Embryology and Reproduction of Ditylenchus destructor Thorne, with Emphasis on Gonad Development*

R. V. Anderson** and H. M. Darling***

Knowledge of the embryonic development of nematodes has been derived largely from studies of permanent mounts or from temporary water mounts of living specimens. Observations thus are restricted to specific developmental stages and lack the continuity needed to correlate the many intricate sequential processes of development. Subtle transitions in development are often difficult to obtain or are obscured by fixation and stains complicating interpretations, particularly relative to function. Prolonged observations of active specimens under high magnifications when supplemented by cytological studies would be preferred. The potato rot nematode feeds and reproduces on fungi and can be aseptically cultured in the laboratory. Opportunity thus is provided for continuous observations at all stages of development. The present study traces the development of Ditylenchus destructor from fertilized egg to adult, with emphasis on gonad development as a means of distinguishing sex and age of larvae.

Materials and Methods

Nematodes used for this study were selected from fungus-reared populations established from single gravid females originally extracted from diseased potato tubers (Faulkner and Darling, 1961). Development of the potato rot nematode from fertilized egg to adult was observed with living specimens grown on the fungus Chaetomium indicum Corda in micro-observation chambers (Anderson and Darling, 1964). These chambers, containing an agar substrate and fungus mycelia, permitted continuous observations at magnifications up to 900 x to 1025 x. Up to the second molt, nematodes tended to remain at the interface between agar and coverslip providing opportunity for uninterrupted observations of individual specimens. Observations of advanced stages were interrupted more often between feeding periods and molting because of more vigorous movements of larvae. Intermediate developmental sequences required repeated and prolonged observations.

*This paper is approved for publication by the Director of the Wisconsin Agricultural Experiment Station.
**Research assistant, Department of Plant Pathology, University of Wisconsin, Madison, now: Assistant Professor, Department of Plant Pathology and Physiology, University of Minnesota, St. Paul.
***Professor of Plant Pathology, University of Wisconsin, Madison.
To assure maximum opportunity for observing all developmental stages, active males and females at different stages were introduced into the chambers. In a single chamber it was possible to see all the transitions between molts, as well as copulation, fertilization, and egg development. Extended observations for periods as long as a month provided a substantial amount of data.

**Embryology**

**Cleavage and development of the embryo:** Egg production of *D. destructor* was lower in micro-observation chambers than in petri plates. To assure maximum egg production, 1 or more young females recently molted and several adult males were added to each chamber. Under these conditions a single female laid as many as 12 eggs within 24 hours. Usually half this number was produced. Older females laid as few as 1 or 2 eggs in 24 hours.

Eggs usually were deposited unsegmented and each contained a large transparent nucleus which sometimes was granular (Fig. 1 D, F). The nucleolus disappeared just prior to mitosis. Nuclei at metaphase were conspicuous and served as a convenient marker for observing cleavage. When at metaphase, the chromatin material was massive and rope-like in appearance (Fig. 1 B, C). Chromosomes aligned in any direction across a nucleus and divided cells were arranged correspondingly (Fig. 1 C, D). Usually cells remained at this stage for 30 minutes or more before the chromosomes split. Within 2 minutes after separation, the chromatin disappeared from view. The cytoplasm soon began to constrict along the equatorial plate which was laid down between the separated chromosomes. Cleavage was completed in 10 minutes. Divisions early in cleavage did not occur consecutively. At the 2-cell stage, mitosis was delayed in one cell while the other blastomere continued to divide. Not before the 5-cell stage did the former cell undergo mitosis (Fig. 1 F, G). Following this stage, divisions were in sequence and 2 or more cells were never observed to divide at the same time. As blastomeres increased in number, there was a corresponding decrease in size of cells, nuclei, and cytoplasmic globules (Fig. 1 A-H). Gastrulation occurred soon after the 16-cell stage. The time interval between divisions up to the 7- or 8-cell stage usually was from 2½ to 5 hours. Sometimes 7 hours lapsed between divisions while other blastomeres cleaved within 1 hour. Beyond 8 cells, the time interval between cleavages decreased to about 1 hour.

Tensions appeared to be produced within the egg during cleavage which caused the entire egg to rotate spasmodically 90°, 180°, and 360°. Occasionally an egg was seen to turn instantly end over end. This phenomenon was observed first at the 6-cell stage and persisted up to cell differentiation.

Cell specialization became obvious soon after the 16-cell stage as evidenced by differential mitotic divisions and cytoplasmic changes between groups of cells. When about 16 celled, most of the peripheral cells divided, became ½ the size of cells in the previous stage, contained fewer and smaller globules, and were less granular (Fig. 1, I). By contrast, the large inner cells became darker and more granular than previously and the globules tended to aggregate. Some enlarged, but all appeared to have thicker, refractive walls. This stage represents a phase in gastrulation.

