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Redial growth and cercarial productivity of *Fasciola hepatica* in three species of young lymnaeid snails

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Abstract

Experimental infections of 1-mm high snails using three populations of *Lymnaea* (*L. glabra*, *L. ovata* and *L. truncatula*) and a cattle strain of *Fasciola hepatica* miracidia were carried out under laboratory conditions to determine if the snail species had an effect on the number of free rediae, their growth, and cercarial productivity in relation to each redial category (R1a, R1b, R2a, or R2b/R3a). The total number of rediae ranged from 6.4 to 7.5 per snail. The mean body length of rediae varied from 1–1.2 mm (R1a) to 0.3–0.4 mm (R2b/R3a). The width of the intrapharyngeal lumen also varied from 26.0–38.8 μm to 3.0–4.2 μm, respectively. The redial category had a significant effect on both measurements, whereas snail species only had a significant influence on body length. The mean number of cercariae produced by all living rediae at day 49 post-exposure ranged from 63.0 in *L. glabra* to 87.2 in *L. truncatula*. In *L. ovata* and *L. truncatula*, 55.8% and 58.6% of cercariae, respectively, were produced by R2a rediae, whereas 53.9% of cercariae in *L. glabra* were formed by the R1b rediae. When young snails were infected with *F. hepatica*, the species of snail had an effect on the number of living rediae, their length and their cercarial productivity.

Introduction

At least three redial generations of *Fasciola hepatica* successively develop in single-miracidium infections of *Lymnaea truncatula*. The number of living rediae in each generation, their growth and larval productivity are dependent on the life or the early death of the first mother redia (R1a) after its exit from the sporocyst, so that two developmental patterns (usual, or unusual) were described for these redial generations (Augot *et al.*, 1998, 1999; Augot & Rondelaud, 2001). However, all these studies were performed using 4-mm high snails and the first objective of the present study is to examine what happens in young snails infected by *F. hepatica*. In the latter snails, there were only small numbers of living rediae and the numbers increased with increasing shell height (Rondelaud & Barthe, 1978), although less than 20 cercariae were produced per snail (Busson *et al.*, 1982). As many *Lymnaea* species from western Europe, other than *L. truncatula*, can sustain full development of *F. hepatica* in very young specimens (Boray, 1978), the second aim was to determine if the species of snail intermediate host influenced the growth and larval productivity of rediae in these young snails. Experimental infections of 1-mm high snails with *F. hepatica* were thus performed using three lymnaeid species: *L. glabra*, *L. ovata* and *L. truncatula* (Glöer & Meier-Brook, 1994).

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Materials and methods

Populations of *L. glabra* and *L. truncatula* originated from ditches along the road D 72 at Berneuil, department of Haute Vienne, central France, whereas the population of *L. ovata* originated from a brook at Thenay, department of Indre. Adult snails were collected from each population in November 1999 and were maintained in aquaria at 20°C under laboratory conditions until they had laid eggs. The progeny of the snails measuring 1 mm in height were subsequently used for miracidial exposure. Eggs of *F. hepatica*, which were collected from the gallbladders of heavily infected cattle, were washed several times with spring water and incubated in complete darkness for 20 days at 20°C.

Three groups of 100 young snails each (one per population) were formed. Each snail was subjected to a bimiracidial exposure for 4 h. The choice of two, rather than one, miracidia per snail was preferred because the infection rate of *L. truncatula* was higher and in 85% of infected snails, only one of the two miracidia developed (Préveraud-Sindou & Rondelaud, 1995). The three groups of snails were then reared for 49 days in polypropylene boxes 1 m by 55 cm and 15 cm high, with 50 snails per box. Each box, containing small stones and a 2-cm deep layer of water, was constantly aerated. Snails were fed with steeped lettuce. Boxes were maintained at 20°C, with a diurnal photophase of 3000–4000 lux light intensity.

