Sex Differentiation in Meloidogyne incognita and Anatomical Evidence of Sex Reversal[1]

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Abstract: Sex differentiation was studied by examining the cellular structure of gonad primordia extracted from second-stage juveniles developing under different environmental conditions. In female juveniles, divisions of the two somatic cells of the primordium occurred in mid-second stage and resulted in 12 cells. Two of them were differentiated as cap cells, two occupied the anterior central and eight the posterior central part of the V-shaped primordium. The two germinal cells divided at the 6–8 somatic-cell stage of the primordium; i.e., earlier than in any other plant-parasitic nematode. In male juveniles of similar developmental stage, divisions of somatic cells resulted in 10 cells: one cap cell at the posterior tip and nine cells at the anterior part of the rod-shaped primordium. Germinal cells divided at the 6–8 somatic-cell stage. On the basis of gonad anatomy it was concluded that some female juveniles undergo sex reversal and proceed with further development as males. The degree of expression of intersexual features depends on the period at which sex reversal occurs. Sex reversal at an early period gives rise to males with one testis, almost indistinguishable from true males. Sex reversal at mid-second stage involves degeneration of the nucleus of one of the cap cells resulting in males with an atrophied testis and a well-developed testis. More delayed sex reversal results in males with two testes of approximately equal size. To explain these patterns of development, it is assumed that sex differentiation is hormonally controlled and that the environment influences hormonal balance by affecting gene expression. *Key words:* sexuality, nematodes, postembryogenesis.

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An interesting feature of the biology of root-knot nematodes is the unbalanced sex ratios occurring in natural populations and in greenhouse cultures of some common species (19). In the same population, males may be rare or absent sometimes and abundant other times. It is generally believed that sex differentiation in the genus *Meloidogyne* is controlled to a large extent by the environment.

Meloidogyne incognita is a mitotically parthenogenetic (apomictic) species which theoretically should be completely thelytokous; *i.e.,* consist of females only (18). Still, males appear frequently in this species and often constitute more than 60% of

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the adult population. The effect of various environmental factors on sexual differentiation of *M. incognita* and other root-knot nematodes has been studied by various investigators, and the topic has been reviewed by Triantaphyllou (16). There is strong evidence that under favorable environmental conditions juveniles develop to become females, but under less favorable conditions the juveniles undergo complete or partial sex reversal and develop as males, many of them with intersexual characteristics (15,16).

The main source of information supporting sex reversal in *Meloidogyne* has come from general observations of the pattern of development of the gonad primordium of second-stage juveniles (3,15). However, such anatomical observations have been difficult and not very precise, mainly because the body of half-grown second-stage, or older, juveniles is thick and opaque, thus obscuring details. Various staining procedures successfully employed in the study of the development of the reproductive system of other nematodes (5,6, 12) have not been very helpful with root-knot nematodes.

In the present investigation we have successfully employed several procedures to study gonad development of second-stage juveniles of *M. incognita* at the cellular level. We have attempted to relate the type of gonad growth with the patterns of sexual differentiation that lead to the development of females, males, and intersexes in this nematode.

MATERIALS AND METHODS

Meloidogyne incognita population E406-Greece of the International *Meloidogyne* Project collection was used in most of these studies. Supplementary information about the process of differentiation of male juveniles was obtained also from studies of *M. arenaria* population E482-Australia. Juvenile inoculum for all the tests was obtained from 50- to 60-day-old stock cultures maintained on Rutgers tomatoes in a greenhouse at 25–28 C.

For the anatomical study of the process of differentiation of female juveniles, 3-wk-old tomato seedlings growing in 10-cm clay pots were each inoculated with 300 freshly hatched juveniles. The roots of six seedlings were washed free of soil 6, 8, 10, 12, and 14 days after inoculation. Small sections of galled roots were stained in boiling acid-fuchsin lactophenol for 3–4 min, destained in clear lactophenol at 45 C for 1–2 min, and stored in clear lactophenol for at least 5 days. The juveniles were then carefully dissected from the galls and mounted in clear lactophenol on ringed slides for microscopic examination. Identification of the developmental stages and sex of the nematodes was based on the morphological characteristics described earlier (19). More precise observations of the cellular structure of the reproductive system of juveniles were made by dissecting the gonad out of the juvenile body and mounting it in clear lactophenol.

To study gonadogenesis of male and sex-reversed juveniles which are produced under crowded conditions, 6-wk-old tomato seedlings were inoculated with 5,000 juveniles. The plants were examined 55–65 days after inoculation. The procedure of obtaining juveniles and preparing them for microscopic examination was the same as described for the study of female juveniles. However, only apical root galls were isolated for this study, since such galls were expected to contain adult males as well as male and sex-reversed juveniles in various developmental stages (15).

A more precise study of early development of gonad primordia was made by direct examination of unfixed, unstained gonad primordia of live juveniles. Juveniles were carefully dissected from root galls in drops of 0.9% NaCl solution on ringed microscope slides. To isolate the gonad primordium, the nematode was cut immediately posterior to the esophageal region with a sharpened needle or eye-knife using a dissecting microscope with a 50 × magnification. Gonad primordia thus obtained were mounted on ringed slides and examined immediately with bright-field, light microscopy and also Normarski differential interference optics, at 1,250 × magnification. Cell divisions, cell migration, and the cellular structure of the primordium were studied during the first 2 h following dissection, while the cells were still alive. Ad-

ditional observations, especially of the spatial distribution of somatic and germinal nuclei, were made shortly after cell death; *i.e.,* 2–6 hours after dissection.

To study early cell divisions of somatic and germinal cells, some gonad primordia were fixed in 1:3 acetic alcohol for 1–2 min, stained in 1% propionic orcein for 25 min, and mounted on slides in 45% acetic acid (17).

OBSERVATIONS

Gonad development in female juveniles: The gonad primordium of infective second-stage juveniles consists of four cells: two large, spherical germinal cells surrounded by two smaller, flat somatic cells (Fig. 1A). No cell divisions take place during the first 5 days following juvenile penetration of the roots, although juvenile and gonad primordium increase slightly in size (Figs. 1B; 2A, B). The sex of juveniles at this stage could not be recognized by any means.

The primordium is located about 65% of body length with its longitudinal axis parallel to the anterior-posterior axis of the juvenile. However, in this study we have arbitrarily designated the anterior and posterior parts of the primordium as left and right, since they eventually become the left and right ovaries. Thus, the first two somatic cells have been designated S_L and S_R; *i.e.,* somatic cells left and right (Fig. 2B). Following a given cell division, the daughter cells, or more precisely the derivative nuclei, are designated numerically with the nucleus closest to the apex of the future gonad (distal) designated as 1 and the one toward the center of the primordium (proximal) designated as 2.

Six days after root penetration, the somatic cells of the primordium divide simultaneously (Fig. 1C) or in close succession. This division generates four similar somatic cells, S_{L1}, S_{L2}, S_{R2}, and S_{R1} (Figs. 2C, 3A). Soon afterwards, two of the somatic cells, presumably the S_{L1} and S_{R1}, divide again and the primordium now has six somatic and two germinal cells (Fig. 2D). At this stage the distal cells of the second division (S_{L11} and S_{R11}) take apical positions and their nuclei change shape from spherical to oval. These distinct cells, which have been observed also in most other nematodes, become the "cap cells" of the ovaries. The same cells have been referred to as "distal tip cells" in gonads of *Caenorhabditis elegans* and *Panagrellus redivirus* (8,13). The cap cells of *M. incognita* do not undergo any further divisions throughout the life of the nematode. No cell divisions take place in the next 2 days during which the S_{L12}, S_{L2}, S_{R12}, and S_{R2} cells migrate toward the center of the primordium. Presumably the S_{L12} and S_{R12} occupy the dorsal side of the primordium and the S_{L2} and S_{R2} the ventral side which eventually becomes the posterior part of the gonad (Fig. 2D).

Between the 8th and 10th days after root penetration, the S_{L2} and S_{R2} cells undergo two consecutive divisions, giving rise to eight cells that occupy the ventral side of the primordium (Figs. 1D, 2E). In the meantime, the two centrally located germinal cells enlarge considerably and subsequently undergo the first and frequently a second division (Figs. 2E, 4A). Therefore, on the 9th or 10th day after root penetration, the primordium has 12 somatic cells and 4–8 germinal cells. Due to the multiplication of the somatic cells of the ventral side, without multiplication of those of the dorsal side, the primordium assumes a "V" shape (Figs. 2G; 4B, C, D). This shape is indicative of the future development of such a primordium into a female reproductive system with two ovaries. Also, at this time, the primordium changes orientation by turning its longitudinal axis perpendicular to the long body axis of the juvenile. Soon afterwards, the gonad primordium migrates ventrally towards the posterior end of the body, close to the hypodermis (Fig. 2F).

