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Miracidial infectivity of *Hypoderaeum conoideum* (Trematoda: Echinostomatidae): differential susceptibility of two lymnaeid species

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Abstract A study was made of the infectivity of *Hypoderaeum conoideum* miracidia to a range of laboratory-reared specimens of freshwater snail species (*Lymnaea peregra*, *L. corvus*, *Physella acuta*, and *Gyraulus chinensis*) that coexist with the parasite in the same natural habitat. *L. peregra* and *L. corvus* were found to be equally susceptible to the parasite when specimens of each snail species were singly exposed to miracidia. However, when miracidia could choose either lymnaeid species, they showed a high degree of specificity toward *L. peregra*. The results obtained suggest that *H. conoideum* miracidia are capable of distinguishing among these lymnaeids in their orientation to the host. This indicates that miracidia might achieve specificity before actually contacting the snail host and suggests that during the host-snail orientation process they respond to signals different from those generated upon snail contact and invasion. The specificity toward *L. peregra* observed in *H. conoideum* miracidia seems to indicate adaptation to the snail community in their natural habitat, resulting in enhancement of their transmission.

Introduction

The miracidial host specificity of digenetic trematodes plays a major role in their transmission and is of great parasitological and epidemiological importance. In recent years, several studies have dealt with the question of how miracidia find, recognize, and invade their snail hosts – particularly for schistosomes (see Christensen 1980; Haas et al. 1995a; Haas and Haberl 1997). There is general agreement that snail susceptibility to miracidia is highly specific, though the published data on how miracidial host specificity is achieved are confusing and even contradictory. For several trematode species, or even strains, the processes occurring prior to contact with the snail host seem to contribute greatly to host specificity, since the former are capable of discriminating between different snail species (Haberl et al. 1995; Kalbe et al. 1996, 1997). However, in other species, miracidia have not been capable of distinguishing between different snail species – including their appropriate hosts – before the actual establishment of contact with them (Haas et al. 1991; Kalbe et al. 1996). In the family Echinostomatidae, only some data concerning the effect of snail-conditioned water and several pure chemicals upon the miracidia of *Echinostoma caproni* Richard, 1964 and *E. trivolvis* (Cort, 1914) are available (Behrens and Nollen 1992; Nollen 1994).

It is known that several species of the genus *Lymnaea* Lamarck, 1799 (Gastropoda: Lymnaeidae), including those from the same geographic locality, act as natural first intermediate hosts of *Hypoderaeum conoideum* (Bloch, 1782) (Trematoda: Echinostomatidae). However, in the natural reference habitat of the present study – the Albufera Natural Park (Valencia, Spain) – only *L. peregra* (Müller, 1774) has been recorded as a natural first intermediate host of this echinostomatid, despite the presence of other lymnaeids (Toledo et al. 1996, 1998).

The purpose of the present study was to establish the infectivity of *H. conoideum* miracidia to a range of sympatric freshwater snail species and to investigate the preference of this miracidium for various susceptible snail species. Due to the miracidial infectivity pattern observed, this work constitutes an initial step in the study of how the miracidial host specificity is achieved.

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Materials and methods

Parasite and hosts

Our laboratory stock of *Hypoderaeum conoideum* was originally isolated from cercariae released from naturally infected *Lymnaea peregra* snails collected in the Albufera Natural Park (Valencia, Spain) and has been cycled in our laboratory for 3 years, with laboratory-reared *L. peregra* serving as the first intermediate host; laboratory-reared *L. peregra*, *L. corvus* (Gmelin, 1791), *Physella acuta* (Draparnaud, 1805), and *Gyraulus chinensis* (Dunker, 1848) serving as second intermediate hosts; and *Gallus gallus* Linnaeus, 1758 serving as the final host (Toledo et al. 1996). Feces of infected chicks were filtered on a series of steel sieves of decreasing pore size (from 500 to 40 μm) to obtain eggs. Miracidia were obtained after hatching of the eggs incubated in darkness in spring water at 20 ± 1 °C.

Only infection-free, laboratory-reared snails from strains originating from the same biotope on the Albufera Natural Park, having a shell size of 3–7 mm at the time of the infection, were used.

