Life History of *Rhabditis (Pelodera) orbitalis*—A Larval Parasite in the Eye Orbits of Arvicolid and Murid Rodents

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**ABSTRACT:** Whereas microbotrophic adults and developmental stages of *Rhabditis (Pelodera) orbitalis* live only in the nesting material of the host, its parasitic third-stage larvae have hitherto been found frequently in the conjunctival sacs of 15 species of mice and voles during the past ~30 years.

In addition to the parasitic (“infective”) larvae, the third stage may exist as dauer larvae as well as normal larvae, differing in morphological as well as ecological details. Only the infective larvae can successfully locate a host by the vibrations of the nesting material and by means of a thermotactic response.

Food is taken up from the lachrymal fluid by endosmosis and is stored as fat in the intestinal cells. After 3–19 days in the conjunctival sac, larvae will leave the host but remain in the nesting material, where they molt twice to the adult stage. The phenomenon designated in this paper as “obligate parasitism” pertains only to the infective larvae.

**KEY WORDS:** nematodes, *Rhabditis (Pelodera) orbitalis*, *Rhabditis (Pelodera) strongyloides*, larval parasitism, parasites of small rodents, parasites of eye orbits, nest fauna, sibling species, life cycle, host finding, thermotactic response.

The lachrymal fluid of conjunctival sacs of voles and mice from Europe and North America (Table 1) often harbors up to 100 or more nematode larvae per eye. According to Osche (1956), Poinar (1965), and Cliff et al. (1978) these third-stage larvae are assumed to belong to the species *Rhabditis (Pelodera) strongyloides* (Schneider, 1860), which is known among nematologists for its easy cultivation and its use as a “potential research tool” in experimental studies (Scott and Whittaker, 1970; Stringfellow, 1974, 1976).

In a more recent revision, however, this well-known nematode was recognized as belonging to a sibling species complex (see Sudhaus and Schulte, 1986; Sudhaus et al., 1987), confusing the biological and ecological patterns of several different species.

1) *Rhabditis (P.) strongyloides* (Schneider, 1860) lives as a microphage in fecal matter of stables and chicken houses. Although without phoretic or parasitic association, third-stage larvae of a separate strain (*Rhabditis (P.) strongyloides dermatitica* Sudhaus and Schulte, 1988) may cause intense dermatitis in warm-blooded animals.

2) *Rhabditis (P.) cutanea* Sudhaus, Schulte, and Hominick, 1987, third-stage larvae can be found coiled in hair follicles of the skin of wood mice (*Apodemus sylvaticus* and *A. flavicollis*).

3) *Rhabditis (P.) nidicolis* Sudhaus and Schulte, 1986, has been found only once in nesting material of a field vole (*Microtus agrestis*). Its life cycle remains to be elucidated.

4) *Rhabditis (P.) orbitalis* Sudhaus and Schulte, 1986, third-stage larvae are regularly found in the conjunctival sacs of lemmings (Cliff et al., 1978), voles (Poinar, 1965; Canning et al., 1973; Prokopić et al., 1974), and, to a lesser extent, of mice and rats (Cross and Santana, 1974). The life history of *Rhabditis orbitalis* is described in this paper.

**Results**

**Cultivation and cross-mating experiments**

All the species of the complex can successfully be cultivated on pure agar plates (2%) with little pieces of uncooked meat (as a substratum for bacterial growth). They are morphologically nearly identical, although they are reproductive isolated.

For interspecific cross-mating experiments, males and females of the above-mentioned species were put on the surface of an agar plate. Mating took place in all combinations, but the development of eggs or larvae soon ceased, indicating that the species are metagametically isolated (see Sudhaus and Schulte, 1986; Sudhaus et al., 1987).

Conspecificity with *R. orbitalis*, on the other hand, was confirmed by the same method for different strains recovered from the orbits or
nesting material of various rodent species (Microtus agrestis, M. arvalis, Clethrionomys glareolus, and Apodemus agrarius).

