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Predaceous Behavior and Life History of *Odontopharynx longicaudata* (Diplogasterida)[1]

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Abstract: The behavior of a California isolate of the predaceous nematode, *Odontopharynx longicaudata* de Man, was studied in water agar culture. When feeding on an *Acrobeloides* sp. the predator completed its life cycle in 13 to 14 days at 25 C. Optimum temperature for reproduction of the predator was 25 C, few individuals survived at 10 C, and 30 C was lethal. Males were necessary for reproduction, and at 25 C the sex ratio was about 1:1. All postembryonic stages were voracious feeders. A single female predator consumed 30 individuals of another *Acrobeloides* sp. in 1.5 days. Juveniles must feed in order to complete their development. Three modes of feeding were observed depending on the prey selected. A high degree of prey selectivity occurred; 6 of 17 nematode prey species were readily consumed by the predator, but there was little or no feeding on the remaining 11 species. Predation percentage varied with prey species. Consumption of *Anguina pacificae* and the two *Acrobeloides* spp. was almost 100%, consumption of *A. amsinckiae, Pratylenchus vulnus,* and *Trichodorus* sp. was 70–78%. Difference in final predator population densities was obtained after feeding on the two species of *Acrobeloides.* Final predator population densities increased linearly with increasing inoculum levels of the first *Acrobeloides* sp.

Key words: Acrobeloides, Diplogasterida, life history, *Odontopharynx longicaudata,* predator, prey selectivity, reproduction, temperature.

In 1985 *Odontopharynx longicaudata* de Man was recovered from soil around the roots of *Poa annua* L. (annual bluegrass) growing on a golf course in San Francisco, California (1). Emended descriptions of the adults and morphology of the juvenile stages have been reported separately (2). De Man (3) suggested that the two adult *O. longicaudata* found in a rotting hyacinth bulb were actually predators of the other free-living nematodes extracted from the bulb, but its biology has remained largely unknown. The objective of our study was to determine the life cycle and examine the predaceous behavior of *O. longicaudata.*

Materials and Methods

Odontopharynx longicaudata was first cultured on a 1.5% water agar dish containing free-living and plant-parasitic nematodes that were extracted with the predator from the original soil sample. Predator populations were subsequently increased and maintained on an unidentified *Acrobeloides* sp. (UCD No. 1) in 1.5% water agar dishes incubated at 25 C.

Two species of *Acrobeloides* in culture were originally obtained from the Department of Nematology, University of California, Riverside. Both species and their unidentified bacterial food source were cultured on oatmeal agar. No special techniques were needed to maintain the bacterial cultures because sufficient numbers of bacteria were transferred from culture to culture on the bodies of the nematodes. Identification of the two *Acrobeloides* species is pending; hereafter, the two will be referred to as UCD *Acrobeloides* No. 1 (close to *A. labiatus* Ivanova) and UCD *Acrobeloides* No. 2 (close to *A. nanus* de Man).

Life cycle: Newly hatched second-stage juveniles (J2) of *O. longicaudata* were obtained from eggs deposited by females on water agar culture dishes. Each dish had been inoculated with four females and one male. Five J2 were transferred to each of 60 35-mm-d petri dishes containing water agar. Approximately 100 UCD *Acrobeloides* No. 1 were transferred to each dish in one

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or two drops of sterilized distilled water. The dishes were sealed with Parafilm and incubated at 25 C. Nematodes were extracted from the agar dishes during a period of 11 days. Extractions were done three times daily at 1000, 1400, and 1800 hours. To extract the nematodes, the agar in each dish was quartered and then blended for 5–8 seconds. All stages of *O. longicaudata* were handpicked from the suspension, processed to glycerin (7), and mounted for microscope examination. This experiment was repeated once.

Reproduction without males was tested by transferring a single J4 female to a water agar dish containing 1,000 UCD *Acrobeloides* No. 1. Four replicates of this treatment were incubated at 25 C for 25 days, and observed daily for egg production.

