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Cytogenetic Status of *Meloidogyne* (*Hypsoperine*) *spartinae* in Relation to Other *Meloidogyne* Species[1]

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Abstract: Four populations of *Meloidogyne spartinae* from the coast of North and South Carolina were identical cytogenetically. Fourteen rod-shaped chromosomes were present in oogonia and spermatogonia, whereas seven bivalents were observed in oocytes and spermatocytes. There were no distinguishable sex chromosomes. Chromosome behavior was similar to that of other *Meloidogyne* species. A slight deviation in morphology of prometaphase bivalents was attributed to an increase in frequency of chiasmata that may be associated with the obligatorily amphimictic reproduction of this nematode. The anatomy of the oviduct–spermatotheca region and most cytogenetic features studied suggested that *M. spartinae* can be regarded as a root-knot nematode. Its position in the genus *Meloidogyne* or *Hypsoperine* can be decided by taxonomists. Its small chromosome number (n = 7) compared to the larger number (n = 13–19) of other *Meloidogyne* species suggests that, cytologically, *M. spartinae* stands closer to the ancestral form from which the present day root-knot nematodes have evolved.
Key words: oogenesis, spermatogenesis, taxonomy, cytotaxonomy, chromosomes, reproduction, *Meloidogyne spartinae.*

The genus *Hypsoperine* was established by Sledge and Golden (5) as an intermediate genus between *Heterodera* and *Meloidogyne* although, admittedly, it showed closer relationship to *Meloidogyne.* The major morphological features distinguishing *Hypsoperine* from *Meloidogyne* were female body oval in shape, very thick cuticle, and the vulva and anus situated posteriorly on a slight protuberance. Females of *Meloidogyne* species have a pear-shaped or flask-shaped body with relatively thin cuticle and no posterior protuberance. *Hypsoperine spartinae,* described by Rau and Fassuliotis (4), was tentatively placed in the genus *Hypsoperine,* although its females were oval to lemon shaped with quite thin cuticle.

The separation of *Hypsoperine* from *Meloidogyne* was eventually rejected by Whitehead (13) because the aforementioned distinguishing morphological characters were also observed independently in several species of *Meloidogyne.* A still later review, (2) suggested that the genus *Hypsoperine* could preferably be maintained as a valid genus.

Subsequent cytogenetic studies revealed that two members of the genus *Hypsoperine,* namely *H. graminis* and *H. ottersoni,* were similar to members of the genus *Meloidogyne* by having 18 chromosomes and by reproducing by facultative, meiotic parthenogenesis (9). Their transfer to the genus *Meloidogyne* (13) therefore was perceived as well justified. However, only seven chromosomes had been observed in females of the original population of *H. spartinae* from which the species was described (4). The

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same chromosome number was observed later in several populations of this species from the coast of North Carolina (8). No detailed cytological study has been conducted since then to evaluate the relationship of the few chromosomes of *H. spartinae* to the 13–19 chromosomes of other *Meloidogyne* species.

This paper reports in more detail the cytogenetic features of *H. spartinae* and discusses the relationship of the karyotype of this species to that of various species of the genus *Meloidogyne* in an effort to elucidate the phyletic relationship of *H. spartinae* and the genus *Meloidogyne.*

Materials and Methods

Three populations of *M. spartinae* from the coastal area of North Carolina were included in this study. Most cytological observations were made on one population from Carolina Beach Inlet near Wilmington, NC, but sufficient observations were made also from the two other populations, from Oregon Inlet–Hatteras Island and Fort Fisher–end of the peninsula, to verify their cytological status. Collections were made at monthly intervals over a 2-year period. Fall collections provided the best nematode material for cytological studies. A fourth population, from South Carolina, supplied by Dr. George Fassuliotis of the U.S. Vegetable Laboratory, USDA ARS, represented the original population from which the species was described.

Whole plants of *Spartina alterniflora* Loisel, with much of their root system, were brought to the lab and kept in plastic bags. Nematode material obtained from such plants over a 2–3 week period was usually satisfactory for cytological studies. The nematode populations could not be maintained in good condition in greenhouse cultures established from such plants and were usually lost within about 6 months.

