

Reproductive Biology and Behavior of *Rhabditis pellio* (Schneider) (Rhabditida: Rhabditidae)

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Abstract: Laboratory studies were conducted on the mating behavior of *Rhabditis pellio* males and females, which were maintained on a culture of *Flavobacterium* sp. bacteria isolated from earthworms. The mean time that elapsed between first contact of the sexes and their ultimate separation was 23.2 min. However, only 5.0 min were required for copulation (the interval during which male spicules were inserted into the female vagina). Three-day-old females that were permitted to mate once on their first day of adult life produced only one-third as many larvae as did females that were permitted unlimited mating. However, the longevity of females was found to decrease with an increase in the number of matings. Both males and females that were permitted to mate daily produced the greatest number of offspring when they were 4 days old. When the initiation of mating was delayed beyond their third day of life, the number of larvae produced by females decreased. In approximately one-half of the copulations, males failed to inseminate their female partners. **Key Words:** Sexual behavior, reproductive potential, fecundity.

The association between *Rhabditis pellio* (Schneider) and the annelid *Aporrectodea trapezoides* (Duges) was recently described by Poinar and Thomas (11). *Rhabditis pellio* is a facultative parasite that enters the earthworms during the dauer stage but does not attain sexual maturity and reproduce until the host dies and the nematodes return to the soil.

Intensive studies of the reproductive biology of nematodes have been conducted on only a few species (7, 12). Investigations of female fecundity often have given misleading results because estimates of oviposition have been based on brief observations, whereas oviposition rates have been shown to vary over time (6). Also, little attention has been given to the reproductive potential of male nematodes.

Some aspects of the reproductive biology of *R. pellio* have been discussed by Otter (10), but his results were based on incomplete data and are not conclusive. Therefore, we have undertaken a detailed study of many characteristics of the reproductive biology and behavior of *R. pellio* males and females.

MATERIALS AND METHODS

Aporrectodea trapezoides were removed from soil in Riverside, California; washed in tap water; and cut into pieces which

were placed on 2.3% nutrient agar in 100-x 15-mm plastic petri plates. *Rhabditis pellio* emerged from these pieces within 2 days, and thereafter they were reared xenically on similar nutrient-agar "colony" plates. All cultures were maintained in a Percival (Model I 30-B) environmental chamber at a 12:12-h light:dark cycle and a temperature of 22 ± 1 C. Two new colony plates were made 5 times each week by transferring a piece of agar containing nematodes from each of two older colony plates onto fresh agar plates.

The microfloral populations found in the earthworms containing *R. pellio* were isolated and colonized separately on 100-x 15-mm plastic petri plates filled with 2.3% nutrient agar. Two of the three constituents found in the colony, a yeast and a bacterium, did not adequately support nematode growth and were not identified. However, the third constituent, *Flavobacterium* sp. (Eubacteriales: Achromobacteraceae), supported abundant growth of *R. pellio*. This genus of bacteria includes gram-negative, rod-shaped species that have proteolytic properties and are commonly found in soil or water (1).

The bacterial colony was maintained under the same conditions as the *R. pellio* colony. New bacterial colony plates were made approximately once a week by applying streaks of bacteria from old plates with a paintbrush (00000 Grumbacher) onto the freshly prepared plates. A small amount of distilled water was poured onto the new plates which were then whirled vigorously

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to distribute the bacteria over the surface of the agar.

Nematodes for experimentation were reared on "brood" plates which were made by applying a streak of bacteria to a 35- x 10-mm petri plate containing 2.25 ml nutrient agar. Five or more brood plates were prepared daily. Five gravid females were removed from colony plates and placed in each brood plate. The females were removed after 24 h and the deposited eggs were left on the brood plates to develop. After an additional 24 h, the progeny had developed to the L₄ stage and were transferred to different "isolation" plates according to sex. On the third day of development when most of the L₄ individuals had molted to the adult stage, each isolation plate was checked to ensure that all nematodes were of the same sex. The nematodes were kept on these plates until needed for experimentation. All experiments were conducted on at least 4 different days to ensure that results reflected the day-to-day variability of the colony.