The embryo was discernible within 2½ hours after cell differentiation was first apparent (Fig. 1 J). The cell walls at this stage began to break down and 2 distinct areas were recognizable. The anterior half of the young embryo was transparent, finely granular, and contained few globules (Fig. 1, J-L).
The ectodermal epithelium and stomodaeum were conspicuous. This region developed into the anterior portion of the larva which contained the esophagus. Within the darker, more coarsely globular half, the midgut entoderm and germinal primordium were recognizable which gave rise to the intestine and reproductive system, respectively. The young embryo was small, revealing the partially collapsed vitelline membrane (Fig. 1 K-L). Movement of the embryo, particularly in the anterior region, was first noted at this time. The ectodermal epithelium and stomodaeum were clearly delimited by a fine arched line (Fig. 1, K-L). Often, a portion of the coeloblastula was visible at the apex of the embryo between the ectodermal epithelium and stomodaeum. The stomodaeum persisted until late in larval development but became indistinct before formation of the esophageal lumen. During this developmental phase, the epithelium developed around the darker posterior portion (midgut entoderm) which became progressively smaller and more compact (Fig. 1 K-L). Small uniformly distributed elliptic nuclei were visible in the stomodaeum mass. At this stage, the embryo soon began to elongate and again filled the vitelline membrane. The posterior region elongated more rapidly than the anterior region and within 20 hours, began to reflex anteriorly (Fig. 2, M-N). Development from the unsegmented egg to this stage took about 48 hours.

Once the tail of the embryo reflexed, the embryo began to elongate rapidly. Most of the increase occurred in the posterior ⅔’s of the body and was accompanied by a decrease in body width. Movements also quickened and, within about 18 hours the first molt began. The body now was not easily accommodated within the vitelline membrane and was tightly compressed and looped 3 or 4 times (Fig. 2, Q-T). For this reason details of its development were not easily discernible. However, during the molt, the body was relaxed and development more easily followed.

The esophagus developed relatively late in first stage larvae (Fig. 2, O-T). Prior to molt, the basal bulb of the esophagus and dorsal gland nucleus were very prominent in first stage larvae as was the nerve ring (Fig. 2, Q-R-S). The dorsal gland duct outlet first became visible after the spear apex had formed. At this stage, the esophageal lumen was faintly visible.

At the beginning of the molt, a short tube within the head became sclerotized (Fig. 2, R). This structure later was shed with the cuticle. Next, the median bulb began to develop and the esophageal lumen became quite distinct. Later in the molt, the median bulb valve appeared and was most conspicuous about the time the spear apex formed. Development of the median bulb, however, was not completed until late in the molt. The excretory duct also was visible at this time. By the time the cuticle was shed, about 20 hours after initiation of the molt, these structures had become functional.

Post-embryonic gonad development: Gonads arose from a genital primordium visible in early first stage larvae (Fig. 1, J). It contained a single germinal nucleus which enlarged and became spherical during first molt. The primordium elongated slightly tapering both anteriorly and posteriorly (Fig. 2, T). A small somatic nucleus appeared anterior and one posterior to the germinal nucleus early in second stage larvae (Fig. 3, A). These divided twice during the second molt and usually became arranged with 3 nuclei anterior to the germinal nucleus, 3 posterior, and 2 along the dorsal side (Fig. 3, C). During the third larval stage, 6 nuclei moved to one end of the primordium (Fig. 3, D). In females, these moved posteriorly while in males the direction was anterior toward the nematode head. Of the 2
removing nuclei, 1 became the cap nucleus while derivatives of the other gave rise to the epithelium of the ovary or testis.

Gonad development at third molt: Female and male reproductive systems are now easily discernible and increased rapidly in size and development during the third molt and later phases of growth. The major regions of the female gonad were distinct at the beginning of the third molt. The ovary was the largest portion of the gonad, was relatively well developed, and contained up to 7 nuclei including the germinal nucleus and cap nucleus (Fig. 3 F). The immature uterus was evident as a short region, ventrally expanded, and contained up to 3 nuclei. The uterus was separated from the ovary by a short narrow region in which the quadriecolumella and spermatheca eventually developed. The post-uterine branch was rudimentary and usually contained 1 nucleus (Fig. 3 F). At this stage, 4 specialized ventral chord nuclei were present along the ventral side of the distal end of the gonad (Fig. 3 F).

