Karyology and Oogenesis of Radopholus similis (Cobb) Thorne[1]

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Abstract: Two morphologically indistinguishable races of *Radopholus similis* from Florida are presently identifiable only by host preference tests. One race attacks banana and other hosts, but not citrus. The other race attacks both citrus and banana, as well as other hosts. The two races normally reproduce by amphimixis. The morphology of the female reproductive systems of both races were examined and appeared to be identical. However, slight differences were observed in developmental stages of oogenesis between the two races. The two races were distinct with respect to karyotype. There were four chromosomes in the banana race and five chromosomes in the citrus race. Karyotypic uniformity was found in the two Florida populations of the citrus race. *Key words:* burrowing nematode, chromosomes, reproduction.

Radopholus similis (Cobb) Thorne, the burrowing nematode, is reported to attack more than 250 species of plants worldwide. Banana (*Musa* spp.) and *Citrus* spp. are among the most important economic hosts affected (3). Differences in host preference of populations of this nematode from banana and citrus led to the description of two physiological races (1). One race has been shown to parasitize banana and other hosts, but not citrus. The other race infects citrus as well as banana and other hosts and has only been described from Florida. The races are morphologically indistinguishable and at present are only identifiable by host preference tests.

Two Florida races of *R. similis* were examined to determine whether cytogenetic differences occur between the races. This approach has been used as an important tool for understanding phylogenetic relationships in nematodes (7). Karyotypic differences between morphologically indistinguishable races within nematode species may be of evolutionary importance (6); however, this has not been throughly investigated in amphimictic species.

Studies were initiated to establish the chromosome numbers of both races and to determine whether there were differences in oogenesis and the mode of reproduction. Comparisons were also made to determine whether there were differences in the morphology of their reproductive systems.

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MATERIALS AND METHODS

The nematode popultaions were maintained in a greenhouse at 22–25 C on either banana roots for the banana race (*Musa* spp.) or citrus (rough lemon and sour orange) and nightshade (*Solanum nigrum* L.) for the citrus race. Nematodes were recovered from washed roots that had been aerated for approximately 48 h. One population of banana race and two populations of the citrus race were examined.

To compare the morphology of the reproductive systems and the developmental stages of oogenesis, adult females were stained *in toto* with 2% propionic orcein acid (2). The karyology was determined by cutting single individuals near the vulva to remove the entire reproductive system from the body wall. A 2% propionic orcein acid stain was applied (5,8). Approximately 5,000 individuals for each race were examined.

OBSERVATIONS

The comparative morphology of the reproductive systems of the citrus and banana race of *R. similis* appeared to be identical. The female reproductive system is amphidelphic, telogonic, and outstretched. Each branch of the system consists of an oviduct, spermatheca, and uterus. The uteri terminate in the vagina. Each ovary consists of a large cap cell, a distal germinal zone, and a proximal growth zone (Figs. 1, 2). The ovaries often are reflexed in young noninseminated females but generally become outstretched after insemination. Occasionally in mature females an ovary was reflexed, generally in the posterior branch.

Mitotic oogonial divisions were observed in the germinal zone of the ovaries of young