Prey selectivity trials: The following 17 nematode species representing four orders were tested as prey for *O. longicaudata.* 1) Order Tylenchida, suborder Tylenchina: *Anguina pacificae* Cid del Prado Vera & Maggenti, *A. amsinckiae* (Steiner & Scott) Thorne, *Criconemella xenoplax* (Raski) Luc & Raski, J2 of *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. hapla* Chitwood, *Pratylenchus vulnus* Allen & Jensen, and *Tylenchulus semipenetrans* Cobb. 2) Order Tylenchida, suborder Aphelenchina: *Aphelenchus avenae* Bastian, *Aphelenchoides fragariae* (Ritzema Bos) Christie, and *Seinura* sp. 3) Order Rhabditida: UCD *Acrobeloides* No. 1 and 2, and *Rhabiditis* sp. 4) Order Dorylaimida: *Trichodorus* sp. and *Xiphinema index* Thorne & Allen. 5) Order Mononchida: *Mononchus* sp.

Most of the species tested were obtained from laboratory and greenhouse cultures maintained in the Department of Nematology, University of California, Davis. *Seinura* sp. and UCD *Acrobeloides* No. 1 and 2 were obtained from the Department of Nematology, University of California, Riverside. *Anguina pacificae* was collected from green stem galls on *Poa annua* L. growing on the same golf course and habitat as *O. longicaudata,* and *A. amsinckiae* came from stem and seed galls on *Amsinckia intermedia* F. & M. growing in Santa Clara County, California.

Prey inocula varied because of limited availability of some species. Treatments included inoculations with 1) five *O. longicaudata* (four females and one male) and a single prey species per 35-mm-d water agar dish, 2) five *O. longicaudata* and no prey per dish, and 3) no *O. longicaudata* and a single prey species per dish. Each treatment for each prey species was replicated four times and incubated at 25 C for 2–23 days depending on the prey. The 23-day trial period allowed 1.5 life cycles of the predator. Shorter trials were necessary with the plant-parasitic nematodes because most survived poorly in the absence of a host. Plant-parasitic nematode prey were surface sterilized with 133 ppm ethoxyethylmercurychloride and 200 ppm dihydrostreptomycin sulfate.

Dishes containing both predator and prey were examined daily for predation. At the end of the experiment, percentage of predation by *O. longicaudata* was calculated from the final prey population densities with and without the predator. To insure accuracy, the final nematode populations were counted before and after extraction from diced agar pieces placed in modified Baermann funnels in a mist chamber for 2 days.

Effects of UCD *Acrobeloides* No. 1 and 2 on final predator population were determined statistically by analysis of variance. Because of differences in inoculum density and trial duration, statistical analyses of data from trials with the other test prey were not possible; therefore, the analyses made were mostly qualitative.

Effect of temperature on final population densities of O. longicaudata: One thousand UCD *Acrobeloides* No. 1 and five *O. longicaudata* adults (four females and one male) were transferred in 2 or 3 drops of sterilized distilled water to water agar in 35-mm-d dishes in the following treatments: 1) prey species and no predator, 2) predator and no prey species, and 3) predator and prey species. Treatments were replicated four times and incubated at 10, 15,

TABLE 1. Time required for the development of the postembryonic stages of *O. longicaudata* at 25 C.

Stage	Day	Hours from hatch
Eggs laid	0	
First molt†	1–2	
J2	3	0–1
Second molt	4	24–25
J3	5	48–49
Third molt	6	75–77
J4	7	96–97
Fourth molt	8	120–124
Adults	9	144–145
Eggs laid	13–14	240–265

† First molt occurs within egg.

20, 25, and 30 C. After 23 days, the nematodes were extracted as in the prey selectivity trials. Prey and predators were counted, and data were subjected to analysis of variance to determine differences in final nematode population densities at different temperatures.

Effect of inoculum levels of UCD Acrobeloides No. 1 on reproduction of O. longicaudata: UCD *Acrobeloides* No. 1 was transferred to water agar in 35-mm-d dishes at rates of 10, 20, 50, and 100 nematodes per dish; four females and one male *O. longicaudata* were immediately added to each dish. Control dishes contained either the prey or predator species alone. Treatments were replicated five times and incubated at 25 C for 15 days. Nematodes were extracted and counted as in the prey selectivity trials, and data were subjected to regression analysis.