No divisions of somatic cells take place during the next 3 or 4 days; *i.e.,* until about the end of the second juvenile stage. During the same period, however, a rapid multiplication of the germinal cells takes place and the two arms of the V-shaped primordium elongate progressively as they are filled with germinal cells (Figs. 2H; 4D, E). The nuclei of the somatic cells maintain their relative position in the primordium. The two cap cells occupy the distal ends of the arms. Two somatic cells (S_{L12} and S_{R12}) are located in the anterior

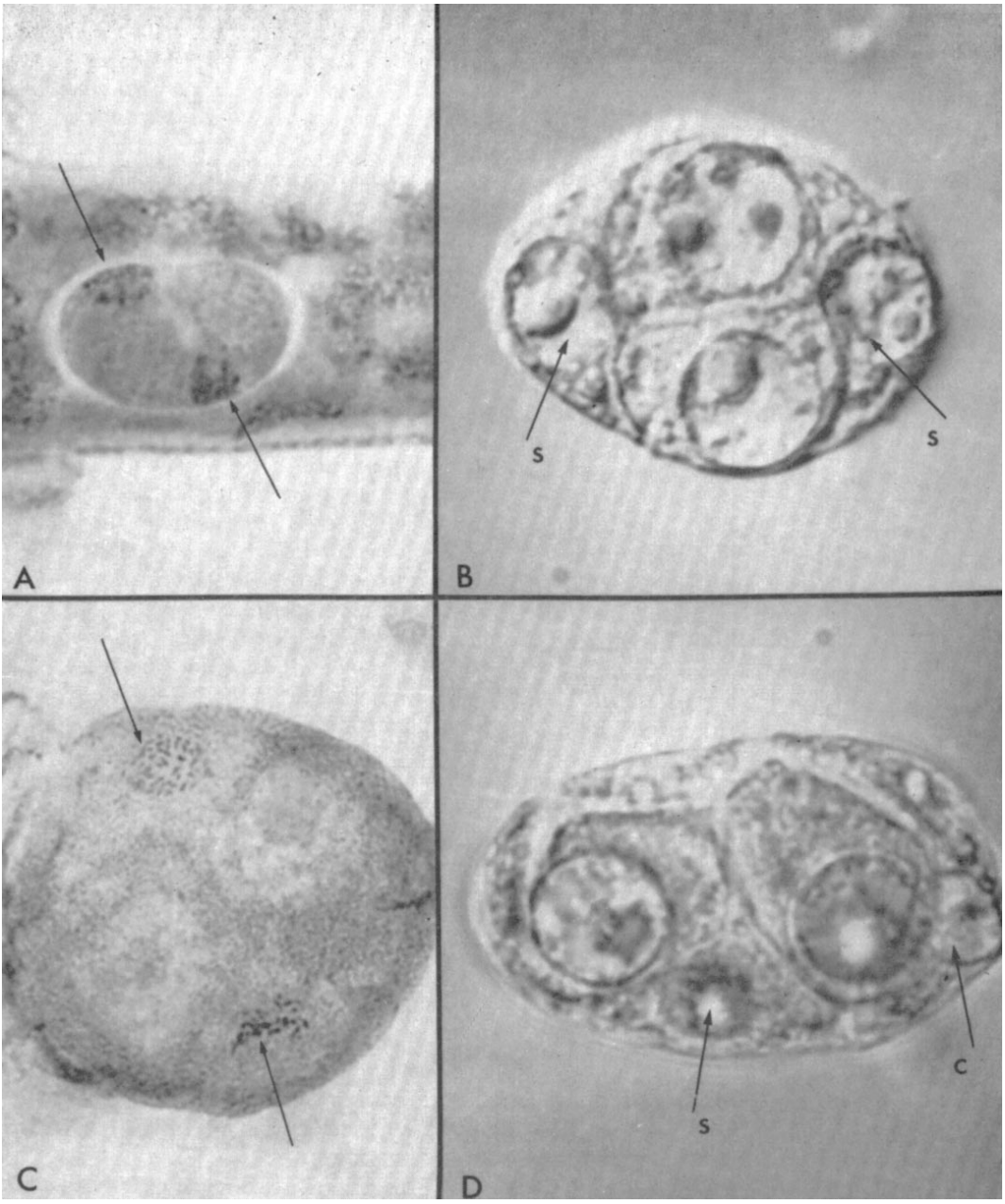

Fig. 1. Anatomy of gonad primordium of second-stage juveniles of *Meloidogyne incognita*. A) Gonad primordium of infective juvenile: two germinal cells with large, spherical nuclei are surrounded by two flat, somatic cells with small darkly orcein stained nuclei (arrows). B) Gonad primordium of a juvenile 6 days after root penetration (interference contrast microscopy); s = somatic cells. C) Gonad primordium of a 6-day-old juvenile: somatic cells (arrows) are at metaphase of the first division (orcein stain). D) Gonad primordium of a 10-day-old female: two large germinal cells are surrounded by somatic cells (s); cap cells (c) are first distinguishable at this stage (interference contrast microscopy).

central part, and another eight somatic cells, descendents of the S_{L2} and S_{R2} cells, occupy the posterior central part and the sides of the primordium (Fig. 2H). The latter cells cannot be recognized individually; however, two of them are assumed to be the S_{L211} and S_{R211}. By the 12th day following root penetration, the nuclei of the S_{L12} and S_{R12} cells move into the anterior side of each gonadal arm (Fig. 2H). The

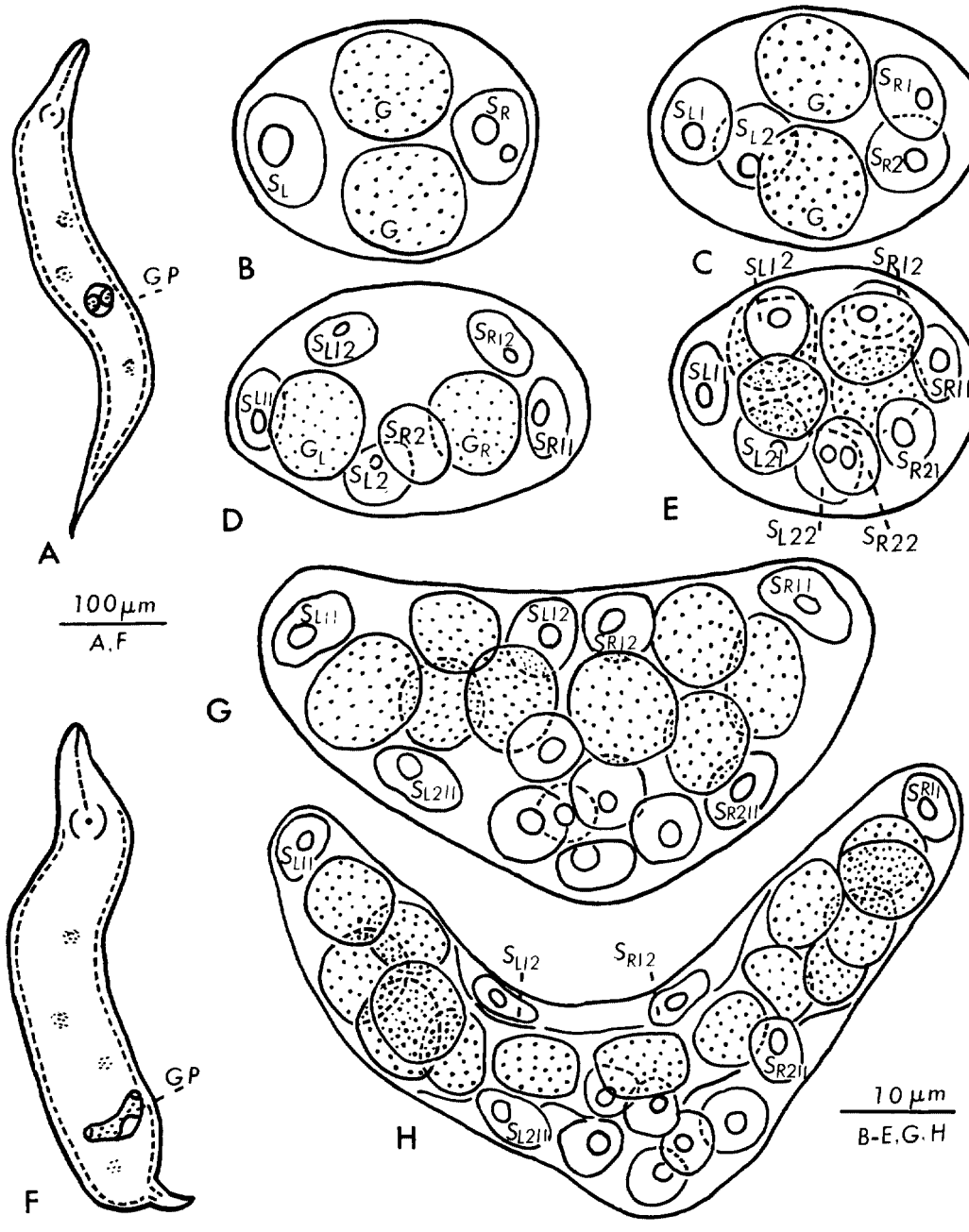

Fig. 2. Camera lucida drawings of somatic and germinal (stippled) nuclei during gonad development of female second-stage juveniles of *Meloidogyne incognita*. A) Six-day-old juvenile: the gonad primordium (GP) is located ventrally, at about 65% of body length. B) Gonad primordium consists of four cells (only nuclei are illustrated): two large germinal cells (G) surrounded by two smaller, somatic cells (S_L and S_R). C) Four-somatic-cell stage. D) Six-somatic-cell stage: cap cells (S_{L11} and S_{R11}) are first distinguishable. E) Eight-somatic-cell stage: germinal cells have divided. F) Mid-second-stage female juvenile: the gonad primordium (GP) is V-shaped and has migrated ventrally towards the posterior end of the body. G) Twelve-somatic-cell stage: the gonad contains eight germinal cells. H) Somatic cells at center of primordium are enlarged and have started displacing the germinal cells into the elongating gonadal arms.

