Host-finding in *Echinostoma caproni*: miracidia and cercariae use different signals to identify the same snail species

B. HABERL*, M. KÖRNER, Y. SPENGLER, J. HERTEL, M. KALBE *and* W. HAAS

Institute for Zoology I, University of Erlangen, Staudtstrasse 5, 91058 Erlangen, Germany

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SUMMARY

The snail host signals releasing host-finding responses in miracidia and cercariae of *Echinostoma caproni* were analysed by fractionation of snail-conditioned water (SCW). Cercariae responded non-specifically to organic and hydrophilic, low molecular weight components of SCW showing their typical turning response. Hydrolysis of peptides in SCW had no effect on cercarial responses. An artificial mixture of amino acids in concentrations determined from SCW as well as glycine alone in a concentration corresponding to the total concentration of amino acids in SCW showed nearly the same efficacy as SCW itself. Miracidia responded to a high molecular weight glycoprotein fraction, which could be isolated from SCW by ion-exchange and size-exclusion chromatography. In contrast to an Egyptian *Schistosoma mansoni* strain, the echinostome miracidia were not able to differentiate between different snail species. The results show for the first time that miracidia and cercariae of the same species may use different signals to identify the same snail host species. This indicates an independent evolution of host-finding mechanisms in the two parasite stages.

Key words: host-finding, *Echinostoma caproni*, miracidia, cercariae, snail host, chemo-orientation.

INTRODUCTION

The free-living larval stages of digeneans show a variety of behavioural adaptations which help them to locate and invade their hosts. After responding to environmental cues during the phases of dispersal and microhabitat-selection, they often use chemical host-signals for short-range orientation, identification of the host species and for invasion and transformation processes (Haas *et al*. 1995*a*; Haas & Haberl, 1997).

The different cercarial species studied so far used a high diversity of chemical host cues during host-finding. Even species infecting the same host genera often rely on different host signals, a phenomenon that might be related to different ecological conditions of the habitats in which the hosts are invaded (Haas, 1994). On the other hand, miracidia of 4 species studied by our group all responded to snail glycoproteins, when approaching the host and at least *Schistosoma mansoni* and *S. haematobium* also when identifying the snail host after contact (Haberl & Haas, 1992; Haberl *et al*. 1995; Kalbe, Haberl & Haas, 1997). This uniform response to similar host glycoproteins may reflect an adaptation to invade snails as hosts. In this context, the echinostomes are of special interest because in this family the cercariae also infect snails, often the same species as the miracidia. However, echinostome cercariae studied up to now used small molecular weight components of snail-conditioned water (SCW), such as amino acids, peptides, sugars, reduced glutathione and lipophilic substances as signals for approach to the snail host (Fried & King, 1989; Haas *et al*. 1995*b*; Fried, Frazer & Reddy, 1997; Reddy, Frazer & Fried, 1997; Körner & Haas, 1998*a*, *b*). Also the echinostome miracidia studied so far were reported to use only small molecular, acidic components of SCW as host-finding signals (Behrens & Nollen, 1992; Nollen, 1994). However, these compounds may not necessarily be identical to the attracting components in snail excretory–secretory (E–S) products as the results were obtained using pure chemicals at high concentrations in accumulation assays, which may easily lead to artifacts (Haberl, 1997; Haas & Haberl, 1997).

Would the cercariae and miracidia of one echinostome species use the same chemical snail signals as host-finding cues? We tried to answer this question by comparative analysis of the host-finding behaviour of *E. caproni* cercariae and miracidia. Our approach was to identify the releasing cues of host-finding behaviour in the two parasite stages by fractionation of SCW rather than by offering pure chemicals as in previous studies (Behrens & Nollen, 1992; Nollen, 1994; Fried *et al*. 1997; Reddy *et al*. 1997).

* Corresponding author. Tel: +49 9131-8528064.
Fax: +499131-8528040.
E-mail: bhaberl@biologie.uni-erlangen.de

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MATERIALS AND METHODS

E. caproni originated from Madagascar and was cycled through its laboratory host snail, a Brazilian albino strain of *Biomphalaria glabrata* and white mice. The snails were kept at 26 °C in aerated standard fresh water (SFW, Meier-Brook, 1978) on a 12 h light 12 h dark cycle and were fed with lettuce, fish food and hamster pellets.

