Fasciola hepatica: an unusual development of redial generations in an isolate of *Lymnaea truncatula*

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Abstract

Single-miracidium infections of *Lymnaea truncatula* by *Fasciola hepatica* were experimentally carried out to identify the redial generations of this trematode when the larval development was unusual (when the first-appearing mother redia, or R1a redia, died after its exit from the sporocyst). Four parameters were measured in the body and pharyngeal region at weekly intervals. At day 49 post-exposure at 20°C, the body of the second mother rediae (R1b) was significantly longer than that of the subsequent generations, R2a and R2b/R3a (a mean of 3.0 mm instead of 1.0 and 0.9 mm, respectively). The body was significantly wider in the R1b and R2a groups than in the R2b/R3a rediae. The pharyngeal lumen was significantly wider in the R1b group than in the R2a and R2b/R3a rediae (a mean of 48.6 μm instead of 10.8 and 3.3 μm at day 49). The thickness of the pharyngeal wall did not differ in the R1b and R2b/R3a groups, but was significantly lower in the R2a group (19.5 μm instead of 23.0–23.6 at day 49). There was better development of R1b and R2b/R3a rediae in the snails when the R1a redia died, compared with normal larval development (with a living R1a redia).

Introduction

At least three redial generations of *Fasciola hepatica* develop in single-miracidium infections of the host snail. Although the measurement of body and pharyngeal dimensions may assist in identification, it is necessary to consider the developmental pattern of redial generations (Rondelaud & Barthe, 1982) for accurate identification. Indeed, in the case of normal development, the first-appearing mother redia (R1a) developed throughout the infection, and the snail harboured an average of 20.6 free rediae on day 30 post-exposure at 20°C. In cases of unusual development, R1a redia died in the course of week 3 after their exit from the sporocyst, so that redial growth (fig. 1) was greatly delayed and the number of free rediae diminished (only 10.6 on day 30). A previous report (Augot *et al.*, 1998) permitted the identification of different redial groups in cases of normal development. The present paper completes the work of Augot *et al.* (1998) and reports on the growth of the different redial groups within the snail in cases of unusual development of the redial generations.

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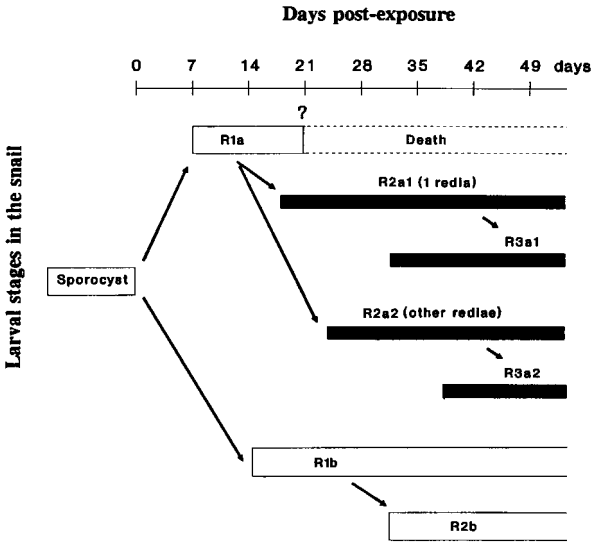

Fig. 1. Developmental pattern of redial generations of *Fasciola hepatica* in *Lymnaea truncatula* (at 20°C) when the first-appearing mother redia of the first generation (R1a) died in the course of week 3 after its exit from the sporocyst. R1b, other second-appearing mother rediae of the first generation; R2a1, first-appearing daughter redia from R1a; R2a2, other second-appearing daughter rediae from R1a; R2b, daughter rediae from R1b; R3a1 and R3a2, grand-daughter rediae from R1a. R2a1 and R2a2 rediae are pooled into a single group named R2a. The R2b, R3a1, and R3a2 rediae are also pooled in a single group named R2b/R3a.

