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SHORT COMMUNICATION

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The presence of uninfected *Omphiscola glabra* in a breeding of infected *Galba truncatula* enhanced the characteristics of snail infections with *Fasciola hepatica*

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Abstract Experimental infections of *Galba truncatula* with *Fasciola hepatica* were carried out under laboratory conditions to determine if the presence of another lymnaeid, *Omphiscola glabra*, during snail breeding had an indirect effect on the growth of *G. truncatula* and to analyse consequences on cercarial production. When the two lymnaeids are raised together, the survival of *G. truncatula* at day 30 post-exposure (PE), the prevalence of snail infections, and the shell height of cercariae-shedding snails at day 45 PE were significantly higher. By contrast, the other parameters characterizing snail infections only showed insignificant variations. The origin of *O.glabra* (living in a snail community, or monospecific population) used for the breeding of *G. truncatula* did not have a significant effect on the values of each parameter. Even if variations in the mean numbers of metacercariae were insignificant, the greater survival of *G. truncatula* at day 30 PE and the higher numbers of cercariae-shedding snails in the groups living with *O. glabra* allowed to obtain a higher total number of larvae than in alone-raised groups of *G. truncatula*.

Live metacercariae of *Fasciola hepatica* are often used in biomedical research to study different aspects of immunology, physiology, and pathology in experimentally infected definitive hosts, or to determine the efficacy and safety of new molecules as anthelminthics in the treatment of fasciolosis in mammals. The cost of commercial production of these larvae needs to be taken into account to propose the lowest sale price possible to researchers and/or institutions. As the cost is closely dependent on the choice of a food source for breeding the intermediate hosts of *F. hepatica* (Rondelaud et al. 2002), it is useful to reduce it by the selection of the best method to feed snails. Several sources of high-quality food have already been proposed by different authors (Kendall 1949, 1965; Boray 1969; Lee et al. 1994; Rondelaud et al. 2004) for *Galba truncatula*, as this amphibious snail is the most used lymnaeid in European countries for experimental infections with *F. hepatica*. However, the use of these food sources often requires the presence of a technician, thus explaining the high cost of metacercariae (Rondelaud et al. 2002).

Romaine lettuce has been used for 25 years by our lab to feed *G. truncatula* (Abrous et al. 1998). As the cost of metacercariae obtained from these lettuce-reared snails was high (Rondelaud et al. 2002), the focus of our team is to reduce it by simplifying the breeding method for *G. truncatula*. The first step of this research is to obtain the best growth possible for these last snails because the mean shell height of experimentally infected *G. truncatula* was often significantly lower than that of unexposed controls (Abrous et al. 1998). As the growth of French *G. truncatula* living in communities with *Omphiscola glabra* was similar to that of *G. truncatula* forming monospecific populations (Rondelaud, unpublished data), two experiments were carried out under laboratory conditions to determine if the presence of several *O. glabra* in a breeding of infected *G. truncatula* had an indirect effect on the shell height of these last snails.

Table 1 gives the geographic origin of lymnaeids, the composition of the ten groups, the number of snails per group at the beginning of the experiments, and the presence or the absence of a snail infection with *F. hepatica*. The first experiment (A) was performed with snails originating from the same community, whereas the second (B) was made using snails from monospecific populations. The choice of four *O. glabra* for 46 *G. truncatula* was based on the results of a preliminary experiment, demonstrating that four *O. glabra* were sufficient to have the best growth for infected *G. truncatula*. All snails were collected from their habitats in November or in April, and measured 4 mm in

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Table 1 The characteristics of the ten groups of snails used for the two experiments

Experiment	Snail group		Total number of snails per group at the beginning of experiment	
	Species	Population	*O. glabra*	*G. truncatula*
A	*O. glabra* and *G. truncatula*	Berneuil	8	92[a]
	G. truncatula	Saint Jouvent	0	100[a]
	O. glabra	Berneuil	50 (controls)	0
	G. truncatula	Berneuil	0	50 (controls)
	G. truncatula	Saint Jouvent	0	50 (controls)
B	*O. glabra* and *G. truncatula*	Blond Saint Jouvent	8	92[a]
	G. truncatula	Saint Jouvent	0	100[a]
	O. glabra	Blond	50 (controls)	0
	G. truncatula	Saint Jouvent	0	50 (controls)

