Oogenesis and Embryology of Two Plant-parasitic Nematodes, *Pratylenchus penetrans* and *P. zeae*[1]

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Abstract: The process of oogenesis was studied in the bisexual species, *Pratylenchus penetrans*, and the monosexual species, *P. zeae*. The nucleus of the oocyte of *P. penetrans* underwent two divisions after sperm penetration. The chromosome number of *P. penetrans* observed at metaphase of the first maturation division was $2n = 10$ and the reduced chromosome number observed at anaphase of the first maturation division was $n = 5$. Two polar bodies were found within the egg, indicating that this species reproduces by amphimixis. The nucleus of the oocyte of *P. zeae* underwent one mitotic division and the chromosome number was $2n = 26$. The presence of only 1 polar body indicates that this species reproduces by mitotic parthenogenesis. The development of the embryo was similar in *P. penetrans* and *P. zeae*. Unsegmented eggs were usually deposited by females. Following the 9-celled stage, the number of cells increased rapidly until a blastula was formed. Cell differentiation immediately followed, as evidenced by the formation of darker and larger inner endodermal cells and smaller ectodermal cells. Six to 7 days after egg deposition, the first stage larva was coiled three to four times within the egg shell. During the first molt, the stylet apex was formed first, then the larva moved frequently and vigorously and the stylet was repeatedly thrust into the egg shell. Finally, the shell was broken and the second stage larva emerged. It took 10 days from the unsegmented egg to hatching at 23 C.

Gametogenesis and cytological aspects of some plant-parasitic nematodes have been studied extensively in recent years (9, 10, 11. 12). Most species have been reported to follow the same pattern of spermatogenesis and oogenesis, i.e. gonial divisions occur in the germinal zone of both male and female gonads. After a period of growth, oocytes and spermatocytes undergo two maturation divisions and eggs start to develop after fertilization. Some species, however, reproduce by mitotic parthenogenesis which consists of a single mitotic division.

The chromosome number was found to vary between different genera and between different species in the same genus suggesting that various degrees of chromosome polyploidy might occur in some populations of nematodes (18).

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Embryos develop similarly in most plant-parasitic nematodes (1, 2, 3, 4, 5, 6, 7, 8, 11). A blastula, formed after a series of repeated divisions of cells, is followed by gastrulation and the first stage larva is formed by the elongation of the embryo. On the other hand, a different developmental pattern in *Ditylenchus dipsaci* (Kuhn) was reported by Yuksel (14). He observed a withdrawal of cells near the anterior end into the interior of the embryo to form a curved head region. A curved tail region was formed by a similar process at the posterior end.

No information concerning the oogenesis and embryology of *Pratylenchus* is available. Therefore, attempts were made to study the process of oogenesis and the development of the embryo of a bisexual species, *P. penetrans* (Cobb), and a monosexual species, *P. zeae* Graham.

MATERIALS AND METHODS

Stock cultures of the bisexual species, *P. penetrans*, and the monosexual species, *P. zeae*, were obtained from the University of Maryland and were subcultured and main-