The gonad increased in length during this molt and its various regions became more discernible. The most marked developmental changes occurred at the uterus. Eight specialized ventral chord nuclei were present now which were ½ the size and double the number (Fig. 3 G) of the previous chord nuclei (Fig. 3 F). These presumably arose by mitotic divisions though none were observed dividing. During this process, somatic nuclei within the uterus enlarged. The one lying most ventral moved from the uterine wall and separated the chord nuclei into 2 groups of 4 nuclei (Fig. 3 H). This nucleus was at first triangular, contained a nucleolus, and its cytoplasm was distinctly granular. It appeared to function in the initiation of the vagina and henceforth is referred to as the vaginal initial. Towards the end of this molt, 16 specialized ventral chord nuclei were present; 8 lying ventrally anterior and 8 posterior to the vaginal initial (Fig. 3 H). The vaginal initial now became spherical, more heavily granular, and began to move into the uterus.

Development of the male gonad during third molt proceeded in the opposite direction to that of the female gonad. The testis, with the proximal end toward the nematode tail (Fig. 3 K), contained a large germinal cell about midway in the testis and was surrounded by numerous small nuclei (Fig. 3 K-L-M). The distal portion of the gonad (gonoduct) consisted of 3 regions early in the molt (Fig. 3 K). The apical segment contained 3 darkly granular nuclei similar in appearance and size to those of the ejaculatory duct of the adult (Fig. 5 B). The adjoining regions of the gonoduct consisted of 2 large granular nuclei, and 2 smaller nuclei, respectively (Fig. 3 K). Serial mitotic divisions of these 4 nuclei throughout the molt resulted in a double row of small nuclei (Fig. 3 L-M). These eventually gave rise to the various regions of the gonoduct. Divisions always were perpendicular to the longitudinal axis of the gonad (Fig. 3 M-N). Development and elongation of the gonad occurred largely in the undifferentiated gonoduct. At the completion of the molt, the elongating portion of the gonad began to reflex and grew posteriorly (Fig. 3 M).

Early phases in the development of the copulatory apparatus were first apparent shortly before the third molt, evident as a concentric compact group of 8-10 nuclei which formed adjacent to the dorsal side of the cloaca and rectum (Fig. 3 P-Q). These nuclei appeared to be contained within a membrane which disappeared during the final molt and after the gubernaculum had formed (Fig. 5 C-D).

Gonad development during the fourth larval stage: During this develop-
mental stage, there was a rapid increase in the length and differentiation of male and female gonads. The somatic nuclei increased in number within the gonoduct which usually showed some structural organization by the fourth molt (Fig. 3 J). Formation of the vagina was initiated just after the third molt and developed slowly until completed late in the fourth molt. Early in the fourth stage larvae, the vaginal initial nucleus moved into the uterus to the dorsal wall (Fig. 3 I). It was followed closely by pairs of specialized ventral chord nuclei, one from each side chain. These nuclei progressed inward and met at the dorsal side of the uterus forming a flask-shaped structure (Fig. 3 J). A cuticular covering now appeared which enclosed the nuclei, separating them from the body cavity and uterine contents. As the vaginal walls developed, the nuclei became arranged at different levels. Shortly before the final molt, all nuclei had moved within the uterus (Fig. 4 A). Most of the nuclei were located within the thicker portions of the immature vagina walls. The walls of the cup-shaped portion of the vagina were continuous at this stage, but the thin base lying against the dorsal wall of the uterus eventually broke down (Fig. 4 A). Two bands of closely associated nuclei encircled the vagina. The nuclei within the vagina eventually disappeared.

The uterus underwent considerable development during this developmental stage. As the gonad lengthened, nuclei in the uterus increased in number. These eventually became compressed within the uterus and post-uterine branch. By the end of the fourth stage, the nuclei began to disappear and a large central cavity formed which extended much of the uterus length and width (Fig. 4 A). The quadricolunella and spermatheca now began to differentiate about midway between the ovary and vagina (Fig. 3 J).

Development of the male gonad proceeded more slowly than the female gonad and usually was not completed until after the final molt. After reversion of the testis was completed, the posterior portion elongated rapidly and grew toward the rectum. Two large hyaline "cells" appeared at the apex of the gonoduct occupying its entire width (Fig. 3 O). Several small granular nuclei were present adjacent to the hyaline cells; the remainder of the gonoduct contained numerous scattered nuclei of different sizes. A gubernaculum was initiated during this molt along the ventral wall of the concentric cluster of nuclei posterior to the rectum (Fig. 5 B, C, D).

GONAD DEVELOPMENT AT FINAL MOLT: Gonad development was completed during the final molt. Somatic cell divisions usually had ceased by the end of the molt and these specialized cells became incorporated into their respective structures. The cells which composed the various structures did not attain their full size until after the molt, about the time the gametes matured.