Exact ages of the cultured nematodes are difficult to specify. Especially with older females, the eggs may be retained in all stages of development and a female carcass may contain in excess of 30 larvae. For this reason and because observations were typically made at 24-h intervals, the estimated age of any one nematode is only accurate to the nearest 24 h. On an average basis, however, estimated ages are much more accurate. In this paper, the stated age of a nematode is the same as the day of culture (the number of days following deposition of eggs by, or release of larvae from, adult females). Also, a 3-day-old nematode is an individual that is in the first day of its adult stage.

Only larval counts were made in experiments that involved determination of the number of offspring; no attempt was made to count the number of eggs oviposited. However, in an earlier experiment (unpublished), the hatch rates of eggs laid by *R. pellio* females reared on nutrient agar and nutrient agar plus liver supplement were 55% and 44%, respectively. These figures were not significantly different ($P \leq 0.05$) from the 44% hatch rate for eggs from females reared on earthworm carcasses in a more natural situation.

During one experiment dealing with male reproductive potential, the nematodes were reared on the nutrient agar plates with 0.1 gm blended calf liver added to the surface. The use of calf liver was discontinued when it became apparent that this material did not increase the reproductive potential of the nematodes but resulted in microfloral contamination and difficulties in observing the nematodes.

Observations of mating behavior: Five-day-old females and four-day-old males were chosen for mating observations after preliminary experiments showed nematodes of these ages to be highly responsive sexually. One male and one female were placed approximately 3 mm apart on a 35- x 10-mm nutrient agar plate which had been inoculated with bacteria 24 h prior to observation. Precopulatory, copulatory, and postcopulatory behaviors were observed on twenty mating pairs under magnifications of up to 90X.

Female mating frequency: Three types of mating regime were imposed on 3-day-old females which were maintained individually on 35- x 10-mm nutrient agar plates: *i*) females were held in isolation, *ii*) females were permitted to mate once, and *iii*) females were permitted unlimited mating. In the third treatment, five new 3-day-old males were added daily to each female and the older, 4-day-old males were removed. Females in the regimes in which mating was allowed were watched carefully at the beginning of the experiment to ensure that each was receptive to mating. Each female was transferred daily at approximately the same time (± 1 h) onto a new plate. Each plate, representing a given day of oviposition in the life of a female, was then checked over a period of 5 days for the number of larvae produced. Each treatment was replicated 19 to 22 times, and the data in this and subsequent experiments were analyzed using Duncan's Multiple Range Test for significance of the 0.05 confidence level.

Interaction of female reproductive potential and age: Virgin females were maintained on isolation plates until the ages of 3, 5, 7, 9, or 11 days, at which times they were placed individually on 35- x 10-mm nutrient agar plates containing ten 3-day-old males. After 24 h, the males were

removed. All females were then transferred to new nutrient agar plates daily until death occurred. Three days following each period during which a female had been confined to a plate, the plate, which then contained F₁ adults, was examined for the number of offspring. Each treatment was replicated 23 to 24 times.

Male reproductive potential: When they were 3 days old, males were placed individually on 35- x 10-mm agar plates for 4 h each day. During these periods, each male was provided with a maximum of ten 3-day-old females. These were placed on the plate one at a time and left there until a mating occurred or 30 min passed. Each plate was observed every 2 min during the 4-h period. Females were transferred singly onto other agar plates after exposure to males. This procedure was repeated daily until the males died. On the third day following mating and for 3 additional days, each plate containing a female was checked for the number of offspring. The experiment consisted of 23 male replicates.

RESULTS AND DISCUSSION

Observations of mating behavior: When a male and female of *Rhabditis pellio* were first placed on a plate, there was a brief increase in the locomotory rate of both sexes which was probably caused by the physical disturbance. After a few seconds, motion decreased to the original rate. The female then grazed on the bacteria while moving about randomly. Both male and female left visible tracks in the bacterial material as they moved. When the male chanced to pass over a track made by a female, he undulated forward and backward over the track for a time and, in some cases, followed the track in either direction. As the male moved closer to the female, he tended to become more active and to display lateral head movements.