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HABITAT AND SAMPLING: All developmental stages could be extracted easily from nesting material of Microtus agrestis and M. arvalis, respectively, by a Baerman funnel: in Berlin (West), 23 out of a total of 33 examined nests (i.e., 70%) revealed larvae and adults of this nematode living there as bacterial feeders. Another way of obtaining R. orbitalis for laboratory studies was to live trap infected voles and put them in cages supplied with sterilized, moist hay or soil, from which all developmental stages could be extracted a few days later (as mentioned by Poinar [1965, 1983] for “P. strongyloides”).

Parasitic larvae were recovered even faster by killing the rodents, flooding the conjunctival sac with 0.22 M saline, and drawing the nematodes up into a pipette. However, only very few of these larvae survived and molted to the adult stage on agar plates.

LIFE CYCLE AND “LARVAL TRIPHENISM”: Once established, R. orbitalis can be grown indefinetely in a laboratory culture, provided that an adequate amount of bacteria is offered as food. The life cycle of one generation takes between 5 and 7 days (at 20°C) with an average of 5.4 days (N = 26). Third-stage larvae of R. orbitalis appear in three morphologically different shapes, which arise simultaneously from a common pre-stage. After 5–10 days in laboratory culture, infective larvae will occur, which are morphologically different from the “normal” third-stage larvae as well as from the dauer larvae, although belonging to the same developmental stage (Fig. 1).

This phenomenon should be referred to as a “larval triphenism,” obviously induced by environmental factors (quality of bacteria available for food). While dauer larvae may tolerate lack of food and desiccation (up to 28 days), only the infective larvae are able to find the rodents’ conjunctival sacs.

Once formed, the infective larvae will not continue their development, not even on fresh agar plates seeded with bacteria (as dauer larvae do). These larval nematodes will only continue their life cycle by finding the eye orbits of an adequate host.

HOST FINDING: Small mammals such as arvicolid rodents possess a short-term rhythm of activity and rest or sleep (Lehmann, 1976). At least every few hours, each rodent will return to its own nest. Before sleeping the rodent causes disturbance of the nesting material by grooming and scratching. Resulting vibrations can be perceived by the infective larvae of R. orbitalis: twirling the petri dish on a table for about 20 sec will cause these larvae to move intensively 3–7 min later. They climb up to the petri dish lid or similar dry areas. Infective larvae in a dish that is not moved will never show such a behavior, but remain quiescent up to 6 wk before dying without further development.

Once activated and exposed to the air, the larvae can locate their host by the heat of its body within a striking distance of about 40 mm, which was shown under experimental conditions (Fig. 2a, b). After the plexiglass triangle had vibrated for about 30 sec, hundreds of infective larvae left the agar surface, wandering directly to the heated metal point and ignoring the unheated reference. While perception of heat stimuli from the host (Fülleborn, 1924, 1932) is well known for moist soil inhabiting infective larvae of Ancylostoma and Strongyloides, infective larvae of R. orbitalis can perceive radiation heat (infrared beams), a capability hitherto best known for certain snakes (Boinae and Crotalinae). Locomotion on the dry plexiglass takes place in a very strange manner: bending of the stoma against the substrate, thus pulling the rest of the body forward (sometimes even a somersault was performed by a moving larva). That mode of locomotion should be considered an adaptation to the dryness of the nesting material of small rodents, which makes the
normal “gliding”-type movement of nematodes on moist substrate impossible. Larvae will reach the warm metal point with a maximum speed of about 1.2 mm/min. Then the nematodes may stand on their tails and wave back and forth or even jump from the metal, ready for a new attack.

INFECTION OF LABORATORY MICE: Quest for a host by the infective larvae was tested under more natural conditions on laboratory mice (*Mus musculus*). The rodents were kept individually in a wire cage (70 × 70 × 125 mm) covered with artificial nesting material containing all developmental stages of *R. orbitalis*. After 12–16 hr, examination of the conjunctival sacs revealed between 11 and 54 larvae per eye (number of mice = 7). This seems to be roughly the “normal” number of larvae counted from a single rodent under field conditions according to Canning et al. (1973), Cross and Santana (1974), Prokopić et al. (1974), and my own findings (min. 1/max. 78 for 12 specimens of *Clethrionomys glareolus* Schreber at Berlin).

