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Gametogenesis and the Chromosomes of *Meloidogyne nataliei*: Not Typical of Other Root-knot Nematodes[1]

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Abstract: Studies of oogenesis and spermatogenesis revealed that *Meloidogyne nataliei* is a diploid, amphimictic species with four (n), relatively large chromosomes, and possibly with an XX ♀–XY ♂ mechanism of sex determination. It differs considerably from all other amphimictic, or meiotically parthenogenetic, species of *Meloidogyne* which have 13–18 smaller chromosomes and from *Meloidogyne* (*Hypsoperine*) *spartinae* which has seven. Consequently, the taxonomic position of *M. nataliei* needs to be re-evaluated. The chromosomes of *M. nataliei* and their behavior during gametogenesis resemble more closely chromosomes of the genus *Heterodera* than those of the genus *Meloidogyne*. This resemblance, however, may not imply a closer phyletic relationship of *M. nataliei* to heteroderid nematodes.

Key words: oogenesis, spermatogenesis, phylogeny, taxonomy, cytotaxonomy.

A nematode attacking grapes in Michigan was described as *Meloidogyne nataliei* on the basis of a number of morphological features of females, males, and juveniles (1). However, certain characters, such as the perineal patterns of females, the stylets of juveniles, and the morphology of the head region of males and juveniles, deviated substantially from those of other *Meloidogyne* species. It is suspected here that this nematode may not belong to the genus *Meloidogyne*. The uncertainty of its taxonomic position was also expressed by the authors of the original description in their statement: "The nematode is described herein as such (*Meloidogyne*), but it is obviously far different from most other root-knot nematode species."

In the past, cytogenetic studies of many *Meloidogyne* species have been helpful in elucidating interrelationships of root-knot nematodes (5). A cytogenetic study of *M. nataliei*, therefore, seemed promising in clarifying its relationship to the other species of *Meloidogyne* and possibly to other related forms.

The present paper provides information about gametogenesis, the chromosomes, and mode of reproduction of *M. nataliei* and discusses the possible cytological relationship of this species to other root-knot nematodes, as well as to the genus *Heterodera*.

Materials and Methods

The *M. nataliei* population in this study, provided by L. M. Rose and G. W. Bird of the University of Michigan, represented the original population from which the species was described (1). Egg masses from infected grape roots were used to inoculate concord grape seedlings which subsequently were kept in a greenhouse at 22–28 C. Five months later the grape roots were examined microscopically. Only adult females were found protruding from the roots, most of them with a few eggs enclosed in a large gelatinous matrix. No juvenile stages were found in the root tissues.

For most of the cytological work, young egg-laying females were smeared on microscope slides and the smears were processed and stained with propionic orcein according to established procedures (3). Young males found inside egg masses were transferred with a fine needle to dry slides and cut immediately by drawing the needle

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across the posterior part of their bodies. The reproductive system was thus spread on the slide and adhered to it as it dried in a few seconds. Slides were processed for staining in the same manner as those with smears of female nematodes.

To study advanced stages of maturation of oocytes, as well as the process of fertilization and cleavage divisions, egg masses from young females were treated for 6 minutes in a mixture of 0.1 N KOH in 0.4% NaOCl. After washing in tap water, the eggs were suspended in 1% plain gelatin solution and the suspension was spread on slides and allowed to dry. The slides were then processed for staining as were slides with smears of females. Better results were obtained by prolonging fixation from the usual 30 minutes to 1 hour.