Cercariae were obtained and washed with buffered tap water (pH 7·0, 5 mM phosphate buffer) as described elsewhere (Haas *et al.* 1995*b*). They were used in the experiments between 1 and 3 h after being shed.

Mice were infected with 35 metacercariae of *E. caproni*, obtained from the pericardium of infected *B. glabrata* serving as second intermediate host. The metacercariae were applied to the mice in 0·9% NaCl solution using a gastric tube.

Eggs were collected from faeces of patent mice and stored for 2 weeks at 26 °C in the dark. Miracidia were obtained by transferring fully embryonated eggs at 9.00 h in the morning in side-arm flasks with water at 26 °C and illuminating. The miracidia have a circadian hatching pattern (Jeyarasasingam *et al.* 1972; Behrens & Nollen, 1993) and a maximal hatching rate was obtained between 13.00 h and 14.00 h. The flask was put in a dark container and the positive photo- and negative geotactical miracidia (Jeyarasasingam *et al.* 1972; Moravec *et al.* 1974; Behrens & Nollen, 1993) accumulated in the illuminated side-arm. They were used for the experiments 1 h after hatching for up to 3 h.

S. mansoni and its intermediate host snail *B. alexandrina* originated from Egypt. The life-cycle was maintained using white mice as definitive hosts as described previously (Kalbe, Haberl & Haas, 1996). *Planorbarius corneus* and *Lymnaea stagnalis* were isolated from fish ponds near Erlangen and bred in our laboratory for several years. Fish (*Leucaspius delineatus*) and tadpoles (*Rana esculenta*) were collected from the same fish ponds.

Quantification of larval behaviour

Chemo-orientation mechanisms of cercariae and miracidia were studied using a one-arm chamber as described previously (Haberl *et al.* 1995; Haas *et al.* 1995*b*). Briefly, test substrates were applied to the end of a straight chamber and the behaviour of miracidia and cercariae entering or leaving the substrate containing section was recorded using a dissecting microscope. In the case of effective substrates, miracidia showed a vigourous increase in the rate of change of direction (RCD) when entering the field of inoculation and a turn-back response when leaving it. Cercariae showed no increase in the rate of change of direction, but did display a marked turn-back response. The ability of the cercariae for directed chemo-orientation was investigated in a T-chamber assay as described elsewhere (Haas *et al.* 1995*b*).

Experiments were carried out at 26 °C under illumination with cold light sources at irradiance intensities between 1 and 2 mW/cm². All media, containing parasites or test substrates were adjusted to the same pH, i.e. pH 7·0 (5 mM phosphate buffer) in experiments with cercariae and pH 7·5 (9 mM Tris buffer) in experiments with miracidia. A blinded protocol was used by giving the test substrates a code which was unknown to the experimenters.

The specificity of host-finding in miracidia was studied by offering identical batches of SCW from different snail species in the same experimental series to the miracidia of *E. caproni* and *S. mansoni*.

SCW and its fractionation

SCW was produced by placing 50 *B. glabrata* (or other snail species for specificity assays) with shell diameters between 5 and 10 mm in 50 ml of tap water for 2 h at 24 °C. The mixture containing snail excretory–secretory products was filtered through glass-fibre pre-filters (AP 25, Millipore, Witten, Germany) and stored at −20 °C. SCW was buffered and used undiluted in experiments with cercariae and diluted 1:10 with tap water in experiments with miracidia. Previous experiments with different dilutions of SCW have shown that these concentrations stimulate maximum host-finding activity in the respective parasitic stages. Water was conditioned with non-gastropod aquatic animals by placing the living organisms in tap water (1 ml per 0·1 g of the organism) for 2 h at 24 °C.