Once in contact with the fur of their host, regardless of the region, larvae will climb among the hairs searching for the rodents’ eyes in a “trial and error” method; similar to their behavior described for the heated metal point (under given conditions) larvae will start jumping from the fur of the host, making a new attempt to find its eyes.

Poinar (1965, 1983) has proposed a hypothesis that the larvae might be picked up by the rodents’ feet and transmitted to their eye orbits while grooming. In order to prove this hypothesis, mice were kept in a special narrow cage, completely
bored far more larvae than non-fixed mice. Thus Poinar's hypothesis cannot be confirmed.

Cliff and Anderson (1980) tried to inoculate lemmings (*Lemmus trimucronatus = L. sibiricus*) with *R. orbitalis* ("P. strongyloides") under laboratory conditions, using dauer larvae instead of infective larvae. They obviously did not know about the larval triphenism as a special character of this species. In their experiments, dauer larvae left the conjunctival sacs of the lemmings within 48 hr, thus leading Cliff and Anderson (1980) to the wrong conclusion that the association might be phoretic only.

"DUMMY" EXPERIMENT: The essential stimuli causing the larvae to search for the host's eyes (vibrations of the nesting material, radiation heat from the host's body, and finally the wetness of its eyes) were affirmed by the aid of a "dummy" (Fig. 2c). The dummy was heated up to 35°C and covered with the artificial nesting material containing *R. orbitalis* for 12 hr. Infective larvae invaded the little tubes filled with tap or saline water, respectively, in nearly the same numbers as they invaded conjunctival sacs of laboratory mice. This result was obtained only when the different stimuli (as mentioned above) were given simultaneously.

**LARVAL PARASITISM IN R. ORBITALIS:** Shortly after the infective larvae gain entrance to the orbit, they free themselves from the previous-stage cuticle and begin to move through the lachrymal fluid.

It had been unclear for a long time whether the heavy incidence of larvae of *R. orbitalis* in the conjunctival sacs of small rodents (confused with "*R. strongyloides*") is part of a phoretic or parasitic association. A considerable amount of information has been published on this problem.

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### Table 2. Body dimensions of *Rhabditis orbitalis* parasitic larvae obtained from the lachrymal fluid of laboratory mice at different times (in µm). Prior to examination, larvae were heat-relaxed and stored in saline (to avoid bursting). Figures in parentheses are means.

<table>
<thead>
<tr>
<th>Age of larvae</th>
<th>1 day</th>
<th>3 days</th>
<th>5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of larvae measured</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Body length</td>
<td>646–720 (648)</td>
<td>711–936 (874)</td>
<td>819–936 (889)</td>
</tr>
<tr>
<td>Body width</td>
<td>30–34 (31)</td>
<td>36–58 (56)</td>
<td>45–65 (58)</td>
</tr>
<tr>
<td>Pharynx length</td>
<td>162–172 (167)</td>
<td>162–188 (179)</td>
<td>162–176 (172)</td>
</tr>
<tr>
<td>Tail length</td>
<td>67–78 (77)</td>
<td>72–97 (82)</td>
<td>90–99 (95)</td>
</tr>
<tr>
<td>Gonad primordium (length)</td>
<td>68–123 (96)</td>
<td>86–144 (103)</td>
<td>84–144 (100)</td>
</tr>
</tbody>
</table>
Osche (1956, 1963), Poinar (1965, 1983), and Prokopic et al. (1974) evaluated the phenomenon involved as facultative parasitism (or phoresis), i.e., preadaptation of the larvae of microbotrophic nematodes to parasitism.

My findings indicated that the only reason why the infective larvae of *R. orbitalis* invade the conjunctival sacs is to obtain their nourishment from the lachrymal fluid. The larvae increase in size while on the host (Table 2) and the intestinal cells are densely packed with lipid droplets, giving the nematodes opaque bodies (Fig. 3). Furthermore, none of the larvae resume development without having stayed in the conjunctival sacs for at least 72 hr.

