

# Reproduction of *Pratylenchus penetrans* (Nematoda: Tylenchida)

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*Abstract:* *Pratylenchus penetrans* did not reproduce without males. Cytological examination indicated that cross-fertilization occurred. Females had a chromosome number of  $2n = 12$ . Virgin females reared in isolation laid eggs, but these failed to undergo cleavage. Males reared in isolation produced sperms. *Key Words:* *Pratylenchus penetrans*, reproduction, oogenesis.

Presence or absence of males and presence or absence of a spermatheca or sperms in the female gonad provide circumstantial evidence of the mode of reproduction of *Pratylenchus* spp. Populations of *P. penetrans* (Cobb) from the field, greenhouse cultures, or monoxenic cultures contain numerous males (1, 2, 5, 8, 10) and the females possess a spermatheca, usually containing sperms. These observations suggest that *P. penetrans* reproduces by cross-fertilization. Cytological studies (6) indicated that *P. penetrans* had a chromosome number of  $2n = 10$  and reproduced by cross fertilization. In other work (1), populations increased in four out of 70 pots of peas each inoculated with one immature female *P. penetrans*, suggesting that hermaphroditic or parthenogenetic reproduction is possible.

In the research reported in this paper, reproduction of *P. penetrans* was investigated by controlled inoculations in monoxenic cultures and by cytological examination.

## MATERIALS AND METHODS

*P. penetrans* was cultured monoxenically on alfalfa, *Medicago sativa* L., 'Du Puits,' callus tissue by the methods of Krusberg (3). Nematodes were extracted by placing callus tissue on facial tissues supported in water.

Nematodes used in controlled inoculations were extracted and transferred under aseptic conditions. Selected individuals were placed close to alfalfa callus tissue established from three seedlings placed 10–14 days previously on 7 ml Krusberg's medium (3) in  $50 \times 12$  mm plastic petri dishes with tight lids. A single second- or third-stage juvenile was placed in each dish in one series, two second- or third-stage juveniles were placed in each dish in a second series, and one second- or third-stage juvenile plus five males were placed in each dish in a third series. In each series, 15–35 dishes were prepared on five different occasions during a 4-month period.

Cultures were kept in the dark at 20–23 C. After 12–16 weeks, agar and callus from individual cultures were placed on a modified Baermann funnel ("piepan") for 24–30 hr at 20–23 C. Extracted nematodes were concentrated in about 10 ml water and males, females, and juveniles were counted. Reproduction was considered to have occurred when both adults and juveniles totalling more than the initial inoculum were extracted. Cultures yielding only adults numbering the same as or less than the initial inoculum were considered non-reproductive.

For cytological work, gravid or non-gravid females were killed in water at 60 C; some females and eggs were killed after eggs had been laid in water at 22 C. Killed specimens were fixed in Carnoy's fluid (absolute alcohol: glacial acetic acid: chloroform:: 6 : 1 : 3 v/v/v) for 4–24 hr. Some specimens were mounted in acetic carmine or

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acetic orcein, 1% (w/v) in 45% (v/v) acetic acid, and observed at intervals during the next 24 hr to detect greatest contrast between chromatin and cytoplasm. Other specimens were transferred to distilled water for 2 hr, held in freshly-prepared 4% (w/v) ferric alum for 2 hr, transferred to distilled water for 5 min, placed in 0.5% hematoxylin for 24 hr, passed twice through tap water, mounted in freshly-prepared saturated picric acid, and examined at intervals during the next 24 hr to detect the optimum amount of destaining.

Eggs or gravid females were processed *in toto* by the lacto-propionic orcein method of LeJambre (4).

### RESULTS

Reproduction did not occur in 127 cultures inoculated with one juvenile, but did occur in 24 of 131 cultures inoculated with two juveniles and in 68 of 90 cultures inoculated with one juvenile plus five males. All or part of the inoculum of two or six nematodes was recovered from 56 non-reproductive cultures; none of these cultures yielded males and females together.

Females possessed no sperm-producing organs and sperms were absent from the spermatheca and other organs of 42 females extracted from non-reproductive cultures (Fig. 1B). Only two of more than 100 females extracted from reproductive cultures had a spermatheca devoid of sperms (Fig. 1A).

Sperms were present in the vas deferens of 16 males extracted from non-reproductive cultures and of numerous males extracted from reproductive cultures.

