Sexual Attraction and Mating Patterns in *Cylindrocorpus longistoma* and *C. curzii* (Nematoda: Cylindrocorporidae)

D. A. CHIN AND D. P. TAYLOR[1]

Abstract: Females of *Cylindrocorpus longistoma* and *C. curzii* excrete attractants which probably function to bring the sexes together before mating. Intraspecific and interspecific heterosexual and homosexual pairing experiments showed the attractants to be species specific as well as sex specific. Observations on mating behavior support the hypothesis that sexual attraction and copulation require independent stimuli.

Chance encounter mating of obligately bisexual nematodes would be inefficient. Lee (10) postulated chemotaxis may be operative in sexual attraction and Anderson and Darling (1) hypothesized *Ditylenchus destructor* amphids probably function as sensory organs enabling males to locate females prior to mating. Recent experimental evidence suggests sex attractants may be involved in mate location in both free-living (7, 9) and parasitic (2, 3, 6, 8) nematodes.

In preliminary studies of mating in *Cylindrocorpus longistoma* Stefanski and *C. curzii* T. Goodey copulation within species was quickly accomplished, but the same or opposite sexes of different species were sexually indifferent and only rarely attempted unsuccessful copulation even though in physical contact. Homosexual copulation attempts were never observed. These observations suggested copulation within this genus was dependent upon specific attraction rather than chance encounter. Experiments involving intraspecific and interspecific heterosexual and homosexual pairing were conducted to determine whether *C. longistoma* and *C. curzii* produce sex attractants or repellents and to establish their specificity.

Received for publication 10 March 1969.

[1] Research Associate and Associate Professor of Nematology, Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801, respectively. This study was supported in part by U. S. Dept. of Interior Grant WP00047. The authors wish to thank Dr. R. B. Malek for critical review of the manuscript.

MATERIALS AND METHODS

C. longistoma and *C. curzii*, initially isolated from the sewage treatment plant at Urbana, Illinois, were cultured monoxenically on *Aerobacter aerogenes* (Kruse) Beijerinck, growing on Chang's medium (4). For these studies, large numbers of fourth-stage male and female larvae were hand-picked from stock cultures and reared separately for 24 hr at room temperature. This procedure ensured the selection of young, vigorous, unmated adults.

Agar migration strips communicating two test chambers to a single inoculation zone were prepared by selective removal of agar from 90 × 15 mm petri dishes containing .5 cm depth of 1.5% water agar (Fig. 1). Test chambers were constructed from 1.5 × .5 cm glass rings affixed with Zut to Whatman No. 1 filter paper disks used to form the base. One chamber was placed at the distal terminus of each migration strip in such a manner that the filter paper disk formed a permeable barrier between the chamber and the agar. Six drops of 1.5% water agar were placed inside each chamber and later inoculated with *Aerobacter aerogenes*.

In both *C. longistoma* and *C. curzii*, responses of the following intraspecific heterosexual and homosexual combinations were tested: (i) males to females; (ii) males to males; (iii) females to males; and (iv) females to females. In heterosexual experi-

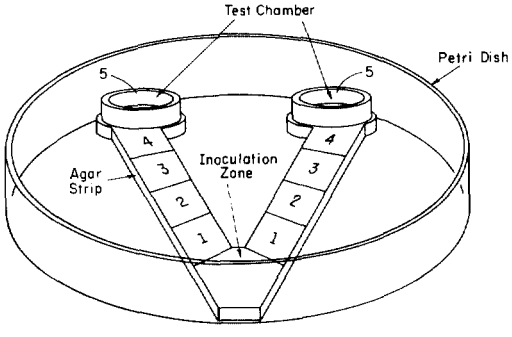


FIG. 1. Diagram of equipment used for studying sex attraction.

ments, one chamber was inoculated with 100 nematodes of one sex and 24 hr later the same number of nematodes of the opposite sex were introduced into the inoculation zone at the base of the "V" formed by the two agar strips. Preliminary trials indicated that 24 hr incubation was sufficient to evoke a sexual response. In homosexual experiments, the same numbers of nematodes of the same sex were similarly placed in the chamber and the inoculation zone. In both heterosexual and homosexual experiments, the second chamber inoculated with bacteria alone served as a control. Each test was replicated three times.