Materials and methods

The population of *Lymnaea truncatula* used in this study originated from Saint Ours, department of Puy de Dôme (central France). This *L. truncatula* isolate was selected due to the high prevalence of unusual development (30% versus 1–15% of snails in other snail populations). Eggs of *F. hepatica* were collected from the gall bladders of heavily infected cattle and were incubated for 20 days at 20°C, in the dark. Single-miracidial infections were performed using 247 *L. truncatula* measuring 4 ± 0.2 mm in height. Snails were subsequently raised in open boxes (1 m × 60 cm × 15 cm deep), with a density of 50 snails per recipient. Each box contained small boulders and a constantly-aerated, 2-cm deep water layer. Snails were fed decayed lettuce, and maintained at 20°C. A variable number of snails (from 10 to 17) were randomly collected in boxes at each observation date as follows: on 7, 14, 21, 28, 35, 42 and 49 days post-exposure. Snails were dissected into tap water to detect a redial burden characterized by dead R1a redia (characterized by a white uniformly-coloured body). The other rediae were classified, based upon their gross pharyngeal morphology and age of infection, into the following categories: mother rediae exiting from the sporocyst (R1b), daughter rediae from R1a before its death (R2a), daughter rediae from R1b (R2b), and grand-daughter rediae from R1a (R3a). As the latter two redial groups could not be differentiated, they were pooled into a single group arbitrarily named R2b/R3a.

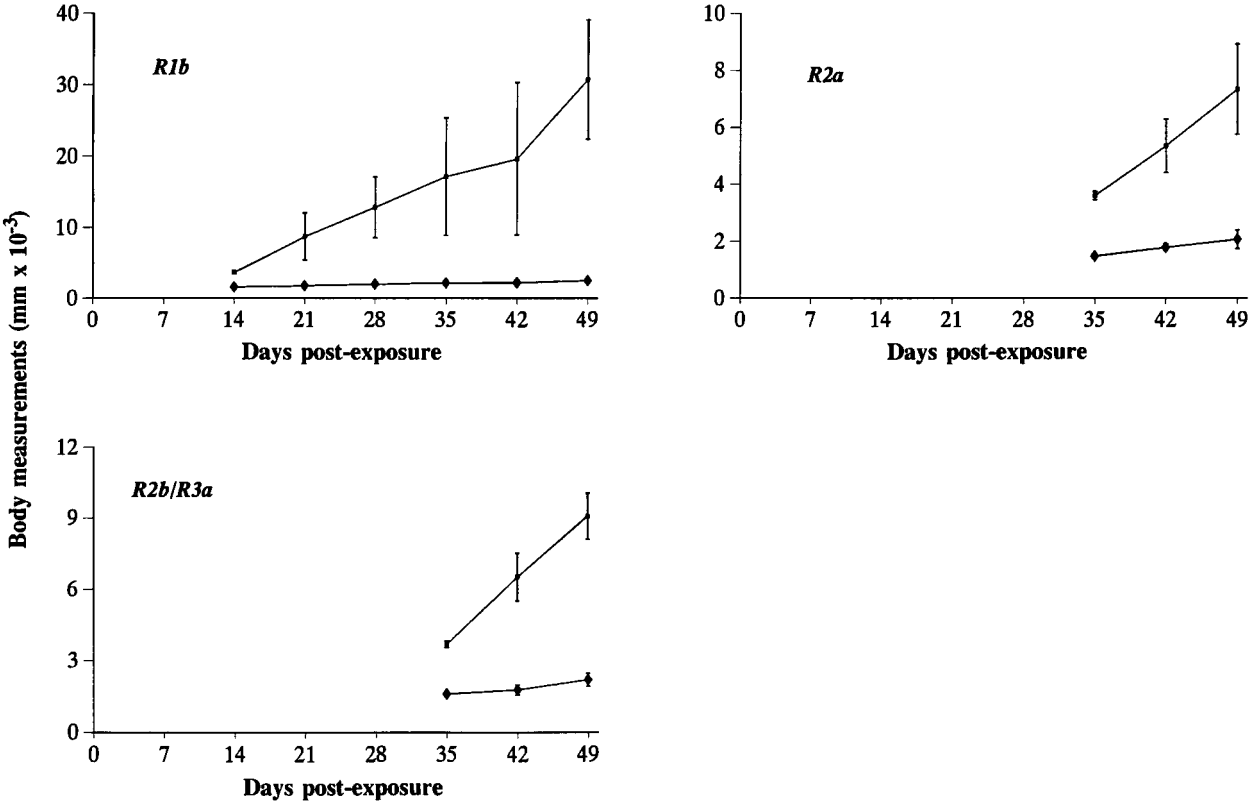

Fig. 2. Mean values (± S.D.) for the length (2a) and width (2b) of the body from living rediae of *Fasciola hepatica*. R1b, second-appearing mother rediae; R2a, daughter rediae from R1a; R2b/R3a, daughter rediae from R1b and grand-daughter rediae from R1a.

