

Natural and experimental associations of *Caenorhabditis remanei* with *Trachelipus rathkii* and other terrestrial isopods

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Summary – *Caenorhabditis remanei* was found in association with the terrestrial isopod *Trachelipus rathkii* at several wooded locations in southwestern Ohio. These associations were as developmentally arrested dauer larvae. The sites of association were the inner surfaces of the dorsal plates and ventral appendages. *C. remanei* associations also were observed with *Armadillidium nasutum*, *Cylisticus convexus*, and *Porcellio scaber*. They were not observed with *Porcellio spinicornis* even though *P. spinicornis* populations were intermingled with infested populations of *T. rathkii*. Consistent with the observed natural associations, *C. remanei* dauers were experimentally able to infest *T. rathkii* and *P. scaber*. Dauer larvae responded to confinement with isopods by nictating and by climbing upon these potential hosts. Experimental infestations were able to persist for at least five days. Long-term infestations were not attempted.

Zusammenfassung – *Natürliche und experimentelle Vergesellschaftungen von Caenorhabditis remanei mit Trachelipus rathkii und anderen terrestrischen Isopoden* – An mehreren waldigen Stellen im Südwesten von Ohio wurde *Caenorhabditis remanei* vergesellschaftet mit dem terrestrischen Isopoden *Trachelipus rathkii* gefunden. Diese Assoziationen waren wie in der Entwicklung gehemmte Dauerlarven. Sie wurden an den inneren Oberflächen der Dorsalplatten und der ventralen Anhänge festgestellt. Vergesellschaftungen von *C. remanei* wurden auch mit *Armadillidium nasutum*, *Cylisticus convexus* und *Porcellio scaber* beobachtet. Nicht beobachtet wurden sie dagegen bei *Porcellio spinicornis*, obwohl Populationen dieser Art mit infizierten Populationen von *T. rathkii* vermischt vorkamen. In Übereinstimmung mit den beobachteten natürlichen Assoziationen konnten Dauerjuvenile von *C. remanei* auch im Versuch *T. rathkii* und *P. scaber* befallen. Die Dauerjuvenile reagierten auf das enge Zusammensein mit den Isopoden durch Nickbewegungen und Klettern auf diese potentiellen Wirte. So ein experimenteller Befall konnte für mindestens fünf Tage bestehen. Langfristiger Befall wurde nicht versucht.

Keywords – Crustacea, host-associate interactions, Nematoda, nematode ecology, Oniscoidea, Rhabditidae.

Many rhabditid nematodes use other soil invertebrates for transport between microenvironments (Poinar, 1983). These phoretic associations form when environmental conditions favour dauer development over continued growth and reproduction. They end when a more favourable environment is reached or created by the death of the host animal. In either case, the associated dauer larvae then resume development and reproduce for one or more generations until dauer development is again favoured.

Among the genera of Rhabditoidea that can form phoretic associations with other soil invertebrates is *Caenorhabditis*. *Caenorhabditis* comprises 18 accepted species (Sudhaus & Kiontke, 1996). Of these, nine have been observed as facultative or obligate associates of other invertebrates; *C. plicata* of carrion beetles (Völk, 1950;

Sudhaus, 1974), *C. bovis* of flies (Msolla *et al.*, 1989, cited in Sudhaus and Kiontke, 1996), *C. formosana* of snails (Yokoo & Okabe, 1968), *C. oncomelaniae* of snails (Yokoo & Okabe, 1968), *C. remanei* of terrestrial molluscs (Sudhaus, 1974; Baird *et al.*, 1994) and of terrestrial isopods (Baird *et al.*, 1994; Sudhaus & Kiontke, 1996), *C. drosophilae* of fruit flies (Kiontke, 1997), *C. sp.* strain CB5161 of palm weevils (Griffith, 1968; Gerber & Giblin-Davis, 1990), *C. elegans* of slugs (Mengert, 1953) and of terrestrial isopods (S.E. Baird, unpubl.), and *C. briggsae* of snails (W.K. Thomas, pers. comm.).