The male, using his oral region, was usually the first to make contact with the female. Contact was usually not broken until after copulation. Following contact, the male moved the length of his body along the sides of the female by using forward and backward undulations. Simultaneously, he probed the vulva with his oral and bursal regions. The spicules were

extended and retracted frequently when the male's bursa neared the vulva. After some probing, the spicules were inserted into the vagina by lateral movements. Then, following a short period of reduced activity, the male released sperm through the opened vulva and into the vagina and uteri of the female. Sperm passage was usually evidenced by a clear area which formed and enlarged in the female uteri. After sperm release, the spicules were retracted but the pair remained in a copulatory position for a few minutes. They parted after the clear area disappeared, an occurrence probably resulting from the distribution of sperm throughout the uteri. Upon separation, the male either initiated courtship again or moved away. A raised area of clear material, the "copulatory plug," was found surrounding the female's vulva after mating.

Of the 20 pairs observed, three did not mate within 60 min following their placement on plates (Table 1). Eight of the 17 females that did mate produced no larvae, and only 2 of the 8 males involved in those unfruitful matings were observed to release sperm.

Several characteristic activities were observed in the mating behavior sequences. Both males and females of *R. pellio* were observed to feed throughout all mating phases. Croll's (3) review of nematode behavior stated that male nematodes do not feed during mating or pursuit of females, whereas females continue to feed. This conclusion led Croll to suggest that an endogenous feeding pacemaker in males is

TABLE 1. Lengths of time for individual phases in the mating behavior of *Rhabditis pellio* males.

Phase of mating behavior	n	Mean time (min) ± SE
Period until initial contact	17	5.2 ± 1.3
Contact to insertion of spicules	17	7.5 ± 1.7
Insertion of spicules to sperm release	10 ^a	1.9 ± 0.5
Sperm release to spicule removal	10 ^a	3.1 ± 0.4
Spicule removal to separation	17	9.9 ± 3.1
Contact to separation	17	23.2 ± 4.1

^aSperm release was not observed in seven of the matings.

inhibited by certain types of sensory input. However, as in *R. pellio*, males of *Cylindrocorpus longistoma* and *C. curzii* have been found to feed during courtship and copulation (2).

Although males and females were simultaneously placed only 3 mm apart, it took over 5 min for the average pair to make contact. This delay might have been caused in part by disturbance during mechanical transfer of nematodes to the plate and a resulting temporary stimulation of escape responses and interruption of normal behavioral patterns. Another possibility is that gradients of sex pheromones have to form before either sex can orient toward the other. However, since both sexes move about, response to a pheromone gradient in this species may be a somewhat inefficient process.

Rhabditis pellio males remained in the copulatory position for about 10 min after retracting their spicules. *Pelodera teres* males also remain in the copulatory position with females for an unspecified time after the retraction of spicules (9). This behavior might prevent sperm from passing out of the female vulva before they have been distributed throughout the uteri. Also, the copulatory plug might be formed during this stage of postcopulatory behavior.

The frequent tendency for males to renew courtship and to copulate again with females immediately after a first mating indicates that recently mated females are still attractive to males and that the so-called copulatory plugs do not deter attraction or prevent mating.

Female mating frequency: Unmated females produced no larvae. Females permitted only one mating produced a mean of 148 larvae, significantly fewer than the 570 larvae for females permitted unlimited mating.

Figure 1 shows the distribution of oviposition over the females' lives under the one- and unlimited-mating regimes. Females permitted only one mating deposited nearly all their viable eggs by the second day following mating. It appears that as the supply of sperm decreases, the oviposition rate also decreases. Therefore, for continuous oviposition of viable eggs, females may have to mate at least once every 2 days. Females permitted unlimited

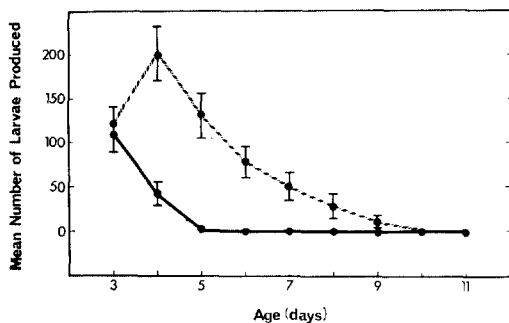


FIG. 1. Daily distributions of mean numbers of larvae produced during the life of *Rhabditis pellio* females which were allowed unlimited mating (broken line) or one mating (solid line). Vertical lines indicate standard errors.

mating reached a peak of larval production at 4 days of age (the second day of mating), and then larval production declined, although the females continued to produce larvae during their entire life spans. A similar distribution of larval production is found in *Aphelenchus avenae* (5). Although *Rhabditis pellio* females readily mate at 3 days of age, the development of most ova to the point of readiness for oviposition may lag behind female mating capabilities. This possibility may account for the fact that the unlimited- and single-mating regimes produced essentially the same number of larvae on the first day of mating.