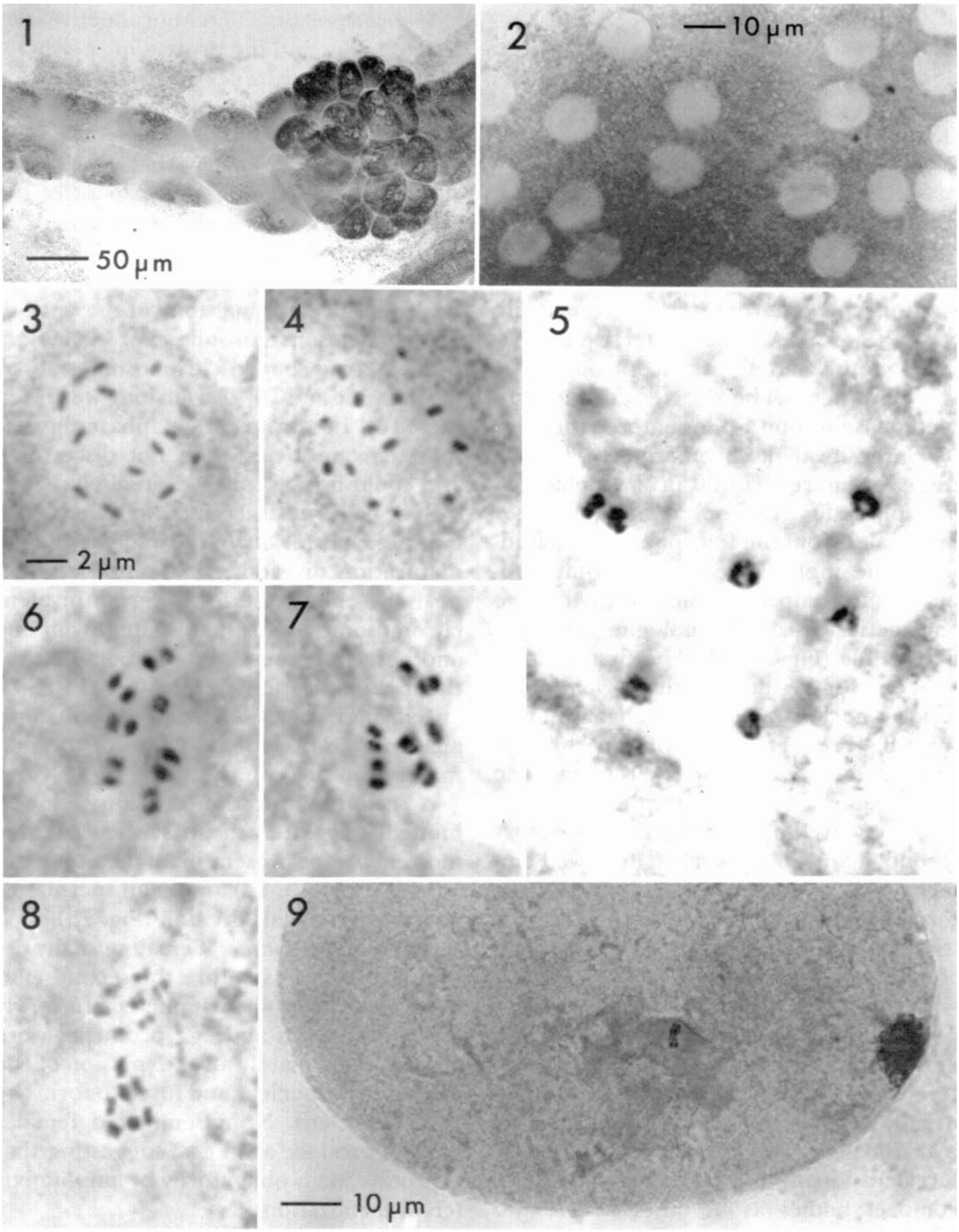
Young egg-laying females extracted from small and medium root galls were smeared on microscope slides, and the smears were processed for staining with propionic orcein according to described methods (11). Young males found associated with the females in the same root galls were transferred with a fine needle to dry microscope slides. The posterior of each body was cut immediately by drawing the needle over it, thus allowing the body contents, including the reproductive system, to spread and adhere to the dry slide surface. Slides were later processed for staining with propionic orcein in the same manner as slides with smears of female nematodes (11).

To study advanced stages of maturation of oocytes as well as the process of fertilization and cleavage divisions, eggs obtained from inside the galls were processed and stained as described for advanced eggs of *M. nataliei* (12).