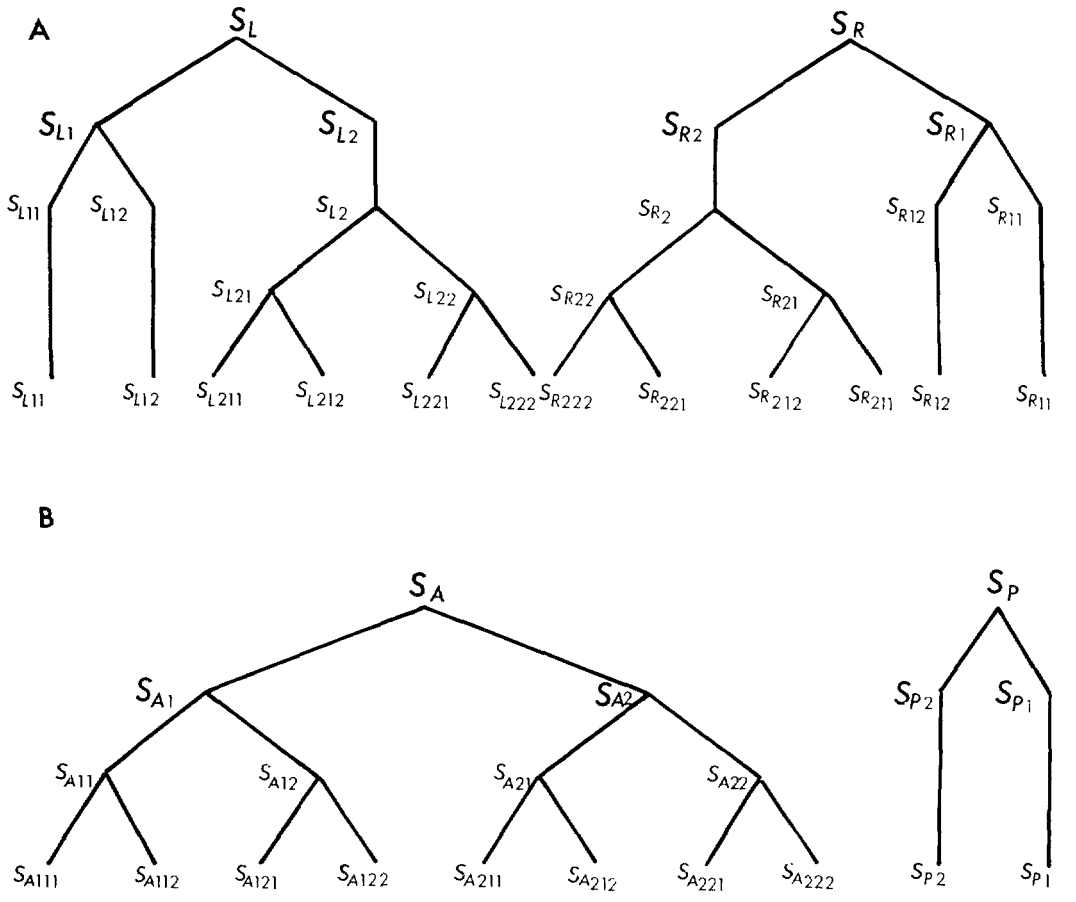

Fig. 3. Somatic-cell lineages reconstructed from the study of developing gonad primordia of female (A) and male (B) second-stage juveniles of *Meloidogyne incognita* (see text).

somatic cells in the posterior central part of the primordium enlarge and start displacing the germinal cells from the central part of the primordium into the elongating arms. There are about 10 germinal cells in each gonadal arm at this stage (Figs. 2H; 4E).

At the end of the second juvenile stage or the beginning of the second molt (*i.e.*, 14 days after root penetration) the somatic cells S_{L12}, S_{R12}, S_{L211}, and S_{R211} (Fig. 2H) divide once, and the derivative cells are positioned along the anterior and posterior sides of the gonadal arms (Fig. 5). These cells form the thin epithelium of the gonads. Two of the somatic cells of the anterior-central part of the primordium also undergo one division. The derivative four cells remain in the central part of the primordium and displace completely the germinal cells into the gonadal arms (Fig. 5). Of the remaining four somatic cells located in the posterior-central part of the primordium, one always takes a position at the posterior tip. Apparently this is the anchor cell, as it has been called in *C. elegans* and *P. redivivus* (8,13), that eventually establishes contact with the specialized hypodermal tissue which later will form the vagina in the adult female. The germinal cells have increased in number, and by the beginning of the second molt, there are about 20 cells in each gonadal arm (Fig. 5). Most of the divisions of germinal cells occur close to the distal end of each gonadal arm.

Further development of the reproductive system beyond the second molt was not followed in detail. General observations, however, during the fourth juvenile stage revealed a rapid multiplication of the somatic cells of the central part of the

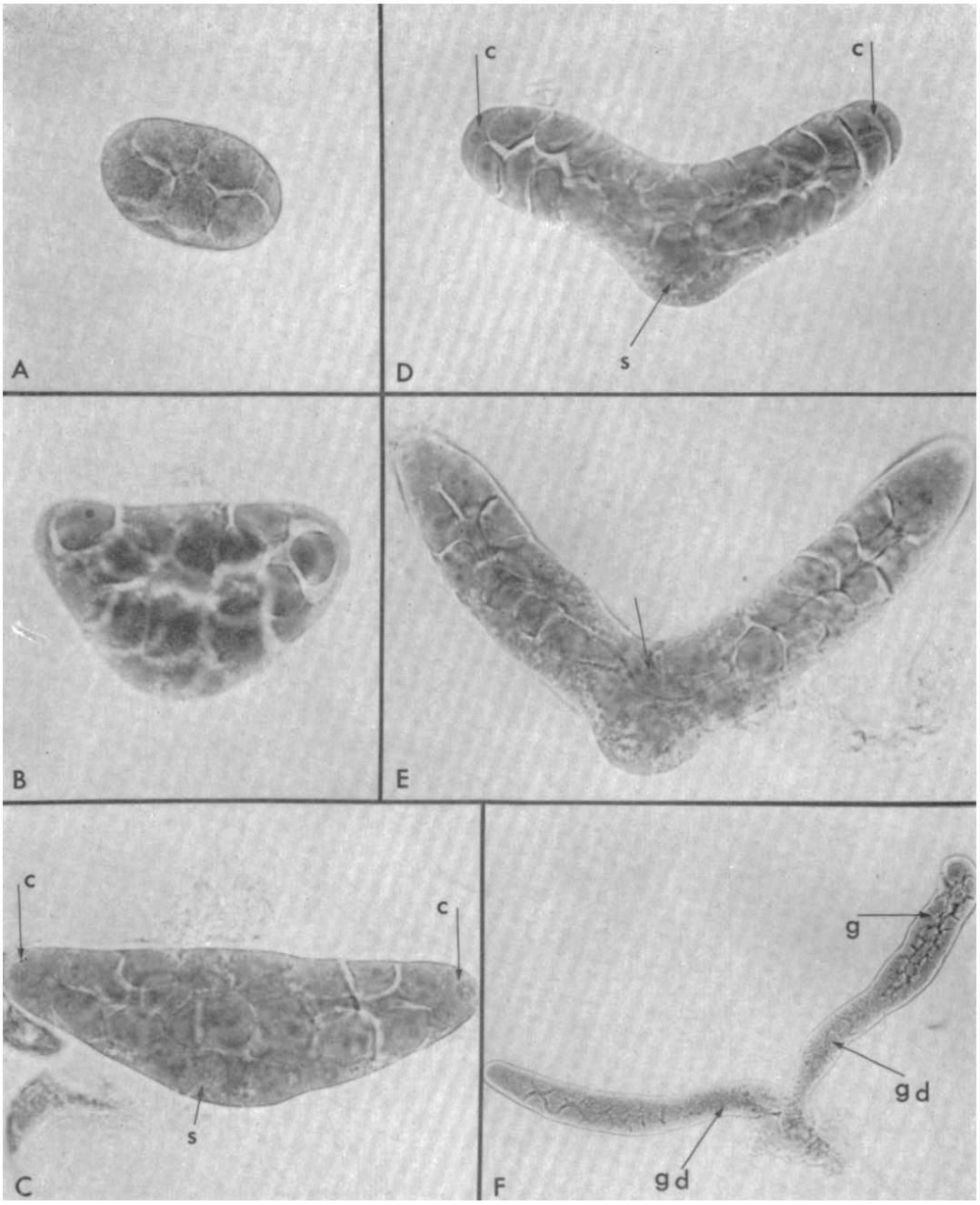

Fig. 4. Early development of gonad of female juveniles of *Meloidogyne incognita* (acid fuchsin lactophenol stain). A) Gonad primordium of an 8-day-old juvenile: five germinal cells are visible. B. Gonad primordium of a 10-day-old juvenile. C and D) Gonad primordia of mid-second-stage juveniles: two cap cells (c) and a region of somatic cells (s) in the posterior central part are visible. E) V-shaped gonad of a late second-stage juvenile: both arms are filled with germinal cells; somatic cells in the center have started displacing germinal cells (arrow) towards the gonadal arms. F) V-shaped gonad of a mid-fourth-stage female juvenile: germinal cells (g) occupy the distal half of each gonadal arm; gonoducts (gd) are forming in the proximal half of each arm.

