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Hermaphroditism in *Meloidogyne hapla*[1]

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Abstract: Hermaphrodites were detected in diploid and polyploid isolates of population 86-Va of *Meloidogyne hapla.* Young hermaphrodites are indistinguishable from normal females. Initially, hermaphrodite ovaries are filled with oocytes at various stages of development. Hermaphroditism is expressed later when young oocytes in the early pachytene region of the growth zone suddenly advance to diakinesis and proceed with maturation divisions, resulting in spermatid production. Spermatogenesis may be initiated shortly after the fourth molt, or later, after a female has produced some eggs. Spermatogenesis may occur in one or both gonads, and it may be initiated in one gonad before the other. Once initiated, spermatogenesis continues for the entire reproductive life of the hermaphrodite. Several thousand spermatozoa accumulate in the ovotestis. Because they do not pass through the oviduct into the spermatotheca, they do not take part in reproduction (nonfunctional hermaphroditism). Among the progeny of hermaphrodites, ca. 50% are hermaphroditic, and the remainder are apparently normal females which, however, produce about 50% hermaphroditic progeny. Two temperature regimes (20–23 C and 27–30 C) did not influence the percentage of hermaphrodites among the progeny. Hermaphroditism could not be transmitted to nonhermaphroditic isolates following attempted crosses between males of hermaphroditic and females of nonhermaphroditic isolates. Although this result suggests cytoplasmic rather than nuclear inheritance, this conclusion is not definitive.

Key words: cytogenetics, hermaphroditism, *Meloidogyne hapla,* nematode, reproduction, spermatogenesis.

More than 50 populations of *Meloidogyne hapla* Chitwood and ca. 900 populations of other *Meloidogyne* species that have been studied cytogenetically in our laboratory have been either bisexual, with males and females, or thelytokous, containing females only. I am not aware of any confirmed case of hermaphroditism in the genus *Meloidogyne,* or any other member of the order Tylenchida. Female intersexes, i.e., females with some male sex characters such as copulatory spicules, have been reported in the genus *Meloidogyne.* Presumably, they are produced as a result of partial sex reversal of second-stage female juveniles during a late developmental period (6). Such female intersexes are not known to produce gametes, oocytes, or sperm.

In the course of a cytogenetic study, involving progeny of crosses between a diploid and a polyploid isolate of population 86-Va of *M. hapla,* some egg- and sperm-producing females (i.e., hermaphrodites) were observed. This peculiarity, interesting as it appeared, was at first attributed to the expected unbalanced chromosomal state of the progeny of such crosses. Subsequent examination, however, revealed that hermaphrodites also occurred in the diploid and polyploid parental isolates. This observation raised the question of how and when hermaphroditism appeared in these isolates. An extensive cytological study of the original stock culture (86-Va) showed no evidence of hermaphroditism. Further examination of all the available isolates derived from 86-Va revealed four kinds of isolates: diploid and polyploid isolates with males and females; and diploid and polyploid isolates with males, females, and hermaphrodites.

These peculiar and totally unexpected observations motivated us to conduct various studies to understand the anatomical and cytological features of hermaphroditism in *M. hapla* and to elucidate the genetic or environmental factors involved in the transformation of *Meloidogyne* females into hermaphrodites.

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Materials and Methods

Populations: Population 86-Va of *Meloidogyne hapla,* from Virginia, USA, has been studied in our laboratory since 1962 (10)

and has served as a standard control in comparative cytological and enzymatic studies of many other *Meloidogyne* species. It exhibits typical *M. hapla* morphological features and infects peanut and strawberry. A polyploid isolate, 86P, was established in 1982 by inoculating a tomato seedling with 40 unusually large second-stage juveniles (J2) obtained from a single egg mass of population 86-Va (11,13). All polyploid isolates in the present study were derived from this original isolate through continuous propagation on tomato with inoculum consisting exclusively of polyploid egg masses, i.e., egg masses that give large juveniles. Some diploid isolates in the present study represent subcultures of population 86-Va that had been maintained continuously for 30 years on tomato, by using for inoculum 10–15 randomly picked egg masses from an old culture. Other diploid isolates were derived secondarily from polyploid isolates by using as inoculum diploid egg masses produced by females of the polyploid isolates that had converted to diploidy. No specific records have been kept about the derivation of diploid isolates; therefore, it is not known whether the hermaphroditic diploid isolate used in this study is a direct descendent of the stock culture or derived from a polyploid isolate.

The following isolates were used in the present study: 86D, a diploid with males and females; 86P, a polyploid with males and females; 86D-Sp, a diploid with males, females, and sperm-producing females (i.e., hermaphrodites); and 86P-Sp, a polyploid with males, females, and hermaphrodites. Throughout this research, each isolate was maintained on tomato (*Lycopersicon esculentum* cv. Rutgers) under normal greenhouse conditions.

Anatomical and cytological observations: Anatomical observations were made on gonads of females and hermaphrodites dissected in physiological salt solution, mounted on microscope slides, and examined immediately after preparation in bright field or interference contrast microscopy at 100 and ×1,250 magnifications. For cytological observations, females that had produced an egg mass or had deposited a large mass of gelatinous matrix were smeared on microscope slides, and the smears were processed for staining with propionic orcein (12).

To determine the presence or the frequency of occurrence of hermaphrodites in a culture, fully grown females were dissected from the roots of greenhouse cultures at least 50 days old. Females were smeared on microscope slides and processed for cytological staining (12). Cytological preparations were examined at ×100 magnification for distinguishing hermaphrodites from females, or at higher magnifications (×1,250) for determining the type of gametogenesis and chromosome number and behavior during maturation divisions.