Results

Oogenesis: The reproductive system of *M. spartinae* is similar to that of *M. javanica* and other *Meloidogyne* species, consisting of an elongate ovary, the oviduct–spermatotheca region, an elongate uterus, and a spherical vagina. The oviduct–spermatotheca region (Fig. 1) in general structure is characteristic and typical of the genus *Meloidogyne.* Thus, as in all *Meloidogyne* species, the oviduct is formed of two rows of four, tightly packed, thick cells. The spermatotheca is also typical of the genus *Meloidogyne* but is composed of approximately 34 lobe-like cells, compared with 16 in *M. hapla* and 24 in *M. incognita* and *M. javanica.*

Oogonial divisions were observed frequently in the apical, germinal zone of the ovary of egg-laying females. The prophase and metaphase chromosomes of dividing oogonia were well condensed and discernible, similar in morphology and behavior to those of other *Meloidogyne* species (Figs. 3, 4). Fourteen chromosomes were observed in each one of more than 50 oogonia at prophase or metaphase. They were rod shaped, about 0.5 μm wide and 0.7–1.5 μm long, depending on the degree of contraction. The zone of synapsis and the growth zone were similar to the corresponding zones of the ovaries of other *Meloidogyne* species. The oocytes in the central region of the growth zone always entered



FIGS. 1–9. Oogenesis in *Meloidogyne spartinae.* 1) Oviduct–spermatotheca region of ovary. 2) Growth zone of ovary with advanced oocytes. The chromatin is diffuse and the nuclei are transparent (diffuse stage). 3, 4) Prophase figures of oogonial cells, each with 14 chromosomes. 5) Oocyte at diakinesis. The bivalents show complex configurations (not clear tetrads), possibly due to nonterminalized chiasmata. 6, 7) Prometaphase or metaphase I figures of oocytes, each with seven bivalents. 8) Metaphase II figure with seven compact chromosomes. The first polar nucleus with seven chromosomes is also visible on the upper side. 9) Primary oocyte at metaphase I and a sperm nucleus visible in the posterior end. The chromatin of the sperm nucleus is loosely spread around the periphery and does not form a compact body as it does in other *Meloidogyne* and *Heterodera* species. Scale for Figures 4–8 as in Figure 3.

a "diffuse state," during which the late-pachytene chromosomes became decondensed and could not be seen. The nuclei appeared as transparent, uniform spheres (Fig. 2).

Oocytes approaching the oviduct–spermatotheca region had advanced to diakinesis. The chromosomes at this stage became visible again and formed complex configurations due to the presence of chiasmata at different stages of terminalization (Fig. 5). A sperm entered each oocyte as the latter passed through the spermatotheca. The oocytes advanced to metaphase as soon as they entered the uterus. Seven bivalent chromosomes were observed in more than 60 prometaphase or metaphase figures from an equal number of females from the four populations studied. The metaphase chromosomes appeared as compact tetrads, with the two chromatids of each homologue not easily discernible (Figs. 6, 7). The first maturation division resulted in the formation of a polar nucleus with seven chromosomes (Fig. 8). Frequently, six of the chromosomes were arranged in a circle and one in the center. The same arrangement was often observed in metaphase II figures. A second maturation division followed immediately after the first and resulted in the formation of a second polar nucleus and the egg pronucleus with seven univalent chromosomes.

During the two maturation divisions the sperm nucleus was located at the posterior end of the egg, i.e., the end that first entered the spermatotheca. Its chromatin usually formed a loose network distributed over the periphery of the nucleus (Fig. 9), but occasionally it formed one or a few compact bodies as in other *Meloidogyne* species.

Only inseminated females, with sperm in their spermatothecae, contained oocytes that had advanced beyond prophase I or had deposited eggs.

Fourteen elongate chromosomes were observed in several dividing blastomeres in embryos at the 2–64-cell stage.

Spermatogenesis: The reproductive system of males and the process of spermatogenesis in *M. spartinae* were found to be similar to those of other *Meloidogyne* species. The study of spermatogenesis, however, was much easier than in other species and revealed more clearly the behavior of the chromosomes during the spermatogonial and maturation divisions.

Some spermatogonial divisions were observed in the germinal zone of the testis of young males. At prophase of such divisions, 14 rod-shaped chromosomes about 0.5 μm wide and 1.0–1.6 μm long were seen (Fig. 10). In advanced metaphase figures, most chromosomes were not discernible and could not be counted precisely.