Single-host experiments

In all, 20 specimens of each snail species (*L. peregra*, *L. corvus*, *P. acuta*, and *G. chinensis*) were singly exposed to 1, 3, and 5 newly hatched miracidia (maximal age 30 min) for 12 h in 3 ml of spring water at 20 ± 1 °C. Exposures were conducted in shallow glass dishes. After exposure, snails were kept under a day:night rhythm of 12:12 h at 20 ± 1 °C and were fed with washed lettuce ad libitum. At 8 weeks after exposure, individual snails were investigated for shed cercariae. Those snails that failed to shed cercariae were crushed and examined for infection.

Two-choice experiments

As the results obtained from the experimental procedure outlined above suggested an interesting pattern of miracidial infectivity of *H. conoideum*, further experiments were conducted with the aim of investigating the transmission patterns when a single miracidium was presented to two specimens of different susceptible snail species. The infection procedure involved the placement of one newly hatched miracidium in a shallow glass dish containing two snails (a single specimen each of *L. peregra* and *L. corvus*) in 3 ml of spring water at 20 ± 1 °C for 12 h. The maintenance conditions for the exposed snails were the same as those described above. In all, 30 replicate exposures of the experimental pairs were conducted.

Results

The miracidial infectivity of *Hypoderaeum conoideum* to four snail species is summarized in Table 1. In single-snail experiments, *Physella acuta* and *Gyraulus chinensis* were not infected, and only *Lymnaea peregra* and *L. corvus* proved susceptible to *H. conoideum* miracidia. A total of 28 (46.7%) specimens of *L. peregra* and 20 (33.3%) *L. corvus* became infected, although the proportions of infected snails belonging to each snail species varied according to the miracidial doses employed (Table 1). However, application of the chi-square test to the numbers of snails infected for each miracidial dose showed the differences between the two lymnaeid species to be nonsignificant [P (χ^2 = 1.21, 0.64, 1.67, and 0.98 for 1, 3, and 5 miracidial doses and for the entire sample, respectively) > 0.05; df = 1]. No significant difference was detected in the number of daily cercariae emitted between the two lymnaeid species.

The results of the experiments conducted to investigate the pattern of miracidial infectivity in two-choice communities showed that only specimens of *L. peregra* (20%) became infected (Table 1). It should be mentioned that the proportion of specimens of *L. peregra* that became infected was identical to that observed in the single-snail exposures to a monomiracidial dose.

Discussion

The first intermediate host spectrum of *Hypoderaeum conoideum* experimentally observed in the present study is restricted to the genus *Lymnaea*. None of the other gastropod species belonging to other genera became infected, which is in agreement with previous literature (Mathias 1925; Rees 1932; Grabda-Kazubska and Kiseliene 1990; Haas et al. 1995b; Toledo et al. 1996, 1998). However, an interesting pattern in *H. conoideum* miracidial infectivity was observed. In single experiments the rate of infection in *L. peregra* and *L. corvus* showed no significant difference. In these experiments the snails were individually exposed to the miracidia in a small volume of water and, hence, the miracidia had no choice. This indicates that *H. conoideum* miracidia are capable of invading both lymnaeids and that these snail species exhibit similar susceptibility. When *L. peregra* was placed together with *L. corvus*, miracidia of *H. conoideum* were infective only to *L. peregra*. Moreover, the observation that the rate of infection obtained under these conditions for *L. peregra* was identical to that observed in the monomiracidial single-snail exposures suggests that the presence of another susceptible host does not reduce the overall level of parasite transmission and points to a high degree of specificity in the two-choice experiments. This finding agrees with the previous field studies carried out by Toledo et al. (1998) in the Albufera

Table 1 Results of the single and choice experiments on the infectivity of miracidia of *Hypoderaeum conoideum* to four laboratory-reared freshwater snail species

Snail species	Miracidial dose	Number of snails infected/ exposed (%)
Lymnaea peregra	1	4/20 (20)
	3	12/20 (60)
	5	12/20 (60)
L. corvus	1	8/20 (40)
	3	8/20 (40)
	5	4/20 (20)
Physella acuta	1	–/20 (0)
	3	–/20 (0)
	5	–/20 (0)
Gyraulus chinensis	1	–/20 (0)
	3	–/20 (0)
	5	–/20 (0)
L. peregra-*L. corvus*	1	6–0/30–30 (20–0)

Natural Park (Valencia, Spain), who found that *L. peregra* acted as the first intermediate host of *H. conoideum*, whereas no specimen of *L. corvus* was infected.