High and low molecular weight compounds of SCW were separated by molecular filtration at 4 °C. Up to 2 l of SCW were filtered using a 150 ml membrane filter stirred cell (Filtron, Northborough, Massachusetts) with a cut-off of 30 kDa at an operating pressure of 3·7 bar. Centriprep centrifugal concentrators (Amicon, Witten, Germany) with cut-offs at 30 kDa and 3 kDa were used for filtration of smaller volumes and for further fractionation of the fraction < 30 kDa. The filtrates were used as obtained, while the retentates were washed twice with 150 ml (stirred cell) or 15 ml (Centriprep concentrators) of tap water to remove low molecular weight residues. The concentrated fraction > 30 kDa was used for subsequent fractionation or adjusted to the initial volume with tap water for behavioural experiments.

The SCW fraction > 30 kDa was further fractionated by anion-exchange chromatography on a column (diameter 2·5 cm) packed with 35 ml of MacroPrep Q (Bio-Rad, Hercules, CA) at 22 °C. Twenty to 35 ml of concentrated SCW > 30 kDa were adjusted to a pH of 8·3 and applied to the column at a flow of 3·5 ml/min. The column was then washed with 70 ml of 50 mM Tris buffer, pH

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8·3, to remove unbound material. Chromatographic separation occurred at a flow rate of 7·5 ml/min running a linear gradient of 0 to 0·5 M NaCl in Tris buffer (300 ml) and 7·5 ml fractions were collected. Heavily bound material was subsequently washed from the column with 150 ml of 1 M NaCl in Tris buffer. Before each run, the column was washed with 150 ml of NaOH and regenerated according to the manufacturer's instructions.

Size-exclusion chromatography of SCW was performed on a column (diameter 1·6 cm, length 66 cm) packed with Sephacryl S-500 HR (Pharmacia LKB, Uppsala, Sweden). A concentrated and desalted fraction from ion-exchange chromatography was applied to the column using a 2·8 ml sample loop. The column was eluted at a flow rate of 1 ml/min with 150 mM ammonium acetate buffer and 5 ml fractions were collected.

The protein profile of all chromatographic separations was recorded at 280 nm. In addition, the sugar content in each collected fraction was determined by the resorcinol-sulfuric acid microtitre plate assay (Monsigny, Petit & Roche, 1988). Collected fractions were pooled according to their sugar content. For behavioural experiments the pooled fractions were adjusted to the initial volume with tap water.

Organic compounds in the SCW fraction < 30 kDa were destroyed by heating over a gas flame for 4 h. The residue was dissolved to the initial volume with deionized water.

Hydrophilic and lipophilic compounds were separated by mixing the SCW fraction < 30 kDa with a double volume of a mixture of chloroform and methanol (2:1). After separation of the 2 phases, the upper hydrophilic phase was removed with a pipette. Both phases were lyophilized and stored at -80 °C.

Peptides in the SCW fraction < 3 kDa were hydrolysed with 6 M HCl *in vacuo* at 110 °C for 22 h. HCl was removed from the samples by repeated lyophilization and redissolving.

Free amino acids in SCW < 3 kDa were analysed by HPLC on a RP-18 column (Lichrospher 100, 5 μm, 250 × 4 mm; Merck, Darmstadt, Germany) applying pre-column derivatization with *O*-phtaldialdehyde-3-mercaptopropionic acid (Graser *et al.* 1985) and fluorescence detection as described elsewhere (Haas *et al.* 1995*b*).

Statistical methods

Statistical analysis was performed using SPSS for Windows 95 (Ver. 7.5). Relative abundancies were arcsin $\sqrt{x}$-transformed to obtain approximately normally distributed data. Mean values and standard errors were computed from the transformed data and were retransformed for graphical visualization. For simplification only the larger of the two different standard errors is indicated in the table. Differences between means were tested for statistical significance by a multiple *t*-test procedure (Tukey-LSD-test;

Table 1. Turn-back responses of the cercariae of *Echinostoma caproni* when leaving fractions of SCW and pure chemicals (75–244 responses, 6–11 replicates)