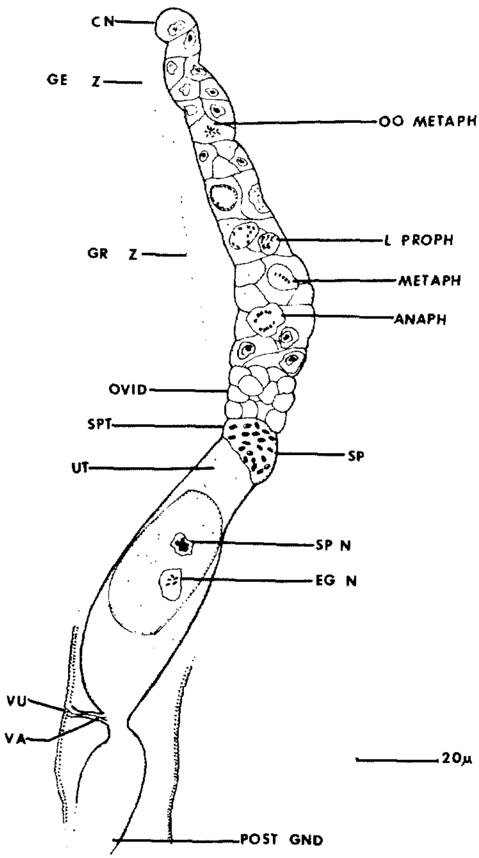


Fig. 1. Illustration of a female gonad of the citrus race of *Radopholus similis*. The morphology of the reproductive system and the stages of meiosis are indicated as follows: ANAPH, anaphase, CN, cap cell nucleus; EG N, egg pronucleus; GE Z, germinal zone; GR Z, growth zone; L PROPH, late prophase; METAPH, metaphase; OO METAPH, metaphase; OVID, oviduct; POST GND, posterior gonad; SP, spermatozoa; SPN, sperm pronucleus; SPT, spermatheca; UT, uterus; VA, vagina; and VU, vulva.

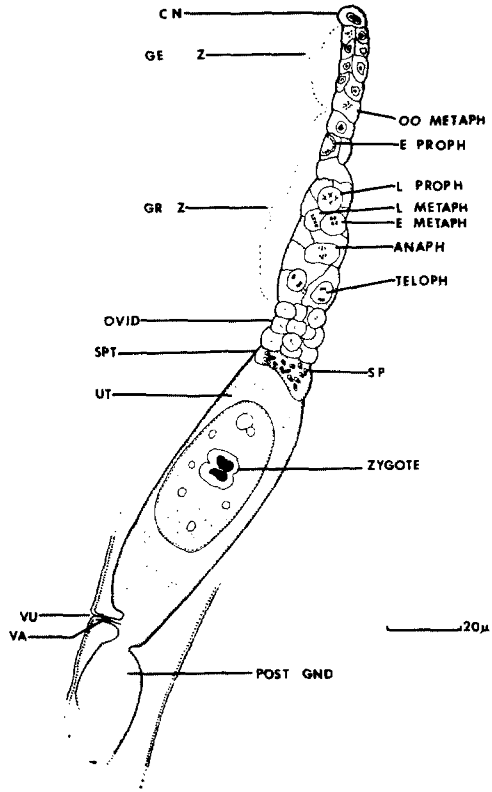


Fig. 2. Illustration of a female gonad of the banana race of *Radopholus similis*. The morphology of the reproductive system and the stages of meiosis are indicated as follows: ANAPH, anaphase; CN, cap cell nucleus; GE Z, germinal zone; GR Z, growth zone; L PROPH, late prophase; METAPH, metaphase; OO METAPH, metaphase; OVID, oviduct; POST GND, posterior gonad; SP, spermatozoa; SPN, sperm pronucleus; SPT, spermatheca; TELOPH, telophase; UT, uterus; VA, vagina; VU, vulva; and ZY, zygote.

females. Mitotic oogonial divisions also occurred in this same zone in inseminated females; early metaphase was observed in this zone in both races of *R. similis*. The chromosome number of the banana race was 2n = 8 (Fig. 3), whereas that of the citrus race was 2n = 10 (Fig. 4).

Leptotene and zygotene chromosomes of the first meiotic division were visible in the nuclei at the distal end of the growth zone. Pachytene chromosomes were also observed in this area as visible strands of heavily stained chromatin in the nuclei of the oocytes (10). This is the stage at which homologous chromosomes fuse to form filaments with chains of distinct chromomeres visible along their length (4,10) (Fig. 5). Diplotene or early diakinesis was observed as faintly stained chromosomes at the periphery of the nucleus (10) (Fig. 6). There is a difference between the two races of *R. similis* in the rate of development of the oocytes beyond the growth zone. The maturation of the oocytes in the two races is discussed separately below.