The mechanisms that generate miracidial host specificity are complex and not fully understood (Haas and Haberl 1997). A wide range of factors related to the parasite, to the snail host, and even to the environmental conditions seems to be involved (Christensen 1980; Haas and Haberl 1997). Much work currently deals with the question of how this specificity is achieved by intrinsic factors of the snail or parasite – such as snail internal-defense systems or parasite immune-evasion mechanisms. However, our results indicate that these intrinsic factors do not determine the miracidial host specificity of *H. conoideum*, since the parasite could successfully develop to the same degree in the two lymnaeids in the single experiments. Thus, *H. conoideum* miracidia might achieve their host specificity before actual invasion of the snail and are probably capable of discriminating between *L. peregra* and *L. corvus* during their period of host-finding behavior.

It is accepted that miracidial host-finding involves several steps – each determining a certain degree of specificity (Haberl et al. 1995). Dispersal and microhabitat selection phases are controlled by internal and environmental factors, but this effect was overlooked in our experiments, in which the snails were exposed to the miracidium in only a few milliliters of water. Consequently, our results are related to orientation to the host and behavior after contact with the host. It is now recognized that these latter steps of the host-finding behavior are stimulated by snail-emitted chemical signals that have been identified as macromolecular glycoconjugates for several miracidial species (Haas et al. 1991, 1995a; Haberl et al. 1995; Kalbe et al. 1996, 1997; Haas and Haberl 1997). Unfortunately, the chemical cues for the orientation of *H. conoideum* miracidia have not been investigated to date. Nevertheless, the results of our choice experiments suggest that a miracidium capable of discriminating between *L. corvus* and *L. peregra* in orientation to the host. This could indicate that the chemoorientation to the host is stimulated by cues other than those implicated in the invasion responses. However, confirmation of this view requires detailed knowledge of the chemical characteristics of the signal substances for *H. conoideum* miracidia. Hence, the signal molecules from *L. peregra* and *L. corvus* should be comparatively analyzed in an attempt to gain further insight into this miracidial behavior.

The ability of miracidia to discriminate among snail species even while approaching them has been observed in *Schistosoma haematobium* (Bilharz, 1852) (Haberl et al. 1995), the Egyptian strain of *S. mansoni* Sambon, 1907 (Kalbe et al. 1996), and *Fasciola hepatica* (Linnaeus, 1758) and *Trichobilharzia ocellata* (La Vallette, 1855) (Kalbe et al. 1997), among others. This ability has been recognized as an ecological advantage for the miracidia of these trematode species, as it allows them to avoid contact with unsusceptible snail species that reduce their chances for future successful infection (Combes and Moné 1987).

However, in our case this cannot been considered an ecological advantage, since both lymnaeids exposed were susceptible to infection and, under our experimental conditions, both species seemed capable of acting as required hosts (Holmes 1979). Although the difficulty of evaluating the correspondence between experimental results and those obtained in the field is recognized, we consider that the preference of the *H. conoideum* miracidium for *L. peregra* observed in the laboratory and in the field (Toledo et al. 1998) could be due to ecological factors. In this sense, the behavior shown by *H. conoideum* miracidia could have been the result of a high degree of adaptation to the snail community structure in their natural habitat, where *L. peregra* is one of the most frequently occurring snail species, whereas *L. corvus* is less commonly encountered (Toledo et al. 1998). Thus, coexistence of the parasite with *L. peregra* could have led the miracidia to acquire a high degree of specificity in host location toward this snail species. However, the ability of *H. conoideum* to develop in several lymnaeid species could enhance parasite transmission when changes in snail populations occur – e.g., after ecological changes in the water system (Toledo et al. 1998).

The possible relationship between miracidial host location and adaptation of the trematodes to the molluscan community structure in a determined geographic locality has previously been suggested. Miracidia of the Brazilian strains of *S. mansoni* have been incapable of distinguishing among several snail species in their host location (Kalbe et al. 1996). These authors have suggested that during adaptation of *S. mansoni* to new intermediate host snails in America the parasite could have lost its original host specificity. This situation is the opposite of that observed in our study, although it reveals that the degree of adaptation of the trematodes to their natural habitat and its ecological conditions plays a major role in miracidial host specificity.

All the above-mentioned data show that *H. conoideum* may represent a suitable laboratory model for the study of miracidial host-finding processes to gain a deeper understanding on the basis of their host specificity.

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