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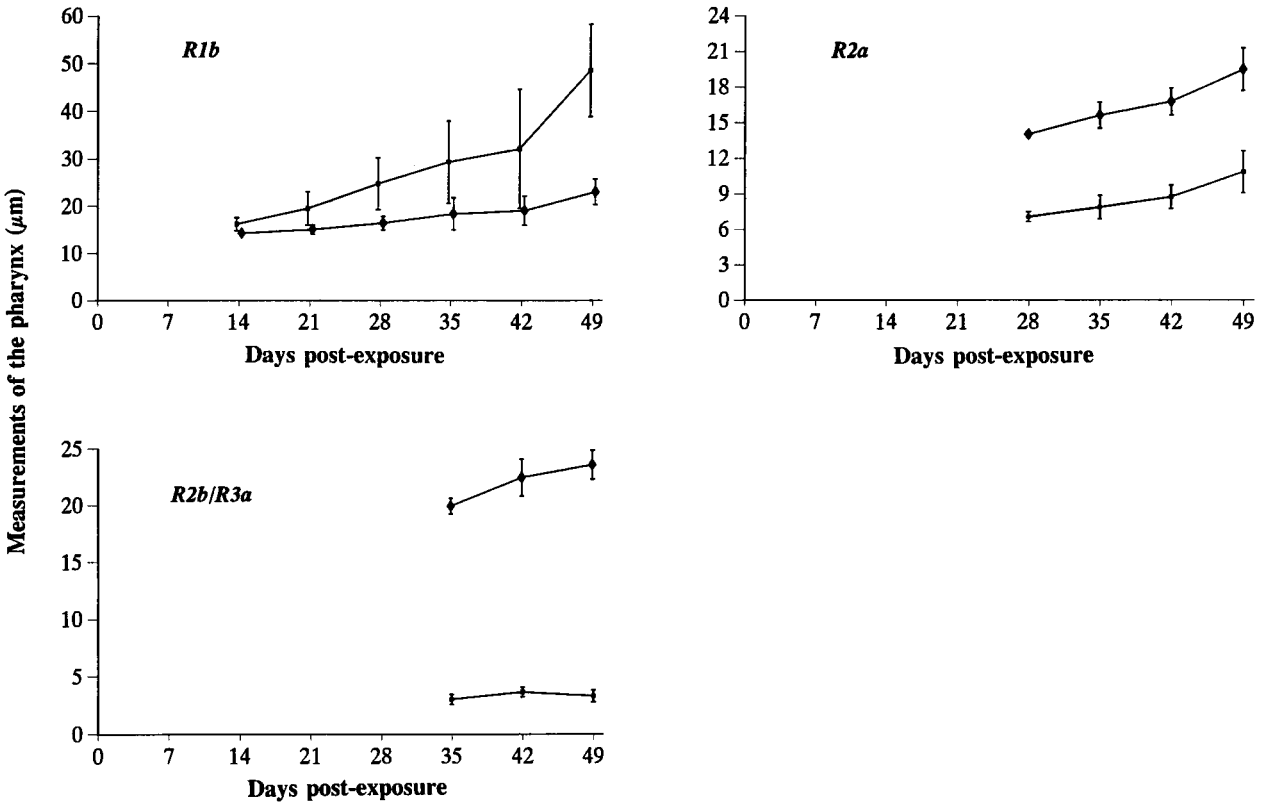

Fig. 3. Mean values (± S.D.) for the width of the pharyngeal lumen (3a) and the thickness of the pharyngeal wall (3b) for living rediae of *Fasciola hepatica*. R1b, second-appearing mother rediae; R2a, daughter rediae from R1a; R2b/R3a, daughter rediae from R1b and granddaughter rediae from R1a.

Four parameters, i.e. the length and width of the redial body, the width of the pharyngeal lumen, and the thickness of the pharyngeal wall, were measured using an image-processor (Aries, Prolabo, France). Mean values ± S.D. for each parameter were calculated, taking into account the redial group and date of sacrifice. Correlation tests and analyses of variance were used to establish levels of significance.