Effect of activity space on predaceous ability of O. longicaudata: Activity spaces of 480 cm^3, 240 cm^3, and 120 cm^3 were obtained in 35-mm-d dishes containing water agar by cutting and removing none, one-half, and three-fourths of the agar. UCD *Acrobeloides* No. 2 were extracted from a stock culture plate as described in the prey selectivity trials. Large adults were extracted by passing the nematode suspension through a 43-μm-pore sieve. Eighty-five UCD *Acrobeloides* No. 2 adults and one nongravid female *O. longicaudata* were transferred in a water drop to each dish. Control dishes contained only UCD *Acrobeloides* No. 2. Treatments were replicated four times and incubated at 25 C. After 36 hours, the nematodes were extracted by blending the agar for 8 seconds in 10 ml water. All postembryonic nematode stages were counted. Population percentage of UCD *Acrobeloides* No. 2 consumed by a single predator was calculated. Data were subjected to analysis of variance.

Voucher specimens of *O. longicaudata* are on deposit in the University of California Davis Nematode Collection (UCDNC).

RESULTS

Life cycle: Odontopharynx longicaudata completed its life cycle (egg to egg) in 13 to 14 days at 25 C (Table 1). The first molt occurred within the egg, and postembryonic stages developed at intervals of 2 days between molts. Different juvenile stages were distinguished by 1) gonad development and length of reflex, 2) total body length, 3) esophagus length, and 4) distance of nerve ring and excretory pore from the anterior end. Sexual differences were first distinguishable in the J4 by the development of the gonad and length of its reflex.

Males were necessary for reproduction; the sex ratio was about 1:1 at 25 C. In tests where a single J4 female was cultured in the absence of males, the nematode developed to the adult stage but failed to produce eggs in 25 days. Mating, first observed 10 days after hatching from eggs, began with coiling of the posterior part of the male body around the female. Often the female moved forward and backward until the male was coiled around the vulvar region. Spicules of *O. longicaudata* are unequal in size, with the left spicule 31–40 μm long and the right spicule 5–7 μm long. The left spicule was extruded and opened the vulva for the introduction of sperm into the uterus. Two female postuterine sacs both serve as seminal receptacles. One or two additional males often were found nearby in the agar or coiled around the anterior part of the female. The nematodes did not feed during mating. Gravid females were present 2 days after mating or 12 days after hatching from eggs. In-

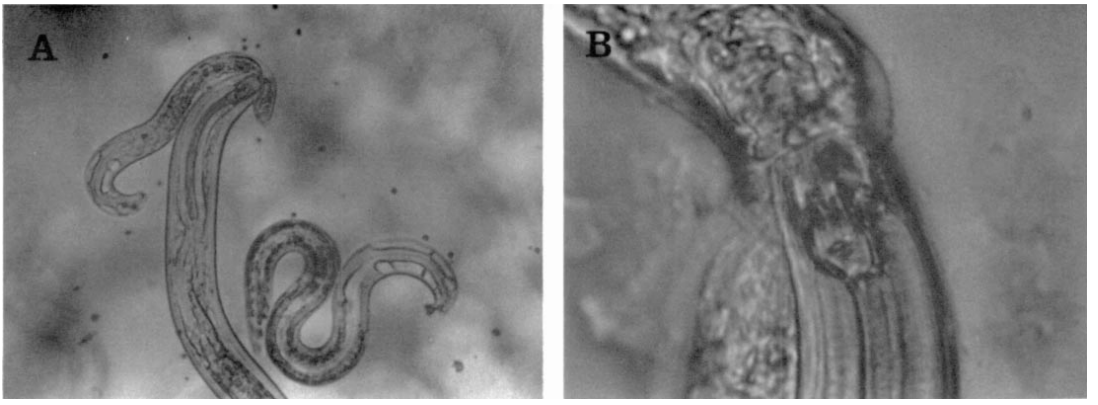

FIG. 1. Light micrographs of *Odontopharynx longicaudata* female. A) Feeding on UCD *Acrobeloides* No. 1. B) Stomatal region during feeding.

trauterine eggs had three or four cells. Females tended to lay more eggs inside the agar medium than on its surface.