Frequency of hermaphrodites among the progeny: To investigate whether normal females (i.e., females producing only eggs) of the hermaphroditic isolate produce any hermaphroditic progeny and, if so, at what rate, the following test was conducted. Sixteen females, each with an egg mass with 30–200 eggs, were isolated from the roots of a tomato plant that 15 days earlier had been inoculated with 2,000 J2 of the hermaphroditic isolate 86D-Sp. The egg masses were kept separately and the corresponding females were examined cytologically to determine whether they had produced sperm. Four of the 16 females had produced no sperm; the remainder contained various numbers of spermatozoa in their ovotestes. Individual tomato seedlings were inoculated with each of the 16 egg masses of each female and, 55 days later, 20 or fewer females recovered from the roots of each plant were studied cytologically to determine whether they had produced eggs, sperm, or both. A similar test was conducted in the next generation using as inoculum egg masses from five females that had produced only eggs and 10 hermaphrodites that had produced eggs and sperm.

Effect of temperature on expression of hermaphroditism: To test whether high temperatures influence the percentage of females that produce sperm, 20 tomato seedlings were inoculated with 1,000 J2 of isolate 86D-Sp and another 20 with isolate 86P-Sp. Ten of the inoculated plants of each set were placed in an environmental chamber at 20–23 C and the other 10 in another chamber at 27–30 C. The plants from the two temperature regimes were washed free of soil 50 or 30 days after inoculation, respectively; then females were manually removed from the root galls. The presence or absence of eggs in the egg mass of each female was recorded. Cytological preparations (12) were examined to identify the females that had produced sperm.

Matings: Crosses were conducted between females of the nonhermaphroditic isolates and males of the hermaphroditic isolates in an attempt to transfer hermaphroditism to the nonhermaphroditic isolates. Five tomato seedlings were each inoculated with 200 J2 of a nonhermaphroditic isolate (86D or 86P). Eighteen days later, ca. 500 males of 86D-Sp or 86P-Sp were added to the soil of each plant. Females with well-developed egg masses were manually removed from each plant 20 days later and their egg masses were kept separately in water in small dishes. Cytological preparations of each female were examined for the presence of sperm in the spermatothecae, which would indicate insemination by a male. Egg masses from sperm-containing females from the same plant were propagated on a tomato seedling. Forty-five days after inoculation, females recovered from the roots (first-generation females) were stained with orcein (12) and examined for evidence of hermaphroditism. In the absence of such evidence, ca. 5,000 J2 from each plant were propagated on another tomato seedling. The second-generation females recovered from these plants were examined for signs of hermaphroditism 50 days later. Eight such crosses were conducted at different times involving the following isolate combinations: 86D × 86D-Sp, 86D × 86P-Sp, 86P × 86D-Sp, and 86P × 86P-Sp.

Results

In general morphology, hermaphrodites and females of *M. hapla* are indistinguishable. Definitive resolution of hermaphrodites from females can be made only by careful microscopic examination of their gonads at high magnifications (see next section). Often, however, old hermaphrodites can tentatively be identified under a stereoscope at 10–20×, because they have produced large, transparent masses of gelatinous matrix that contain very few eggs or none. In contrast, large egg masses deposited by females appear opaque, since they contain many nontransparent eggs.

Anatomical observations: The reproductive systems of females and hermaphrodites of *M. hapla* are indistinguishable morphologically. They consist of two gonads, each formed by the gonad proper (ovary or ovotestis) and the gonoducts (oviduct, spermatotheca, and uterus). The two uteri join in a common vagina.

The first indication that an adult is a hermaphrodite is the onset of sperm production, which initially appears as a lengthening of the transparent, apical portion of one or both ovaries, as can be observed with a ×10 objective (Fig. 1A,B). In a female, this transparent portion corresponds to the apical oogonial region, the zone of synapsis, and the early pachytene region of the growth zone. The rest of the ovary is nontransparent because of the presence of globular storage materials (proteins and lipids) in the cytoplasm of oocytes in a more advanced pachytene stage. In young hermaphrodites, spermatogenesis starts at the posterior region of the transparent portion of one or both of their ovaries (henceforth referred to as ovotestes). The sperm that is produced posteriorly is also transparent and contributes to the lengthening of the transparent portion of each ovotestis (Fig. 1B). As sper-