The uterus proper of the young adult consisted of 2 distinct regions: a large celled anterior portion and a non-cellular homogeneous portion continuous with the post-uterine branch (Fig. 4 B). In heat relaxed specimens, the contents of this portion broke down and moved freely throughout its length when pressure was applied to the coverslip. Though the uterus was discernible in second stage larvae, it did not begin to develop until the vagina initiated during the fourth larval stage. The uterus was practically empty early in the fourth molt with only a thin layer of cytoplasm lining its walls. Several nuclei soon appeared within the layer. Cytoplasm began to accumulate around them and slowly progressed inward (Fig. 4 A). Eventually, the cytoplasm met at the center and fused to form an unsegmented structure (Fig. 4 B). The nuclei had disappeared by this time. The anterior portion
Abbreviations for Figures: ej—ejaculatory duct; gm z—germinal zone; gr z—growth zone; m ret—molting rectum; m z—maturation zone; ov det—oviduct; q c—quadricolomella; sp—spermatozoa; spi p—spicular pouch; spthpc—spermatheca; ut—uterus proper; v det—vas deferens; vag—vagina; v.i.—vaginal initial.

of the uterus proper was composed of large thin-walled epithelial cells.

Early in development of the uterus, the thin base of the immature vagina disappeared (Fig. 4 A). The thick-walled vagina began to shorten in depth, became cylindrical, and its ventral surface flattened against the sub-cuticle. Prior to molting, the cuticle dissolved along the narrow compressed vaginal lumen forming the gonopore. The quadricolumella, spermatheca, and oviduct had formed by this time.

Spicules began to form just prior to the last molt within 2 pouches located between the molting rectum and gubernaculum (Fig. 5 C, D). Within each pouch there were at least 3 nuclei which at first were similar in size and appeared enclosed within a membrane. At the beginning of the molt, fine refractive strands appeared in the distal tapered end of each pouch (Fig. 5 C). Within 2 hours, a long tapering cell had formed around the most distal nucleus of the pouch (Fig. 5 D). The fine refractive strands were present also within this cell, but soon disappeared. This portion of the spicular pouches corresponded to the position and shape of the spicule apices (Fig. 5 C-D).

The ventral side of the spicules formed adjacent to the rectum wall, the distal portion developing first. The proximal portion of the spicules developed later in a ventromedian direction (Fig. 5 A). In the adult the dorsal curved part of the spicules was more lightly sclerotized than the ventral side (Fig. 5 B). The proximal part to which the muscles are attached became as heavily sclerotized as the ventral side of the spicule. A cluster of nuclei of unknown function surrounded the spicular primordium during development of the spicules.

Towards the end of the molt, a lumen became discernible through the center of the ejaculatory duct and vas deferens. Free, globular-like bodies and nuclei within this region soon became arranged throughout its length. Their development was not completed until after the molt. In the adult, the ejaculatory duct contained 12 granular, hemispherically-shaped nuclei. These nuclei were arranged in rows of 3 around the lumen and adjacent nuclei were staggered (Fig. 5 B). At fourth molt, and shortly after, these nuclei may be spherical and opposite, but always remained arranged in a similar grouping.

The vas deferens consisted of 2 regions (Fig. 5 B). The region adjoining the ejaculatory duct contained 8 large, clear globules which formed progressively from smaller ones. As the male ages, each may contain 2 to 4 vacuole-like bodies. In heat relaxed specimens, these globules were oblong and the contents granular. The adjoining portion of the vas deferens was expanded and contained at least 6 similar globules that were about half the size of these in the posterior portion of the vas deferens (Fig. 5 B). An undetermined number of smaller globules of unknown function also were present throughout this region.

**Spermatogenesis**: Spermatogonia evolved from mitotic divisions of the germinal nucleus which remained inactive until about midway between the third and the final molt. Certain small epithelial cells were seen to migrate within the testis and became attached to the posterior and anterior ends of the spermatogonia (Fig. 3 O, Fig. 5 A). Larger epithelial nuclei were also observed in the testis. Spermatogonia matured as they moved down the testis; their nuclei and nucleoli increased in size (Fig. 5 A). After molt, when the testis was full of spermatoocytes, mitosis ceased and all appeared alike. Meiosis of the posterior spermatoocyte began shortly after the molt.
Prior to the first meiotic division, elongate globules developed; first within the posterior spermatocyte and later in the adjacent anterior cell. These refractive globules developed around the nucleus first and gradually spread out to fill the entire cytoplasm (Fig. 5 B). During meiosis, these shortened, but were retained in the cytoplasm of the spermatocytes.