In order to develop to mature adults, the juveniles must feed. Of 30 newly hatched J2, two survived 40 days without prey but developed only to the J3 stage. Survival and development to J3 by these two juveniles may have been due to their ingestion of the decomposed contents of the other dead juveniles.

Feeding behavior: The feeding apparatus of *O. longicaudata* is composed of a large open stoma containing one large dorsal tooth and 12 smaller teeth. The anterior part of the stoma is globose shaped, and the posterior one-third is funnel shaped. The large dorsal tooth is located about midstoma and is movable in a dorsoventral plane within the stoma. The 12 smaller teeth are nonmovable; six are positioned opposite the large tooth and the other six are in the posterior region of the stoma.

Odontopharynx longicaudata moved slowly through the agar and made contact with the prey by probing (pressing) its lip region against the body. This probing action occurred once or twice before the prey was attacked, but once selected for feeding, the prey was attacked quickly. If the prey was not selected for feeding, the predator moved away, often without probing.

The basic feeding action after probing involved drawing part of the prey's cuticle and body into the stoma by pumping action of the muscular anterior part of the esophagus, then rupturing the cuticle with the large dorsal tooth (Fig. 1A, B). Depending largely upon the size of the prey, feeding was achieved by one of three modes: 1) piercing the prey's body, while maintaining continuous contact, and ingesting the body contents; 2) piercing and rupturing the prey, without maintaining continuous contact, then ingesting the oozing body contents; or 3) ingestion of the entire body of the prey. The predator showed no preference for a particular feeding site on the prey's body; however, when it ingested the entire body, the prey was attacked near the tail.

Time required to consume a prey was 1–30 minutes for all postembryonic stages of the predator. The voracious feeding behavior of *O. longicaudata* was evident when a newly hatched J2 completely ingested a UCD *Acrobeloides* No. 2 juvenile and immediately severed another prey into two pieces and consumed half of that body. Cannibalism was observed only twice, each time in a 4–5-week-old starved culture where an adult female ingested a J2. Without prey, males usually died within a few days, but the females survived for a much longer time. Cultures without prey lasted about 4 weeks before the predator population began to decrease.

Prey selectivity: Odontopharynx longicaudata was very selective in the nematode species upon which it preyed (Table 2). Of

TABLE 2. Predation by *Odontopharynx longicaudata*† on selected nematode species.

Nematode prey species	Period (days)	Prey inoculum (no./dish)	Predation (%)	Final predator population‡
UCD *Acrobeloides* No. 1	23	100	99.7	80 a
UCD *Acrobeloides* No. 2	23	100	99.9	132 b
Anguina amsinckiae	11	100	78.0	50
A. pacificae				
Females	2	20	100.0	5
J2	2	20	100.0	5
Aphelenchoides fragariae	10	40	0.0	4
Aphelenchus avenae	23	100	0.0	3
Criconemella xenoplax	23	50	0.0	2
Meloidogyne hapla J2	23	100	0.0	0
M. incognita J2	23	100	0.0	0
M. javanica J2	23	100	0.0	1
Mononchus sp.§	2	38		1
Pratylenchus vulnus	2	20	70.0	5
Rhabditis sp.§	23	100		2
Seinura sp.	10	20	0.0	1
Trichodorus sp.	11	20	71.0	6
Tylenchulus semipenetrans	23	100	0.0	0
Xiphinema index	5	30	0.0	0

† Four females and one male *O. longicaudata* were added per trial.

‡ Means of four replicates per treatment. Means followed by different letters are significantly different ($P = 0.01$) according to Duncan's multiple-range test. Remaining nonlettered means were not statistically analyzed.

§ Predation observed but not quantified.

17 nematode species tested, six were readily consumed. High prey selectivity was represented by differences in prey population consumed. Consumption of *Trichodorus* sp., *Pratylenchus vulnus,* and *Anguina amsinckiae* was relatively high (70–78%), whereas it was almost 100% for UCD *Acrobeloides* No. 1, UCD *Acrobeloides* No. 2, and *Anguina pacificae.* In only 2 days, *Anguina pacificae* was completely eliminated by the predator. Predation percentage of either the *Mononchus* sp. or the *Rhabditis* sp. could not be determined. *Odontopharynx longicaudata* and the *Mononchus* sp. were observed feeding on each other. Only two or three *Rhabditis* sp. were attacked, and their bodies were only partly consumed. Sometimes feeding on *Rhabditis* sp. was unsuccessful because of the rapid movement of the prey after being attacked by *O. longicaudata.*

