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On the Geographical Distribution and Parasitism of *Rhabditis* (*Pelodera*) *orbitalis* (Nematoda: Rhabditidae)

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ABSTRACT: Successful crossings between Californian and European populations proved the nematode *Rhabditis* (*Pelodera*) *orbitalis* to be of holarctic distribution. Larvae of this species occur in the lachrymal fluid of small rodents, whereas its free-living (microbivorous) stages live in the nesting material. No pathogenic effects were observed in rodents infected by the nematode larvae. Interfertility of the 2 separated populations is discussed on the basis of stabilizing conditions in the nematode's microhabitat in both the Old and the New Worlds.

KEY WORDS: nematodes, *Rhabditis* (*Pelodera*) *orbitalis,* larval parasitism, nest fauna, hybridization, biogeography.

Rhabditis (*Pelodera*) *orbitalis* Sudhaus and Schulte, 1986, are microbotrophic nematodes in the nests of arvicolid and, to a lesser extent, murid rodents. Adults, developmental stages, and dauer juveniles can easily be extracted from that habitat and grown in the laboratory (Schulte, 1988). Third-stage infective juveniles, however, enter the orbital fluid of the rodents and remain as parasites for several days (Schulte, 1989). The phenomenon has attracted interest since its first discovery (Morozov, 1955; Stammer, 1956) and additional reports have documented similar eye nematodes resembling *R. orbitalis* (often confused with *R. strongyloides* [Schneider, 1860]) from numerous different hosts and sites in the Northern Hemisphere.

During the summer of 1989, the authors were able to establish laboratory cultures of 2 Californian and 1 European isolates of this species. The present paper reports on the results of experimental crosses between Californian and German isolates.

Materials and Methods

Adults of the suspected *R. orbitalis* were extracted using a Baermann funnel from material collected from nests of voles (*Microtus* sp.) at the University of California Field Station in Hopland, California (about 700 m elevation).

Cultures of the nematode were established on 2% agar plates containing small pieces of calf liver and maintained at room temperature. Additional nematodes were removed from the conjunctival sacs of a specimen of *Microtus californicus* (Peale) livetrapped near the Sagehen Creek Field Station near Truckee, California (elevation approx. 2,000 m) in the Sierra Nevada. Nematodes from the lachrymal fluid of both eyes (left eye 43, right eye 56) were transferred to agar plates. Two mature females survived and were crossbred with males from the Hopland strain.

The European isolate was collected by the Baermann method from nest material of the European meadow vole, *Microtus arvalis* (Pallas), at the Attendorn area (Northrhine-Westfalia, Western Germany; elevation about 350 m). It contained adults and developmental stages of *R. orbitalis* in great numbers that were used to start a laboratory population.

To test for interfertility, males and females of each strain were raised separately and males of one strain were combined with females of another strain in the ratio of 5 females to 10 males on an agar dish in a droplet of bacterial suspension. Mating and egg-laying were monitored with a dissecting microscope. Five replicates were conducted of each of 2 reciprocal crosses: American strain males × European strain females and European strain males × American strain females.

Results

Appearance of eye infection

With a little experience it is possible to determine the presence of the nematodes in situ in the field. Eyes parasitized by rhabditid nematode larvae in California have exactly the same appearance as those of European voles: the moving nematodes appear as an undulating glistening mass on the eye's surface and can be seen readily with a low power hand lens, especially when the skin at the corner of the eye is drawn back. Occasionally, some larvae near the inner angle of the eye leave the lachrymal fluid and wave one-third of their bodies back and forth in the air. These larvae nearly always resume development when removed from the eye and transferred to an agar plate seeded with bacteria. In general, "older" larvae (i.e., living in the orbital fluid for more than 5 days) show a tendency to stay at the

Table 1. Reports of parasitic larvae of *Rhabditis orbitalis* in the lachrymal fluid of small rodents.