A total of 217 rediae from 32 snails each with a dead R1a redia were measured. These rediae were grouped as follows: R1b (118 rediae), R2a (62) or R2b/R3a (37) groups.

Results and Discussion

The mean lengths of the redial bodies (fig. 2a) increased throughout the experiment and were significantly correlated with the age of infection. This was recorded in groups R1b ($r = 0.69$, $P < 0.001$), R2a ($r = 0.80$, $P < 0.01$) and R2b/R3a ($r = 0.91$, $P < 0.001$). On day 49, the mean values were 3.0, 1.0 and 0.9 mm, respectively. A length of 4.5 mm was noted for one R1b redia on this date. A significant difference between the R1b and R2a groups ($F = 23.45$, $P < 0.01$) was also noted. The enlargement of the width of the body (fig. 2b) was significantly correlated with the age of infection (R1b, $r = 0.64$, $P < 0.001$; R2a, $r = 0.82$, $P < 0.001$; R2b/R3a, $r = 0.72$, $P < 0.001$). At day 49, the mean values were 245, 248 and 220 μm, respectively. A significant difference ($F = 7.80$, $P < 0.01$) was only noted between the R2b/R3a rediae and the other two redial groups.

A significant correlation between the enlargement of the width of the pharyngeal lumen (fig. 3a) and the age of infection was noted only in the R1b ($r = 0.71$, $P < 0.001$) and the R2a ($r = 0.70$, $P < 0.001$) redial groups. At day 49, the mean widths from the R1b, R2a, and R2b/R3a groups were 48.6, 10.8 and 3.3 μm, respectively. There were significant differences between the R1b and R2a groups ($F = 225.88$, $P < 0.001$) as between the R2a and R2b/R3a groups ($F = 213.80$, $P < 0.001$). Lastly, the thickness of the pharynx wall (fig. 3b) increased and was significantly correlated with the age of infection (R1b, $r = 0.70$, $P < 0.001$; R2a, $r = 0.78$, $P < 0.001$; R2b/R3a, $r = 0.68$; $P < 0.001$). On day 49, the mean values were 23.0, 19.5 and 23.6 μm, respectively. The thickness of the R2a group was significantly less ($F = 14.93$, $P < 0.001$) than those of the two other groups of rediae studied.

A comparison of these results was performed with the body dimensions measured in the same redial groups in which the R1a redia lived throughout the infection period (Augot *et al.*, 1998). On day 49, the body was significantly longer in the R1b group from the *L. truncatula* harbouring a dead R1a redia (3.0 mm vs. 1.4 mm in snails with a

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normally-developed R1a redia) and the R2b/R3a rediae (0.9 mm vs. 0.6 mm). On the other hand, in the R2a group, there was no significant difference between the mean lengths of the body. The width of the body on day 49 did not differ in the R2b/R3a rediae, but was significantly lower in the R1b and R2a groups when the R1a redia died (245 and 248 μm, respectively, instead of 298 μm).

Our data demonstrate a better development in the R1b and R2b/R3a rediae when the R1a redia died. However, in the R2b/R3a group, it is logical to assume that this increase in growth particularly concerned the R2b rediae, as they were more numerous than the R3a larvae within the snail (Rondelaud & Barthe, 1982). Further studies will be necessary to determine whether the normal development of R1a mother redia or their death had an impact on cercarial production in relation to the specific redial generation.

The frequency of mortality of R1a redia among snail populations is still unknown. Dead R1a rediae (one per single-miracidium infected snail) were often situated in the intervisceral spaces of the snail's digestive gland as were most of the normally-developing R1a rediae. Therefore, the location of this larva during its development within the intermediate host cannot explain its death in numerous cases. The death of R1a redia is more probably related to an exit of a daughter redia in an unusual site through the wall, inducing a severe, and subsequently mortal, injury to the mother redia. This assumption is in part based upon the work by Augot *et al.* (1997) concerning the *in vitro* culture of *F. hepatica* rediae. According to these authors, the exit of daughter rediae occurred in unusual sites, such as through the wall in the posterior third of the mother redia, or sometimes at the base of the mother pharynx.

Acknowledgements

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