Citrus race: Metaphase I chromosomes were visible immediately after the oocytes entered the growth zone. Early and late metaphase I, with five bivalent chromosomes aligned on the equatorial plate, were observed in oocytes before they entered the oviduct (Figs. 7, 8). Anaphase and telophase

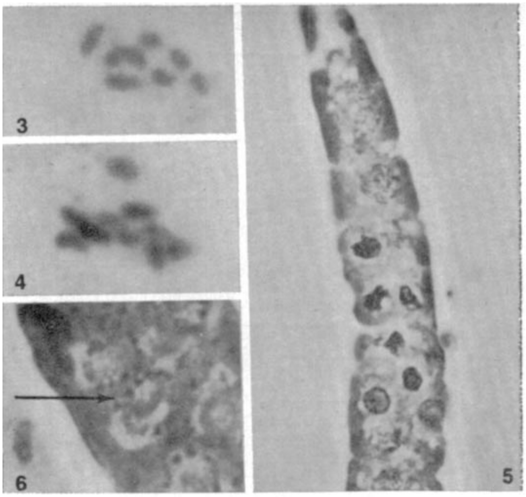
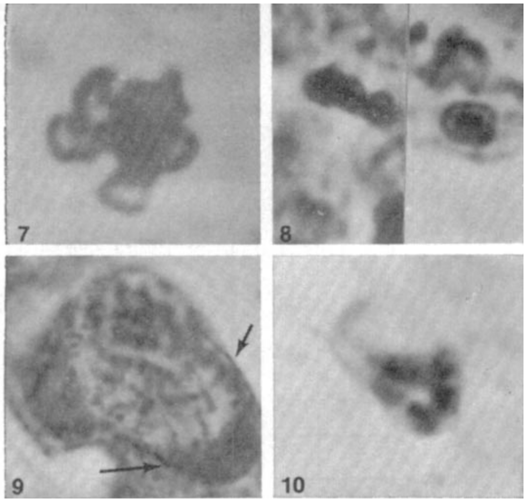


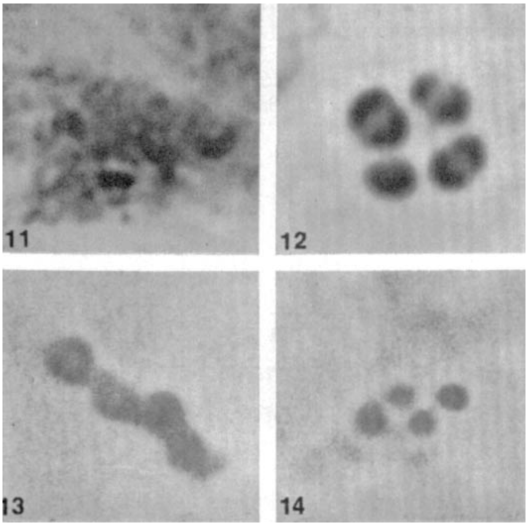
Fig. 3–6. Oogonial metaphase observed in the germinal zone of the ovary. 3) Chromosomes in the banana race of *Radopholus similis* (2n = 8). 4) Chromosomes in the citrus race of *R. similis* (2n = 10). 5) The growth zone of the ovary of the citrus race of *R. similis*. The upper nuclei that are granular in appearance are leptotene and zygotene chromosomes of the first meiotic division. The darkly staining nuclei in the middle zone are pachytene chromosomes. The lower nuclei with a ringed appearance are diplotene and diakinesis chromsomes. 6) Chromosomes in diakinesis in the first meiotic division of the citrus race of *R. similis*.



Figs. 7–10. Chromosomes of the citrus race of *Radopholus similis*. 7) Metaphase I chromosomes; 2n = 10 (phase-contrast microscopy). 8) Late metaphase I chromosomes (phase-contrast microscopy). 9) An oblong oocyte is shown in the medial portion of the growth zone. The margin was outlined to increase contrast. 10) Metaphase II chromosomes observed in an oocyte in the uterus. Five univalent chromosomes are visible and outlined to clarify their structure.