The associations of *C. remanei* are of interest because they occur with such divergent hosts. However, as noted by Sudhaus and Kiontke (1996), associations between *C. remanei* and terrestrial isopods have been reported only with isopods obtained from compost heaps. As com-

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post heaps are anthropogenic, they cannot represent the natural habitat of *C. remanei*. Thus, associations of *C. remanei* with terrestrial isopods merit further investigation.

In this report, associations of *C. remanei* with *Trachelipus rathkii* and with other terrestrial isopods are described. These associations were observed at several wooded locations in southwestern Ohio and were also established experimentally. Among the collection sites where *C. remanei*-isopod associations were observed were relatively undisturbed sections of woods that had never been clear cut or extensively logged. These sites represent a more natural habitat for *C. remanei* than compost heaps.

Materials and methods

ISOPOD COLLECTION, MAINTENANCE, AND SACRIFICE

Isopods were collected in woodland locations from rotting logs, from under rocks, and from under leaf litter. Species identifications were made following Pratt (1935), Van Name (1936), and Schmidt (1997). Collected isopods were sacrificed by evisceration. Eviscerated animals were placed on bacteriological plates seeded with *E. coli* strain OP50 (Baird *et al.*, 1994). Plates were periodically scored for the appearance of nematodes using a dissecting microscope (magnification 12-50 \times) for up to 24 h post-evisceration.

DISSECTIONS OF *T. RATHKII*

Dissections involved cutting off the head, eviscerating the animals by pulling the abdomen away from the thorax, and separating the abdomen from the viscera. In some cases, the thorax and abdomen was further dissected to separate the ventral appendages from the dorsal plates.

NEMATODE STRAINS, STRAIN MAINTENANCE AND SPECIES IDENTIFICATION

Nematodes were grown on bacteriological plates seeded with *E. coli* strain OP50 (Brenner, 1974). Worms obtained from isopods were transferred to fresh plates as they were observed. Several isofemale strains were established from collected nematodes. These strains are available from the *Caenorhabditis* Genetics Center (gopher://elegans.cbs.umn.edu:70/1). Species identifications were made from microscopic observations (magnification

400-1000 \times , differential interference contrast optics) according to Andr assy (1983) and Sudhaus and Kiontke (1996). Identification of *C. remanei* isolates were confirmed by mating tests with *C. remanei* strains SB146 or EM464 (Baird *et al.*, 1992).

EXPERIMENTAL INFESTATIONS

In each experiment, isopods were infested by confining them overnight to a single 60 mm agar plate that contained a population of *C. remanei* dauer larvae. Responses of dauer larvae to potential hosts were observed by stereo microscopy (12-50 \times). Following overnight confinement, host isopods were removed to a dry container and placed inside a humid chamber for 1 to 5 days prior to sacrifice. Infestation levels of experimental hosts were determined as described above. The *C. remanei* strains used in these studies, EM464, PB218, and PB205, were originally obtained from *A. vulgare*, *T. rathkii*, and *P. scaber*, respectively (Baird *et al.*, 1994; this work).

STATISTICAL ANALYSIS OF HOST PREFERENCE

Infestation frequencies of *A. nasatum*, *C. convexus*, *P. scaber*, and *P. spiniornis* were compared to infestation frequencies of sympatric (intermingled) populations of *T. rathkii* using a chi-squared test. The *A. nasatum*, *P. scaber*, and *P. spiniornis* comparisons were made using data from Huffman MetroPark. The *C. convexus* comparison was made using data from Eastwood MetroPark.

Results

C. remanei was found in association with terrestrial isopods at nine of eleven woodland locations in and around Dayton Ohio (Table 1). At all locations, *T. rathkii* was either the predominant or the only isopod observed. Less abundant isopod species included *Armadillidium nasatum*, *Cylisticus convexus*, *Porcellio scaber*, and *Porcellio spiniornis*. Infestation frequencies of *A. nasatum*, *C. convexus*, and *P. scaber* were not significantly different from that of sympatric populations of *T. rathkii* ($P = 0.7$, 0.05 , and 0.2 , respectively). Infestation of *P. spiniornis* was not observed. This lack of infestation of is significant ($P < 0.001$).