Snail sampling was only made at day 49 post-exposure (p.e.) to have 20–30 infected snails per population. Surviving snails were measured under the stereomicroscope before being dissected in tap water to determine if R1a rediae were alive or dead. Rediae originating from snails with living R1a stages were examined using an image-processor (Aries, Châtillon, France) to determine the maximum length of each redia, the maximum width of the intrapharyngeal lumen, and also quantify intraredial morulae, daughter rediae, and procercarial embryos. A comparison test of experimental frequencies, and a two- or three-way analysis of variance (Stat Itcf, 1988) were used to establish levels of significance. Cercarial productivity of rediae (Augot & Rondelaud, 2001) was estimated using the difference between the highest mean number of morulae (N1) in a 0.3–0.5 mm long redia and the mean number of morulae and procercarial embryos (N2) remaining in the body of the longest rediae at day 49 p.e. (daughter rediae were also counted in N2).

Living rediae were also classified, according to the gross morphology of the pharynx, into the following categories (Augot *et al.*, 1998): R1a, the first appearance of mother redia from the sporocyst; R1b, the second appearance of mother rediae from the sporocyst; R2a, daughter rediae from R1a; R2b, daughter rediae from R1b: and R3a, grand-daughter rediae from R1a. Because the two last groups could not be differentiated, they were pooled into a single group R2b/R3a.

Results

Table 1 gives the values of four parameters at day 49 p.e. First, the survival rates of snails ranged from 39% to 52% and no significant differences were evident between the three groups. Secondly, the prevalence of infection ranged from 36% to 63% and a significant difference ($P < 0.05$) only occurred between *L. ovata* and *L. truncatula*. Thirdly, the growth of infected *L. truncatula* was significantly lower ($F = 17.35$, $P < 0.001$) than those recorded in the two other snail groups. Finally, the total number of living rediae ranged from 6.4 to 7.5 per snail and no significant differences were evident between the snail groups.

Table 2 gives the body and pharyngeal measurements at day 49 p.e. The mean body lengths varied from 1–1.2 mm (R1a rediae) to 0.3–0.4 mm (R2b/R3a rediae). The redial category ($F = 38.47$, $P < 0.001$) and snail species ($F = 3.93$, $P < 0.05$) had significant effects on the body lengths of the rediae. The width of the intrapharyngeal lumen also varied from 34.9–38.8 μm (R1a rediae) to 3.0–4.2 μm (R2b/R3a rediae). However, only the redial category had a significant influence ($F = 38.47$, $P < 0.001$) on the width of the intrapharyngeal lumen.

Table 3 gives, for each living redia, the number of morulae differentiating into cercariae for each redial category and each lymnaeid species. In *L. ovata* and *L. truncatula*, most cercariae were produced by the R2a rediae (12.4 and 14.2 cercariae per redia, respectively) and corresponded to 55.5% and 58.6% of cercariae formed by all living rediae, whatever their category (table 3). In *L. glabra*, most cercariae were produced by R1b rediae and corresponded to 53.9% of all cercariae. In all snail groups, the number of cercariae, differentiating in the body of R2b/R3a rediae, was low. If the numbers of cercariae produced by living rediae (table 3) are added in each snail group, rediae from each *L. glabra* produced 63.0 cercariae, whereas the total number of cercariae in each of *L. ovata* and *L. truncatula* was 82.5 and 87.2, respectively.

Discussion

The heavy mortality of young snails on day 49 p.e., the growth of young infected snails, and the total number of living rediae verified the first findings of Kendall (1950), Rondelaud & Barthe (1978) and Busson *et al.* (1982). The results obtained for the body and pharyngeal measurements of living rediae agreed with those of Augot *et al.* (1998, 1999) in preadult *L. truncatula* and demonstrated that the growth of living rediae within the intermediate host was mainly dependent on redial category. A

Table 1. General characteristics of *Fasciola hepatica* infections in the three groups of young *Lymnaea* species at day 49 p.e.

Parameters	*L. glabra*	*L. ovata*	*L. truncatula*
Survival rate of snails (%)	39.0	52.0	44.0
No. of snails with *F. hepatica* larvae (and prevalence* in %)	22 (56.4)	19 (36.5)	28 (63.6)
Height growth** in mm: mean ± SD	2.5 ± 0.8	2.9 ± 0.7	1.6 ± 0.7
No. of snails with a living R1a redia	17	14	20
Total number of living rediae: mean ± SD	6.4 ± 1.8	7.5 ± 2.7	6.8 ± 5.2

* No. of snails with *F. hepatica* larvae/no. of surviving snails.
** Measurements of snails with larval forms of *F. hepatica*.