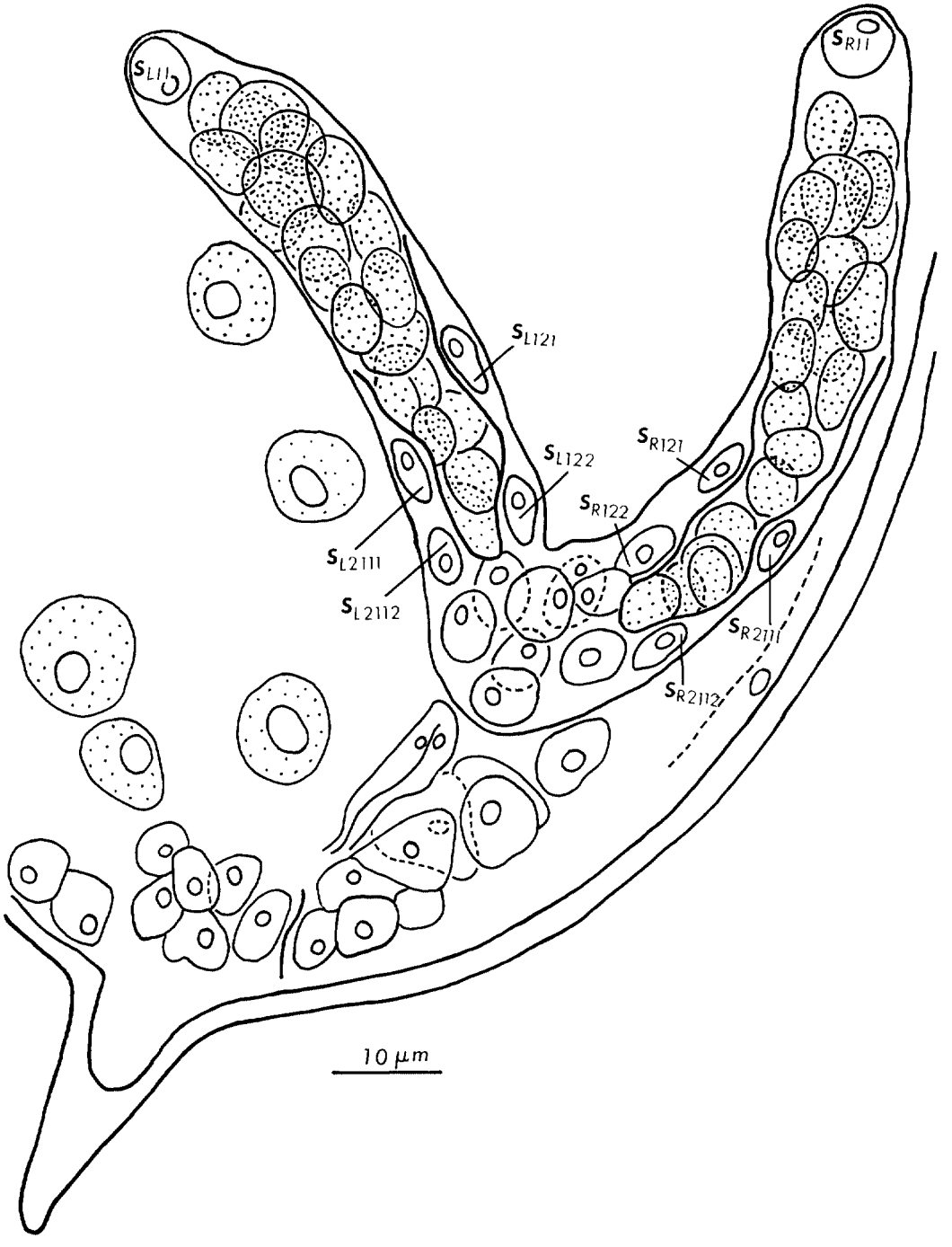

Fig. 5. Arrangement of somatic and germinal (stippled) nuclei in the gonad of a female juvenile of *Meloidogyne incognita* during the second molt. Multiplication and enlargement of somatic cells in the central part have displaced germinal cells into the gonadal arms.

primordium and the proximal parts of each gonadal arm. These divisions resulted in a large number of cells that formed the gonoducts (Fig. 4F) which later were differentiated into distinct regions: oviduct, spermatotheca, and uterus. Few divisions of germinal cells were observed during the third and early fourth juvenile stages.

Gonad development in male juveniles: Male juveniles, as well as sex-reversed juveniles, were found only in roots of old plants with second generation infections. Therefore, the exact age of such juveniles was not known, and the sequence of cell divisions was deduced following examination of many juveniles at various developmental stages. Based on anatomy of the genital primordium, less than 1% of these juveniles were classified as male juveniles. The remaining 99% were characterized as sex-reversed juveniles and are discussed in the next section.

The gonad primordium of young second-stage male juveniles is identical with that of female juveniles (Figs. 6A, B). For reasons that will become obvious in the following paragraph, the two somatic cells in male primordia are designated as anterior (S_A) and posterior (S_P) (Fig. 6B) according to their orientation. In subsequent divisions, derivative cells are designated numerically as in female juveniles.

The first somatic cell divisions generate four cells; S_{A1} and S_{A2} in the anterior, and S_{P2} and S_{P1} in the posterior part of the primordium (Fig. 6C). Shortly afterward, differences in symmetry emerge between male and female patterns of gonad development. The posterior distal cell S_{P1} differentiates as a "cap cell," similar to the cap cells described in female gonads. The anterior somatic cells S_{A1} and S_{A2} undergo two consecutive divisions giving rise to eight cells, which remain in the anterior part of the primordium, but none is transformed into a cap cell (Figs. 6D, 3B). Thus, the male primordium has only one cap cell—at the posterior distal tip. At this stage the two germinal cells divide once or twice. Soon thereafter the S_{P2} cell apparently migrates anteriorly (Fig. 6F). The primordium now takes a certain shape and exhibits a cell distribution typical of the male sex. It is rod-shaped with nine somatic cells at the anterior blunt end, a cap cell at the posterior, more pointed tip, and 6–8 germinal cells in the central part (Figs. 6F, G; 7A). A juvenile with this type of primordium is at mid-second stage (Fig. 6E). No further divisions of somatic cells take place until the beginning of the fourth juvenile stage. Divisions of germinal cells, however, continue along the entire primordium during the second half of the second juvenile stage. The number of germinal cells varies from 30 to 50 in gonadal primordia of male juveniles undergoing the second molt (Fig. 7B).

After the completion of the second juvenile stage, the anterior part of the gonad bends so that the anterior tip makes a 180° turn and becomes directed posteriorly (Fig. 7C). This part continues to elongate posteriorly during the fourth juvenile stage when repeated divisions of the group of nine somatic cells give rise to the male gonoduct (Fig. 7D). At the same time, the germinal cells undergo rapid multiplication and the testis elongates considerably (Fig. 7D). Later the gonoduct connects with the cloacal primordium and becomes differentiated into seminal vesicle and vas deferens.

Gonad development in sex-reversed juveniles: In addition to the female and male juveniles already described, second-stage juveniles with atypical gonads were also observed in these studies. The latter were considered earlier as female juveniles (15), which undergo sex reversal due to the influence of various environmental conditions and proceed to develop into males. The present, more detailed observations confirm this interpretation.

Sex-reversed juveniles can be recognized at mid-second stage on the basis of gonad primordium anatomy and can be classified into the following three types.

Type A: Mid-second-stage juveniles of this type possess a V-shaped gonad primordium, similar to that of female juveniles. The two arms of each primordium are of approximately equal length, and each arm has distally a normal cap cell and about 6–8 germinal cells.