	Substrate	Turn-back (% ± S.E.M.)
(A)	Inorganic components of SCW < 30 kDa	
	SCW, ashed	27·5 ± 4·8
	SCW	84·3 ± 2·8*
	Control	28·0 ± 4·7
(B)	Hydrophilic and lipophilic fractions of SCW < 30 kDa	
	Hydrophilic fraction	68·2 ± 8·5*
	Lipophilic fraction	44·4 ± 3·2
	Mixture hydrophilic + lipophilic fraction	77·2 ± 2·3*
	SCW	83·1 ± 2·2*
	Control	40·0 ± 7·5
(C)	SCW < 3 kDa after hydrolysis of peptides	
	SCW, hydrolysed	79·0 ± 3·2*
	SCW, similarly treated	82·5 ± 2·7*
	Control	41·8 ± 3·9
(D)	Amino acids (pure chemicals)	
	Amino acid mixture as identified in SCW‡	81·7 ± 4·0*†
	Glycine 5 mM	72·4 ± 6·1*†
	SCW < 30 kDa	92·9 ± 2·8*
	Control	54·1 ± 6·5

* Significant versus control.
† Significant versus SCW < 30 kDa.
‡ Concentrations (μM): ser 1·11; gly/thr 1·10; ile/phe/leu 0·52; ala 0·38; val/met 0·36; asn 0·32; asp 0·27; glu 0·19; orn 0·19; tyr 0·12; lys 0·09; his 0·08; arg 0·07; γ-aminobutyric acid 0·06.

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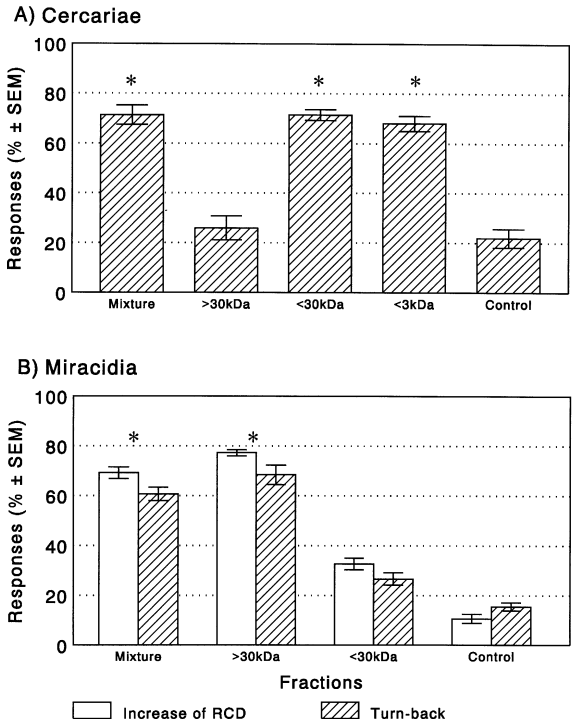

Fig. 1. Responses (%) of cercariae (A) and miracidia (B) of *Echinostoma caproni* to fractions of *Biomphalaria glabrata* SCW, obtained by molecular filtration; *, significant versus control; 95–138 responses, 10 replicates.