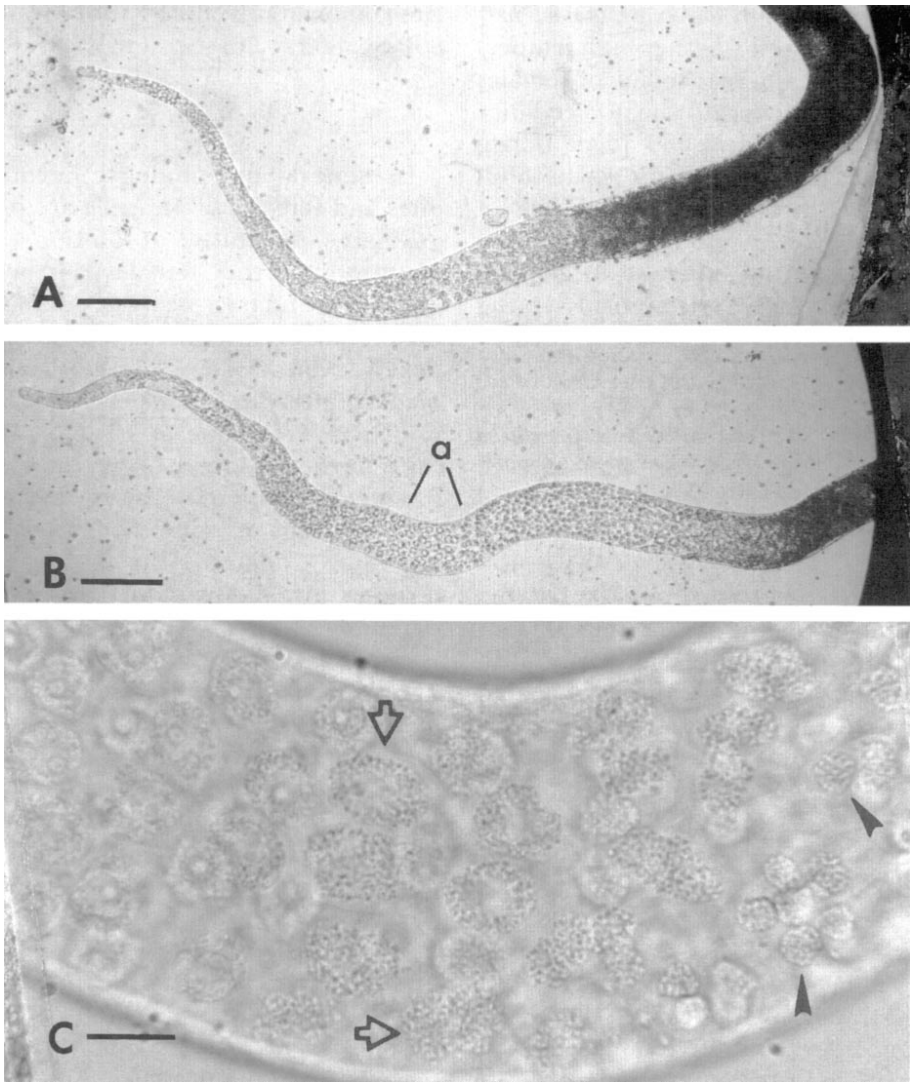

FIG. 1. Photomicrographs of the apical portions of the two gonads (one ovary and one ovotestis) of a young hermaphrodite of *Meloidogyne hapla*. A) Ovary with short transparent apical portion corresponding to the oogonial region, the zone of synapsis, and the early pachytene region of the growth zone. The dark region on the right corresponds to the area filled with oocytes at advanced pachytene. B) Ovotestis with long, transparent apical portion. The posterior third of the transparent region, which is occupied by transparent spermatids is the area where spermatogenesis is occurring. C) Higher magnification of region (a), showing gametocytes on the left, granular spermatocytes (arrows) undergoing maturation divisions in the middle, and spermatids (arrowheads) on the right. Scale bars: A, B = 100 μm; C = 15 μm.

matogenesis proceeds further, the sperm occupies an increasingly greater proportion of the length of the ovotestis, and finally, the entire ovotestis becomes transparent, as in old hermaphrodites (Fig. 2A).

Spermatogenesis can be detected more readily in microscopic preparations of extracted gonads viewed with a ×100 objective in bright field or, preferably, with interference contrast optics. The main sign is a sudden increase in size of a small number of oocytes located in the early-pachytene region of the growth zone of the ovary. Thus, oocytes of about 12 μm in diameter suddenly increase to about 15-μm-d and become granular, an indication that they have transformed into primary spermatocytes and are undergoing maturation divi-