One female from a culture inoculated with one juvenile was gravid. One to nine eggs were teased from callus tissue in seven cultures from which one female was extracted. These eggs had not undergone cleavage and contents were brown. In con-

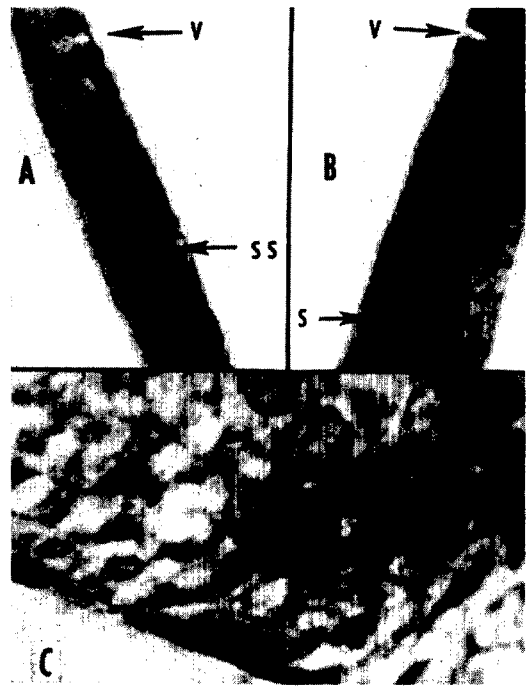


FIG. 1. *Pratylenchus penetrans* from monoxenic culture: A, impregnated female showing vulva (v) and spermatheca containing sperms (ss); B, virgin female (reared in isolation) showing vulva (v) and spermatheca devoid of sperms (s); C, primary oocyte with six tetrads at metaphase I.

trast, numerous eggs teased from reproductive cultures had undergone cleavage and had gray contents.

Several hundred gravid females were examined cytologically. No cell divisions were observed in the cap cell region of the ovary. Primary oocytes enlarged as they moved toward the oviduct; those adjacent to the spermatheca were about the size of an egg. At this stage, chromosomes were visible as long, faintly-staining threads. Passage of an oocyte through the spermatheca was not observed, nor was penetration of an oocyte by a sperm. Many oocytes in the uterus, however, contained a sperm nucleus at or near the proximal tip of the oocyte (Fig. 2).

The chromosomes of the oocyte were

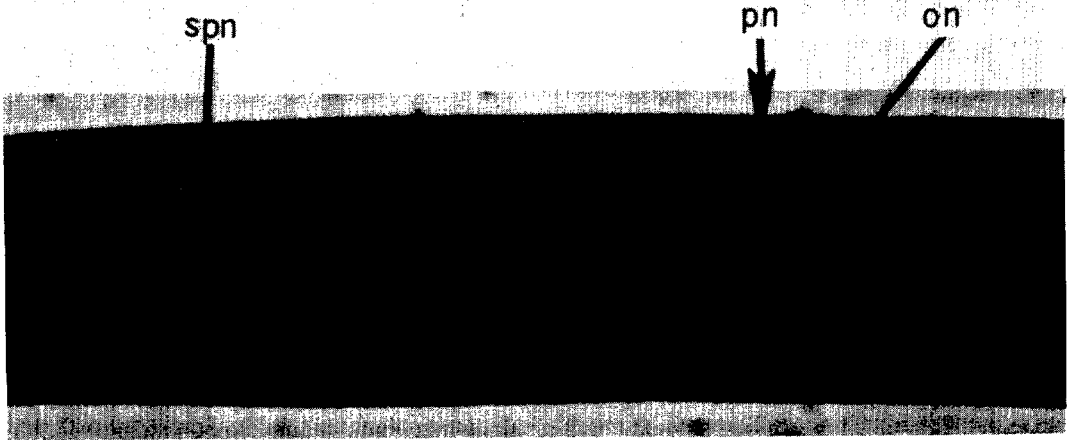


FIG. 2. *Pratylenchus penetrans* from monoxenic culture. Primary oocyte at telophase I of oogenesis. Chromosomes of first polar nucleus (p n) at periphery of oocyte, chromosomes deeper within oocyte which later undergo second maturation division (o n), sperm nucleus (sp n) at proximal pole of oocyte.

observed near its center and, in the two best preparations, 12 chromosomes grouped fairly closely in six pairs were counted. At metaphase I, six deeply-staining tetrads appeared (Fig. 1C). Numerous examples of anaphase I and telophase I were seen; the spindle was roughly perpendicular to the long axis of the oocyte (Fig. 2). Six dyads of the first

polar nucleus were distinguished clearly at the periphery of the oocyte. The other dyads were grouped closely within the oocyte; occasionally, six dyads were counted individually.