Parallel lines were etched across the bottom of the petri dish to divide each agar strip into five 1.5-cm sections. At hourly intervals, for three hours after introduction of the nematodes into the inoculation zone, nematodes in each section were counted with a dissecting microscope to determine their distribution. It was assumed that in the absence of an attractant or repellent the nematodes would migrate from the inoculation zone equally in both directions. Half the number of nematodes remaining in the inoculation zone (usually less than 10%) were assigned to the first 1.5-cm sector at the base of each strip. Thus, the observed data could be statistically compared with a normal distribution by application of the Chi-square test.

RESULTS

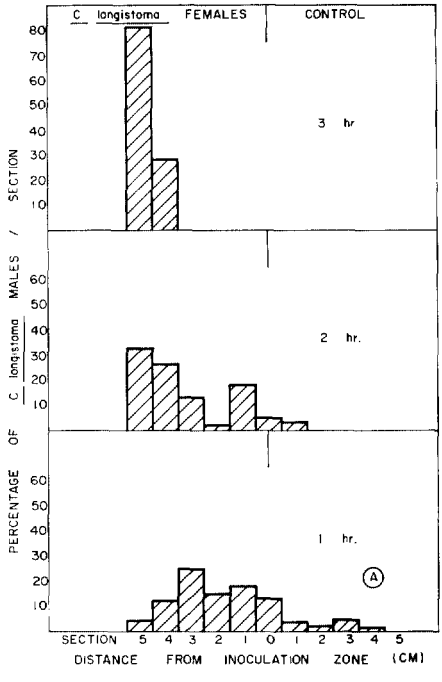
INTRASPECIFIC PAIRING: The response of *C. longistoma* and *C. curzii* males to their respective females is illustrated in Fig. 2, A and B. Within 1 hr after inoculation, the number of males of both species which migrated towards the chamber containing females was significantly greater ($P < 0.01$) than the number migrating towards the chamber without females. After three hr, 80% of *C. longistoma* males and 63% of *C. curzii* males had congregated directly under the filter paper disk of the test chamber containing the females.

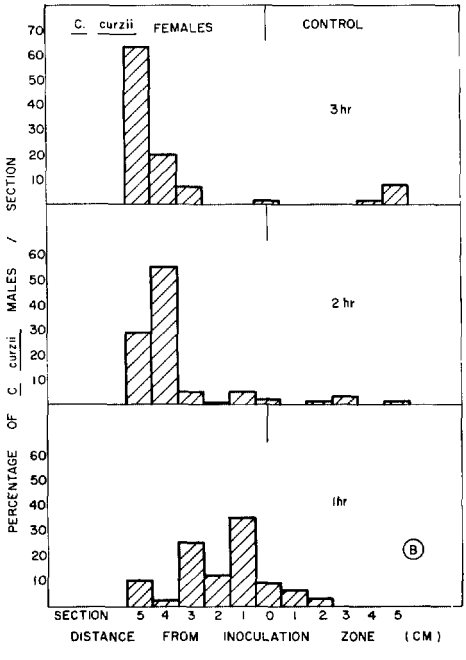
The response of *C. longistoma* and *C. curzii* females to males in intraspecific heterosexual experiments is summarized in Fig. 2, C and D. Few nematodes migrated from the inoculation zone during the first hour after inoculation. Within the second hour, females of both species migrated in both strips in approximately equal numbers. Three hr after inoculation, the number of females of both species which migrated towards the chamber containing males of the same species was not significantly different ($P > 0.05$) from the