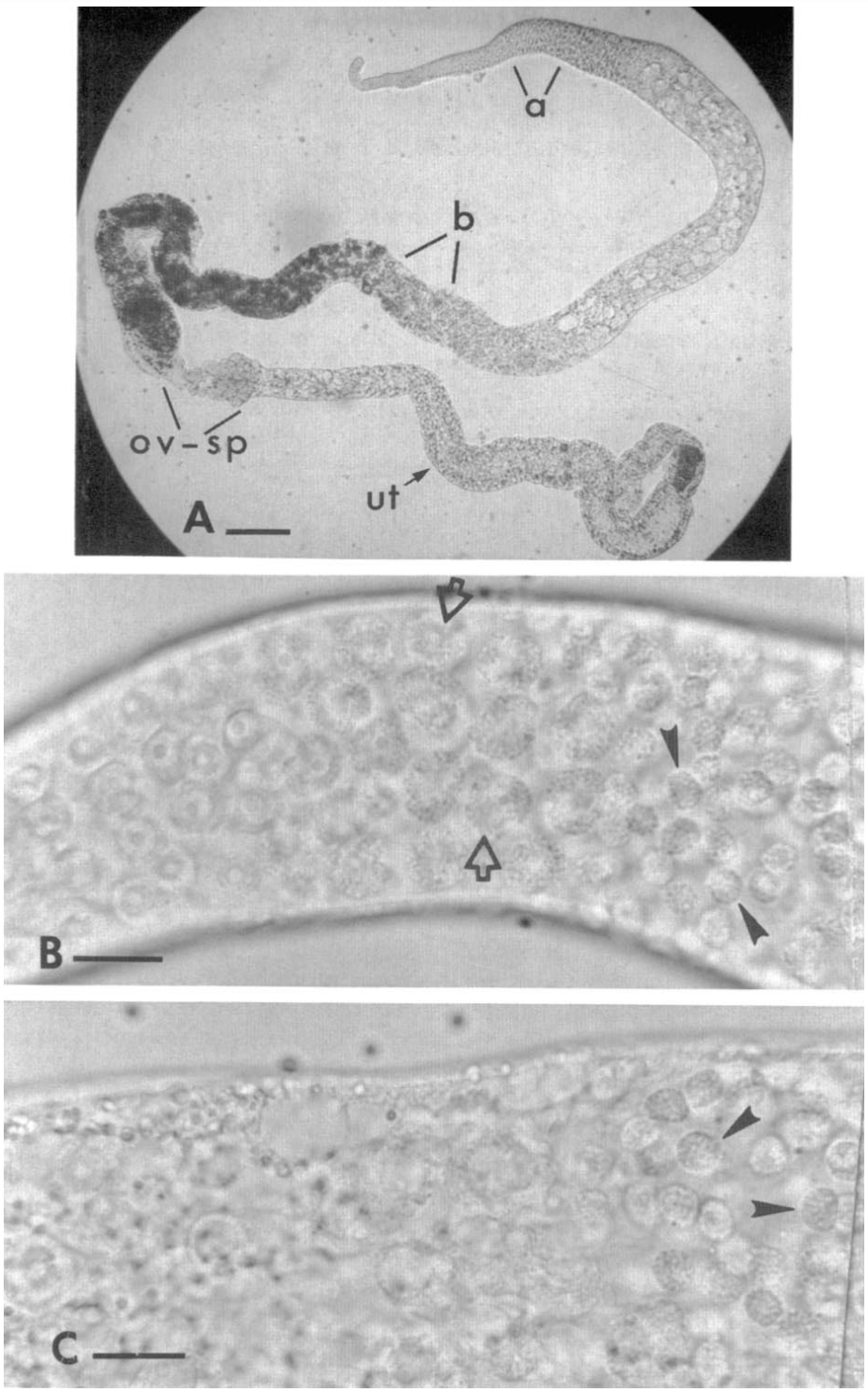

FIG. 2. Spermatogenesis in hermaphrodites of *Meloidogyne hapla*. A) Photomicrograph of one of the two gonads of an old hermaphrodite showing the ovotestis filled with transparent sperm along two-thirds of its length and with degenerating oocytes (darker area) in the posterior third. The ovotestis is connected to the oviduct–spermatotheca region (ov–sp) and the uterus (ut). B) Higher magnification of region (a), showing gametocytes on the left, granular spermatocytes (arrows) undergoing maturation divisions in the middle, and spermatids (arrowheads) in the right (posterior) region. C) Higher magnification of region (b), showing spermatids (arrowheads) on the right (end of the region where spermatogenesis has occurred), and oocytes at various stages of degradation on the left (posterior region). Scale bars: A = 100 μm; B,C = 15 μm.

sions (Figs. 1C,2B). These spermatocytes are followed by a number of spermatids of about 7-μm-d (Figs. 1C,2B). In females, the same area of the growth zone of the ovary is occupied by oocytes of progressively larger size.

Oocytes located posterior to the zone where spermatogenesis has occurred cease to grow and start degenerating as they become granular and may burst open. Degeneration gradually extends posteriorly so that all oocytes in the growth zone of the ovotestis eventually degenerate (Fig. 2A,C). Only a few fully grown oocytes, located close to the oviduct during initiation of spermatogenesis, may pass through the oviduct–spermatotheca region into the uterus and become normal eggs. Their fate was determined in cytological preparations.

Cytological observations: Hermaphroditism in *M. hapla* was detected originally and confirmed during microscopic examination of cytological preparations of gonads with stained nuclei. This examination revealed maturation divisions in small oocytes (gametocytes) located a short distance behind the zone of synapsis in gonads of females that apparently had converted to hermaphroditism (Fig. 3A).

Subsequent cytological investigation revealed the following details of gametogenesis in hermaphrodites. The gonial divisions in the germinal zone of hermaphrodite gonads and the gametocyte behavior in the zone of synapsis and the anterior part of the growth zone of the ovotestis are similar to those described for ovaries of females of *M. hapla* (10) and other *Meloidogyne* species (9). In hermaphrodites, a few oocytes located a short distance behind the zone of synapsis suddenly advance from early pachytene, with no distinct chromosomes, to diakinesis, with distinct diakinetic chromosomes (Fig. 3B,C). Apparently, these oocytes transform into spermatocytes, which subsequently advance to metaphase of the first maturation division (Fig. 3B–D). Spermatogenesis advances as the first and second maturation divisions are completed (Fig. 3C), and small spermatids are formed (Fig. 3D). Spermatocytes at late prophase clearly contain bivalent chromosomes (17 in the diploid, and 34 in the tetraploid isolate), each forming a typical tetrad, characteristic of meiotic chromosomes of *M. hapla*. They are spread throughout the large nucleus of each spermatocyte and are quite discrete (Fig. 4A). In later stages of maturation, the individual chromosomes are not as distinct. The four spermatids that derive from each spermatocyte (Fig. 4B) are closely associated with each other and often maintain this association even in later stages. The chromatin of each spermatid at first is distributed in a small number of chromatin bodies (Fig. 4B) but usually becomes compacted into a small spherical body later, when spermatids transform into spermatozoa (Fig. 4C).

As spermatogenesis progresses, spermatids that are produced posteriorly occupy an increasingly larger proportion of the ovotestis. The posterior part of the ovotestis may remain filled with dark particulate materials, the remnants of the degenerating oocytes, but this region progressively shortens and becomes more transparent as some of these materials appear to be adsorbed. Eventually, the entire ovotestis up to the oviduct may be filled with spermatozoa intermingled with remnants of the degenerating oocytes and residual cytoplasmic bodies cast off during the formation of individual spermatids (Fig. 5A). Many hermaphrodites produce 4,000–8,000 spermatozoa, which remain inside the ovotestis. Spermatozoa were not observed to pass through the oviduct into the spermatotheca and uterus.