Associations of *C. remanei* with terrestrial isopods were as developmentally arrested dauer larvae. When first observed, *C. remanei* individuals were either dauer larvae (if observed within 10 h of sacrifice) or L4 larvae (if observed between 10 and 24 h after sacrifice). The appearance of L4 larvae between 10 and 24 h post sacrifice is

Table 1. Association of *Caenorhabditis remanei* with terrestrial isopods.

Collection site	Host species	Number sacrificed	Number infested	Worm load ¹
Aullwood Audubon Center	<i>T. rathkii</i>	27	7	3.4 ± 1.4
	<i>C. convexus</i>	2	0	–
Carriage Hill MetroPark	<i>T. rathkii</i>	18	1	3
Cox Arboretum	<i>T. rathkii</i>	60	2	2.0 ± 1.0
	<i>A. nasatum</i>	27	0	–
Eastwood MetroPark	<i>T. rathkii</i>	30	6	3.7 ± 1.5
	<i>P. spinicornis</i>	3	0	–
	<i>C. convexus</i>	15	6	4.8 ± 2.0
Englewood MetroPark	<i>T. rathkii</i>	30	9	14.3 ± 6.9
Germantown MetroPark	<i>T. rathkii</i>	18	0	–
Huffman MetroPark	<i>T. rathkii</i>	90	16	5.5 ± 2.3
	<i>P. spinicornis</i>	59	0	–
	<i>A. nasatum</i>	20	3	2.0 ± 0.0
	<i>P. scaber</i>	15	1	1
John Bryan State Park	<i>T. rathkii</i>	21	15	8.3 ± 1.2
Possum Creek MetroPark	<i>T. rathkii</i>	15	0	–
Sugarcreek MetroPark	<i>T. rathkii</i>	47	5	2.2 ± 0.7
Wright State University Biology Preserve	<i>T. rathkii</i>	66	28	5.9 ± 1.8
	<i>C. convexus</i>	2	1	1
	<i>A. nasatum</i>	2	1	1

¹ Mean ± sem, excluding uninfested isopods.

consistent with the dauer to L4 recovery period of *C. remanei* (data not shown).

C. remanei infestations were primarily in the thoracic and abdominal segments of *T. rathkii*. Forty *C. remanei* dauers were obtained from dissected isopods. The distribution of these dauers among the head, thorax, abdomen, and viscera was 1, 15, 23, and 1, respectively. Attempts were made to separate the ventral appendages from the dorsal plates of the thorax and abdomen. These attempts were only partially successful as *C. remanei* dauer larvae are motile and often appeared during secondary dissections. Five dauers were obtained following secondary dissections of the thorax. All of these were associated with the dorsal plates. Three dauers were obtained following secondary dissections of the abdomen. One of these was associated with the dorsal plates and two with the ventral appendages.

T. rathkii and *P. scaber* have been experimentally infested by confining them to agar plates containing populations of *C. remanei* dauer larvae. *C. remanei* dauers

responded to the presence of potential hosts by nictating and by moving up on the legs, antennae, and dorsal plates of these isopods. Nictation usually was apparent within one minute of isopod confinement and most frequently occurred on isopod frass. Nictation also was observed following the transfer of frass to dauer-laden plates and, to a lesser extent, following the transfer of glass beads to dauer-laden plates. Dauer larvae usually were observed on experimental hosts within five minutes of isopod confinement.

Although *C. remanei* dauers readily mounted experimental hosts, establishment of a persistent infestation was problematic. When host isopods were transferred to a damp environment immediately following confinement, dauer larvae abandoned their host in favour of the new environment. When host isopods were transferred to a dry environment following confinement, dauer associations were maintained for periods of up to five days (Table 2). However, isopods are sensitive to desiccation and few hosts survived beyond five days in a dry environment.

Table 2. Experimental infestation of *Trachelipus rathkii* and *Porcellio scaber* by *Caenorhabditis remanei*.