During the second half of the second juvenile stage, the arms of the primordium elongate, following multiplication of the germinal cells, in a similar manner as in female juveniles. Many germinal cells, however, still occupy the central part of the primordium, while the somatic cells of this region are displaced at the posterior most central part (Fig. 8A). The presence of many germinal cells in the central region is indicative of the process of sex reversal

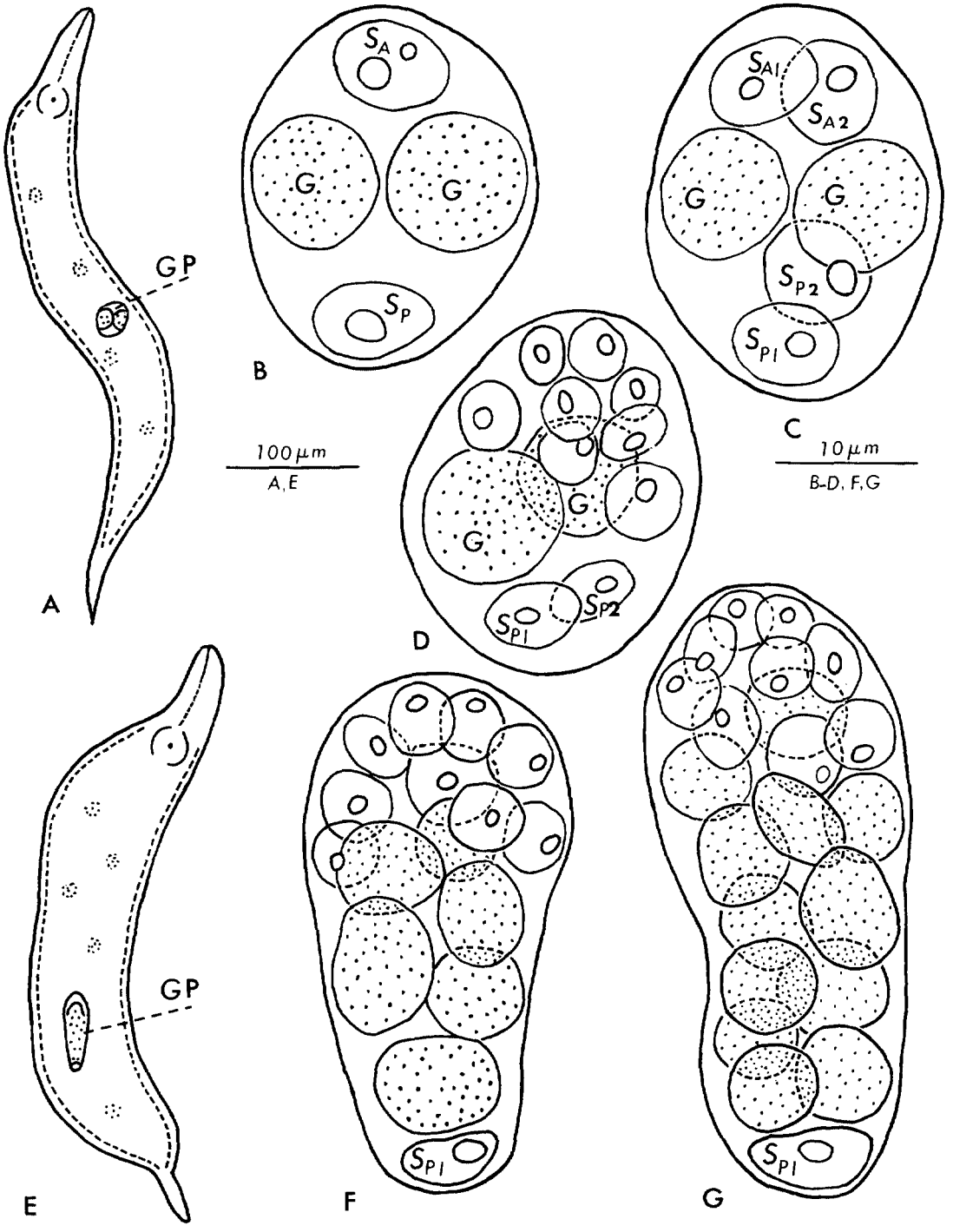

Fig. 6. Camera lucida drawings of somatic and germinal (stippled) nuclei during gonad development of male second-stage juveniles of *Meloidogyne incognita*. A) Six-day-old juvenile: the genital primordium (GN) is located ventrally, at about 65% of body length. B) Genital primordium consists of four cells (only nuclei are illustrated): two large germinal cells (G) and two smaller somatic cells (S_A and S_P). C) Four-somatic-cell stage. D) Ten-somatic-cell stage: only one cap cell (S_{P1}) is differentiated. E) Mid-second-stage, male juvenile: the gonad primordium (GN) is rod-shaped and has migrated towards the posterior end of the body. F and G) Gonad primordia of mid- and late-second-stage juveniles, respectively: nine somatic cells are located anteriorly and one cap cell (S_{P1}) is located posteriorly.

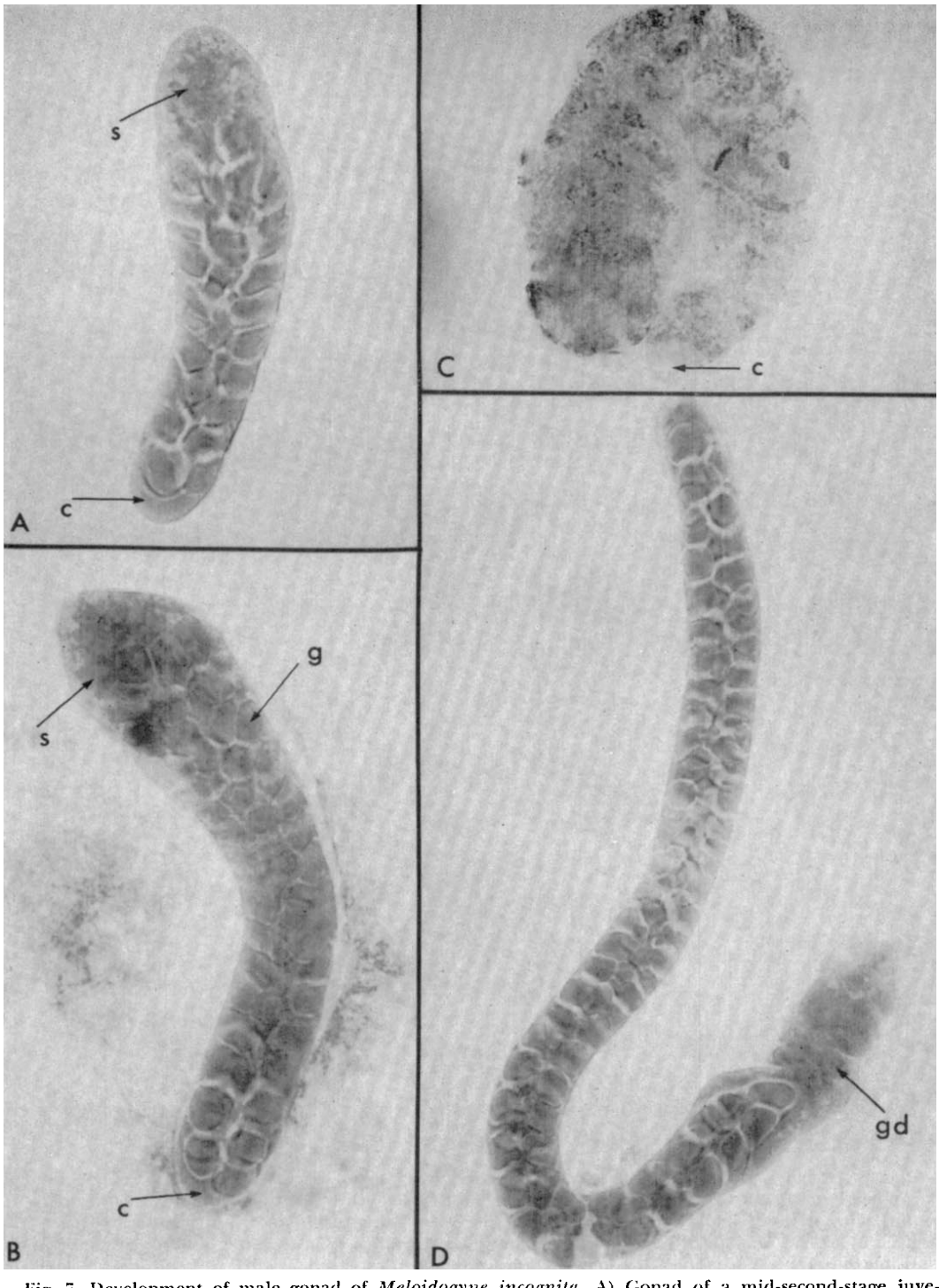

Fig. 7. Development of male gonad of *Meloidogyne incognita.* A) Gonad of a mid-second-stage juvenile: the anterior, broad part contains the somatic cells (s); the posterior, narrow part contains the cap cell (c); the rest of the primordium is filled with germinal cells. B) Gonad of a third-stage juvenile containing about 50 germinal cells (g). C) Gonad of a juvenile undergoing the third molt: the anterior part of the gonad bends so that the anterior tip makes a 180° turn and becomes directed posteriorly. D) Gonad of a fourth-stage juvenile with developing gonoduct (gd). (A, B, D–acid fuchsin lactophenol stain; C–orcein stain.)