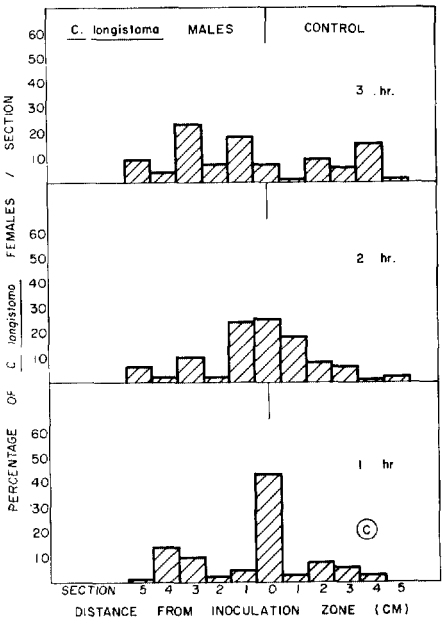
→

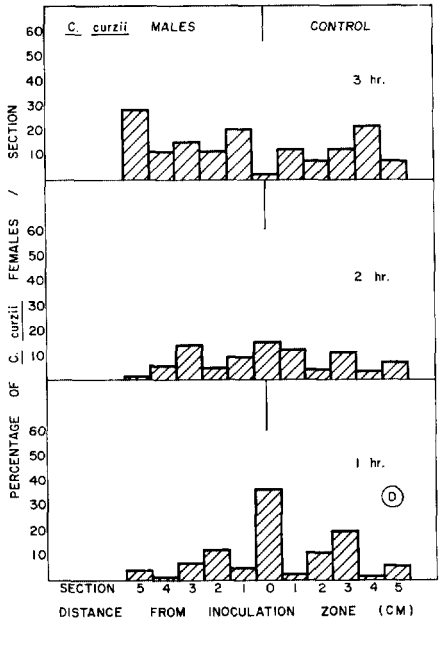
FIG. 2. The distribution of *C. longistoma* and *C. curzii* males and females in agar strips at hourly intervals during three hours in intraspecific heterosexual experiments. Each histogram is the mean of three replicates.

Section 0 = inoculation zone.
Section 1 = less than 1.5 cm from inoculation zone.
Section 2 = 1.5 to 3.0 cm from inoculation zone.
Section 3 = 3.0 to 4.5 cm from inoculation zone.
Section 4 = 4.5 to 6.0 cm from inoculation zone.
Section 5 = 6.0 to 7.5 cm from inoculation zone.


C. longistoma FEMALES
CONTROL
3 hr
2 hr.
1 hr.
A
PERCENTAGE OF C. longistoma MALES / SECTION
SECTION 5 4 3 2 1 0 1 2 3 4 5
DISTANCE FROM INOCULATION ZONE (CM)


C. curzii FEMALES
CONTROL
3hr
2 hr
1hr
B
PERCENTAGE OF C. curzii MALES / SECTION
SECTION 5 4 3 2 1 0 1 2 3 4 5
DISTANCE FROM INOCULATION ZONE (CM)


C. longistoma MALES
CONTROL
3 hr.
2 hr.
1 hr
C
PERCENTAGE OF C. longistoma FEMALES / SECTION
SECTION 5 4 3 2 1 0 1 2 3 4 5
DISTANCE FROM INOCULATION ZONE (CM)


C. curzii MALES
CONTROL
3 hr.
2 hr.
1 hr.
D
PERCENTAGE OF C. curzii FEMALES / SECTION
SECTION 5 4 3 2 1 0 1 2 3 4 5
DISTANCE FROM INOCULATION ZONE (CM)

number migrating towards the chamber without males.

No significant attraction was detected in intraspecific male and female homosexual experiments.

INTERSPECIFIC PAIRING: Using techniques similar to those described for intraspecific experiments, the response of *C. curzii* males to *C. longistoma* females and *C. longistoma* males to *C. curzii* females was tested in repeated experiments.

In seven experiments, the number of *C. curzii* males which migrated towards the chamber containing *C. longistoma* females was not significantly different ($P > 0.05$) from the number migrating in the other direction. Similarly, no significant attraction was shown by *C. longistoma* males to *C. curzii* females in five experiments.