Host isopod	<i>C. remanei</i> strain	Infestation load (N) ¹		Fraction infested (N) ²	
		Days post-confinement		1	5
		1	5		
<i>T. rathkii</i>	EM464	25 ± 15 (5)	10 ± 6 (5)	1.0 (5)	1.0 (5)
	PB218	72 ± 7 (5)	17 ± 2 (5)	1.0 (5)	1.0 (5)
	control ³	11.2 ± 15.8 (27)		0.37 (27)	
	control ⁴	3.5 ± 4.0 (26)			
<i>P. scaber</i>	PB205	129 ± 32	102 ± 66	1.0 (3)	1.0 (3)
	control ⁵	0	0	0.0 (3)	0.0 (3)

¹ Infestation load = mean number of *C. remanei* dauers per host ± twice the sem.

² Fraction infested = number of infested hosts/number of hosts sacrificed.

^{3,4} Control isopods were sacrificed the day of host confinement. Most of these were either uninfested or infested with fewer than ten nematodes. One host was infested with 212 *C. remanei* dauers. This infestation load is greater than two standard deviations higher than the mean control infestation load. Infestation load control values (³) including and (⁴) excluding this host are given.

⁵ *P. scaber* control animals were mock infested (confined to an empty agar plate) and sacrificed on the same schedule as experimental animals.

Discussion

The results presented here confirm and broaden knowledge of the associations of *C. remanei* with terrestrial isopods. Previously, knowledge of these associations had been restricted to *A. vulgare* and *A. nasatum* (Baird *et al.*, 1994). It now has been expanded to include associations with *T. rathkii*, *C. convexus*, and *P. scaber*.

T. rathkii was an especially abundant host of *C. remanei*. Association of *C. remanei* with *T. rathkii* was observed at nine of eleven locations surveyed. These locations are separated from each other by up to 50 km and there are no contiguous woodlands connecting most of these locations. Significant migration of isopods between these locations is unlikely (McNamara, 1980). Thus, the results presented here represent associations of *C. remanei* with multiple distinct populations of *T. rathkii*.

The isopods used in this study were obtained from wooded collection sites. Unlike compost heaps (Sudhaus & Kiontke, 1996) these habitats are not anthropogenic. This is especially true of the WSU Biology Preserve, which is an old growth stand that has never been clear cut or extensively logged (DeMars & Runkle, 1992). Thus, the woodland association of *C. remanei* with *T. rathkii* must be considered a normal part of the natural history of *C. remanei*. The only caveat to this conclusion is that

T. rathkii is native to Europe, not North America. In Europe *T. rathkii* is not found in woodlands (Van Name, 1936; Rapp, 1988; Schmidt, 1997). It will be of interest to determine whether or not *C. remanei* is associated with non-woodland populations of *T. rathkii* in Europe and/or with other species of *Trachelipus* in European woodlands.

The host range of *C. remanei* includes members of four families, Armadillidiidae, Cylisticidae, Porcellionidae, and Trachelipodidae, within the superfamily Porcellionioidea (Suborder Oniscidea). However, *C. remanei* does exhibit host preferences within Porcellionioidea. *P. spinicornis*, a member of Porcellionidae and a close relative of *P. scaber*, is not a host for *C. remanei*. Presumably, *C. remanei* either cannot initiate or cannot maintain infestations in *P. spinicornis*. Experiments to address the bases of *C. remanei* host preferences require an appropriate experimental infestation protocol.

Short-term experimental infestations of *T. rathkii* by *C. remanei* dauers, although achieved, were not entirely satisfactory. In part, this is because a suitable post-confinement regimen has not been defined; immediate transfer to a damp environment results in host abandonment, long-term transfer to a dry environment results in host mortality. Most likely, a suitable post-confinement regimen will consist of a brief transfer to a dry environment followed by transfer to a damp environment.

Experiments to define an appropriate infestation protocol have not been conducted because of statistical problems resulting from rare, heavily infested control hosts. To eliminate these problems, uninfested laboratory populations of host isopods are required.

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