Locality	Host species	Author(s)
A) North America		
1. Eskimo Point (Hudson Bay)	*Dicrostonyx groenlandicus* (Traill), *Lemmus sibiricus* (Kerr)	Cliff et al. (1978)
2. Missoula (Montana)	*Clethrionomys gapperi* (Vigors), *Microtus longicaudus* (Merriam)	Kinsella (1967)
3. California	*Microtus californicus* (Peale)	Poinar (1965), present study
4. Point Barrow (Alaska)	*Lemmus sibiricus*	Rausch (1952)
5. Black Hills (S. Dakota)	*Clethrionomys* sp.	Trapido, pers. comm.
B) Europe		
6. Central Europe (Western Bohemia [CSSR]; Berlin, Erlangen, Vogelsberg, Freiburg, Sauerland [Germany])	*Apodemus flavicollis* (Melchior), *A. sylvaticus* (L.), *A. agrarius* (L.), *Arvicola terrestris* (L.), *Clethrionomys glareolus* (Schreber), *Mus musculus* (L.), *Pitymus subterraneus* (de Selys-Longchamps), *Microtus agrestis* (L.), *M. arvalis* (Pallas)	Helm (1974), Prokopič et al. (1974), Stammer (1956), Schulte (1989), present study
7. Great Britain (Oxford, Slapton Ley, Ascot)	*Apodemus sylvaticus, Clethrionomys glareolus, Microtus agrestis, M. arvalis*	Canning et al., 1973, Hominick and Aston (1981), Poinar (1965), Trapido, pers. comm.
C) Asia		
8. Nantou Hsien (Taiwan)	*Apodemus sylvaticus, Rattus norvegicus* (Berkenhout)	Cross and Santana (1974)
9. Novosibirsk Province (USSR)	*Apodemus flavicollis, Arvicola terrestris, Clethrionomys glareolus, Lagurus lagurus* Pallas, *Micromys minutus* Pallas, *Microtus agrestis, M. arvalis, M. oeconomus* (Pallas), *Pitymus subterraneus*	Morozov (1955)

bottom of the conjunctival sac while "younger" larvae can be seen moving across the surface of the eye.

Under natural conditions, there is no sign of inflammation of the eyes or surrounding tissues caused by the nematode larvae even in hosts with hundreds of nematodes present. Since the nematode microhabitat is the "outside world" of the orbital fluid surface film rather than the host tissue, even high numbers of larvae may be tolerated after being sterilized by exsheathment (Schulte, 1989). It is not known whether the host's vision is handicapped by the nematodes themselves or their movements. However, no noticeable changes were observed in the behavior of infected voles or mice kept in the laboratory.

Cross-mating experiments

Females reared from eye larvae from the Sagehen Creek area were fertilized by males from Hopland, resulting in a laboratory culture which is now referred to as the "American strain." Individually raised males and females were crossbred with the European *R. orbitalis*. All crosses were successful except for one of American males with European females where worms were overcome by fungal infection.

These results confirm the conspecificity of the different strains and, furthermore, the holarctic distribution of the species, *Rhabditis* (*Pelodera*) *orbitalis*.

Geographical distribution by earlier records

Unidentified small nematode "eye larvae" were first briefly mentioned by Rausch (1952) in a survey on the parasites of lemmings, *Lemmus sibiricus* (Kerr). First detailed descriptions came from Morozov (1955), who found similar larvae in 9 different species of small rodents in the Novosibirsk Province (USSR). Further records hitherto published are listed in Table 1.

With reference to the successful crossings between European and North American populations presented above, we would further expect to find *R. orbitalis* throughout the Northern Hemisphere wherever environmental conditions are suitable.

Discussion

Rhabditis orbitalis is a larval parasite that is strictly associated with a rodent host: without the opportunity of entering the orbital fluid, populations of this nematode would become "trapped" in the decaying nesting material and sooner or later perish. However, its highly specialized third-stage (infective) larvae allow this species to use the orbital fluid as an unexploited nutrient source and become distributed to new habitats (i.e., rodents' nests) at the same time (Schulte, 1989).

Although the parasitism of *R. orbitalis* is obligatory in this particular respect, the species can be grown indefinitely on any bacteria in the laboratory like many other nematodes of the genus *Rhabditis*. This offers the rare opportunity to cross-breed populations from widely separated localities and/or different hosts. Apart from morphological identification characters, conspecificity can thus be tested directly. This becomes a matter of particular interest as several species closely related to *R. orbitalis* proved to be morphologically nearly identical sibling species (Sudhaus et al., 1987).

Because *R. orbitalis* is found in both parts of the Northern Hemisphere, Eurasia and North America, the association must have evolved before the continents were separated by the Bering strait. As in many other patterns of holarctic animal distribution, closely related species of arvicolid rodent species (*Microtus* spp., *Lemmus* spp., *Clethrionomys* spp.), occur in both the Old and the New Worlds (Thenius, 1980). Since ecological conditions in the separated continents are very much the same, there is no noticeable divergence in the evolution of the 2 populations of *R. orbitalis,* as shown by the hybridization studies reported here.

The place of origin of *R. orbitalis* remains unknown, but a possible hypothesis is that this parasitic association may have evolved among colonies of arctic lemmings. This is probable on the basis of a continuously high level of humidity offered there for the microbotrophic stages.

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