MATING: The mating patterns of *C. longistoma* and *C. curzii* in bacterial cultures were similar and are described as one. Females became receptive to males on completion of the final molt. Frequently the lip region of the male was the first part of the body to make contact with a female, yet the tactile response which led to copulation occurred only when the posterior halves of the bodies of both sexes made contact. Once contact was made, the posterior region of the male looped ventrally around the body of the female. The male then swung its body away from the female until their bodies were at right angles to each other. During this time, the female continued to feed, moving backwards and forwards inside the loop. Males also continued to feed while their spicules were periodically projected. Eventually, the spicules came in contact with the vulva and penetrated the opening and rapid spicular action was accompanied by ejaculation. Copulation was very brief, lasting only 10–15 seconds and terminated after the grip of the spicules was broken. If the female moved out of the loop before copulation was achieved the male made no attempt to follow her. However, a single male would mate with the same female if contact was made again. Occasionally several males would attempt to mate with the same female at the same time.

DISCUSSION

The results indicate that *C. longistoma* and *C. curzii* females secrete attractants which probably serve to bring nematodes of opposite sexes together prior to mating. There was no evidence of repulsion in experiments with either heterosexual or homosexual pairing. The attractants appear to be species specific, as well as sex specific, since the attractant emitted by females was attractive only to males of the same species. These observations are in partial agreement with those of Jones (9) who reported that males of *Pelodera teres* are attracted to their females but males do not attract females. On the contrary, nematodes of opposite sexes of *Panagrolaimus rigidus* (7) and certain ancylostomid nematodes (2) produce different specific substances and are equally attracted to each other. Bonner and Etges (3) found that males of *Trichinella spiralis* are attracted to females, but not nearly as strongly as females are attracted to males. In *Heterodera* species the female is sedentary and by necessity the male is the attracted sex (6). From these few examples it appears that sexual response varies among different nematode species. Thus, if sex attractants are to be exploited as a means of nematode control the attractive sex or sexes and the specific compounds involved should be determined for each species.

In both *C. longistoma* and *C. curzii*, the act of copulation was initiated only when the posterior halves of nematodes of opposite sexes made contact. Thus, the responsive region is located in the region of the body where minute body papillae of the female (5) and the genital papillae of male are located.

Papillae are generally considered to be structures for sensory perception. These observations therefore support the hypothesis of Greet (7) that sexual attraction and copulation are independent events requiring separate stimuli. It may be, as suggested by Greet, that the attractant serves only to bring nematodes of opposite sexes close to each other and subsequently a tactile stimulus differentiates sex and species.

LITERATURE CITED

1. ANDERSON, R. V., and H. M. DARLING. 1964. Embryology and reproduction of *Ditylenchus destructor* Thorne, with emphasis on gonad development. Proc. Helminthol. Soc. Wash. 31:240–256.
2. BEAVER, P. C., Y. YOSHIDA, and L. R. ASH. 1964. Mating of *Ancylostoma caninum* in relation to blood loss in the host. J. Parasitol. 50:286–293.
3. BONNER, T. P., and F. J. ETGES. 1967. Chemically mediated sexual attraction in *Trichinella spiralis*. Exptl. Parasitol. 21: 53–60.
4. CHANG, S. L. 1958. Cultural, cytological and ecological observations on the amoeba stages of *Naegleria gruberi*. J. Gen. Microbiol. 18, 565–578.
5. CHIN, D. A. 1969. Developmental biology and factors influencing development and reproduction of *Cylindrocorpus longistoma* and *C. curzii* (Nematoda: Cylindrocorporidae). Ph.D. Thesis. University of Illinois, Urbana, Illinois. 145 pp.
6. GREEN, C. D. 1966. Orientation of male *Heterodera rostochiensis* Woll. and *H. Schachtii* Schm. to their females. Ann. Appl. Biol. 58:327–339.
7. GREET, D. N. 1964. Observations on sexual attraction and copulation in the nematode *Panagrolaimus rigidus* (Schneider). Nature (London) 204:96–97.
8. GREET, D. N., C. D. GREEN, and M. E. POULTON. 1968. Extraction, standardization and assessment of the volatility of the sex attractants of *Heterodera rostochiensis* Woll. and *H. Schachtii* Schmidt. Ann. Appl. Biol. 61:511–519.
9. JONES, T. P. 1966. Sex attraction and copulation in *Pelodera teres*. Nematologica 12: 518–522.
10. LEE, D. L. 1965. The physiology of nematodes. W. H. Freeman and Company, San Francisco. 154 pp.