The final population density of *O. longicaudata* was significantly greater when the predator fed on UCD *Acrobeloides* No. 2, than when it fed on UCD *Acrobeloides* No. 1 ($P = 0.01$). There were no significant differences, however, between these trials in either percentage of predation or final control populations. Final population densities in controls of predator and prey were 4 *O. longicaudata,* 7,635 UCD *Acrobeloides* No. 1, and 5,863 UCD *Acrobeloides* No. 2.

Effect of temperature: Odontopharynx longicaudata did not survive at 30 C with or without the prey UCD *Acrobeloides* No. 1 (Table 3). The prey not only survived at 30 C, the population levels increased in numbers equivalent to the final control.

In treatments with prey, final population densities were significantly greater at 25 C than at the other temperatures ($P = 0.01$). Few predators survived at 10 C, but their numbers increased significantly at 15 and 20 C. Prey populations decreased as the number of predators increased. Even though there was no difference in the numbers of prey recovered at 15, 20, and 25 C, their populations were significantly lower at 20 and 25 C than at 10 C.

Predator population densities decreased in the absence of prey, and there were no significant differences among population densities at the different temperatures. At

TABLE 3. Effects of temperature on reproduction (final population densities) of the predator, *Odontopharynx longicaudata,* and prey, UCD *Acrobeloides* No. 1.

Treatment	Final population densities†				
	10 C	15 C	20 C	25 C	30 C
Prey (alone)	810 a	1,540 a	1,655 a	4,600 b	5,600 b
Predator (alone)	2 a	1 a	1 a	1 a	0 a
Combined					
Prey	645 a	263 ab	27 b	53 b	6,105 a
Predator	2 a	78 b	81 b	165 c	0 a

† Numbers are means of four replications. Analyses were done on square root transformed values. Means in horizontal rows followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple-range test.

the termination of the experiment, no males of *O. longicaudata* were recovered at any temperature.

Prey population densities increased with temperature in the absence of the predator. Final population densities at 25 and 30 C were significantly greater than at the other temperatures ($P = 0.01$). At 15 and 20 C, prey population densities were similar and were significantly greater than populations at 10 C ($P = 0.05$).

Effect of prey inoculum levels: Final population densities of the predator increased linearly with increasing initial density of the prey, explaining 65% of the variation and describing the relationship (Fig. 2). The predator eliminated the prey at the 10 and 20 inoculum levels. At the 50 inoculum level, final prey population density was reduced to $\frac{1}{17}$th (129 nematodes) of the control prey population density; at the 100 inoculum level, it was reduced to $\frac{1}{34}$th (106 nematodes) of the control prey population density.

Effect of activity space on predaceous ability: After 36 hours, the predator had consumed about the same number of UCD *Acrobeloides* No. 2 regardless of activity space. During the 36 hours, *O. longicaudata* consumed 35% (30 nematodes) of the prey population (data not shown).

DISCUSSION

The present study is the first investigation of the biology of *O. longicaudata* since its description by de Man in 1912 (3). Re-