Since the stoma remains closed at its beginning and the pharynx musculature is reduced or degenerated (Fig. 3b), proteins from the lachrymal fluid are apparently taken up through the modified cuticle ("endosmosis"). That mode of nutrition is common in some parasitic nematodes from the body cavity of invertebrates.

If stored, namely in an auto-sterile saline solution (containing Na-EDTA) for several hours, injury inside the epidermis became visible all over the body surface. Immersion of older parasitic larvae recovered from the lachrymal fluid in tap or distilled water led to rapid bursting.

A special feature connected with the inevitable uptake of inorganic salts from the lachrymal fluid (mainly NaCl) is the enlargement of the phasmids (Fig. 3d). This suggests that they may serve as excretion organs. The hypertrophy is not caused by the salt, since it will occur even when the infective larvae are obtained from the culture and stored at 30°C in a tap water film on the surface of an agar plate.

Former investigators in this association were often amazed by the fact that the rodents do not show pathological effects caused by the nematodes, although the lachrymal fluid of one eye can harbor up to some 100 larvae (Stammer, 1956; Poinar, 1965). Most probably, the larvae become sterile with their exsheathment, thus becoming more tolerable to their host. Furthermore, the nematodes never cause damage of surrounding tissues, but only use the lachrymal fluid in the "mild" endosmotic manner.

Several experiments were run to determine the time period of larval parasitism: laboratory mice were infected with *R. orbitalis* for 12 hr as described above. Infected mice were kept singly in a plexiglass box (95 x 95 x 60 mm) containing sterilized moist hay (artificial nesting material), which was put in a glass aquarium with a layer of sawdust. Emigrated larvae were extracted from the hay daily by taking samples which were then examined by the Baermann method. Parasitic larvae remained in the conjunctival sac between a minimum of 3 and a maximum of 19 days (average 8/N = 9) before leaving the host and resuming development (Fig. 4).

![Figure 3. Morphological characters of *Rhabditis orbitalis* parasitic larvae obtained from the lachrymal fluid of a vole (*Microtus agrestis*). a. Larva in toto (lateral). b. Pharynx with bend (indicated by arrow). c. Anterior region of the body (lateral). d. Caudal region with enlarged phasmids.](image-url)
LEAVING THE HOST: Leaving the conjunctival sac as well as resuming the microbotrophic mode of life must take place obligatorily within the rodents’ nest. Since *R. orbitalis* is a bisexual species, only the nesting material provides the chance to find a mate and thus continue its lifecycle. While still in the lachrymal fluid, the larva has to perceive internal stimuli from the host connected with its periodic stay in the nest. In fact, emigrated larvae from laboratory mice could only be extracted from nesting material itself, and never from the surrounding substratum.

Possibly oscillation of endothermy will give this internal stimulus, but the exact mechanism remains unknown.

In contrast to the results presented by Poinar (1965), 2 molts were always observed in post-parasitic larvae before they became adults.

**Discussion**

*Rhabditis orbitalis* is only found in the scanty nesting material, where its infective larvae can invade the conjunctival sacs of mice and voles. The infective larvae exhibit a number of unique, specialized features connected with their obligate parasitism in the lachrymal fluid. However, *R. orbitalis* still has the ability to undergo a complete free-living (i.e., microbotrophic) cycle similar to that of the closely related *R. strongyloides*. Periods of adversity, such as lack of food or drought, may be tolerated with the aid of the dauer larva stage. That is the reason why *R. orbitalis* can be grown indefinitely in laboratory culture.

Infective larvae of *R. orbitalis* were able to persist in the nesting material up to 6 wk, thus increasing their chance to meet a host and obtain their nourishment from the lachrymal fluid.

Since rodents’ nests are like small islands in the surrounding soil, decomposing organic material of a nest will only persist for a comparatively short time. If the parasitic larvae are not distributed by their host and transmitted to a new habitat, populations of *R. orbitalis* would soon perish without reproduction.

This complicated association only would have evolved in connection with clear advantages even for the post-parasitic generation. Possibly this could mean better fitness for the offspring (depending on the food stored in the intestinal cells...
of the parasitic larva) or the predictability for completing the life cycle.

**Literature Cited**