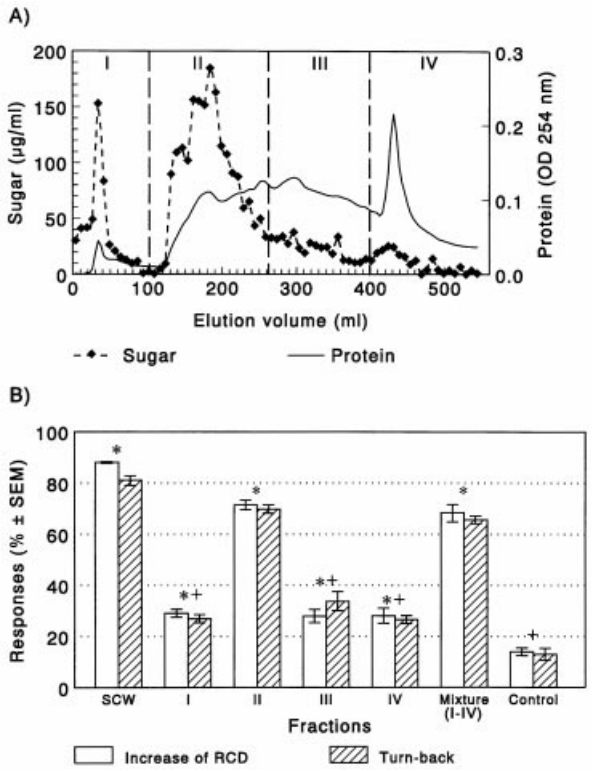

Fig. 2. Anion-exchange chromatographic separation of a SCW fraction > 30 kDa from *Biomphalaria glabrata* (A) and responses (%) of the miracidia of *Echinostoma caproni* to pooled fractions (B). Fraction I, eluted with Tris buffer 50 mM; fraction II, eluted with a linear gradient from 0 M NaCl to 0·28 M NaCl in Tris buffer; fraction III, eluted with a linear gradient of 0·28 M NaCl to 0·5 M NaCl in Tris buffer; fraction IV, eluted with 1 M NaCl in Tris buffer; *, significant versus control; +, significant versus SCW; (B), 85–135 responses, 8 replicates.

$P < 0{\cdot}001$). A χ^2-procedure with $P < 0{\cdot}001$ was used to test for statistically significant differences from an equal distribution in experiments on chemotactic orientation.

RESULTS

Cercariae of *E. caproni* showed no directed chemotactic orientation towards *B. glabrata*-SCW in the T-chamber, $56{\cdot}8 \pm 3{\cdot}3$ % entered the SCW-containing arm (control $53{\cdot}8 \pm 5{\cdot}5$ %, $n = 95$–145, 10 replicates, not significant versus equal distribution by χ^2-test). However, they performed turn-back responses in decreasing concentration gradients of SCW. Turn-back swimming was stimulated only by the low molecular weight SCW fractions < 30 kDa and < 3 kDa (Fig. 1A). The active components of SCW seem to be organic in nature, as ashing of SCW < 30 kDa fully abolished the stimulating activity (Table 1A). The cercariae responded only to the hydrophilic fraction of SCW < 30 kDa, the lipophilic fraction had no effect, neither did it have an effect in combination with the hydrophilic fraction (Table 1B). Peptides seem not to be responsible for the biological activity of SCW < 3 kDa as it was not altered by acid hydrolysis (Table 1C). An artificial amino acid mixture prepared according to an analysis of SCW had a similar effect on the cercariae as the low molecular weight fraction of SCW itself (Table 1D). However, glycine in a concentration similar to the total amount of amino acids in SCW, also stimulated a significant increase of the turning response in cercariae.

In contrast to cercariae, the miracidia responded with both orientation behaviours to the high molecular weight fraction of SCW (Fig. 1B). The fraction < 30 kDa had no effect, neither did it increase the efficacy of the high molecular weight compounds. After anion-exchange chromatography of the SCW fraction > 30 kDa a carbohydrate and protein rich fraction (fraction II) which was released from the column with up to 0·28 M NaCl stimulated maximum miracidia attracting activity (Fig. 2A, B). Further fractionation by size-exclusion chromatography resulted in 3 poorly separated protein peaks (Fig. 3A). Two of these peaks were heavily glycosylated. The activity on the miracidia was restricted to fraction III which contained most of the carbohydrate material (Fig. 3B).

The echinostome cercariae responded with a similar turn-back rate to water conditioned with different snail species, fish and tadpoles when the

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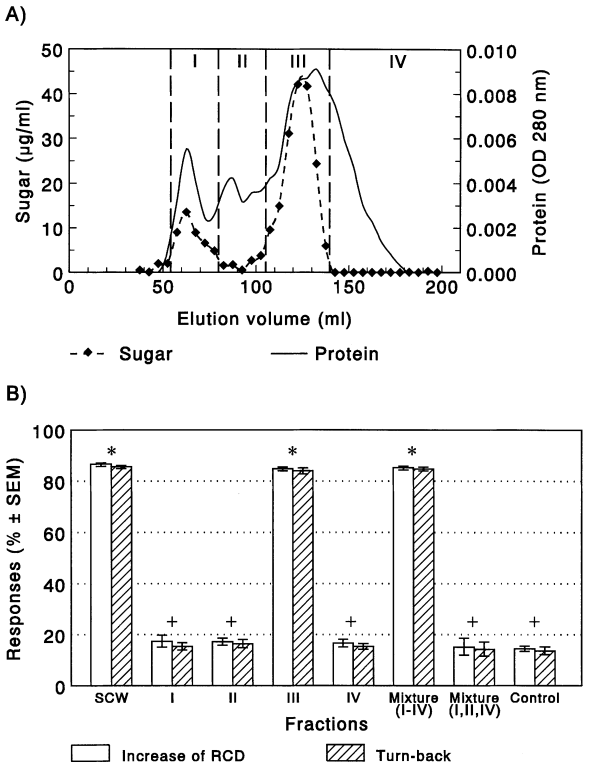

