 1991).

The evolutionary and epidemiological significance of species-specific miracidial host-finding behaviour is often overlooked in investigations focussing on parasite–host interactions inside the snail. Yet, host-finding preferences seem to be strongly conserved and probably reveal much more reliable information about phylogeny and biogeographic history than sole observation of cercarial shedding, especially in laboratory-adapted strains. Neglecting the behavioural ability of miracidia to discriminate host snails from non-host snails underestimates the potential of such organism in which the central nervous system occupies as much as 8% of the whole body mass (Wilson & Denison, 1970). Nevertheless, a switch from high to low host-finding specificity as suggested by the findings of this study would be an adequate behavioural adaptation in order to acquire new intermediate hosts.

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