The first meiotic division occurred perpendicular to the longitudinal body axis. The second divisions were parallel to the longitudinal body axis resulting in 4 spermatids of equal size. The posterior most cell from the first meiotic division usually divided first. Spermatids accumulated in a zone (maturation zone) where they became arranged in a double row (Fig. 5 B). Here they underwent maturation and asymmetrical nuclei appeared. Mature spermatids were stored in a seminal vesicle and were arranged in a single row. As sperms accumulated, they became compressed and discoid. If not soon ejected at copulation, they began to accumulate in the maturation zone and may eventually form a double row throughout much of the seminal vesicle. Spermatocytes moved down the testis to replace those that divided.

Oogenesis: The germinal nucleus began to divide in fourth stage larvae and subsequent divisions continued through the fourth molt. Oögonia appeared to form in the same way as spermatogonia. Small free epithelial cells became attached to the oögonia apparently providing their cytoplasmic shells. Nuclei and nucleoli of oögonia increased in size as they moved down the

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ovary and their cytoplasmic contents became progressively more granular. By the end of the final molt, the ovary was full of oocytes. In young fertilized females, the posterior oocytes increased in size and globules began to form within the cytoplasm. The first meiotic division occurred prior to its passage into the spermatheca as judged by disappearance of the nucleoli.

**REPRODUCTION**

Copulation and egg production were most frequent shortly after final molt. Females thus were selected soon after or prior to molt for this study, being detected easily under the dissecting microscope. Only males containing sperms were selected. Despite numerous observations, actual copulation and subsequent migration of sperms within the female were seen in relatively few instances.

Females became receptive to males for about a week after final molt. Males remained sexually active for a longer time and were capable of copulation at least 3 weeks after final molt. A single female may copulate several times during her receptive period and with more than one male. Several hours usually lapsed between copulations.

Amphids of the male probably function in part as sensory organs for locating the female and orientation prior to copulation. As the male approached the female, he proceeded directly to the perineum. The male head first touched the vulva, then twisted away and began to revolve in a spiral parallel to the female with the ventral side in contact with the female perineum. The bursa partially encircled the female and functioned to hold the male in place as well as a guide for orientation prior to copulation. The female tail was bent frequently ventrad which assisted also in guiding the male over the vulva. Several passes of the male generally occurred before the vulva and spicules became aligned and copulation took place. After each pass, the head again moved to the vulva, then twisted away with its body revolving against the female. If copulation did not take place within 5 or 10 minutes, the male moved away.

At copulation, the male and female heads were never parallel, but away from each other. The duration of copulation was very short. The curved spicules, held in place by the bursa, were inserted deeply into the vagina with the apices extending a short distance into the uterus. Insertion of the spicules was followed immediately by ejection of the sperms into the posterior portion of the uterus. As few as 6 or as many as 20 sperms were transferred within 1 second. The male moved away immediately after copulation.

**STRUCTURE AND FUNCTION OF THE SPERMATHECA:** The spermatheca served as a storage organ for sperms and was composed of thin-walled epithelial cells capable of considerable expansion. The inner walls protruded into the lumen and appeared thinner than the outer walls. Those cells surrounding the posterior and anterior ends of the spermatheca were flattened.

Sperms became attached to the spermathecal walls as they entered singly from the quadricolumella and remained attached to one another by mucous-like membranes (Fig. 4, B). Sperms were arranged in tandem within the spermatheca and when more than 7 or 8 sperms were present, the row was reflexed and opposite with at least one side of the ellipsoidal sperms in contact with the inner walls of the spermatheca (Fig. 4, B). The inner ends of the sperms were attached to a thin membrane which separated the 2 rows. Sometimes the reflexed row of sperms was on a different level and then both sperm ends adhered to the inner spermathecal walls. Most anterior sperms
appeared to be fixed, but those in the posterior portion of the spermatheca sometimes migrated slowly from side to side though always moving as a unit. Their movement was more rapid and erratic during the entrance of an oocyte.

The inner walls of the spermatheca rhythmically rippled between the sperms. Movement resulted from an invagination that formed just below an

attached sperm and moved slowly toward the lower sperm. The invagination first extended practically the width of the spermathecal cell and was \( \frac{1}{2} \) to \( \frac{3}{2} \) as wide as it was long; becoming smaller as it approached a lower sperm. A small spheroid globule sometimes was seen discharged from the wall at the termination of each “peristaltic” wave. In young females about 6 waves occurred a minute. The lower sperm was pulled forward at its point of attachment as the wave approached and returned to its original position when the wave terminated. This created the impression that the sperms moved rhythmically back and forth. No movement of the inner spermathecal walls was observed when devoid of sperms.