All snail populations were living in the department of Haute Vienne, central France. Berneuil (snail community with *G. truncatula* and *O. glabra*, living in a road ditch near the village of Berneuil). Blond (monospecific population of *O. glabra*, inhabiting an open drainage furrow at La Rouze, commune of Blond). Saint Jouvent (monospecific population of *G. truncatula* originating from a road ditch at La Petite Châtre, commune of Saint Jouvent).[a]Snail infections, with two miracidia of *F. hepatica* per snail at the beginning of experiment

height at the beginning of the experiments. Routine bimiracidial exposures were performed in four groups of *G. truncatula*, whereas the other groups of this last species and *O. glabra* were unexposed. All snail groups were raised during the first 30 days using the method by Abrous et al. (1998). Food was only constituted by leaves of cos lettuce, faded after their stay under wet conditions at 4–6°C. Some dead leaves of *Glyceria fluitans* (Poaceae) were also added in the boxes containing *O. glabra*, as this snail often fed on this grass. The breeding boxes were maintained at 20°C, under a natural photoperiod of 12 h. At day 30 post-exposure (PE), the surviving *G. truncatula* from infected groups were individually placed at 20°C in 35-mm-diameter Petri dishes, with 2–3 ml of spring water and a piece of faded-lettuce leaf per recipient. A routine daily surveillance was performed to change the water and to count metacercariae before their removal from dishes. The other snails were only counted at day 30. By contrast, all surviving snails were measured at day 45 under a stereomicroscope coupled with an image-processor (Aries, Châtillon, France) using the Esilab software.

The parameters studied were snail survival at day 30 of the experiment, the prevalence of *F. hepatica* infections (calculated using the ratio between the number of cercariae-shedding snails and that of surviving snails at day 30), the shell height at day 45, the date of the first cercarial shedding, the length of the patent period, and the number of metacercariae recorded for each group containing infected snails. A χ^2 test and one-way analysis of variance (Stat Itcf 1988) were used to determine levels of significance.

In experiment A, the survival rate of *G. truncatula* at day 30 of the experiment (Table 2) was significantly greater in unexposed controls than in exposed snails (Berneuil: χ^2=5.31, P<0.05; Saint Jouvent: χ^2=33.14, P<0.001). A similar finding was also noted in the B experiment (Saint Jouvent: χ^2=10.73, P<0.01 in the case of *G. truncatula* raised with *O. glabra*, and χ^2=26.08, P<0.001 for alone

Table 2 Several characteristics in the ten groups of snails in relation to the experiment and the lymnaeid species

Experiment	Population of *G. truncatula*	Number of surviving snails at day 30 (rate in %)	Number of CS snails (prevalence in %)	Shell height of snails (mm) at day 45: mean±SD
A	Berneuil (raised with *O. glabra*)	73 (79.3)	66 (90,4)	8.8±1.2[a]
	Saint-Jouvent	51 (51.0)	31 (60.7)	7.2±1.1[a]
	Berneuil (controls)	47 (94.0)	0	9.0±1.2
	Saint-Jouvent (controls)	49 (98.0)	0	8.9±1.0
B	Blond (with *O. glabra*)	71 (77.1)	65 (91.5)	8.6±0.9[a]
	Saint-Jouvent	58 (58.0)	27 (46.5)	7.1±1.1[a]
	Saint-Jouvent (controls)	49 (98.0)	0	8.8±1.1

CS snails Cercariae-shedding snails
[a]The measures only concerned CS snails

Table 3 Mean values (SD) for three parameters characterizing snail infections with *F. hepatica* in four groups of *G. truncatula*

Experiment	Population of *G. truncatula*	Date of the first cercarial shedding (days)	Length of the patent period (days)	Number of metacercariae per CS snail
A	Berneuil (with *O. glabra*)	49.2 (7.6)	15.6 (5.7)	127.4 (72.5)
	Saint-Jouvent	47.3 (8.2)	19.2 (8.4)	105.5 (62.3)
B	Blond (with *O. glabra*)	48.5 (6.0)	16.3 (8.5)	133.4 (55.2)
	Saint-Jouvent	47.7 (7.2)	17.5 (8.4)	112.7 (73.0)

CS snail Cercariae-shedding snail

raised snails). Comparison of exposed *G. truncatula* demonstrated significantly greater survivals when snails are raised with *O. glabra* (experiment A: χ^2=16.83, P<0.001; experiment B: χ^2=7.99, P<0.01). By contrast, for each category of exposed snails separately considered (snails living with *O. glabra*, or alone raised), no significant differences between the rates recorded in both experiments were found. The prevalence of snail infections with *F. hepatica* was significantly greater when *G. truncatula* was bred together with *O. glabra* (experiment A: χ^2=15.67, P<0.001; experiment B: χ^2=31.60, P<0.001). Lastly, the shell height of cercariae-shedding snails at day 45 post-exposure did not significantly differ from that of unexposed controls, whereas the shell heights of snails living with *O. glabra* were significantly greater (experiment A: F=4.06, P<0.05; experiment B: F=4.57, P<0.05) than those raised alone.