Table 2. The body length and maximum width of the intrapharyngeal lumen of rediae in three groups of *Lymnaea* species infected with *Fasciola hepatica*.

Parameter (μm)	Redial category	Mean values ± SD for each group of *Lymnaea* snails*		
		L. glabra	*L. ovata*	*L. truncatula*
Body length	R1a	1209 ± 160 (17)	1231 ± 218 (14)	1093 ± 197 (20)
	R1b	1113 ± 407 (44)	1111 ± 396 (34)	1002 ± 353 (50)
	R2a	974 ± 387 (40)	942 ± 381 (44)	942 ± 338 (61)
	R2b/R3a	497 ± 149 (8)	419 ± 144 (13)	344 ± 69 (6)
Maximum width of pharyngeal lumen	R1a	36.7 ± 9.6	34.9 ± 11.0	38.8 ± 5.9
	R1b	26.3 ± 8.1	27.7 ± 8.1	26.0 ± 7.5
	R2a	10.2 ± 2.2	10.1 ± 2.1	9.1 ± 1.8
	R2b/R3a	4.2 ± 0.2	3.1 ± 0.2	3.0 ± 0.1

* The number of living rediae measured is given in parentheses.

Table 3. *Fasciola hepatica*: larval productivity of each redial category for each *Lymnaea* species.

Snail species	Redial category*	No. of free rediae at day 49 p.e.	No. of morulae differentiating into cercariae in a living redia		No. of cercariae produced by living rediae in a snail (and %)***
			N1–N2**	Difference	
L. glabra	R1b	3.2	12.7–2.1	10.6	33.9 (53.9)
	R2a	2.6	18.3–7.7	10.6	27.5 (43.6)
	R2b/R3a	0.6	14.5–10.3	4.2	1.6 (2.5)
L. ovata	R1b	2.9	13.8–2.4	11.4	33.0 (40.0)
	R2a	3.7	19.6–7.2	12.4	45.8 (55.5)
	R2b/R3a	0.9	13.2–9.0	4.2	3.7 (4.5)
L. truncatula	R1b	2.9	14.1–2.2	11.9	34.5 (39.6)
	R2a	3.6	20.5–6.3	14.2	51.1 (58.6)
	R2b/R3a	0.3	15.1–9.6	5.5	1.6 (1.8)

* R1a rediae produced only daughter rediae.
** N1, mean number of morulae in a 0.3–0.5 mm long redia. N2, mean number of morulae and procercarial embryos remaining in the body of the longest rediae at day 49 p.e. (daughter rediae are also counted in N2). Number of morulae which developed into daughter rediae in *L. glabra*: R1b (1.3 per redia), R2a (1.6), R2b/R3a (0); in *L. ovata*: R1b (1.2 per redia), R2a (1.7), R2b/R3a (0.1); in *L. truncatula*: R1b (1.3 per redia), R2a (1.5), R2b/R3a (0).
*** (N1–N2) × mean number of free rediae.

significant reduction in the body length of rediae in *L. truncatula* is likely to be related to the lower growth of this snail species (a mean of 1.6 mm only for 49 days), whereas 6.8 living rediae developed in these snails. The fact that the width of the redial pharyngeal lumen did not vary in either snail species suggests that this parameter can be used to differentiate redial generations of *F. hepatica*.

However, more variability occurred in cercarial output, with the majority of cercariae being produced by R1b or R2a rediae. On the other hand, the proportion of redial categories in each snail group was similar, i.e. 43% and 53% in *L. glabra* (table 3), as the R2b/R3a rediae were few in number. In preadult snails, there was a greater difference in the proportion of cercariae produced by R1b and R2a rediae, as shown by Augot & Rondelaud (2001) for preadult *L. truncatula*. The low mean number of R2a rediae (2.6–3.7) in these young snails compared with 13.5 in preadult *L. truncatula* is possibly due to more intense competition for space between the R1b and R2a rediae in the younger, small-sized snails. This competition would lead to a predominance of R1b over the R2a rediae (or vice versa) and would limit the development of other intraredial germinal masses, thus explaining the low number of morulae (less than 90 in each young snail) capable of differentiating into cercariae.

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