The 569 larvae produced/female in the unlimited mating group is considerably higher than the earlier estimate of Otter (10) of 150-300 offspring/female under natural conditions. Females permitted unlimited mating had a significantly shorter life span (8.4 days) than did females that mated once (10.9 days) or not at all (11.6 days). The differences in longevity might be related to nutrition, in that the unlimited-mating females may have used a large amount of their nutrient reserves to form and oviposit viable eggs. It is also possible that females ovipositing few or no eggs might have reabsorbed nutrients from their infertile eggs.

Interaction of female reproductive potential and age: The mean numbers of larvae produced by females which were each permitted unlimited mating with 10 males for a 24-h period varied with age at mating. Females which were allowed unlimited mating when 3 days old produced 253

larvae and oviposited the greatest number of viable eggs on the fourth day of age (Fig. 2), a phenomenon similar to that observed earlier for females that were permitted unlimited mating throughout their lives. The number of offspring produced decreased drastically when the females were delayed from mating for 2 to 8 days after they had attained sexual maturity. Thus, females that mated at 5, 7, 9, and 11 days of age produced 71, 37, 15, and 4 larvae, respectively. A similar result was observed by Fisher (7) in amphimictic populations of *Aphelenchus avenae*. He suggested that oocytes are produced in all females at some normal rate, but that in unmated females complete oocyte development is prevented and decomposition occurs. Once mating is initiated, only those oocytes being currently produced are available for fertilization.

Male reproductive potential: The data concerning mating and larval production by *Rhabditis pellio* males are presented in Table 2. The mean longevity of males (7.9 days) provided with up to 10 females daily was very near that of the females discussed earlier (8.4 days) which were provided with 5 males each day. Otter (10) had reported

that *R. pellio* males maintained on a decaying earthworm lived 1 to 3 days following maturity.

Only 11.8 matings out of the mean total of 21.3 matings engaged in by each male were effective in producing offspring in their partners. This phenomenon was noted earlier during observations of mating behavior when only 9 of 17 matings resulted in larvae. Green et al. (8) observed that *Heterodera rostochiensis* and *H. schachtii* populations contained large proportions of ineffective males (42 and 36%, respectively); however, those ineffective males were assumed to be unable to fertilize females, whereas individual *R. pellio* males were ineffective only during some of their populations.

Although the mean number of matings per day tended to decrease with increasing male age from the third day (Table 2), the number of larvae produced by 4-day-old males was significantly greater than that produced by males of any other age. This fact indicates, perhaps, that maximum male sperm production lags behind the onset of male sexual maturity.