**Sperm morphology and migration:** Spermatozoa were spherical within the seminal vesicle at first, but became compressed and discoid as they accumulated (Fig. 5 B). Cytoplasm of the sperms was densely packed with large refractive elongate globule-like bodies. Each sperm contained an asymmetrical nucleus, which varied in shape: some consisting of 2 to 4 distinct segments, whereas others were unsegmented and U-shaped (Fig. 5 B).

At copulation, sperms passed rapidly through the lumen of the vas deferens, ejaculatory duct, cloaca, and through the vagina into the uterus. As they passed into the uterus, the sperms became tightly compressed and discoid. They remained in the uterus about a half hour where they underwent certain changes before migration anteriorly. They soon separated from one another and for the first time, were seen to be connected by a thin, transparent mucous-like membrane. Sperms were now ellipsoidal and the globules within the cytoplasm were fewer, smaller, and less refractive than in the seminal vesicle. Sperms then moved slowly with an amoeboid motion into the thin-wall portion of the uterus proper where they became attached to the walls by mucous-like extensions as in the spermatheca (Fig. 4 B). After 30 minutes, sperms began to move slowly anteriorly through the quadricolumella into the spermatheca. Migration proceeded without interruption for about 2 hours. If an egg was present in the gonoduct, the sperms easily moved around it. Migration of sperms terminated in the spermatheca. The first to enter became attached to the inner walls near the oviduct.

Sperms lost by impregnation of oöcytes were replenished by sperms stored in the quadricolumella, uterus proper, or sometimes post-uterine branch. Occasionally young females were seen with groups of 10 or more sperms in each of these structures; attached to their walls as in the spermatheca. They migrated forward when those in the quadricolumella moved into the spermatheca. Migrations of sperms stored in the quadricolumella usually occurred when sperms in the spermatheca were reduced to eight or less. The first to enter from the quadricolumella joined with the posterior sperm of the existing chain. When all sperms had moved into the posterior end of the spermatheca, the free end moved along the existing chain to a position opposite the anterior most sperms.

**Impregnation and development of the egg within the female:** Development of the oöcytes and their subsequent fertilization and expulsion from the uterus were followed in 25 females and substantiated by numerous observations made at each of the various phases in egg maturation. An oöcyte moved into the spermatheca when it had attained the approximate size of a fully developed egg, usually within 2 hours after the nucleolus disappeared. When fully developed, its cytoplasm was largely globular. Passage of an oöcyte into the spermatheca took 1 or 2 minutes. The oviduct expanded little, allowing only a narrow stream of globules to pass. Oöcyte contents were thus disorganized as it entered the spermatheca. The contents reorgan-
Fig. 4. Female gonads of *D. destructor*. A—fourth molt. A germinal nucleus has just completed division. B—mature gonad with spermatozoa adhering to the spermathecal walls, ×2000.
ized to the shape of the spermatheca and the nucleus remained visible as a clear area about midway in the oöcyte, but to one side. The remaining oöcytes in the ovary slowly moved down to fill the vacated space and the posterior most cells began to increase in size. A second oöcyte moved into the spermatheca within 4 or 5 hours.

Impregnation of oöcytes always was observed to take place in the spermatheca from a single sperm which became detached from the end of the row of sperms. The sperm lost its identity within 1 or 2 minutes after penetrating the anterior end of the oöcyte and a clear area formed in its place. The impregnated oöcyte remained within the spermatheca about 5 minutes before moving out. One sperm was always missing from the spermatheca after passage of an oöcyte.

Subsequent migration of the egg was slow and usually it did not arrive in the quadricolumella for 5 to 10 minutes. Fertilization apparently caused a hardening of the egg membrane as it retained its shape during migration through the narrow tube from the spermatheca and into the quadricolumella despite pressures exerted by these expanded structures. Frequently 3 or 4 sperms followed the egg into the quadricolumella, but these eventually moved back into the spermatheca. The membrane connecting the sperms was most clearly seen at this point as it became stretched during the sperms passage through the narrow tube connecting the spermatheca and quadricolumella.

The larger granular cells of the quadricolumella expanded greatly as the egg advanced. After entrance, the end walls closed over the egg and completely encased it. Those cells covering the ends of the egg were stretched greatly in length and became flattened. Most of the tension was on the median cells which separated slightly to accommodate the egg. The egg usually remained in the quadricolumella for at least 1 hour. During this period, secretions were observed flowing between the egg and walls of the quadricolumella. Soon after entrance of the egg, the sperm nucleus began to migrate slowly, increasing in size as it approached the egg pronucleus. Migration of the sperm pronucleus was not always seen and fusion of the pronuclei was not observed. In one case, however, the sperm nucleus was observed opposite the egg pronucleus prior to migration of the egg into the uterus. Both nuclei were the same size, appearing as large vacuoles.