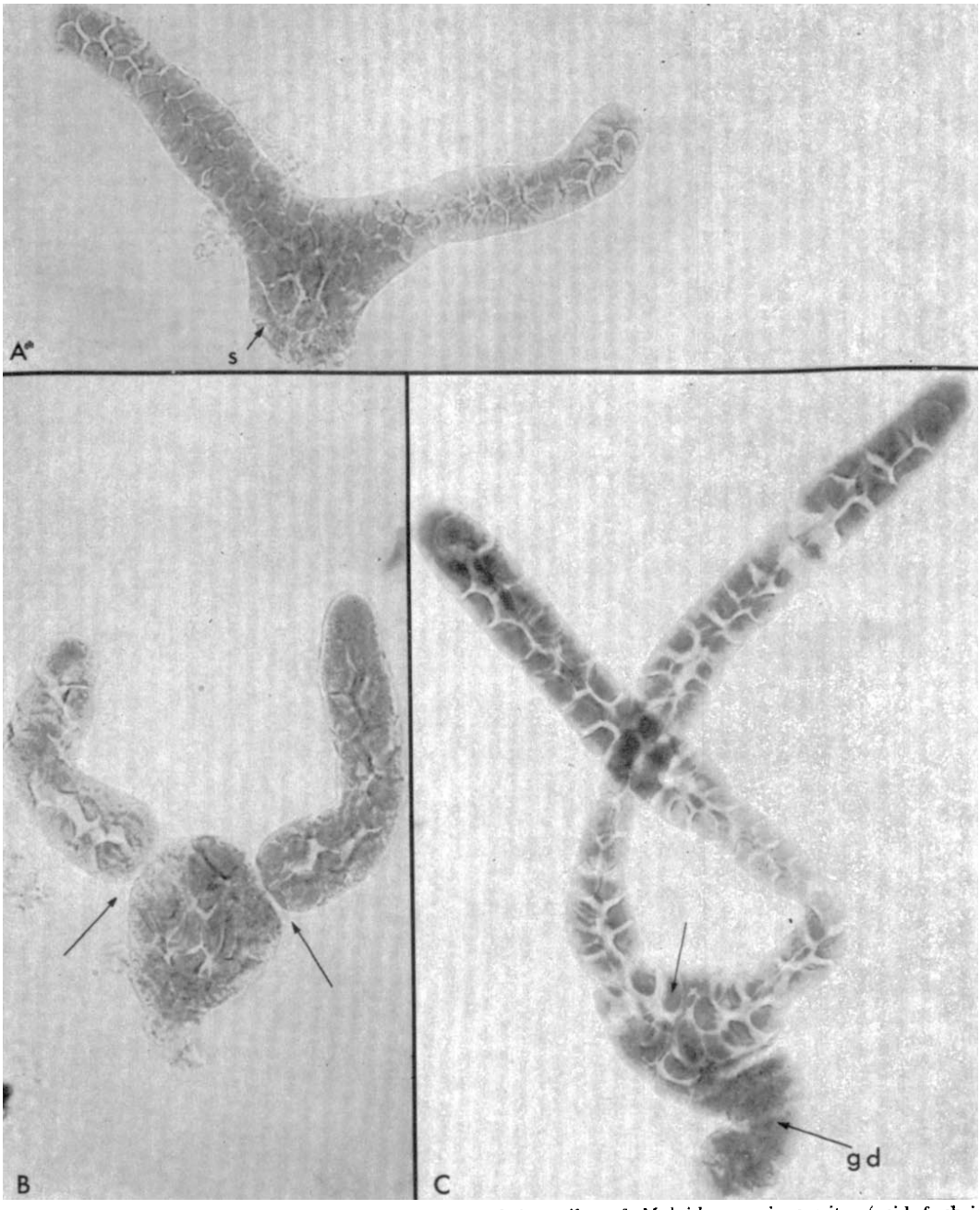

Fig. 8. Gonad morphology of type-A, sex-reversed juveniles of *Meloidogyne incognita* (acid fuchsin lactophenol stain). A) Gonad of an advanced, second-stage juvenile: many germinal cells still occupy the central part of the primordium, while the somatic cells of this region occupy the posterior most central part(s). B) Gonad of a third-stage juvenile: a constriction (arrow) between the central part and each arm is visible; the central part and the gonadal arms are filled with germinal cells. C) Gonad of a fourth-stage juvenile: the central part (arrow) is filled with germinal cells; a single gonoduct (gd) is forming posteriorly.

taking place in these juveniles. Most germinal cells would have been displaced into the gonadal arms at this stage of development in normal female juveniles (Fig. 4E). The difference becomes more apparent shortly after the second molt, or in early fourth stage, when about 10–15 germinal cells are present in the central region of the primordium in sex-reversed juveniles (Fig. 8B) but are absent in female juveniles (Fig.

5). Furthermore, a constriction often separates the arms from the central part of the primordium in sex-reversed juveniles (Fig. 8B).

A second cycle of somatic cell divisions occurs in the central part of the primordium during the fourth juvenile stage and gives rise to the male gonoduct (Fig. 8C). It appears that the central region of the primordium becomes part of the seminal vesicle in adults, while the arms of the primordium become the testes. By late fourth juvenile stage, the vas deferens connects with the cloacal primordium.

Type B: Mid-second-stage juveniles of this type have a V-shaped gonad primordium, similar to that of female juveniles and type-A, sex-reversed juveniles. However, one of the arms of the primordium is shorter than the other and terminates in a large cell that resembles a cap cell but has no distinct nucleus (degenerate cap cell). Distribution of somatic cells is identical to that of female gonad primordia, and the 10–20 germinal cells are spread along the entire length of the primordium, including the central region.

Identification of this type of primordium is easier during the second half of the second juvenile stage when the gonadal arm with the cap cell increases in size, whereas the arm with the degenerate cap cell remains short, apparently due to the lack of multiplication of the germinal cells (Fig. 9A, B, C). Adult males developing from such juveniles have two testes of unequal length.

Type C: Mid-second-stage juveniles of this type have a small V-shaped primordium similar to that of female juveniles, but only one arm has a cap cell and has undergone some growth. The other arm lacks a cap cell and is atrophied (Fig. 10C, arrow). The developed arm is directed anteriorly and has 8–12 germinal cells in addition to the cap cell. It is usually separated from the proximal, posterior part of the primordium by a distinct constriction (Fig. 10C). The region posterior to the constriction corresponds to the central part of a female gonad primordium and contains approximately 10 somatic cells and a small number of germinal cells. During the second half of the second juvenile stage, the anterior part of the primordium elongates following multiplication of the germinal cells, while the posterior part becomes thicker through an enlargement of germinal and somatic cells (Fig. 10D, E). Usually only a trace of the undeveloped, second gonadal arm is seen.

A second cycle of divisions of the somatic cells located at the posterior most part of the primordium takes place following the second molt. It results in the formation of the gonoduct that differentiates into seminal vesicle and vas deferens. Adult males of this type have one outstretched testis and a somewhat swollen seminal vesicle. Occasionally, mid-second-stage juveniles of this type have a rod-shaped primordium similar to that of true male juveniles, without a trace of an atrophied gonadal arm (Fig. 10A). A study of the distribution of somatic cells in such juveniles revealed that the single cap cell is located in the anterior end of the primordium. In a true male juvenile of similar developmental stage, the cap cell would be located in the posterior end. Furthermore, the posterior part of the primordium that includes about 10 somatic cells and a few germinal cells frequently is separated from the rest of the gonad by a distinct constriction (Fig. 10A). Such a constriction has not been observed in true male primordia. During the latter half of the second juvenile stage, the primordium elongates considerably, following multiplication of the germinal cells and enlargement of the somatic cells (Fig. 10B). Presumably males developing from such juveniles are indistinguishable from true males. They have one testis, always outstretched.

DISCUSSION

The gonad primordium of second-stage juveniles of *Meloidogyne incognita* consists of two germinal cells and two somatic cells. The somatic cells undergo the first division at mid-second stage; *i.e.*, about 6 days following initiation of feeding of the juveniles. After the first somatic cell division, gonad development follows a different pattern in females and males. In female juveniles the first cycle of divisions results in the formation of a V-shaped primordium with 12 symmetrically arranged somatic cells. Two