The uteri of young hermaphrodites may contain a variable number of oocytes in various stages of maturation or in early cleavage. In older hermaphrodites, however, the uteri are often empty or may contain few (1–20) oocytes, with most or all in the pronucleus stage (Fig. 5B). Such oocytes have undergone two maturation divisions and are now haploid, as evidenced by the presence of two polar bodies and by the reduced number of chromo-

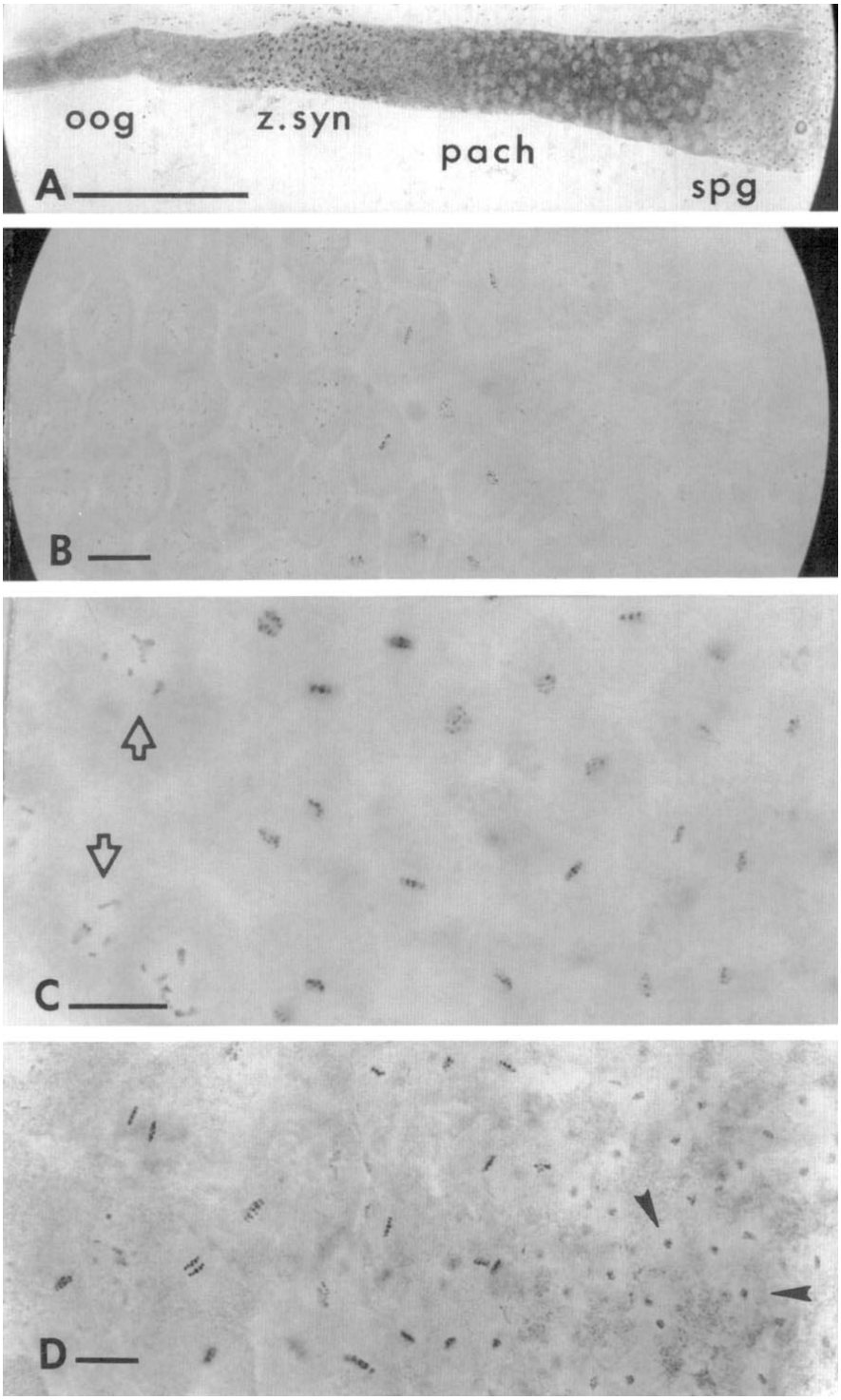

FIG. 3. Spermatogenesis in *Meloidogyne hapla* hermaphrodites. A) Apical portion of the ovotestis of a hermaphrodite, showing oogonial region (oog), zone of synapsis (z. syn), early pachytene region (pach), and spermatogenesis region (spg). B) Initiation of spermatogenesis in the ovary of a female that is becoming hermaphroditic. On the left, young oocytes that have transformed into spermatocytes and have advanced to diakinetic stage, with discrete chromosomes. In the middle, spermatocytes undergoing the first maturation division. On the right, oocytes at pachytene. C) Same region of ovotestis as in Figure 3B in a more advanced hermaphrodite. On the left, spermatocytes at diakinesis (arrows); on the right, spermatocytes undergoing the first and second maturation divisions. D) Same region of ovotestis as in Figure 3C, but in a more advanced hermaphrodite. Spermatids have already been produced posteriorly (arrowheads), following the two maturation divisions seen on the left. Scale bars: A = 100 μm; B = 15 μm; C,D = 10 μm.