Migration of the egg from the quadricolumella into the uterus took about 15 minutes in young females where it may remain 30 minutes before deposition. The uterine walls were expanded by the egg, particularly the thinner-walled anterior portion (Fig. 4 B). This structure may be stretched to twice its size by an egg, but returns to its original size about 3 hours after the egg is expelled from the uteri.

The walls of the vagina began to twitch periodically as the egg approached and advanced slightly beyond the vagina. When in this position, muscular contractions began which opened the vagina slightly for periods of several seconds. With each contraction, a small portion of the egg wall protruded into the vagina. Usually several contractions occurred before the egg was deposited. Expulsion requires about 1 second during which time the egg flowed out of the vagina which opened to only 1/4 of the egg diameter. Egg contents appeared disorganized for a time after its expulsion, apparently due to its passage through the narrow vagina.

As females age, the rate of egg migration through the gonoduct and egg oviposition decreased. Consequently, several eggs may accumulate in the uterus in different stages of cleavage. One female observed after 2 weeks, for exam-
Fig. 5. Male gonads of *D. destructor*. A—fourth molt, note small epithelial cells adhering to a germinal nucleus. B—adult gonad with spermatocytes undergoing meiosis. C—spicular development at beginning of the fourth molt before the spear shaft fades. D—Spicular development 2 hours later. × 2000.
ple, contained 3 eggs in the uterus with another in the quadricolubella. One egg over the vagina contained 3 blastomeres. The other 2 laid tandem in the anterior portion of the uterus proper. One of these contained 2 blastomeres while the other was unsegmented. In another female, an egg was found in the uterus which contained a fully developed larva. It was eventually deposited after 5 hours.

Size of the oocytes appeared to be reduced in females approaching senility which may result in deformed larvae that die before the first molt within the egg. One such female, for example, deposited 2 eggs that were half the normal egg size. Both eggs were able to cleave, but died soon after gastrulation. This female was observed for 1 week and did not lay additional eggs.

Senility in females was characterized largely by dark, densely globular intestinal cells and arrested reproduction. The intestinal walls of old females became distended and turgid, exerting considerable pressure on the reproductive system. When the body bent, the intestine was pushed against the gonad forcing it out of position. Movements of nematodes at this stage were slow which in part may be due to turgidity of the distended intestine. Though senile females lost their ability to lay eggs, oocytes continued to be produced for a time which accumulated in the distended base of the ovary. These were never observed to enlarge or move into the spermatheca.

**DISCUSSION**

Recent studies of gonad development have provided reliable criteria for distinguishing the sex and various stages of larval development (Raski, 1950; van Gundy, 1958; Triantaphyllou and Hirschmann, 1960; van Weerdt, 1960; Yuksel, 1960; Hirschmann, 1962). Sex in some nematodes has been discernible in second stage larvae by the presence or absence of spicular primordia (van Gundy, 1958) and by the number of gonads (Triantaphyllou and Hirschmann, 1960). Hirschmann (1962) first reported the role of specialized ventral chord nuclei in vaginal development. Their presence late in second stage larvae of *Ditylenchus tricornis* separates females from males. She found that differences in the structure and orientation of male and female gonads were conspicuous at second molt and at subsequent stages of larval development. Sexual differentiation can be expected to vary to some extent with the species or environmental conditions. In *D. destructor*, differential development of gonads did not occur until shortly after second molt. The specialized ventral chord nuclei were seen first just prior to the third molt.

Development of the vagina begins during the third molt and appears to be initiated by a specialized nucleus within the uterus. During third molt, this nucleus separates from the ventral uterine wall midway between the specialized ventral chord nuclei. As it moves into the uterus, it is followed closely by 8 pairs of chord nuclei thus appearing to provide the (entrance and) establishment of the vagina. Subsequent development of the vagina proceeds in much the same manner as described in *D. tricornis* by Hirschmann (1962). Development of the gubernaculum and spicules appears similar to their development in other nematodes (Chitwood and Chitwood, 1950).

Genital primordia are recognizable in first stage larvae and may contain 1 germinal nucleus (Hirschmann, 1962) or 2 germinal nuclei (Chitwood and Chitwood, 1950; van Weerdt, 1960; Chuang, 1962; Hechler, 1963). At this developmental stage, 2 somatic nuclei are present within the primordium: 1 anterior to the germinal nucleus and 1 posterior. Chuang (1962) determined that these were mesodermal cells acquired early in first stage larvae of *Pelodera* (*Pelodera* teres Schneider, 1866) (Dougherty, 1953). In *D.
*destructor* at first molt, the ovate genital primordium contains 1 germinal nucleus, enclosed by a membrane which becomes continuous with the epithelium of the reproductive system. Somatic nuclei appear within the primordium only in second stage larvae. These divide twice during the second molt and become specialized in function during the third larval stage.