Fig. 3. Size-exclusion chromatographic separation of anion-exchange fraction II (Fig. 2) from *Biomphalaria glabrata* (A) and responses (%) of the miracidia of *Echinostoma caproni* to pooled fractions (B). Fractions were pooled according to their sugar content; *, significant versus control; +, significant versus SCW; (B), 63–153 responses, 11 replicates.

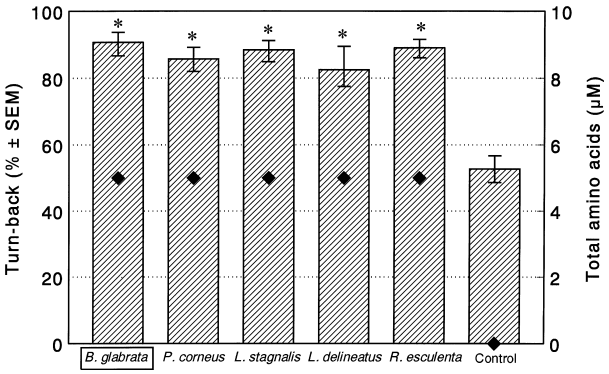

Fig. 4. Turn-back responses (%) of the cercariae of *Echinostoma caproni* when leaving water conditioned with different snail species, fish (*Leucaspius delineatus*) and tadpoles (*Rana esculenta*). The conditioned media were adjusted to equal total amino acid concentrations (5 µM) after HPLC analyses; *, significant versus control; 56–85 responses, 6 replicates.

conditioned water was diluted to similar total concentrations of amino acids (Fig. 4). Also, the echinostome miracidia could not differentiate between different snail species during host-finding, while the *S. mansoni* miracidia responded only to SCW from their host snail *B. alexandrina* (Fig. 5A, B).

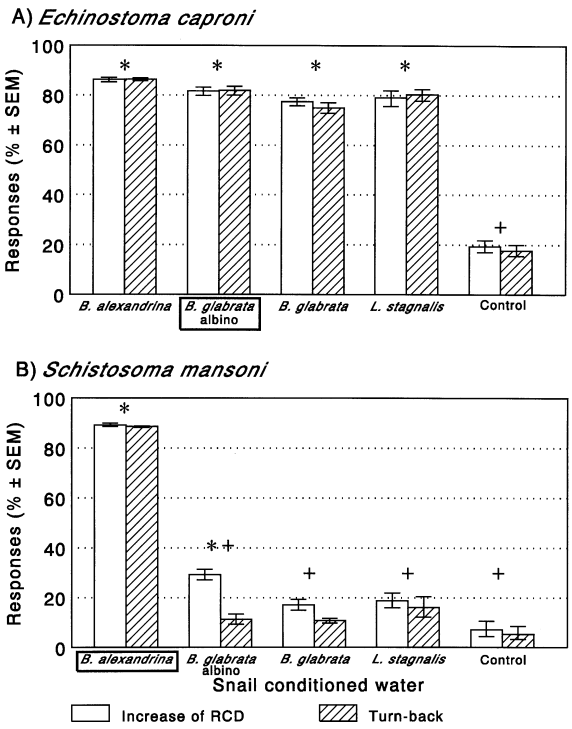

Fig. 5. Responses (%) of miracidia of *Echinostoma caproni* (A) and of an Egyptian *Schistosoma mansoni* strain (B) to identical batches of water conditioned with different snail species; *, significant versus control; +, significant versus host snail (boxed); 53–70 responses, 5 replicates.