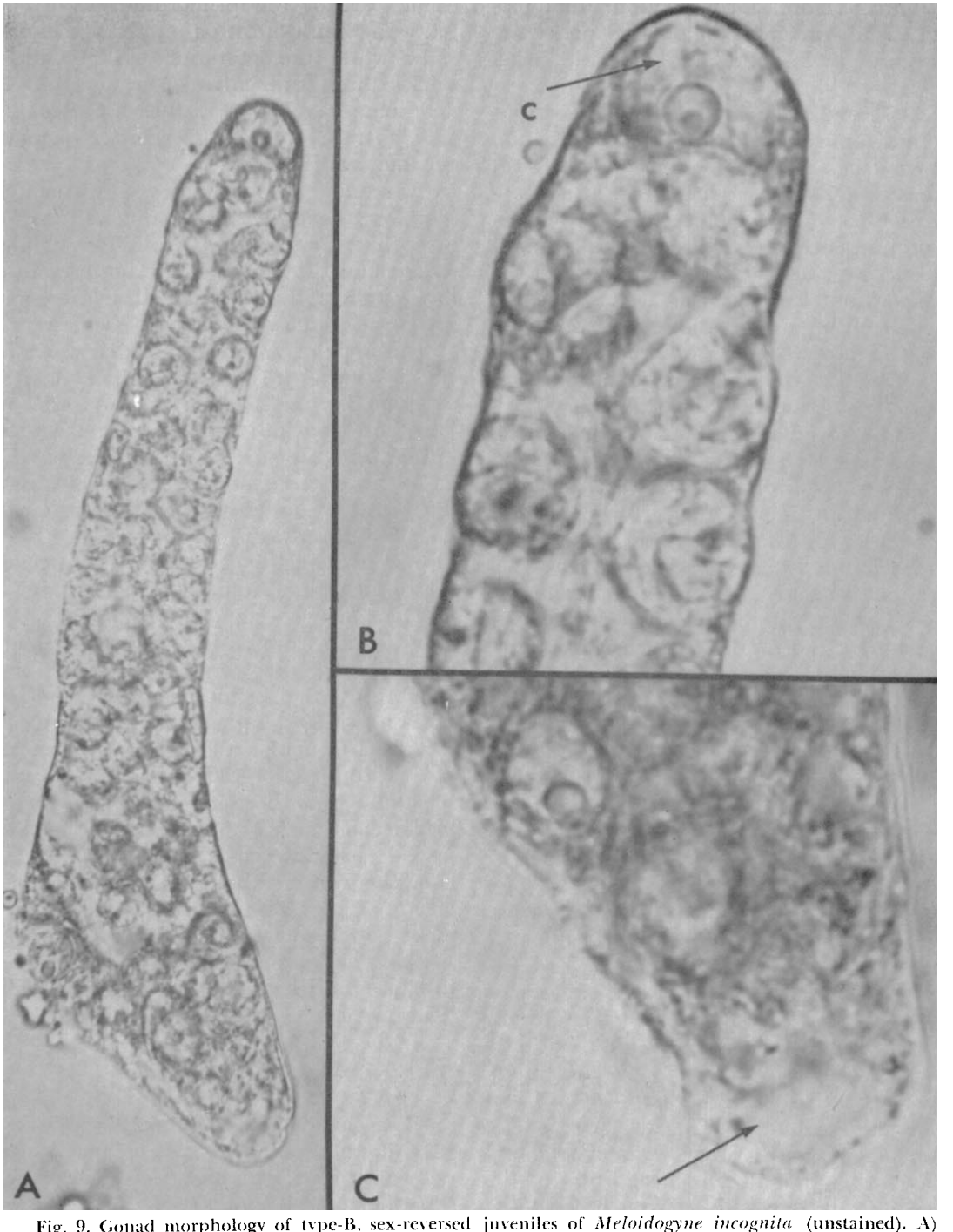

Fig. 9. Gonad morphology of type-B, sex-reversed juveniles of *Meloidogyne incognita* (unstained). A) Gonad of a third-stage juvenile: one of the arms is longer than the other. B) Enlargement of tip of longer arm of (A) showing cap cell (c) with large nucleus and nucleolus. C) Enlargement of shorter arm of (A) showing a cap cell with degenerated nucleus (arrow).

of them become differentiated into cap cells, two occupy the dorsal-central side, and eight the ventral-central side of the primordium. In male juveniles the first cycle of somatic-cell divisions results in the formation of a rod-shaped primordium with one cap cell in the posterior end and a group of nine somatic cells in the anterior

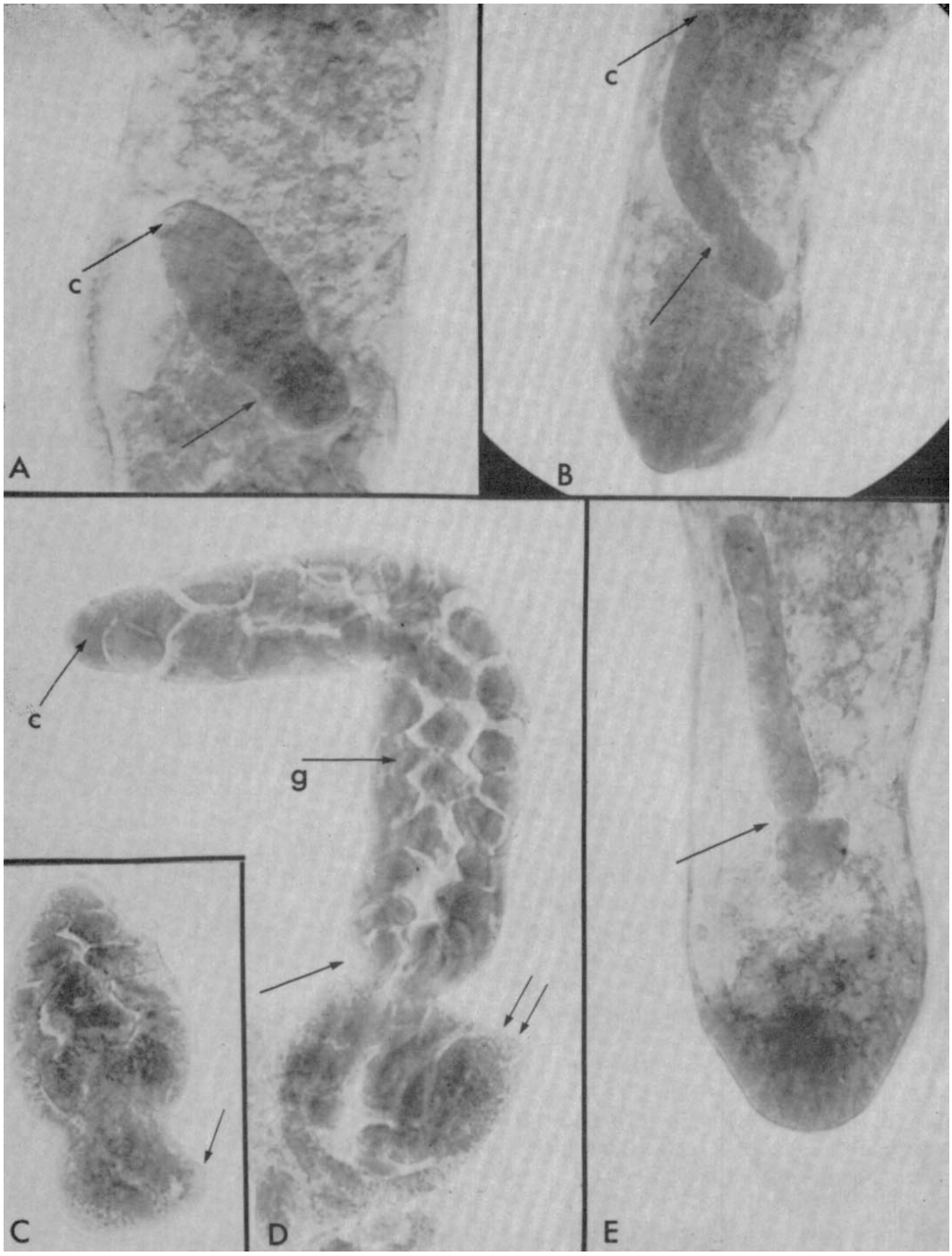

Fig. 10. Gonad morphology of type-C, sex-reversed juveniles of *Meloidogyne incognita* (acid fuchsin lactophenol stain). A) Gonad of a mid-second-stage juvenile: a cap cell (c) in the anterior part and a constriction (arrow) near the posterior part are visible. B) Gonad of an early, fourth-stage juvenile (posterior constriction–arrow). C) Gonad of a mid-second-stage juvenile: a trace of a second gonadal arm (arrow) is visible. D and E) Gonads of third-stage juveniles: a cap cell (c) at the anterior part, a constriction (arrow) between the long arm and the central region, and a trace of a second arm (double arrow) are visible; g = germinal cell.

end. This pattern of gonad development in females and males of *Meloidogyne* is analogous to that reported for hermaphrodites and males of *Caenorhabditis elegans* (8) as well as females and males of *Panagrellus redivivus* (13). Somatic-cell lineages follow a standard pattern with respect to sequence of cell division, cell arrangement, and cell migration. As in *C. elegans* and *P. redivivus,* there are two periods of somatic-cell divisions in *Meloidogyne,* and they are separated by a period of cell growth. The first cycle of divisions occurs at mid-second stage in juveniles of both sexes. The second cycle starts at the beginning of the second molt in female juveniles and during the third molt in male juveniles. It is extended through the fourth juvenile stage in both sexes.

The first divisions of germinal cells of the gonad primordium of *M. incognita* occur in mid-second-stage juveniles, when the primordium has 6–8 somatic cells. This early multiplication of germinal cells in *Meloidogyne* presents a situation analogous to that of *C. elegans* but much different from the one reported for many plant-parasitic nematodes. In all plant-parasitic nematodes studied thus far, the first division of germinal cells occurs much later—during the third or fourth juvenile stage (1,2,5,6, 12). The precocious multiplication of germinal cells in *Meloidogyne* may be related to the specialized pattern of post-embryogenesis in this genus. Only second-stage juveniles of *Meloidogyne* feed; the third- and fourth-stage juveniles undergo development without feeding. Furthermore, half-grown, second-stage juveniles of *Meloidogyne* can develop to adults without further feeding. It is possible that multiplication of germinal cells is initiated soon after the juveniles have attained sufficient growth to be able to develop to adulthood without additional feeding. A parallel situation exists in *Aphelenchus avenae,* where divisions of the germinal cells in fourth-stage juveniles begin shortly after the juveniles have acquired the ability to molt without additional feeding (4).