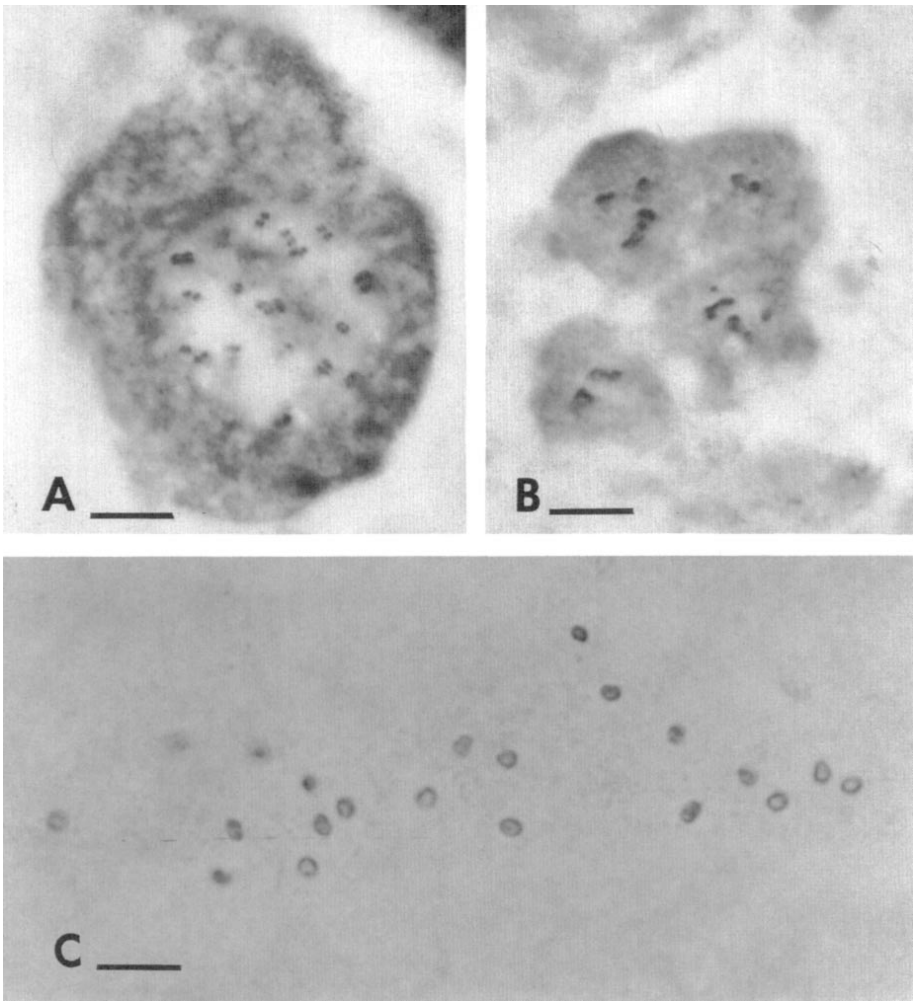

FIG. 4. Spermatogenesis in *Meloidogyne hapla* hermaphrodites. A) Spermatocyte at late prophase I with distinct bivalent chromosomes. B) The four spermatids resulting from the two maturation divisions of a spermatocyte. C) Spermatozoa spread along the posterior part of the ovotestis, near the oviduct–spermatotheca region of an old hermaphrodite. Scale bars: A–C = 5 μm.

somes visible in some pronuclei. Although many of these oocytes have well-developed eggshells, none has advanced to cleavage.

Young hermaphrodites that have been inseminated by a male do contain a small number of spermatozoa in their spermatothecae. All these spermatozoa are used later for fertilization of the first oocytes produced by the young hermaphrodites. Older hermaphrodites, whose ovotestes are filled with spermatozoa, do not contain sperm in their spermatothecae.

Frequency of hermaphroditism among progeny: Hermaphroditic progeny appeared among the progeny of all 16 females (four normal and 12 hermaphroditic) examined cytologically in the first test. Of the 65 progeny of the four normal females, 30 (46%, range 31–62%) were hermaphroditic and 35 produced only eggs. Of the hermaphroditic progeny, 53% produced only sperm and 47% produced sperm and eggs. In the same test, of 192 progeny of the 12 hermaphroditic females, 90 (47%, range 25–62%) were hermaphroditic and 50% of them produced only sperm. Similar results were obtained from the second test, conducted with normal and hermaphroditic females of the following generation, in which 56% of a total of 224 prog-

