RESEARCH NOTES

Cross-mating of Romanomermis culicivorax and R. communensis (Nematoda: Mermithidae)

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Journal of Nematology 14(2):274-276. 1982.

The genus *Romanomermis* presently contains seven recognized species, six of which parasitize mosquitoes (2). These species differ morphologically, are geographically isolated, and exhibit different biological characteristics from one another (e.g., optimal host species, development times, and temperatures) (1,3). The study conducted by Petersen (3) represents the only published attempt at cross-mating *Romanomermis* spp. He crossed *R. nielseni* (Tsai and Grundman) from Wyoming and *R. culicivorax* Ross and Smith from Louisiana but did not observe egg development or oviposition in any cross-mated females.

During investigations of mermithid parasites of mosquitoes in Manitoba, we encountered *R. communensis* Galloway and Brust parasitizing larvae of *Aedes communis* (DeGeer) at Goose Creek (2). Sufficient numbers of *R. communensis* were available to conduct cross-mating experiments with *R. culicivorax* from Louisiana.

For experiment 1, in 1975, *R. communensis* juveniles emerged in the laboratory from field-collected larvae. Juveniles of *R. culicivorax* were obtained from our laboratory stock culture. Juveniles were sexed and separated and *R. culicivorax* were held up to 4 days at 25 C while *R. communensis* were held up to 7 days at 20 C to ensure that both species would moult at the same time. Nematodes were then placed in moist silica sand (280 g/60-ml glass jar) in the following combinations: *R. culicivorax* 30 ♂ × 30 ♀, *R. communensis* 30 ♂ × 30 ♀, *R. communensis* × *R. culicivorax* 30 ♂ × 30 ♀, *R. culicivorax* × *R. communensis* 30 ♂ × 30 ♀, *R. communensis* 30 ♀, and *R. culicivorax* 30 ♀.

Received for publication 7 July 1981.

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Funding was provided by a NRC postgraduate scholarship to the senior author and a Manitoba Northern Studies grant to the second author. Jack Harlos, Dave Thornton, Carol Galloway, and Eileen Adams provided assistance both in the field and laboratory.

Survival was high in all groups after 56 days (Table 1). All survivors had moulted to adults, and all females in the presence of males contained eggs. Most *R. communensis* females were nearly depleted of eggs, while *R. culicivorax* females still had the trophosome largely intact. Parthenogenesis was not observed in isolated females of either *R. communensis* or *R. culicivorax*.

All survivors were returned to their respective containers for an additional 56 days at 20 C before being flooded with chlorine-free tap water. Preparasites were collected and numbers estimated 24 h later. The reciprocal crosses of *R. communensis* × *R. culicivorax* and the *R. communensis* groups each produced approximately 100 preparasites, while the *R. culicivorax* group produced ca. 2,700 preparasites. The *R. culicivorax* culture produced more preparasites than the crosses or the culture of *R. communensis*. However, *R. communensis* eggs do not hatch synchronously in the laboratory (1,5). Many fully embryonated, viable eggs were present in our culture. Cross-mated *R. culicivorax* females and *R. communensis* females laid approximately as many eggs as did female *R. culicivorax* but they could not be stimulated to hatch when flooded.

Approximately 100 preparasites from each reciprocal cross were placed in separate 15-cm-d plastic pans containing 100 ml of chlorine-free tap water. One-day-old *Aedes aegypti* L. larvae (250) and liver powder were added to each pan and kept at 25 C. Seven male postparasites from both crosses were recovered 8–11 days after preparasite introduction. No female juveniles were obtained. The development time closely resembled that of *R. culicivorax;* male *R. communensis* take 12–14 days to complete parasitic development at 25 C (Galloway, unpublished).

Another cross-mating experiment was conducted in 1976 (experiment 2). Three

Table 1. Percent survival of *Romanomermis culicivorax* (CUL) and *R. communensis* (COM) in two cross-mating experiments and percent surviving females gravid at each inspection time (days).

	% survival ♂			% survival ♀			% gravid		
	26	56	112	21	56	112	21	56	112
Experiment 1 (1975)									
CUL ♂ × CUL ♀*	...	90.0	70.0	...	93.3	0.0	...	100	...
CUL ♂ × COM ♀*	...	96.7	60.0	...	93.3	0.0	...	100	...
COM ♂ × CUL ♀*	...	96.7	56.7	...	90.0	0.0	...	100	...
COM ♂ × COM ♀*	...	83.3	50.0	...	83.3	0.0	...	100	...
CUL ♀†	...	...	...	...	90.0	66.7	...	0.0	0.0
COM ♀†	...	...	...	...	86.7	63.3	...	0.0	0.0
Experiment 2 (1976)									
CUL ♂ × CUL ♀‡	73.2	70.0	56.7	91.7	88.3	73.3	0.0	0.7	2.0
CUL ♂ × COM ♀‡	86.7	86.7	75.0	91.7	0.0	0.0	98.1	...	...
COM ♂ × CUL ♀‡	90.0	70.0	58.3	93.3	90.0	86.7	0.0	0.0	0.7
COM ♂ × COM ♀‡	96.7	85.0	80.0	100	0.0	0.0	100	...	...
CUL ♀§	...	...	...	80.0	80.0	75.0	0.0	0.0	0.0
COM ♀§	...	...	...	90.0	90.0	85.0	0.0	0.0	0.0

*One replicate of 30 ♂ × 30 ♀.
†One replicate of 30 ♀.
‡Mean of 3 replicates of 20 ♂ × 20 ♀.
§One replicate of 20 ♀.

replicates each of 20 ♂ and 20 ♀ were established as before for all combinations of *R. communensis* and *R. culicivorax*. Two containers with 20 ♀ per container of each species were also prepared. Juveniles of *R. communensis* emerged from mosquito larvae in the field and were 3–8 days old at the beginning of the experiment. Juveniles of *R. culicivorax* were only 1–2 days old (25 C). Thereafter, all cultures were kept at 20 C and examined at 21, 56, and 112 days.

All but one of the *R. communensis* females were gravid after 21 days, and by 56 days all females had laid their full complement of eggs and died. Egg development in *R. culicivorax* females was retarded although all had moulted to adults after 21 days (Table 1). The reason for this delay is unclear since *R. culicivorax* males were observed mating with *R. communensis* and conspecific females after 21 days. As in 1975, no parthenogenesis was observed in either species. Sand containing eggs from reciprocal-cross females was flooded after 180 days but too few preparasites were recovered to attempt infection trials.

The successful crossing of *R. communensis* and *R. culicivorax* is a challenge to the taxonomic status of these allopatric species. However, cross-mating does not prescribe conspecificity, especially when manipulation of animals in the laboratory is involved. Considering morphological and biological differences and the possibility of pseudogamy, *R. communensis* and *R. culicivorax* must be maintained as distinct species.

The genus *Romanomermis* in North America poses some very difficult problems for mermithid taxonomists. There is little information available on factors affecting morphological variation within species, and differences between species are often small (2,4). However, the dispersal ability of these nematodes is limited since parasitic development is largely confined to larval mosquitoes. Consequently, populations in close proximity to one another may in fact have been reproductively isolated for a long time. For example, populations of *R. communensis* and *R. hermaphrodita* Ross and Smith near Churchill, Manitoba, are found only 25 km apart. Considerable research into variation within species is necessary before further taxonomic relationships between *Romanomermis* spp. can be established.

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