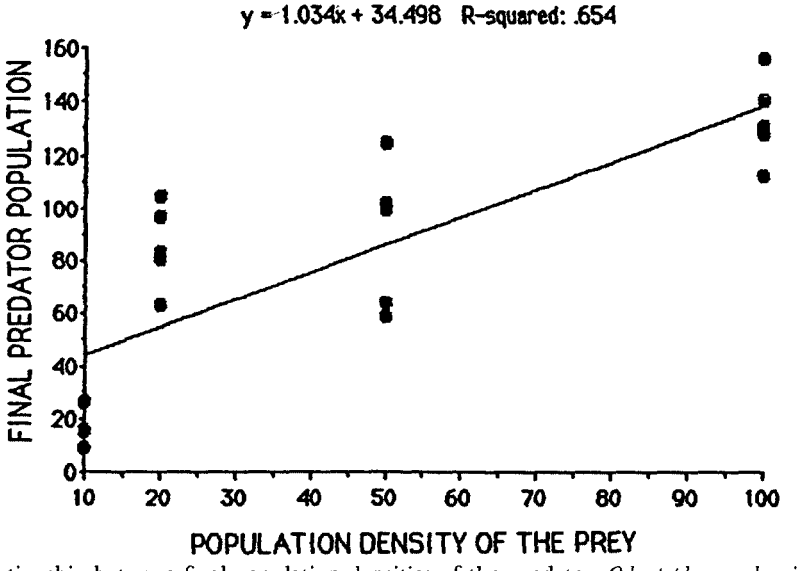

FIG. 2. Relationship between final population densities of the predator, *Odontopharynx longicaudata,* and initial population densities of the prey, UCD *Acrobeloides* No. 1.

ports on the behavior of other predaceous diplogasterids are limited to the genera *Mononchoides* (9) and *Butlerius* (5,6). Studies of the quantitative behavior of certain species of *Mononchoides* are the most commonly reported.

Odontopharynx longicaudata, an obligate predator, is very prey selective. The ability of *O. longicaudata* to eliminate populations of *Anguina pacificae* is of particular interest because it suggests that a target prey–predator relationship existed in the field habitat from which both species were collected. In our study, endoparasitic plant nematodes were attacked more readily than the ectoparasitic ones. This is generally true of predaceous nematodes (4); however, not all endoparasitic nematodes are susceptible to attack by *O. longicaudata,* as was demonstrated with *Meloidogyne* J2 and *Aphelenchoides fragariae.* The ability to attack and feed on the ectoparasitic *Trichodorus* sp. and the predator *Mononchus* sp. indicates a more vigorous feeding behavior than that reported for the predaceous diplogasterid *Butlerius* sp. which did not feed on *T. sparsus* Szczygiel and adults of *M. aquaticus* Coetzee (5). This may be due, in part, to the elaborate stomatal apparatus of *O. longicaudata* and (or) the presence or absence of specific chemical stimuli.

Prey selectivity has been reported in *Butlerius* sp.; however, all of the endoparasitic prey tested were susceptible to the predator, namely, *Pratylenchus* sp., *Meloidogyne naasi* Franklin, and *A. fragariae* (8,9). *Mononchoides potohikus* Yeates, is not a prey-selective species (9).

Differences in final predator population densities in trials with UCD *Acrobeloides* No. 1 and 2 indicate that different prey species may affect the rate of reproduction of the predator. The difference in reproduction was observed even when percentage of predation on those prey species was similar.

Increase in final populations of *Odontopharynx* was directly related to prey density. As the inoculum levels increased, the number of prey per unit area of predator increased, thereby increasing the number of contacts between predator and prey. Esser reported that prey density can affect predation by affecting the number of contacts (4). Predation in *Mononchoides potohikus* is also directly related to prey density (9). One generation of *O. longicaudata* is able to eliminate populations of UCD *Acrobeloides* No. 1 at initial nematode levels of 10 and 20. At initial nematode levels of 50 and above, the predator reduces prey population progressively as the number of predator–prey encounters increase.

Attraction between predaceous nematodes and prey has been suggested by some workers but has not been proven. Predation by *O. longicaudata* may occur by chance encounter with its prey, but the possible presence of an attractant needs investigation. High numbers of predators were observed near concentrations of UCD *Acrobeloides* No. 1 contained in an agar wedge within 1 hour of transferring the wedge to a water agar dish. Pheromone activity is suggested by the frequent presence of two or more males near each female during copulation.

At 25 C, *O. longicaudata* has a rather short life cycle of 13 to 14 days. All of the postembryonic life stages are voracious feeders, and a single female is capable of consuming 30 *Acrobeloides* No. 2 in 1.5 days. Among the other diplogasterid predators reported, *Butlerius* sp. has a shorter life cycle (8.5–9 days at 25 C), will eat 12 or 13 *Panagrellus* sp. in 1 day, and frequently is cannibalistic (5). *Mononchoides potohikus,* however, is reported to have consumed 180–380 *Mesorhabditis littoralis* (now *Bursilla littoralis* (Yeates) Andrássy) in 24 hours, and only one case of cannibalism was observed (9).

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