The second maturation division occurred quickly. The two polar nuclei rapidly lost their stainability and the chromosomes in

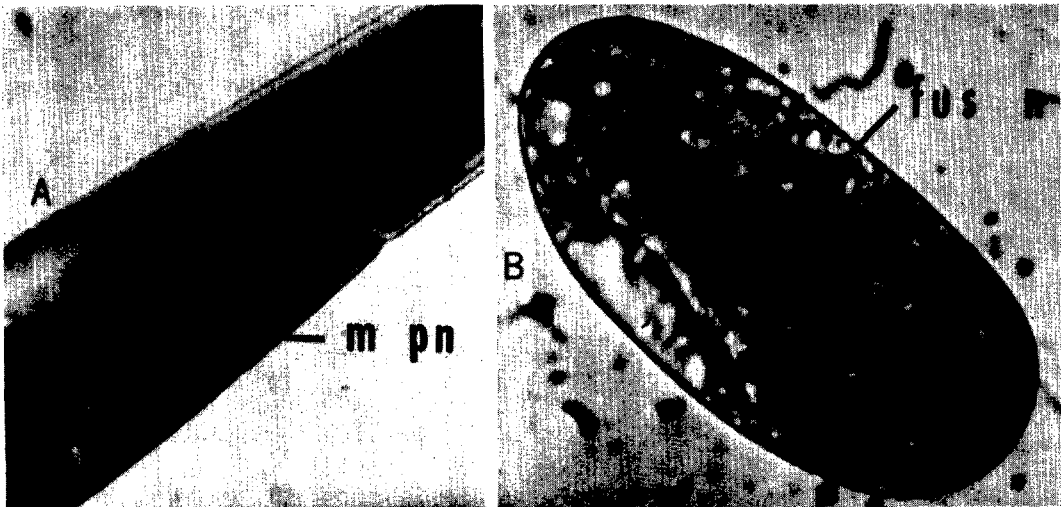


FIG. 3. *Pratylenchus penetrans* from monoxenic culture: A, ovum in utero showing male pronucleus (m pn); B, zygote showing fusion nucleus (fus n).

the female pronucleus became very diffuse. At about this stage, the male pronucleus was formed. It was about four times the diameter of the sperm nucleus and was less deeply stained than the sperm nucleus (Fig. 3A). The male pronucleus migrated towards the center of the ovum where the female pronucleus was located. Discrete chromosomes were not seen in the male pronucleus and spermatogenesis was not investigated.

Fusion of male and female pronuclei occurred after eggs were laid (Fig. 3B). Chromosomes in eggs undergoing cleavage could not be distinguished clearly, but there seemed to be more than six.

Completion of maturation divisions in oocytes was associated closely with penetration by a sperm. Of 48 oocytes (*in utero*) with a dividing and well-defined nucleus, 46 contained a sperm nucleus and two contained no identifiable sperm nucleus. Of 21 oocytes with a nucleus not detected or poorly stained and not dividing, only three contained a sperm nucleus. Each of the 69 females involved had numerous sperms in the spermatheca.

Most observations were of specimens stained with acetic carmine or acetic orcein and good preparations were obtained. The hematoxylin schedule was laborious, but gave good preparations. The lacto-propionic orcein stain (4) failed to penetrate eggs or oocytes *in utero* or eggs extracted from callus tissue.

#### DISCUSSION

From the results of controlled inoculations I concluded that reproduction did not occur in the absence of males. Two polar nuclei were formed in maturing oocytes and chromosome division figures indicated that meiosis occurred. The evidence was that *P. penetrans* reproduced by cross-fertilization. Females had a chromosome number of  $2n = 12$ . Only one population of *P. penetrans* was used. Other workers reported a chro-

mosome number of  $2n = 10$  and that reproduction was by cross-fertilization (6) or gave evidence that *P. penetrans* can reproduce in the absence of males (1). Other species of *Pratylenchus* (6) or other genera (9) were reported to have different populations with different chromosome numbers, or different methods of reproduction, or both.

Many eggs of *P. penetrans* from monoxenic cultures on excised corn roots failed to develop to the first cleavage division (2) and a progressive increase with time in the proportion of malformed, discolored, non-viable eggs in alfalfa callus cultures was reported (7). A high proportion (20 of 68) of oocytes examined *in utero* had not been penetrated by a sperm and, in most cases, the oocyte nucleus was not undergoing maturation divisions. Presumably such eggs are non-viable, like those laid by virgin females, and accumulate in monoxenic cultures.

Results of inoculations with single juveniles indicated that individuals of *P. penetrans* can live at least 4 months. In other similar experiments, some individuals lived 6 months.

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