The germinal nucleus, first apparent during gastrulation, divides in fourth stage larvae giving rise to the oögonia and spermatogonia. Usually no more than 3 mitotic divisions occur in males at this stage of development and no more than 1 in females. In no case did the cap cell nucleus undergo division and apparently does not function in gametogenesis in this species. This agrees with the observations of other workers (Chitwood and Chitwood, 1950; Hirschmann, 1962). Epithelial cells are conspicuous between the germ cells in the germinal zones of the gonads. These vary in size and function. The large epithelial cells contribute to the development and expansion of the epithelium (Hirschmann, 1962). The smaller epithelial cells, as seen in *D. destructor*, adhere posteriorly and anteriorly to the germinal nuclei and may add to the cytoplasmic mass of the oögonia and spermatogonia. Subsequent development of the germ cells is similar in males and females.

Meiosis is observed easily in males with the compound microscope. Prior to meiosis, elongate refractive globules appear in the cytoplasm which are similar to those described in *Spirina parasitifer* by Cobb (1928). These shorten during maturation of the spermatids, but are retained in the spermatozoa. Spermatids temporarily accumulate within the gonoduct anterior to the seminal vesicle where they undergo maturation before moving into the seminal vesicle. The posterior portion of the gonoduct consists of a vas deferens of 2 sections and an ejaculatory duct terminated by 2 large hyaline cell-like bodies. Copulation and subsequent amoeboid migration of sperms in the female gonoduct are described.

Sperms stored within the spermatheca are bathed in secretions exuded from its moving inner walls with which the sperms are intimately associated. These secretions probably serve as nourishment for the sperms and prolong their longevity. The quadricolumella, described by Wu (1958), and the uterus proper function in a similar manner when containing sperms. When an egg is present, fluids were seen to move around it, particularly in the quadricolumella. These fluids may serve as a “lubricant” which aids in the passage of the eggs.

**Summary**

Unsegmented eggs are deposited by young females. Cleavage and embryonic development occasionally may be found within the body of older females. Cleavage normally begins soon after the egg is laid and is delayed in one blastomere until the 5 cell stage. Divisions occur during a 2½ to 5 hour period up to the 8 cell stage, but occur within an hour in subsequent division. Contents of eggs often rotate spasmodically during cleavage and sometimes the eggs turn end over end. Gastrulation is evident soon after the 16 cell stage and the moving embryo is discernible within 2½ hours. Developmental stages of first stage larvae is described. Larval development is complete in about 48 hours from first cleavage.

Sex is discernible in third stage larvae by the presence or absence of specialized ventral cord nuclei and by orientation, size, and structure of the gonad and germinal nucleus. In females, 4 specialized ventral chord nuclei appear late in development of third stage larvae and increase to 16 by the
end of the third molt. These move into the uteru in pairs during fourth stage. A specialized uterine nucleus separates from the ventral uterine wall, providing an entrance for the specialized ventral chord nuclei. Vaginal development is completed during final molt. Testes develop first toward the tail of the nematode, reflex during the third molt and early fourth stage, and by late in the fourth stage are orientated anteriorly. Spicules and the gubernaculum develop during the fourth stage and is completed during final molt. The gubernaculum forms along the ventral side of a concentric group of nuclei first seen during the third molt. Spicules form during final molt, each within a pouch of nuclei located between the molting rectum and gubernaculum. The spicule apex appears first along the rectum wall. The proximal portion develops in a ventromedian direction from this region. The morphology of the adult male and female reproductive system is described.

Gametes arise from a germinal nucleus which divides during the fourth larval stage and into the fourth molt. Soon after mitosis certain small epithelial cells become attached to the germinal nuclei synchronous with the appearance of the membranes enclosing the oögonia and spermatogonia. Meiosis follows distinct changes in the cytoplasm and nuclei of the germ cells. Sperm are stored primarily in the spermatheca, but may be present in the quadricolumella, uterus proper, and post-uterine branch. Following copulation, they migrate with an amoeboid movement within the female gonoduct as a group, connected to one another by mucous membranes. Sperm adhere to the inner walls of the spermatheca which rhythmically ripple, giving off secretions which bathe the sperms. Oocytes are impregnated by 1 sperm as they move into the spermatheca.

LITERATURE CITED