Results

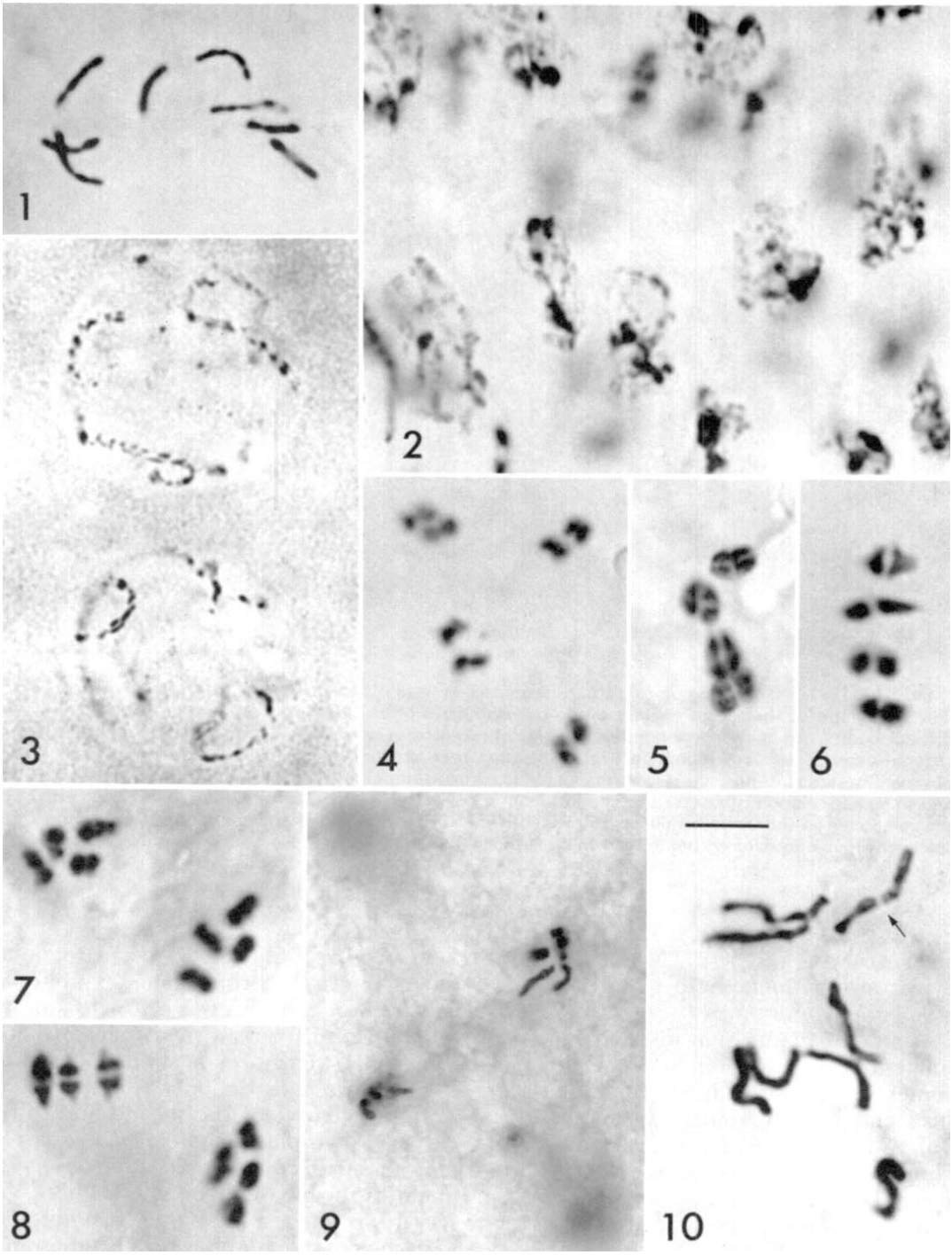
Oogenesis: Few oogonial divisions (fewer than five) were observed in the short, apical oogonial region of the ovary of egg-laying females. Eight discrete chromosomes, 2.5–4 μm long, were observed in several oogonia at mitotic mid-prophase (Fig. 1). In more advanced oogonia, approaching metaphase or later stages of division, the chromosomes were positioned very close to each other and were no longer discrete.