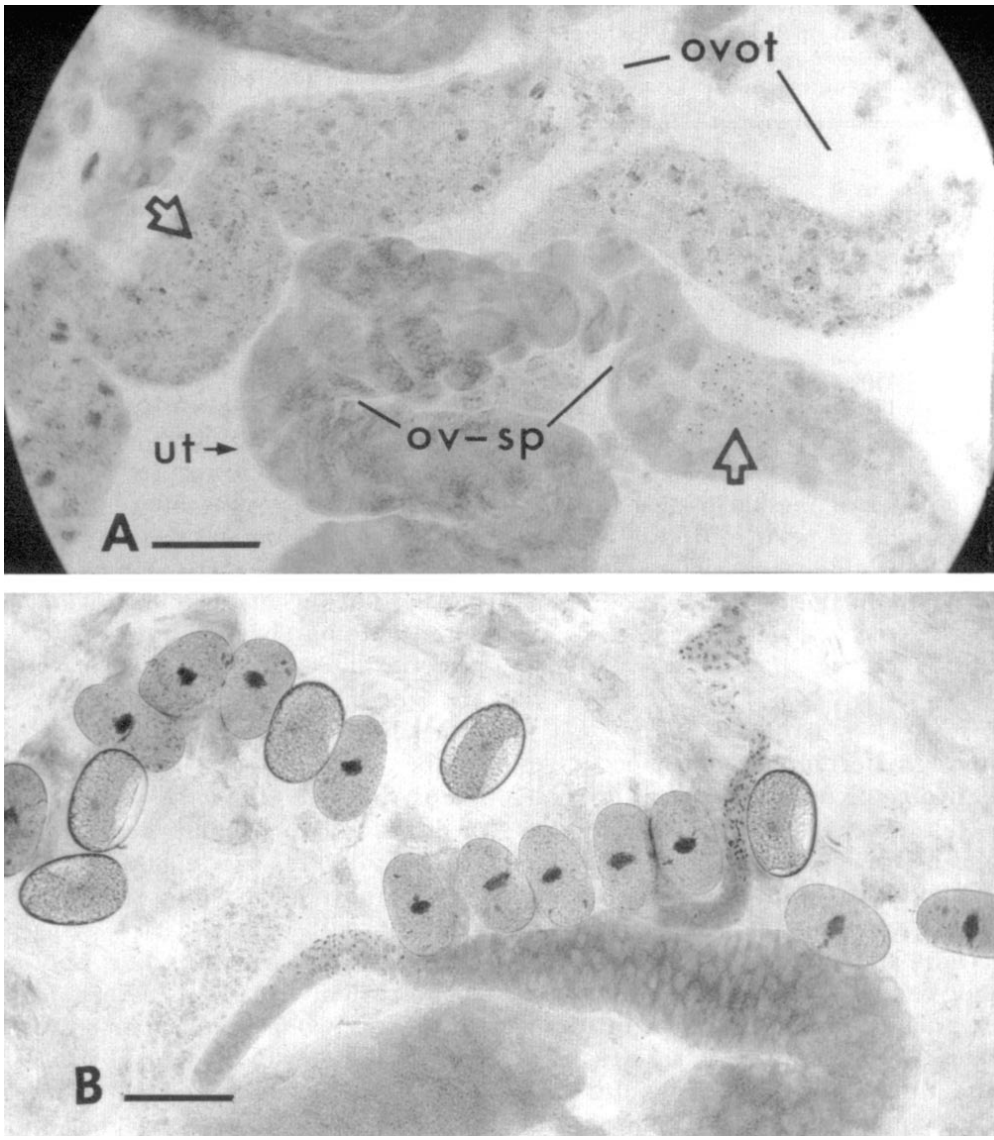

FIG. 5. Gametogenesis in *Meloidogyne hapla* hermaphrodites. A) part of the ovotestis (ovot) of a very old hermaphrodite filled with spermatozoa (pepper-like inclusions—arrows), intermingled with remnants of degenerating oocytes and residual bodies (condensed inclusions); ov–sp = the oviduct–spermatotheca region; ut = part of the uterus. B) Unfertilized eggs at the pronucleus stage released from the uterus of an old hermaphrodite. Scale bars: A = 50 μm; B = 100 μm.

eny studied were hermaphroditic and 54% of them had produced only sperm. There was no difference in the percentages of hermaphroditic females among the 64 progeny of 5 normal females and the 160 progeny of 10 hermaphroditic females in this test (in each case, average 56%, range 37–81%).

Effect of temperature on expression of hermaphroditism: Development at low (20–23 C) or high (27–30 C) temperature had no effect on the reproductive behavior of females. Thus, about 53% of the females of the diploid isolate and 59% of the polyploid isolate produced only eggs at both temperatures, whereas the remainder expressed their hermaphroditic trait by either producing sperm and eggs or sperm only (Table 1).

Matings: In eight attempted crosses be-

TABLE 1. Reproductive behavior of females of a diploid (86D-Sp) and a polyploid (86P-Sp) hermaphroditic isolate of *Meloidogyne hapla* reared at two temperature regimes on *Lycopersicon esculentum*.

Isolate	Number of females studied	Percentage of females producing		
		Sperm	Sperm + eggs	Eggs
			20–23 C	
86D-Sp	177	24	24	52
86P-Sp	138	18	22	60
			27–30 C	
86D-Sp	180	25	22	53
86P-Sp	179	29	12	59

tween females of nonhermaphroditic isolates and males of hermaphroditic isolates, no hermaphrodites were detected among 900 progeny of the first and second generation.

DISCUSSION

Although hermaphroditism occurs in some groups of nematodes such as rhabditids (5), aphelenchs (1), and mononchs (7), and is suspected in criconematids (14) and trichodorids (8), to my knowledge this is the first report of hermaphroditism in *Meloidogyne* and in the Tylenchida. Understanding the genetic or other mechanisms that regulate hermaphroditism in *Meloidogyne* would be of great significance, because it may provide clues about the evolutionary interrelationships of hermaphroditism and other modes of nematode reproduction. The generally accepted viewpoint is that animal hermaphorditism is the original state from which gonochorism (separate sexes) evolved. For nematodes, however, arguments since 1900 (5) have held that nematodes evolved as gonochoristic animals and that hermaphroditism, in the few groups where it occurs, represents a derived condition. Triantaphyllou and Hirschmann (14) in 1964 evaluated the evidence supporting this view and concluded that this seems to be a reasonable theory. Researchers who have studied exhaustively the genetic mechanism of sex determination of *Caenorhabditis elegans* (2) similarly believe that hermaphroditism in *C. elegans* is secondarily derived.