DISCUSSION

The aim of this study was to compare the snail-host finding of miracidia and cercariae of *E. caproni* using similar experimental designs. However, the results obtained seem to be contradictory to previous results.

The miracidia of *E. caproni* responded exclusively to high molecular weight glycoproteins of SCW, like 4 other miracidial species studied by our group (Haberl & Haas, 1992; Haberl *et al.* 1995; Kalbe *et al.* 1996, 1997) and probably also like *S. japonicum* miracidia (Haas *et al.* 1991). These results are contradictory to those of Behrens & Nollen (1992), who reported acidic components, such as glutamic acid, aspartic acid, acetic acid and H_2SO_4, to cause accumulation in choice chambers. However, such an accumulation could be due to toxic effects of the acidic components. We have demonstrated that toxic chemicals, such as ethanol, cause comparable accumulation of miracidia in choice chambers as SCW (Haberl, 1997; Haas & Haberl, 1997; Kalbe & Haberl, unpublished results).

Glycoproteins have been shown to enable the miracidia to determine their host snail species with high precision (Kalbe *et al.* 1996, 1997). The miracidia of *E. caproni*, however, were not able to differentiate their host snail from other snail species. This might be an artifact due to prolonged maintenance of the parasite cycle in the laboratory in a

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host snail that is not natural for *E. caproni*. We found non-specific host-finding also in miracidia of 2 Brazilian strains of *S. mansoni*, one of which has been maintained in the laboratory for more than 30 years. There is some evidence from infection studies that echinostome miracidia are capable of distinguishing between different snail species. Miracidia of *Hypoderaeum conoideum* produced comparable infection rates in *L. peregra* and *L. corvus*, but they showed a clear preference for *L. peregra*, when they had the opportunity to choose between the 2 snail species (Toledo *et al*. 1999). Studies with freshly isolated parasite strains would be necessary to elucidate, whether non-specificity in *E. caproni* miracidial host-finding and in the Brazilian schistosome strains is a natural strategy or just a side-effect of laboratory maintenance.

Most species of cercariae studied so far seem not to show chemo-orientation when swimming free in the water (reviewed by Haas, 1994; Haas & Haberl, 1997). However, chemo-orientation may be typical for echinostome cercariae which invade slowly moving snail hosts. The mechanisms of chemo-orientation are known for 3 species. *E. revolutum* and *Pseudechinoparyphium echinatum* show turn-back swimming in decreasing concentration gradients of attractants while *H. conoideum* swims directed chemotactically along increasing gradients (Haas *et al*. 1995*a*, *b*). *E. caproni* displays a similar orientation behaviour as *E. revolutum* and *P. echinatum* and responds to similar cues in SCW. Amino acids account for most of the orientation stimulating activity in the 3 species. In *E. revolutum* and *P. echinatum* the full activity of SCW was obtained by a mixture of amino acids, ammonia, and urea in concentrations as determined in SCW (Körner & Haas, 1998*b*). The same may occur in *E. caproni* as the turn-back rate of the cercariae in response to a mixture of amino acids as contained in SCW was still 10% lower than in response to SCW. Lipophilic components and peptides of SCW did not stimulate turn-back responses in *E. caproni* cercariae. This is in contrast to the results of Fried *et al*. (1997) who reported that *E. caproni* cercariae aggregate around agar plugs containing lipophilic snail components. As stated before this discrepancy might be due to the fact that Fried *et al*. (1997) used an aggregation assay, which does not differentiate between orientation and, for example, penetration stimuli. Also toxic effects or pH differences might lead to an aggregation of the cercariae.