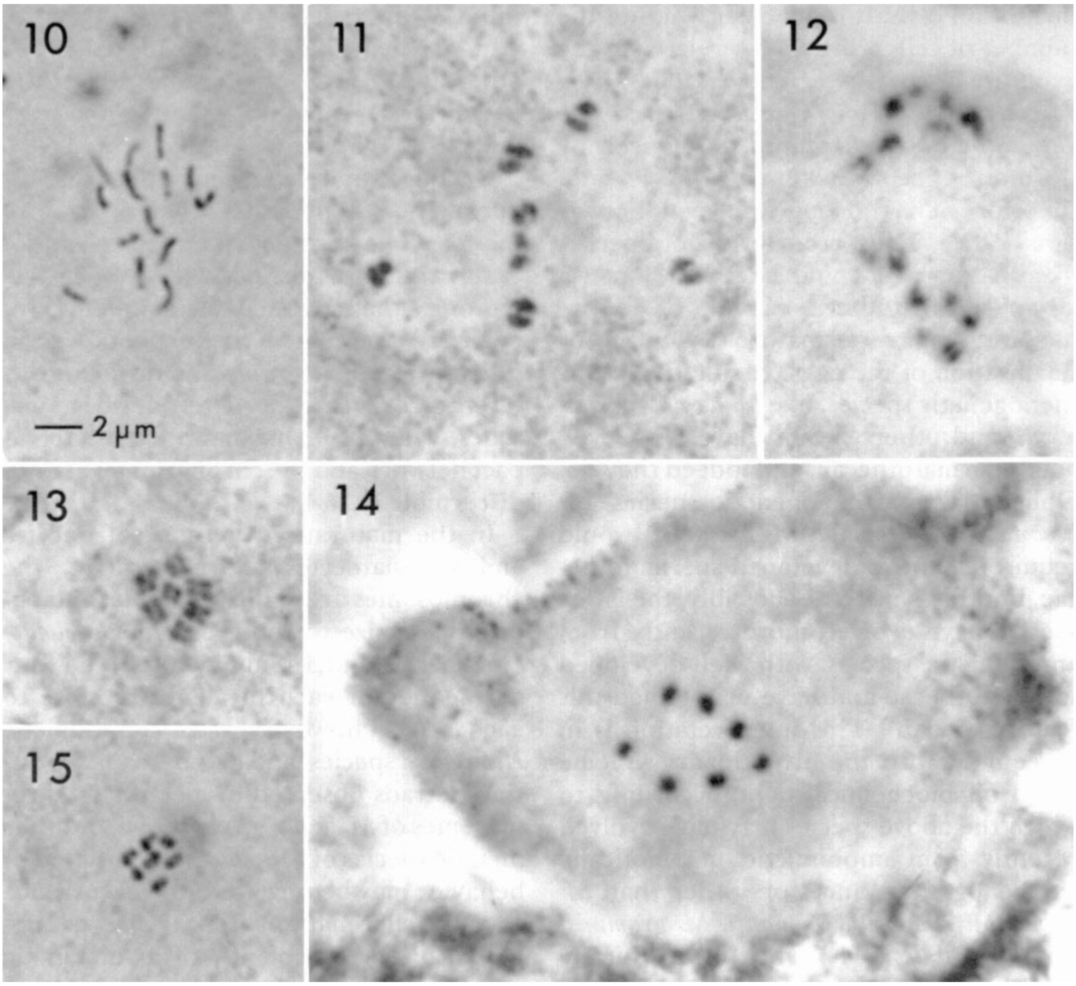
Spermatocytes increased in size as they migrated posteriorly and underwent two maturation divisions, at close succession, near the end of the testis. Seven bivalent chromosomes, indistinguishable from one another were observed at prometaphase of the first maturation division (Fig. 11). Seven chromosomes were also seen in both anaphase and telophase plates of the first maturation division (Fig. 12).

A second maturation division followed immediately after the first. Seven chromosomes were present in all metaphase II figures (Figs. 13, 14) and in all spermatids resulting from this division (Fig. 15).

Mode of reproduction: Females usually deposited their eggs before the second maturation division was completed. In newly laid eggs, sperm and egg pronuclei were formed immediately after expulsion of the second polar nucleus and fused to form the zygote nucleus. Noninseminated females did not produce any eggs, suggesting that reproduction is obligatorily by amphimixis (cross-fertilization).

Discussion

The present cytogenetic study was conducted primarily in the hope that it could clarify the following two questions: 1) Is *M. spartinae* cytogenetically another *Meloidogyne* species or does it belong to a different genus? 2) Can cytogenetic information



FIGS. 10–15. Chromosomes during spermatogenesis of *Meloidogyne spartinae.* 10) The 14 rod-shaped prophase chromosomes of a spermatogonium in the germinal zone of the testis. 11) The seven prometaphase I, bivalent chromosomes of a spermatocyte. 12) Telophase I figure with seven chromosomes in each plate. 13) Early metaphase II figure before contraction of the chromosomes. 14) Secondary spermatocyte at metaphase II. 15) The seven chromosomes of a spermatid shortly before they become diffuse. Scale for Figures 11–15 as in Figure 10.

about *M. spartinae* assist in the interpretation of the evolutionary relationships of root-knot nematodes?