could not be detected. These stages may have taken place as the oocyte passed through the oviduct and spermatheca. Occasionally, however, oocytes entering into the oviduct had two visible nuclei. One nucleus was slightly larger than the other. The smaller nucleus was presumed to be a polar body. These oocytes appeared to have an outer membrane and were oblong in shape (Fig. 9). They retained their shape when removed from the reproductive system. The oocyte then passed from the oviduct through the spermatheca which contained a large number of spermatozoa. The second maturation division took place after the oocyte entered the uterus (Fig. 10). Metaphase II, with five univalent chromosomes, was observed in the oocyte while it was still in the uterus. A sperm pronucleus with chromosomes was observed in the oocyte. However, due to the thickness of the cytoplasm in the ovum, an exact number of chromosomes in the sperm pronucleus could not be determined. Also, fusion of the sperm and egg pronuclei was not observed. The developmental stages of meiosis in the citrus race are illustrated in Fig. 1.

Banana race: Late prophase I with ring- or rod-shaped chromosomes was observed in large oocytes (Fig. 11). Unlike the citrus race, the oocytes of the banana race did not maintain their shape when removed from the reproductive system. Early metaphase I, with eight chromosomes that were tetrapartite in arrangement, was observed in the amorphous oocytes (Fig. 12). Late metaphase I and anaphase were also observed in those oocytes (Figs. 13, 14). The first meiotic divisions may have occurred in the region of the growth zone. Metaphase II, with four univalent chromosomes, was also observed in the growth zone before the oocyte had passed through the spermatheca. Seldom were oblong oocytes observed before they had passed through the spermatheca. After entering the uterus the ovum contained dense chromatin material indicating that meiosis had been completed. Occasionally, two dense nuclei were observed as well as a smaller nucleus that was presumed to be the second polar body. Spermatozoa presumed to penetrate the ovum, but this was not observed. The developmental stages of meiosis in the banana race are illustrated in Fig .2.



Figs. 11–14. Chromosomes of the banana race of *Radopholus similis.* 11) Late prophase I of the first meiotic division (2n = 8). 12) Early metaphase I tetrapartite chromosomes. 13) Late metaphase I chromosomes. 14) Anaphase I chromosomes with only one plane of chromosomes in view.

DISCUSSION

The classical developmental stages of oogenesis, as have been demonstrated in other diploid amphimictic nematodes (10), were also observed in *R. similis.* The two races, however, were found to have different rates of oocyte maturation. Oocyte maturation in the banana race was similar to that of other plant-parasitic nematodes that have been studied (9). All meiotic divisions were observed in amorphous oocytes before they reached the oviduct region of the gonad. In the citrus race the divisions appeared to take place at a much slower rate. Metaphase II was not observed until the ovum had been formed. Although the sperm pronucleus was observed in the ovum of the citrus race, actual fusion of the sperm and egg pronuclei were not observed.

The observed differences in oocyte development indicate the possibility that these two races are separate species. The difference in rate of oogonial maturation between the races is probably genetically controlled, and the possibility exists that it could form the basis of a genetic isolation mechanism (12). These differences in ovum formation may also be of diagnostic importance in the separation of the races. The citrus race was observed to have large occytes formed before they reached the spermatheca, whereas the banana race had small, cuboidal shaped oocytes in the same location in the gonad. These differences are detectable with the propionic orcein acid stain and light microscopy.

A difference in the chromosome numbers of these two races indicates that either a dissociation or a centric fusion may have taken place in one race to give rise to the other (11). This difference in chromosome number adds strength to the possibility that these two races are, in fact, sibling species.

Karyotypic uniformity was observed in two populations of the citrus race, but other populations need to be examined. Consistency in chromosome numbers seems to be characteristic in other bisexual nematode genera (4). Roman and Triantaphyllou (4) demonstrated karyotypic uniformity in many populations of *Pratylenchus* from thoroughout the world. Several populations of each race of *R. similis* need to be examined to demonstrate the consistency of the chromosome numbers. The banana race particularly needs to be examined on a worldwide basis. The chromosome numbers of *R. similis* should be determined from populations from other hosts, such as black pepper and ornamentals, to clarify the status of these populations.

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