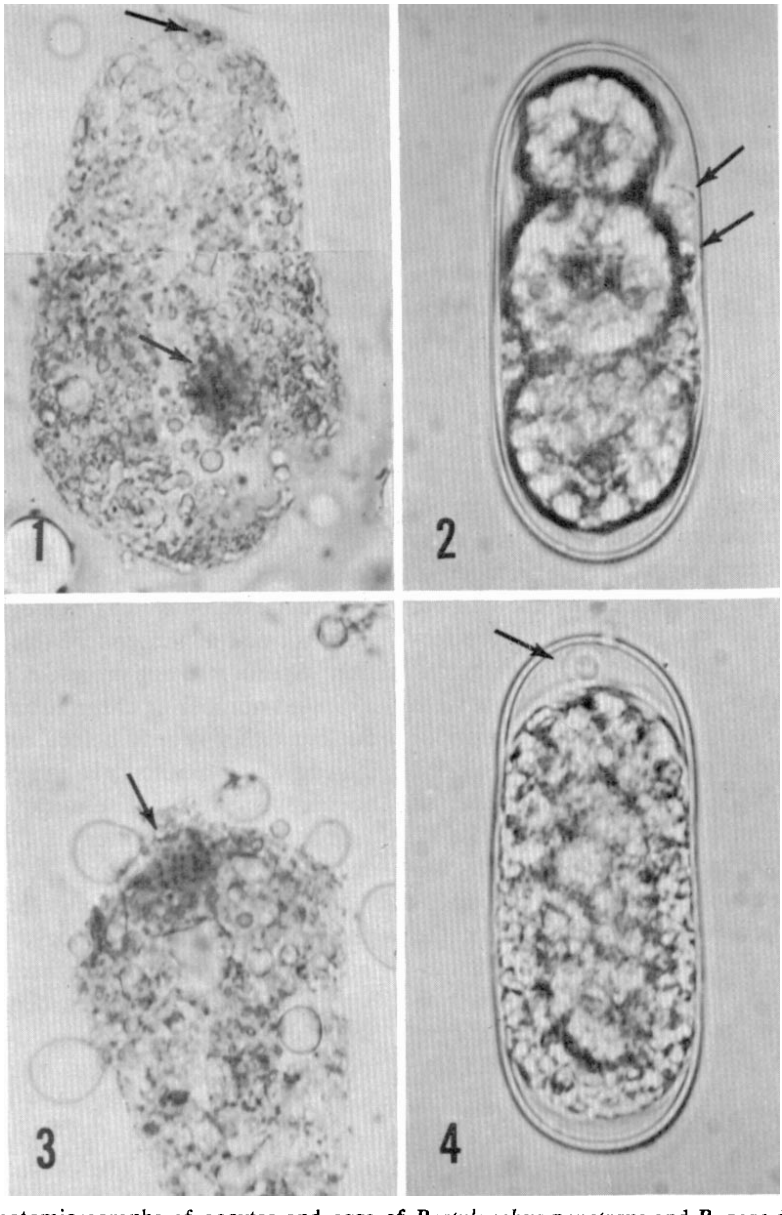

FIG. 1–4. Photomicrographs of oocytes and eggs of *Pratylenchus penetrans* and *P. zeae* during oogenesis and embryonic development: (1) A large oocyte of *P. penetrans* stained with aceto-orcein. A sperm (upper arrow) is shown penetrating the oocyte. The nucleus with 10 duplicated chromosomes underwent metaphase of the first maturation reduction division (lower arrow). (2) Two polar bodies (arrows) at the center of the egg of the 5-celled embryonic stage of *P. penetrans*. (3) Twenty-six chromosomes (arrow) at the metaphase of the maturation division in a large oocyte of *P. zeae* stained with aceto-orcein. (4) One polar body present at one end of the egg (arrow) during the cell differentiation stage of the embryo of *P. zeae*. × 2800.

tained in alfalfa (*Medicago sativa* L. var. *Du Puits*) callus tissue cultures at 23 C.

OOGENESIS: Females of *P. penetrans* and *P. zeae* were removed from the callus tissue cultures and either large oocytes in the oviduct, eggs without a shell or with a thin shell in the area from the spermatheca to the uterus, or mature eggs with a shell in the uterus were excised from females. They were then placed in a drop of 1% aceto-orcein on a slide. By this means, the chromosomes were stained a dark red color in a lighter red cytoplasm. Some specimens were squeezed by the application of gentle pressure on the cover glass and then observed. The chromosome number was determined during either mitosis or meiosis at late prophase, early metaphase, or early anaphase. The mode of reproduction was determined by the number of nuclear reduction divisions or by the formation of polar bodies.

EMBRYONATION: Gravid females were picked from concentrated suspensions of nematodes and kept in watch glasses filled with water. Newly laid eggs were taken up in a fine pipette and mounted in water under 12 mm coverslips supported by glass rods and sealed with petroleum jelly. The best preparations resulted when the diameter of the glass rods was slightly larger than the diameter of the egg. This method of mounting permitted satisfactory observation of the eggs for 10 days at a room temperature of 23 C.

RESULTS

OOGENESIS: Oogonial divisons were observed at the apical portion of the gonad of the young females of *P. penetrans* and *P. zeae*. Oocytes rapidly increased in size and reached full size after entering the oviduct.

In *P. penetrans*, it appeared that only 1 sperm entered each oocyte as it passed through the spermatheca. When this sperm had penetrated one end of the oocyte, the oocyte nucleus moved toward the center position and underwent maturation division (Fig. 1).

The chromosome number determined in early metaphase was $2n = 10$ in *P. penetrans*. At the metaphase of the first meiotic division, chromosomes appeared in five pairs and each pair was found to be composed of two homologs. Shortly after the oocyte reached the uterus, it advanced to the anaphase stage and the first polar body which contained the haploid chromosome number of $n = 5$ was formed. At this time, a thin membranous shell was observed on the egg.

Shortly after this first reduction division, the second mitotic division took place but the prophase of the second division was not observed. During metaphase, the spindle fiber was perpendicular to the cell membrane. The second polar body and the egg pronucleus were formed at the end of this mitotic division. Sperm and egg pronuclei fused to form a diploid number of chromosomes in the egg nucleus either shortly before or shortly after the egg was deposited. In the egg, two polar bodies were formed outside the vitelline membrane at the center of the developing embryo (Fig. 2).