With regard to the first question, anatomical observations of the structure of the reproductive system provided very strong evidence that *M. spartinae* is indeed a species of *Meloidogyne. Meloidogyne* species have a characteristic oviduct–spermatotheca region (3,7), different from that of any other nematode genus. The oviduct is tubular, consisting of two rows of four thick cells, whereas the spermatotheca is spherical and is formed by a variable number of thick, lobe-like cells. Members of the genus *Meloidogyne* are strongly unified by this characteristic anatomical feature, and *M. spartinae* is no exception.

Furthermore, Tchizhov (6) reported that *M. hapla* has a primitive spermatotheca, composed of 16 large cells, whereas *M. incognita* and *M. javanica* have spermatothecae with 24 smaller cells. The spermatotheca of *M. spartinae* consists of approximately 34 cells. It is not clear whether the number of cells is in any way related to the evolutionary status of the various *Meloidogyne* species. It may represent a more recent functional adaptation influenced by the mode of reproduction of a species and, therefore, it has no long-term evolutionary significance. Thus, cross-fertilizing species, like *M. spartinae* could have developed a larger spermatotheca, capable of accommodating many spermatozoa required for fertilization of the eggs. Facultatively parthenogenetic species, like *M. hapla, M. chitwoodi,* and others, would have less developed spermatothecae, and indeed they do. Obligatorily parthenogenetic species (*M. javanica, M. incognita, M. arenaria*) would require even less developed spermatothecae, but this is not true. Possibly, the latter species have evolved more recently from amphimictic species with well-developed spermatothecae and have maintained this feature to date. This interpretation is in agreement with the evolutionary scheme based on biochemical data, according to which the above species may have evolved recently from amphimictic or meiotically parthenogenetic ancestors other than *M. hapla, M. microtyla, M. naasi, M. graminis,* or *M. graminicola* (1). Only the mitotically parthenogenetic race of *M. hapla* appears to be biochemically and, presumably, phyletically close to the meiotic form of *M. hapla*; indeed, the two forms have similar spermatothecae (unpubl.).

In sperm nuclei observed inside the oocytes of *M. spartinae,* the chromatin is spread along the periphery of the nucleus and appears as a network of thin threads with small knobs. Only rarely is the chromatin compact, forming a single or a few interconnected knobs in the center of the nucleus, which is the norm for other *Meloidogyne* and also for *Heterodera* species. The significance of this deviation in distribution of the sperm chromatin of *M. spartinae* is not clear at present.

Seven chromosomes were observed in oocytes and in all spermatids of *M. spartinae.* This observation suggests that, if sex chromosomes are present, they must be of the XX–XY type, with the X and Y chromosomes being indistinguishable from each other and from the autosomes. The situation is the same in other *Meloidogyne* species.

During prophase and metaphase of oogonial and spermatogonial divisions of *M. spartinae,* the chromatin condenses considerably and forms distinct, rod-shaped chromosomes that can be easily counted. This behavior is similar to that encountered in other *Meloidogyne* species and distinctly different from that of heteroderid nematodes, where the chromatin forms a compact network and the chromosomes are not discernible.

In the maturation divisions of oocytes and spermatocytes of *M. spartinae,* the chromosomes are similar in shape and approximate size to those of other *Meloidogyne* species undergoing meiosis. The four chromatids of each bivalent are close to each other, however, as in amphimictic *Heterodera* species, and do not form the distinct tetrads observed in metaphase chromosomes of *M. hapla, M. chitwoodi,* or other meiotic species of *Meloidogyne.* This kind of behavior may be related to the occurrence of more chiasmata in *M. spartinae,* as suggested by the complexity of its diakinetic chromosomes (Fig. 5). A higher frequency of chiasmata formation could be expected in an obligatorily amphimictic species like *M. spartinae,* as compared with the facultatively parthenogenetic species, in which crossing over may be less important. Therefore, the deviation in bivalent chromosome morphology of *M. spartinae* from that of other *Meloidogyne* species may be the result of its amphimictic reproduction.

In conclusion, *M. spartinae* can be considered a member of the group commonly referred to as root-knot nematodes. Whether it is placed in the genus *Meloidogyne* or in a different genus, *Hypsoperine,* may be only an academic question that could be decided by nematode taxonomists. Cytogenetic data suggest that the seven chromosomes of *M. spartinae* are evolutionarily related to the 13–19 chromosomes of other *Meloidogyne* species. Behavioral differences during gametogenesis, as discussed in this paper, may be the result of the obligatorily amphimictic reproduction of *M. spartinae.* The small chromosome number of this species undoubtedly is closer to the ancestral chromosome number from which the genus *Meloidogyne* has evolved, possibly through polyploidization during a parthenogenetic phase as has been suggested earlier (10).

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