The oogonial zone of the ovary is followed by a short "zone of synapsis" which is distinct because of the characteristic arrangement of the chromatin in the nucleus of the young oocytes (Fig. 2). Such an arrangement is typical of zygotene and early pachytene oocytes of root-knot and cyst nematodes (5). Beyond the zone of synapsis, the oocytes increased progressively in size and advanced to diplotene stage. The chromatin inside the nucleus was arranged in long double filaments which were visible in oocytes along the entire length of the growth zone of the ovary (Fig. 3).

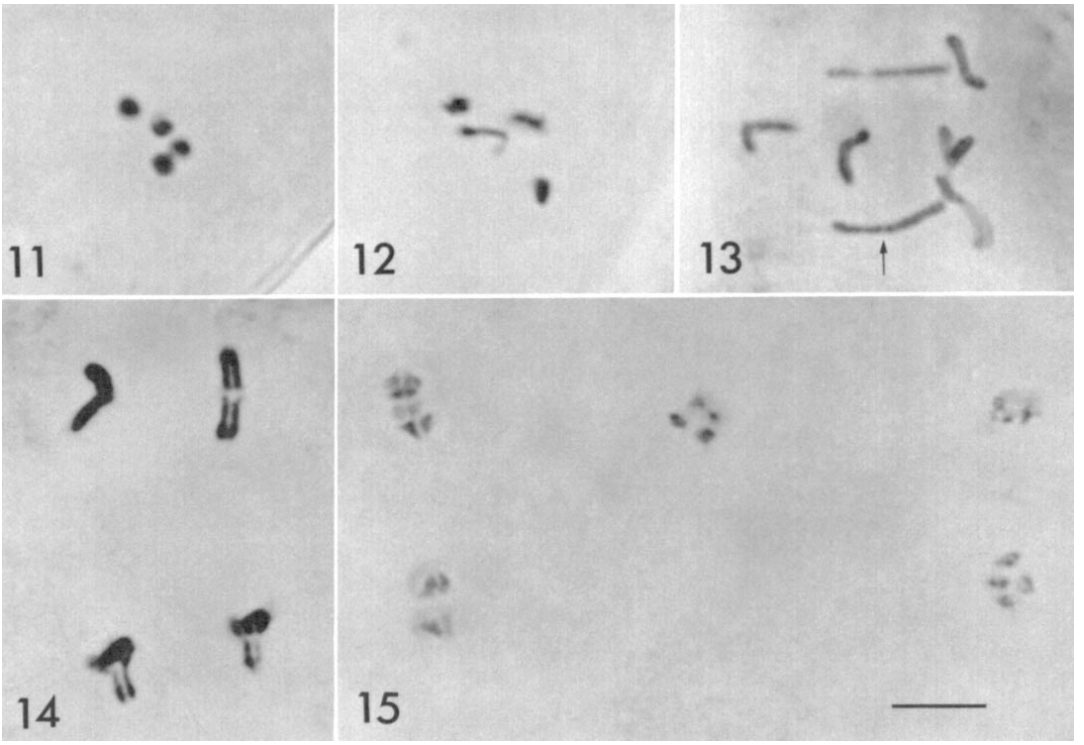
One spermatozoon entered each oocyte as the oocytes passed through the spermatotheca. The sperm nucleus always remained close to one pole of the egg, away from the egg nucleus. Its chromatin was condensed in a single mass or in two or three interconnected chromatin bodies. The oocytes advanced to metaphase I as they entered the uterus. Four bivalent chromosomes were observed in many oocytes at prometaphase or metaphase I (Figs. 4–6). At telophase I, four chromosomes could be observed in each telophase plate (Fig. 7). The first maturation division was followed by a second division (Fig. 8) which resulted in the formation of a second polar nucleus and the egg pronucleus. At the completion of the second division, the sperm nucleus was transformed gradually into a sperm pronucleus. Occasionally, during the early stages of this process, the chromatin of the sperm nucleus resolved into four bodies, apparently corresponding to four individual chromosomes (Figs. 11, 12). Eventually, the chromatin decondensed completely and became invisible as the sperm was transformed into the sperm pronucleus. Egg and sperm pronuclei fused to form the zygote nucleus which soon underwent the first cleavage division. During the first cleavage, the egg and sperm chromosomes often were arranged in two separate groups on the metaphase plate (Fig. 9). Eight chromosomes were observed at prophase of the second and third cleavage divisions (Fig. 10). Chromosomes at late prophase varied in length from 5 to 8 μm; however, the individual chromosomes or the various homologous chromosome pairs could not be recognized consistently on the basis of their length. A constriction was often observed in one of the large chromosomes at a position about 40% of its length (Fig. 10, arrow). Chromosome length decreased rapidly as condensation progressed from early prophase to metaphase. Late prophase figures were the best for chromosome study; at metaphase the chromosomes were no longer discrete.