Thomas & Eaton (1993) reported that the amino acid composition of SCW from different snail species is species-specific if the snails are kept under constant conditions. They speculated that this might be used by conspecifics, predators or trematode parasites to identify the snail species. Indeed, cercariae of *P. echinatum*, which also respond to amino acids, showed clear differences in the turn-back response to living *L. stagnalis* and *L. palustris* (Haas *et al*. 1995*b*). Also Fried *et al*. (1997) and Reddy *et al*. (1997) suggested that cercariae of *E. caproni* and *E. trivolvis* might exhibit a certain snail host specificity during host-finding. This was based on the findings that cercariae of *E. caproni* and *E. trivolvis* showed differences in their responses to different low molecular weight chemicals and that *E. caproni* cercariae differentiated between snail species, but only if the snails were constrained in dialysis membrane sacs. However, our results show that *E. caproni* as well as *P. echinatum* and *E. revolutum* cercariae (Körner & Haas, 1998*a*) responded mainly to the total amount of host-emitted amino acids and they could not differentiate between E–S products of different snail species, fish or tadpoles if the amino acid content was adjusted to the same total concentration. The cercariae seem not to differentiate between amino acid types, and for *E. revolutum* and *P. echinatum* it was shown that the cercariae respond mainly to the primary α-amino group and the α-carboxyl group of the amino acids (Körner & Haas, 1998*b*). These findings, together with the fact that amino acid composition of SCW differs considerably among individual snails (Körner & Haas, 1998*a*), make it very unlikely that the echinostome cercariae studied exhibit a narrow host-species specificity during host-finding. This view is supported by the fact that encysted metacercariae of these echinostomes may be found in a variety of different snail species, as well as in tadpoles (Yamaguti, 1975; Huffman & Fried, 1990; Fried & Huffman, 1996). However, there is evidence for some degree of differential compatibility between echinostome cercariae and second intermediate host snails, that is at least in part determined by host-finding or host-invasion mechanisms (Evans, Whitfield & Dobson, 1981; Evans & Gordon, 1983; McCarthy & Kanev, 1990). A certain host-specificity was found at the attachment and enduring contact phases (Haas *et al*. 1995*b*) and might also occur in the penetration behaviour.

Miracidia and cercariae of *E. caproni*, which share the same genome, use different signals and thereby different receptors to fulfil the same task, finding and recognizing a suitable snail host. The trematode miracidia have evolved sophisticated mechanisms, which at least bear the possibility of identifying the appropriate host snail species with great accuracy with the help of complex glycoprotein signals. Also the miracidia of *E. caproni*, even if obviously not host-specific in our experiments, use such signals during host-finding. Why are these receptors not used by the cercariae? The answer might come from current concepts of trematode life-cycle evolution (Gibson, 1987; Rhode, 1994). The miracidia are thought to have evolved very early as ciliated larvae of a turbellarian-like parasite of molluscs to facilitate the transmission to the molluscan hosts. For up to

200 million years trematodes might have been exclusively parasites of molluscs. This explains the intimate relationship of miracidia and sporocysts with their snail host. Sporocyst development is usually only possible in one or a few particular snail species and many trematode species are able to exploit their first intermediate host in a highly efficient way, undergoing an enormous multiplication (Whitfield & Evans, 1983). In contrast, the cercariae have been introduced in the trematode life-cycle very late, after vertebrate definitive hosts have already been acquired. Their task is to facilitate transmission to the early definitive fish host by encysting in the environment or in other prey of the host. In such a situation a restricted host specificity similar to that of the miracidia would not efficiently increase the chance of transmission for the parasite, but would lead to intraspecific competition of sporocyst and metacercarial stages. Therefore, one would expect that the cercariae have evolved mechanisms to avoid penetration of already infected hosts (Christensen, Frandsen & Roushdy, 1980; McCarthy & Kanev, 1990). On the other hand it might have been an advantage for the parasite to introduce a larval stage with a relatively low host specificity, to overcome the disadvantages of the miracidial dependency on one certain host species. The thousands of cercariae, which are genetically identical with the miracidium from which they descend (Whitfield & Evans, 1983), could be the 'experimental stage', responsible for the acquisition of new hosts and thereby increase the chance of reaching the reproductive stage in the definitive host, even if the first intermediate host is rare. The combination of these two strategies, a very narrow host specificity in miracidia and a relatively broad specificity in cercariae, i.e. a broad encounter filter (Combes, 1991), could be evolutionary stable as it assures reasonably high infection levels in the definitive host more or less independent of fluctuations in the intermediate host populations.

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