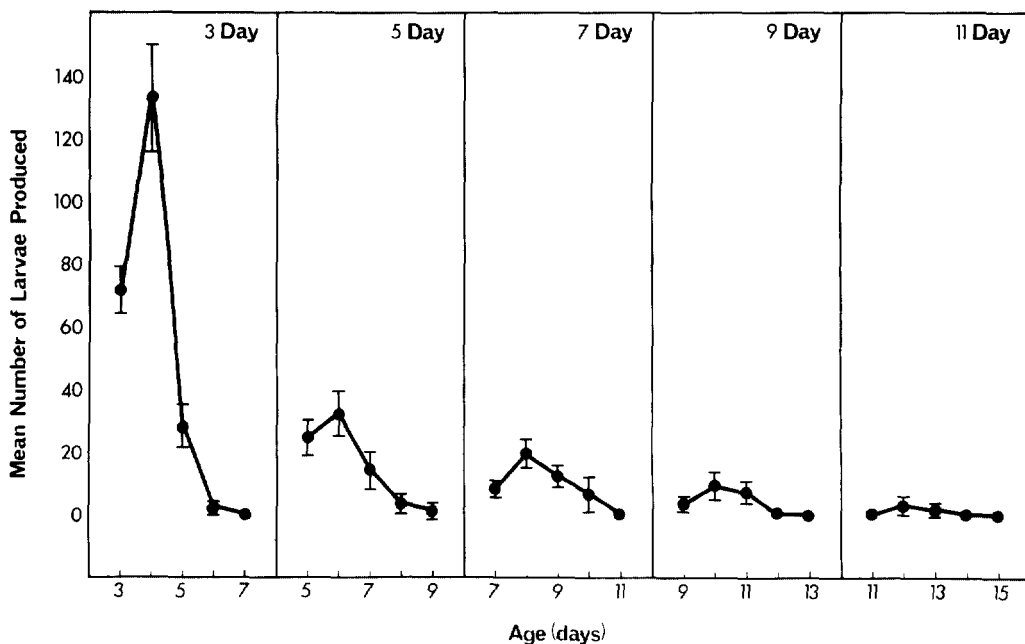


FIG. 2. Larval production by *Rhabditis pellio* females for 5 days following mating at age 3, 5, 7, 9, and 11 days. Vertical lines indicate standard errors.

TABLE 2. Mating frequency and fecundity of *Rhabditis pellio* males which were provided with eight to ten females daily.

Male age (days)	n	Mean number of matings ^a	Mean number of matings producing larvae ^a	Mean number of larvae produced ^a
3	22-23	5.8 a	3.0 ab	131 bc
4	21-23	5.4 a	3.9 a	350 a
5	22-23	4.1 ab	2.3 ab	190 b
6	17-20	3.6 bc	2.2 ab	114 bc
7	10-15	2.3 cd	2.4 ab	56 bc
8	7-11	1.2 d	1.0 ab	28 c
9	4-8	1.4 d	1.5 ab	14 c
10	3-6	1.2 d	0.3 b	1 c

^aMeans in the same vertical column followed by the same letter are not significantly different as determined by analysis of variance and Duncan's multiple range tests ($P \leq 0.05$).

LITERATURE CITED

- BREED, R. S., E. G. D. MURRAY, and N. R. SMITH. 1957. *Bergey's manual of determinative bacteriology*. Williams and Wilkins Co., Baltimore. pp. 309-322.
- CHIN, D. A., and D. P. TAYLOR. 1969. Sexual attraction and mating patterns in *Cylindrocorpus longistoma* and *C. curzii*. *J. Nematol.* 1:313-317.
- CROLL, N. A. 1972. Behavioural activities of nematodes. *Helminthol. Abstr. B. Plant Nematol.* 41:87-106.
- CROLL, N. A., and J. M. SMITH. 1970. The sensitivity and responses of *Rhabditis* sp. to peripheral mechanical stimulation. *Proc. Helminthol. Soc. Wash.* 37:1-5.
- FISHER, J. M. 1968. Fecundity of *Aphelenchus avenae*. *Bastian. Aust. J. Biol. Sci.* 21:169-171.
- FISHER, J. M. 1969. Investigations on fecundity of *Aphelenchus avenae*. *Nematologica* 15: 22-28.
- FISHER, J. M. 1972. Observations on the effect of males on reproduction and fecundity of *Aphelenchus avenae*. *Nematologica* 18:179-189.
- GREEN, C. D., D. N. GREET, and F. G. W. JONES. 1970. The influence of multiple mating on the reproduction and genetics of *Heterodera rostochiensis* and *H. schachtii*. *Nematologica* 16:309-326.
- JONES, T. P. 1966. Sex attraction and copulation in *Pelodera teres*. *Nematologica* 12:518-522.
- OTTER, G. W. 1933. On the biology and life history of *Rhabditis pellio*. *Parasitology* 25: 296-307.
- POINAR, G. O., JR., and G. M. THOMAS. 1975. *Rhabditis pellio* Schneider (Nematoda) from the earthworm, *Aporrectodea trapezoides* Duges (Annelida). *J. Nematol.* 7:374-379.
- VAN GUNDY, S. D. 1965. Nematode behavior. *Nematologica* 11:19-32.