Spermatogenesis: A few spermatogonial divisions (fewer than five) were observed close to the tip of the testis in young males. Eight chromosomes, 3–5 μm long, could be seen in late mitotic prophase figures. One long chromosome showed a distinct constriction at a point about 40% of its length (Fig. 13, arrow).

A short zone of synapsis was distinct in young males but not in males of advanced age. Maturation divisions took place at the posterior part of the testis, just before it opens into the seminal vesicle. Usually, two or three spermatocytes were undergoing the first maturation division. Four diakinetic chromosomes were observed in a few



FIGS. 1–10. Oogenesis in *Meloidogyne nataliei*. 1) The eight mid-prophase chromosomes of an oogonial division in the germinal zone of the ovary. 2) Young oocytes in the "zone of synapsis" with the chromatin highly condensed, forming a network. 3) Two oocytes at pachytene-diplotene stage. 4–6) Respectively, prometaphase, metaphase, and early anaphase figures showing the four bivalents in oocytes located in the anterior part of the uterus. 7) Telophase I. 8) Metaphase II (upper left) and first polar nucleus (lower right) in a secondary oocyte. 9) First cleavage division with the sperm and egg chromosomes in two distinct groups. The spindle poles are visible in the upper left and lower right corners. 10) The eight prophase chromosomes of an egg during the second cleavage division. A distinct constriction (arrow) was often observed in one chromosome. Bar = 4 μm.



FIGS. 11–15. The chromosomes during spermatogenesis and fertilization of *Meloidogyne nataliei*. 11–12) The chromatin of the sperm nucleus inside the cytoplasm of an oocyte is occasionally resolved into four distinct bodies, probably corresponding to four chromosomes of the male karyotype. 13) The prophase chromosomes of a spermatogonium in the germinal zone of the testis. A constriction (arrow) was always observed in one chromosome. 14) Four diakinetic or prometaphase I chromosomes of a spermatocyte. An end-to-end association is observed in one bivalent (upper right), whereas terminalization of chiasmata is not yet completed in the other bivalents. 15) Metaphase II figures (left side) and spermatids (center and right side) with four univalent chromosomes. Bar = 4 μm.

spermatocytes (Fig. 14). Most spermatocytes were at metaphase I and had four bivalent chromosomes.

A second maturation division followed immediately after the first. Four chromosomes were observed in all telophase II figures and in very young spermatids (Fig. 15). Soon, however, the chromosomes clumped together to a single compact chromatin body that could be seen thereafter in more advanced spermatids and in spermatozoa that filled the vas deferens.

DISCUSSION

Gametogenesis in *M. nataliei* is by regular meiosis. Two maturation divisions lead to the production of haploid male and female gametes, both with four chromosomes. Reproduction is exclusively by amphimixis (cross-fertilization).