Female and type-A, sex-reversed juveniles have a similar pattern of gonad development almost up to the end of the second stage (10–12 days after root penetration). With the completion of the second stage, the germinal cells that earlier occupied the central region of the V-shaped primordium are completely displaced into the gonadal arms in female juveniles, but remain in the central region in sex-reversed juveniles. The somatic cells of the central region of the primordium of female juveniles multiply and migrate anteriorly into the gonadal arms, giving rise to two gonoducts; each gonoduct eventually differentiates into an oviduct, spermatotheca, and uterus. In sex-reversed juveniles, however, the somatic cells of the central region multiply and give rise to a single gonoduct which extends posteriorly. Later, this gonoduct differentiates into seminal vesicle and vas deferens. The central region of the primordium, which is still filled with germinal cells, becomes part of the seminal vesicle, whereas, the arms of the primordium become testes. Our evaluation of these two patterns of development suggests that type-A juveniles derive from female, second-stage juveniles which undergo sex reversal after they have completed about 2/3 of their development in this stage.

Strong anatomical evidence that males develop from female juveniles following sex reversal is provided by type-B, sex-reversed juveniles. These juveniles follow the female pattern of development until their gonad primordium attains the V-shape. Then, one of the gonadal arms stops elongating as the nucleus of its cap cell degenerates and multiplication of its germinal cells ceases. The other gonadal arm has a normal cap cell and continues to elongate. Soon, such juveniles have two gonadal arms of unequal size and eventually develop into males with one well-developed testis and a small branch of a second testis. The relative size of the second testis depends on the degree of development of the gonadal arm at the time when degeneration of the cap-cell nucleus occurred.

Degeneration of the cap-cell nucleus appears to be associated with cessation of multiplication of germinal cells and of elongation of the gonadal arm. A possible function of the cap cell may be to regulate gonial cell divisions and gonad growth. This function apparently is lost when the cap-cell nucleus degenerates. A type of

"programmed cell death" appears to be part of the normal process of development and differentiation of *C. elegans* (14). Also, it has been shown that the cap cell is necessary for normal gonadal growth in *C. elegans* (9) and that the cap cell of the posterior gonad branch of *Panagrellus redivivus* undergoes programmed cell death, resulting in inhibition of gonad growth and the development of a small post-vulval sac (13). It is very likely that monodelphic forms of many nematodes have evolved from didelphic ones through similar changes of the pattern of post-embryogenesis, involving early death of the cap cell of one of the gonadal arms.

Degeneration or death of the cap-cell nucleus in *M. incognita* does not involve complete degeneration of the cap cell. The latter persists in later stages without an organized nucleus. Furthermore, death of the cap-cell nucleus in *M. incognita* is not programmed and probably is induced by the same environmental factors that induce sex reversal. It occurs in young, female juveniles before their V-shaped gonad primordium reaches a certain state of development. Beyond that point, sex reversal is likely to result in type-A, sex-reversed juveniles.

Early sex reversal associated with concurrent death of the cap-cell nucleus can explain also the appearance of type-C, sex-reversed juveniles. Only an indication of a second gonadal arm is present in juveniles of this type, probably because of early death of the cap-cell nucleus and the cessation of multiplication of the germinal cells of that gonadal arm. In all other respects, gonad development of type-C juveniles is similar to that of type-B juveniles.

Gonad primordia of type-C, sex-reversed juveniles are similar to those of true-male juveniles, but can be distinguished by the following features:

1. In many type-C juveniles, the gonad primordium shows a trace of a second gonadal arm (Fig. 10C, D, E).

2. In most type-C juveniles, the gonad primordium is subdivided into two parts by a distinct constriction (Fig. 10A–E). The anterior part corresponds to the gonadal arm, whereas, the posterior part corresponds to the central region of a female gonad primordium. No constriction has been observed in true male juveniles (Fig. 7A, B).

3. The single cap cell of the primordium in type-C juveniles is always oriented anteriorly, whereas it is directed posteriorly in true male juveniles.

These distinguishing features support the contention that true male juveniles represent a distinct category, and possibly their development has a cytogenetic basis. However, one may argue that such males represent a similar case of sex reversal occurring during the first 5 days following initiation of feeding of female juveniles. Sex reversal could be induced by various environmental factors which conceivably modify gene activity and thus influence various physiological and developmental processes. Indeed, a true male pattern of development could be expected, if it is assumed that sexual differentiation of juveniles is hormonally regulated and that hormonal balance is modified during an early period of development of juveniles.

Modifications in sex expression from the normal pattern (XX–XO) leading to partial or complete sex reversal have been studied in more detail in *C. elegans* (7,11). Phenotypic males, *e.g.*, devoloped from hermaphroditic (XX) nematodes carrying a temperature-sensitive, sex-transformer mutation under certain temperature conditions, and various types of intersexual individuals were produced with certain manipulation of the temperature regimen at critical periods of juvenile development (10).

No information is available about the genetic basis of sex determination in *Meloidogyne*. This aspect needs to be studied in amphimictic, or at least in facultatively amphimictic, species. Considering the apomictic and, therefore, thelytokous nature of *M. incognita*, we can speculate that environment affects genetic expression to such an extent that it, in essence, controls sexual differentiation of developing juveniles.

The genetic implications of the developmental peculiarities of *M. incognita* are not clearly understood at present. Males of all types, except those representing severely aberrant intersexual forms, have well-developed sexual instincts and produce viable

sperm. Following insemination, the sperm penetrates oocytes but degenerates in the cytoplasm without participating in actual fertilization (18). It is assumed, therefore, that sexual developmental peculiarities of *M. incognita* are of limited genetic significance. Probably they play an important biological role in terms of population density adjustments and, therefore, ecological adaptation (16).

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. Bhatti, D. S., H. Hirschmann, and J. N. Sasser. 1972. Post-infection development of Heterodera lespedezae. J. Nematol. 4:104-112.

3. Davide, R. G., and A. C. Triantaphyllou. 1967. Influence of the environment on development and sex differentiation of root-knot nematodes. III. Effect of foliar application of maleic hydrazide. Nematologica 14:37-46.

4. Fisher, J. M., and A. C. Triantaphyllou. 1976. Observations on development of the gonad and on reproduction in Aphelenchus avenae. J. Nematol. 8:248-255.

5. Hirschmann, H. 1962. The life cycle of Ditylenchus triformis (Nematoda: Tylenchida) with emphasis on post-embryonic development. Proc. Helminthol. Soc. Wash. 29:30-43.

6. Hirschmann, H., and A. C. Triantaphyllou. 1967. Mode of reproduction and development of the reproductive system of Helicotylenchus dihystera. Nematologica 13:558-574.

7. Hodgkin, J. A., and S. Brenner. 1977. Mutations causing transformation of sexual phenotype in the nematode Caenorhabditis elegans. Genetics 86:275-287.

8. Kimble, J., and D. Hirsh. 1979. The post embryonic cell lineages of the hermaphrodite and male gonads in Caenorhabditis elegans. Develop. Biol. 70:396-417.

9. Kimble, J. E., and J. G. White. 1981. On the control of germ cell development in Caenorhabditis elegans. Develop. Biol. 81:208-219.

10. Klass, M., N. Wolf, and D. Hirsh. 1976. Development of the male reproductive system and sexual transformation in the nematode Caenorhabditis elegans. Develop. Biol. 52:1-18.

11. Nelson, G. A., K. K. Lew, and S. Ward. 1978. Intersex, a temperature-sensitive mutant of the nematode Caenorhabditis elegans. Develop. Biol. 66:386-409.

12. Roman, J., and H. Hirschmann. 1969. Embryogenesis and postembryogenesis in species of Pratylenchus (Nematoda: Tylenchidae). Proc. Helminthol. Soc. Wash. 36:164-174.

13. Sternberg, P. W., and H. R. Horvitz. 1981. Gonadal cell lineages of the nematode Panagrellus redivivus and implications for evolution by the modification of cell lineages. Develop. Biol. 88:147-166.

14. Sulston, J. E., and H. R. Horvitz. 1977. Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Develop. Biol. 56:110-156.

15. Triantaphyllou, A. C. 1960. Sex determination in Meloidogyne incognita Chitwood, 1949, and intersexuality in M. javanica (Treub, 1885) Chitwood, 1949. Ann. Inst. Phytopathol. Benaki, N.S. 3:12-31.

16. Triantaphyllou, A. C. 1973. Environmental sex differentiation of nematodes in relation to pest management. Ann. Rev. Phytopathol. 11:441-462.

17. Triantaphyllou, A. C. 1979. Cytogenetics of root-knot nematodes. Pp. 85-109 *in* F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (Meloidogyne species). Systematics, Biology and Control. London & New York: Academic Press.

18. Triantaphyllou, A. C. 1981. Oogenesis and the chromosomes of the parthenogenetic root-knot nematode Meloidogyne incognita. J. Nematol. 13: 95-104.

19. Triantaphyllou, A. C., and H. Hirschmann. 1960. Post-infection development of Meloidogyne incognita Chitwood 1949 (Nematoda: Heteroderidae). Ann. Inst. Phytopathol. Benaki, N.S. 3:1-11.