Substantial differences in the expression of hermaphroditism in *M. hapla* and *C. elegans* makes comparison of the two systems and their genetic controls difficult. Because females and hermaphrodites in either species are anatomically identical, hermaphrodites are regarded as females whose gonads have the capacity to undergo both oogenesis and spermatogenesis. In *C. elegans,* spermatogenesis occurs during a short period in the fourth juvenile stage and oogenesis follows in the adult stage (protandric hermaphroditism). In *M. hapla* hermaphrodites, both oogenesis and spermatogenesis occur in adults. Thus, shortly after the fourth molt, young females and hermaphrodites of *M. hapla* have anatomically identical gonads. The posterior half of their ovaries or ovotestes are filled with immature gametocytes which, because of their relatively large size, can be characterized as oocytes, not spermatocytes. Therefore, the young individual destined to become hermaphrodite starts its adult life as a female and converts to hermaphroditism later on (deuterandric hermaphroditism).

In *M. hapla* hermaphrodites, spermatogenesis usually occurs in both gonads simultaneously but often occurs only in one gonad or earlier in one gonad than in the other. This variable pattern of occurrence may indicate that the signal that initiates spermatogenesis is not a generalized but a localized signal affecting a small sector of the ovarian zone containing meiotic oocytes at early pachytene. In *C. elegans,* because spermatogenesis always occurs simultaneously in the two gonads of a hermaphrodite, it can be assumed that a generalized trigger mechanism operates. This dissimilarity between *M. hapla* and *C. elegans,* however, may not be as large as it appears. The mechanism that triggers spermatogenesis in *C. elegans* indeed involves only a specific region of the gonads (i.e., a localized effect) while, concurrently, the juvenile develops somatically towards a female. This two-directional developmen-

tal process has recently been clarified to a large extent in *C. elegans* as expression of hermaphroditism (i.e., spermatogenesis) has been shown to depend on the outcome of interactions among seven autosomal genes (3,4). These genes are organized in an ordered, four-step cascade of negative regulatory interactions that act downstream of one to three master regulatory sex genes.

Spermatogenesis in *M. hapla* is irreversible and it continues for the rest of the reproductive cycle of the hermaphrodite, resulting in the production of several thousand spermatozoa. Conversely, spermatogenesis in *C. elegans* is arrested permanently after production of ca. 300–600 spermatozoa and is followed by oogenesis, which then continues for the rest of the reproductive cycle of the hermaphrodite.

Hermaphroditism in *M. hapla* is an aberration of the natural reproductive process. Because sperm does not pass into the spermatotheca, where it could contact oocytes, hermaphrodite spermatozoa do not function in reproduction. Furthermore, observations indicate that by the time hermaphrodite sperm matures, oocytes in the uterus have developed a thick eggshell that presumably cannot be penetrated by spermatozoa.

Fully grown oocytes and eggs with a well-formed eggshell located in the uteri of hermaphroditic females had advanced up to the pronucleus stage, but none had undergone cleavage divisions. Apparently, fertilization is required before such eggs can proceed with the first cleavage division. The state of ploidy of the pronucleus of such eggs does not seem to be the limiting factor, because eggs of the polyploid form, which should be in the diploid state, still fail to advance to cleavage. It is likely that physiological or other changes that enable a female to produce sperm render the eggs of the hermaphrodite incapable of developing parthenogenetically. In contrast, eggs produced by a hermaphrodite before initiation of spermatogenesis can develop by parthenogenesis or can be fertilized if the female has been inseminated by a male before initiation of spermatogenesis.

Normal and hermaphroditic females of the hermaphroditic isolates produced approximately the same percentages of hermaphroditic progeny (about 45 to 65%). This observation suggests that although normal females do not express hermaphroditism, they have the same genetic potential for hermaphroditism as do hermaphrodites of the same isolate. What influences the expression of hermaphroditism among the progeny of both females and hermaphrodites is not clear. Two temperature regimes applied during the developmental cycle of diploid or polyploid females and hermaphrodites did not influence the percentages of hermaphrodites among their progeny. Other, unknown environmental factors, however, may play a role as considerable deviations in the frequency of hermaphrodites (i.e., 10 or 90% hermaphrodites) have been noticed in some greenhouse cultures during regular semiannual or annual evaluations. Further study of the possible effects of different temperature regimes and other environmental factors on expression of hermaphroditism in *M. hapla* is needed, especially because certain temperature-sensitive mutants of *C. elegans* display variability in cell- or tissue-autonomous sex expression (2). In the latter case, different sexual phenotypes can be expressed in adjacent tissues within an animal (mosaic intersexual phenotypes) with a given mutation when temperature shifts are imposed at various periods of the developmental cycle.

Hermaphroditism could not be transmitted through controlled matings of males of the hermaphroditic lines with females of the nonhermaphroditic lines. In eight such attempts involving 900 progeny, only normal females were obtained as progeny in the first and second generations. This failure suggests that hermaphroditism in *M. hapla* may not be controlled by a nuclear gene but instead may be cytoplasmically inherited through the females and hermaphrodites of the hermaphro-

ditic isolates. Nonetheless, one should consider that *M. hapla* is facultatively parthenogenetic and that no genetic markers were available to verify that any of the 900 progeny did indeed result from cross-fertilization. Although it is quite unlikely, all 900 progeny could have developed parthenogenetically and thus would not have expressed hermaphroditism. Therefore, the specific mechanism that controls hermaphroditism in *M. hapla* is unknown.

Because about 50% of the females of a hermaphroditic population or isolate are hermaphroditic and produce few progeny, hermaphroditism substantially reduces the reproductive rate of *M. hapla*. Consequently, if transmitting hermaphroditism from hermaphroditic to nonhermaphroditic populations through crosses or other means were possible, such a procedure could effectively be used as a method for controlling nematode population densities. Further research on this matter may be well justified.

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