In prophase figures of spermatogonia, one of the large chromosomes exhibited a constriction and thus was distinct from its homolog and from all the other chromosomes. Furthermore, a similar chromosome with a constriction was observed in prophase figures of the second and third cleavage divisions of some eggs. No chromosomes with constrictions were observed in mitotic prophases of oogonia. These observations suggest that the chromosome with the constriction is a sex chromosome (Y); consequently, its homolog, which is not recognizable from the other chromosomes, is an X chromosome. The chromosomal mechanism of sex determination, therefore, could be XX ♀–XY ♂; i.e., with the male being the heterogametic sex. The presence of a Y chromosome in some cleaving eggs, and its absence in others, appar-

ently reveals the existence of two types of eggs which should give rise to males and females.

The basic chromosomal complement of the genus *Meloidogyne* consists of 18 (n) relatively small chromosomes. This number is observed in most amphimictic or facultatively parthenogenetic species of *Meloidogyne*; e.g., *M. exigua, M. naasi, M. graminis,* and *M. carolinensis* (3,5). *Meloidogyne nataliei,* with four considerably larger chromosomes, deviates so much from *Meloidogyne* that evaluation of its cytological relationship to the genus *Meloidogyne* becomes very difficult.

Meloidogyne (*Hypsoperine*) *spartinae* is the only root-knot nematode whose chromosomal complement consists of a small number (seven). Its relationship to the other *Meloidogyne* species is not clear, but cytologically it may represent an ancestral chromosomal form of *Meloidogyne* (4). It has been pointed out that the basic chromosomal complement of all nonspecialized nematodes comprises about six, and certainly fewer than nine, small-sized chromosomes. Consequently, the 18 chromosomes of the genus *Meloidogyne* must have evolved secondarily from ancestral forms with smaller chromosome numbers, possibly similar to that of *M. spartinae.* A cytogenetic mechanism involving polyploidization during a parthenogenetic phase and subsequent conversion to amphimixis has been proposed to explain this type of cytogenetic evolution in the genus *Meloidogyne* (2,4). Assuming that this theory has some merit, one may find it easier to relate the chromosomal complement of *M. nataliei* (four) to that of *M. spartinae* (seven) than to the remaining species of the genus *Meloidogyne.* A number of alternative interpretations of this relationship could be discussed, but such an evaluation preferably should be postponed until a more detailed cytological study of *M. spartinae* is carried out. Similarly, *M. nataliei* should be compared to the genus *Meloinema,* another member of the family Meloidogynidae, for which, however, no cytological information is available at present.

Actually, the morphology and behavior of the chromosomes of *M. nataliei* during gametogenesis are more similar to those of the genus *Heterodera* than the genus *Meloidogyne.* Oogonial divisions occurred in *M. nataliei* at a slightly higher frequency than in *Heterodera* but substantially lower frequency than in *Meloidogyne.* The midprophase chromosomes of oogonial divisions of *M. nataliei* were quite discrete compared with those of *Heterodera,* but, like the *Heterodera* chromosomes of that stage, they were long and thread-like. The *Meloidogyne* chromosomes at this stage are short, and most of them are rod-shaped. At metaphase of oogonial divisions of *M. nataliei* and *Heterodera* spp., the chromosomes are not discrete, whereas those of *Meloidogyne* species are always quite discrete. Similarly, the large meiotic chromosomes of *M. nataliei* and the configurations of the bivalents at prometaphase of the first maturation division resemble more closely those of the genus *Heterodera* than those of the genus *Meloidogyne.* Still, in spite of these cytological similarities, one may not assume a closer phyletic relationship of *M. nataliei* to the heteroderids than to the meloidogynids.

In conclusion, the chromosomal complement of *M. nataliei* is sufficiently different from that of *Meloidogyne* to warrant reevaluation of the taxonomic position of this species. However, additional morphological, anatomical, and biological information about *M. nataliei* will be needed before this species can be compared to the other members of the families Meloidogynidae or Heteroderidae and possibly to other families of plant-parasitic nematodes.

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