In *P. zeae*, only one mitotic division occurred at one end of the oocyte as it entered the uterus (Fig. 3). The chromosome number of $2n = 26$ was clearly identified at prophase and at early metaphase. No sperm was found in the spermatheca. Only one polar body was found at one end of the egg (Fig. 4).

EMBRYONATION: The average size of 25 viable eggs of *P. penetrans* was 60.6 μ (52.8–73.6 μ) in length and 24 μ (20.8–25.6 μ) in width. Eggs of *P. penetrans* averaged 62.4 μ (56–57.2 μ) in length and 22.2 μ (19.8–24 μ) in width. Embryonic development was found to be identical in both *P. penetrans* and *P. zeae* and similar to that reported previously for other nematodes.

Eggs were usually laid prior to the first

division. The protoplasm of the newly laid egg was granular, usually irregular in shape, and apparently bounded by a vitelline membrane which contacted the egg shell at all points, except at each end. The protoplasm streamed continuously and gradually developed into a slightly kidney shaped, 1-celled stage. Each egg contained a single large transparent nucleus.

Development of the embryo resulted from unequal divisions over a period up to 5 to 5½ days at which time larval movement developed. Elongation continued during 1 day giving rise to the first larval stage which was coiled three to four times within the egg shell.

During this stage of development, movement of the larva was rapid. For closer observation, the larva was released without injury from the egg by applying gentle pressure on the coverslip. Average measurements of 10 liberated first stage larvae of *P. penetrans* were: 243.7 μ (207.2–294 μ) in length and 16.3 μ (14.7–17.4 μ) in width. For *P. zeae* larvae: 224.5 μ in length and 15.8 μ (14.7–16.2 μ) in width.

Molting of the first stage larvae occurred about 7 to 8 days after egg deposition by the female. During this molt, the differentiation of the esophagus and development of the stylet were completed. Just prior to hatch, movement of the larva was rapid and stylet thrusts frequent. Development and hatching took about 10 days at 23 C from the newly laid egg.

DISCUSSION

Sperm were observed in the spermatheca of *P. penetrans*, one penetrating the oocyte as it entered the spermatheca. Two polar bodies were formed as a result of two maturation divisions within the oocyte. In *P. zeae*, however, no sperm were found in the spermatheca and only one maturation division occurred in the oocyte while only one polar body was formed. These findings indicate that reproduction in *P. penetrans* is by amphimixis and is by mitotic parthenogenesis in *P. zeae*.

The chromosome number of *P. penetrans*, determined at early metaphase, was $2n = 10$ whereas the chromosome number of *P. zeae* identified at prophase and at early metaphase was $2n = 26$. These differences suggest that polyploidy might exist in the genus *Pratylenchus*. The basic chromosome number in this genus could be postulated as $n = 5$. *P. zeae*, with 26 chromosomes could be regarded as pentaploid. However, only two species of *Pratylenchus* have been examined. The subject could be clarified further as more information on the cytology of other species of *Pratylenchus* becomes available.

The study of embryonation of *P. penetrans* and *P. zeae* indicated that the cleavage pattern of these two species was similar to the pattern known for other plant-parasitic and predaceous nematodes in general (1, 2, 3, 4, 5, 8, 13). In *Criconemoides xenoplax* Raski and *Nacobbus serendipiticus* Franklin (2, 8), a polar body was reported outside the vitelline membrane near the center of the egg while a polar body was situated at the anterior pole of the egg in *Radopholus similis* (13). Two polar bodies were observed outside the vitelline membrane near the center of the egg in *P. penetrans* while one polar body was located at one pole of the egg of *P. zeae*.

Rotation of cells has been reported in the six-celled stage in *Ditylenchus destructor* Thorne (1), but not in *Nacobbus serendipiticus* (2). In this study, rotation of cells was observed in the four-celled stage in both *P. penetrans* and *P. zeae*.

According to Anderson and Darling (1), the appearance of the smaller, lighter, outer cells and the larger, darker, inner cells represented a phase in gastrulation. The actual process of gastrulation was not followed in the present study. To understand this

process, the internal structure of the gastrula will need to be investigated.

Anderson and Darling (1) also reported that at the begginning of the molt a short tube within the head of *Ditylenchus destructor* became sclerotized and that this structure later was shed with the cuticle. A short sclerotized tube was shed with the old cuticle during molting in *P. penetrans* and *P. zeae*, indicating that an undifferentiated stylet was present in the anterior end of the first stage larva of these two species.

The first molt and the formation of the stylet during molting in *P. penetrans* and *P. zeae* followed the usual pattern known for other plant-parasitic nematodes (1, 2, 14). The stylet started to function immediately after it completed its development. Thrusting of the stylet helped to break the old cuticle and egg shell.

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