Table 3 gives the values of the other three parameters. The date of the first cercarial shedding and the length of the patent period did not show any significant variations, whatever mode of comparison. Even if the number of metacercariae recorded in the case of snails living with *O. glabra* was slightly higher, no significant difference was found.

When the two lymnaeids were bred together during the first 30 days of experiment, greater snail survivals, higher prevalences of *F. hepatica* infections and bigger shells for cercariae-shedding snails were noted for *G. truncatula*. Moreover, the origin of *O. glabra* (living in a snail community, or monospecific population) had no significant effect on the characteristics of snail infections in *G. truncatula*. These results indicated that the presence of several unexposed *O. glabra* in each box of *G. truncatula* had indirect effects on three characteristics of snail infections, and one may wonder about the causes of such changes for *G. truncatula.* In our opinion, the main reason would be of dietary nature. As *O. glabra* fed more willingly on lettuce than *G. truncatula*, the former species would facilitate the dietary pattern of the latter snail by damaging and eroding lettuce, so that *G. truncatula* might more easily eat the pieces of these leaves. An argument supporting this approach is the presence of numerous *G. truncatula* on lettuce in the boxes containing *O. glabra* as the first days of the experiment, whereas a few snails were only observed on lettuce when *G. truncatula* is raised without *O. glabra*.

Even if the variations in the mean numbers of metacercariae are insignificant, the greater survival of *G. truncatula* at day 30 and the higher number of cercariae-shedding snails in the two groups living with *O. glabra* allowed to obtain a higher total number of larvae than in groups of *G. truncatula* raised alone (a total of 8,409 and 8,671 metacercariae in the experiments A and B, respectively, instead of 3,271 and 3,043 larvae). The cercarial production of *F. hepatica* metacercariae was so highly increased in the case of mixed breeding of snails. However, these results can only be obtained with a 30-day cohabitation of two lymnaeids measuring 4 mm in height at the beginning of the experiment and the subsequent isolation of *G. truncatula* from the other species. Indeed, beyond day 30 at 20°C, there was a concurrence between the two lymnaeids in breeding boxes, as *O. glabra*, measuring 11–12 mm at day 30 PE, forced the other species to emerge from water and to take refuge on box walls, thus, entailing the death of most *G. truncatula*.

References

Abrous M, Roumieux L, Dreyfuss G, Rondelaud D, Mage C (1998) Proposition d'une technique simple pour la production métacercarienne de *Fasciola hepatica* Linné à partir du mollusque *Lymnaea truncatula* Müller. Rev Med Vet (Toul) 149:943–948

Boray JC (1969) Experimental fascioliasis in Australia. Adv Parasitol 7:95–210

Kendall SB (1949) Nutritional factors affecting the rate of development of *Fasciola hepatica* in *Limnaea truncatula*. J Helminthol 23:179–190

Kendall SB (1965) Relationships between the species of *Fasciola* and their molluscan hosts. Adv Parasitol 3:59–98

Lee CG, Kim SK, Lee CY (1994) Rapid growth of *Lymnaea viridis*, the intermediate host of *Fasciola hepatica*, under laboratory conditions. Vet Parasitol 51:327–331

Rondelaud D, Abrous M, Dreyfuss G (2002) The influence of different food sources on cercarial production in *Lymnaea truncatula* experimentally infected with digenea. Vet Res 33:95–100

Rondelaud D, Denève C, Belfaiza M, Mekroud A, Abrous M, Moncef M, Dreyfuss G (2004) Variability in the prevalences of infections and cercarial production in *Galba truncatula* raised on a high quality diet. Parasitol Res 92:242–245

Stat Itcf (1988) Manuel d'utilisation. Institut technique des céréales et des fourrages, Service des études statistiques, Boigneville