Oogenesis and the Chromosomes of the Cystoid Nematode, *Meloidodera floridensis*[1]

A. C. TRIANTAPHYLLOU[2]

Abstract: Three *Meloidodera floridensis* populations of different geographic or host origin all reproduced by mitotic parthenogenesis. One of them from pine had a somatic chromosome number of 26, whereas, another population from pine and one from azalea had $2n = 27$ chromosomes. All are considered to be triploid forms derived from an amphimictic ancestor with $n = 9$ chromosomes, the basic number in the closely related genus *Heterodera*. Evidence is presented which suggests that during division the chromosomes of the germ-line cells of the developing embryo behave differently than the chromosomes of all other blastomeres. *Key Words:* Reproduction, Cytology, Germ-line cells.

The genus *Meloidodera* Chitwood, Hannon and Esser has been recognized as a link between the genera *Meloidogyne* Goeldi and *Heterodera* A. Schmidt because it exhibits several morphological characteristics of both these genera (1). Furthermore, the genus *Cryphodera* Colbran appears to morphologically link *Heterodera* and *Meloidodera* (2). The mode of reproduction of some species of *Heterodera* and *Meloidogyne* already has been studied cytologically and this information has facilitated considerably elucidation of the phylogenetic relationships of these organisms (5). Similar studies of members of the genus *Meloidodera* could prove equally valuable in defining the relationship of this genus to the other genera of the family Heteroderidae. With this objective in mind, we cytologically studied three populations of *M. floridensis* Chitwood, Hannon and Esser, the type species of the genus. The other two described species, namely *M. charis* Hopper, and *M. armeniaca* Pogosyan, could not be obtained in live cultures which are necessary for cytological study.

Received for publication 22 October 1970.

[1] Paper number 3310 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina. Supported in part by the National Science Foundation Grant GB-7214. Appreciation is extended to Dr. J. L. Ruehle of the Forestry Science Laboratory, Athens, Georgia, who kindly supplied the Florida population of *M. floridensis*.

[2] Professor, Department of Genetics, North Carolina State University, Raleigh, North Carolina 27607.

MATERIALS AND METHODS

Population 21-NC and 264-Fla were obtained originally from loblolly pine (*Pinus taeda*) from North Carolina and Florida, respectively. They were maintained in the greenhouse for at least 5 years before they were used for cytological study. Population 250-NC was obtained from azalea (*Rhododendron* sp.) from North Carolina.

Oogenesis of all three populations was studied in young, egg-producing females obtained from roots of loblolly pine seedlings inoculated with eggs and larvae 4 to 6 months earlier. The females were smeared on slides, treated for 5 min with 1N HCL, fixed in 3:1 (v/v) alcohol-acetic acid and stained in propionic orcein as described earlier (6).

Divisions of somatic cells were studied in embryos at the blastula and gastrula stages. Females containing eggs in all stages of development in their uteri were dissected in distilled water. The eggs thus released were treated 5 to 6 min in 1.5% sodium hypochlorite solution and were then processed for staining with orcein as described earlier (6).

Most of the observations reported herein were made with population 21-NC. The other two populations were studied only with regard to the chromosome number and the general process of oogenesis.

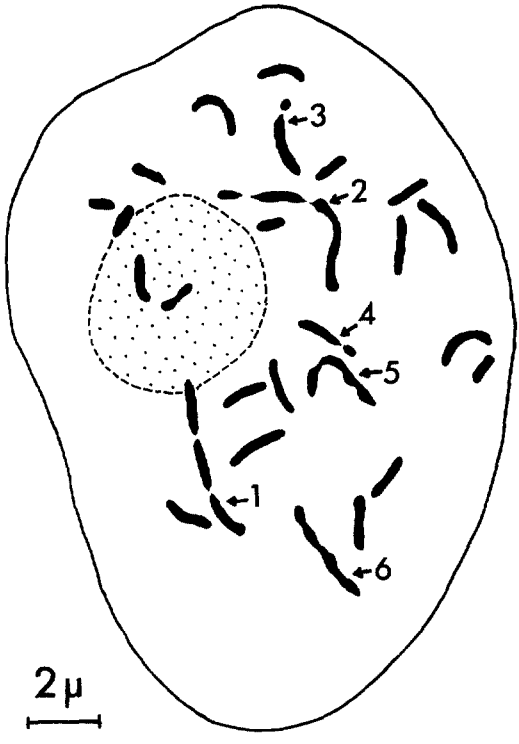

FIG. 1. Camera lucida drawing of an oogonium of *M. floridensis* at midprophase showing details of the karyotype. Chromosomes No. 1 to No. 6 are identified.

OBSERVATIONS

MULTIPLICATION OF OOGONIAL CELLS: Very few oogonial divisions were observed in the germinal zone of egg-producing females. Most oogonial divisions take place during the 4th molt and in very young females. At midprophase, when the nucleolus is still well visible, 26 distinct chromosomes are scattered in the nucleus (Fig. 1). Four, and in some cases six chromosomes could be identified in several oogonia according to their size and distribution of various constrictions. No. 1 and No. 2 chromosomes are clearly the largest and have two constrictions each. They do not appear to be completely homologous: in No. 1 chromosome the two constrictions divide the chromosome in three approximately equal sections; whereas, in No. 2 chromosome the three segments are of unequal size (Fig. 1). No. 3 and No. 4 chromosomes are of medium size and have one satellite each. Although they are very similar in general shape, No. 3 chromosome appears to be slightly larger than No. 4, and in most oogonia it has a distinctly smaller satellite. Occasionally No. 5 and No. 6 chromosomes could be distinguished as the next largest chromosomes after No. 1 and No. 2, but distinction between them could not be made. All the other chromosomes are of small or medium size with no constrictions and cannot be identified. All chromosomes at midprophase and up to metaphase appear as single strands. Individual chromatids are not visible until early anaphase.

MATURATION OF OOCYTES AND MODE OF REPRODUCTION: Following the last oogonial division and through the growth period of the oocytes, the chromatin behaves as described for *Meloidogyne arenaria* (Neal) Chitwood (4). Near the end of the growth zone, each oocyte contains a large nucleus with one to three (and occasionally more) nucleoli and with the chromatin condensed in small, dark-staining spheres which are arranged in tandem, forming long, twisted, knotted threads (Fig. 2). Later as the oocytes approach the oviduct-spermatotheca area, these chromatic threads contract and form definite chromosomes which, however, are not clearly discernible because they are closely associated with each other (Fig. 3). At prometaphase the chromosomes spread in a relatively large area and at the same time become arranged in one plane. Thus a large metaphase plate with quite distinct chromosomes is formed. At this stage the chromosomes can be counted accurately but only a few exceptionally good chromosomal figures (Fig. 4–7). All the chromosomes are univalent, each one consisting of two chromatids

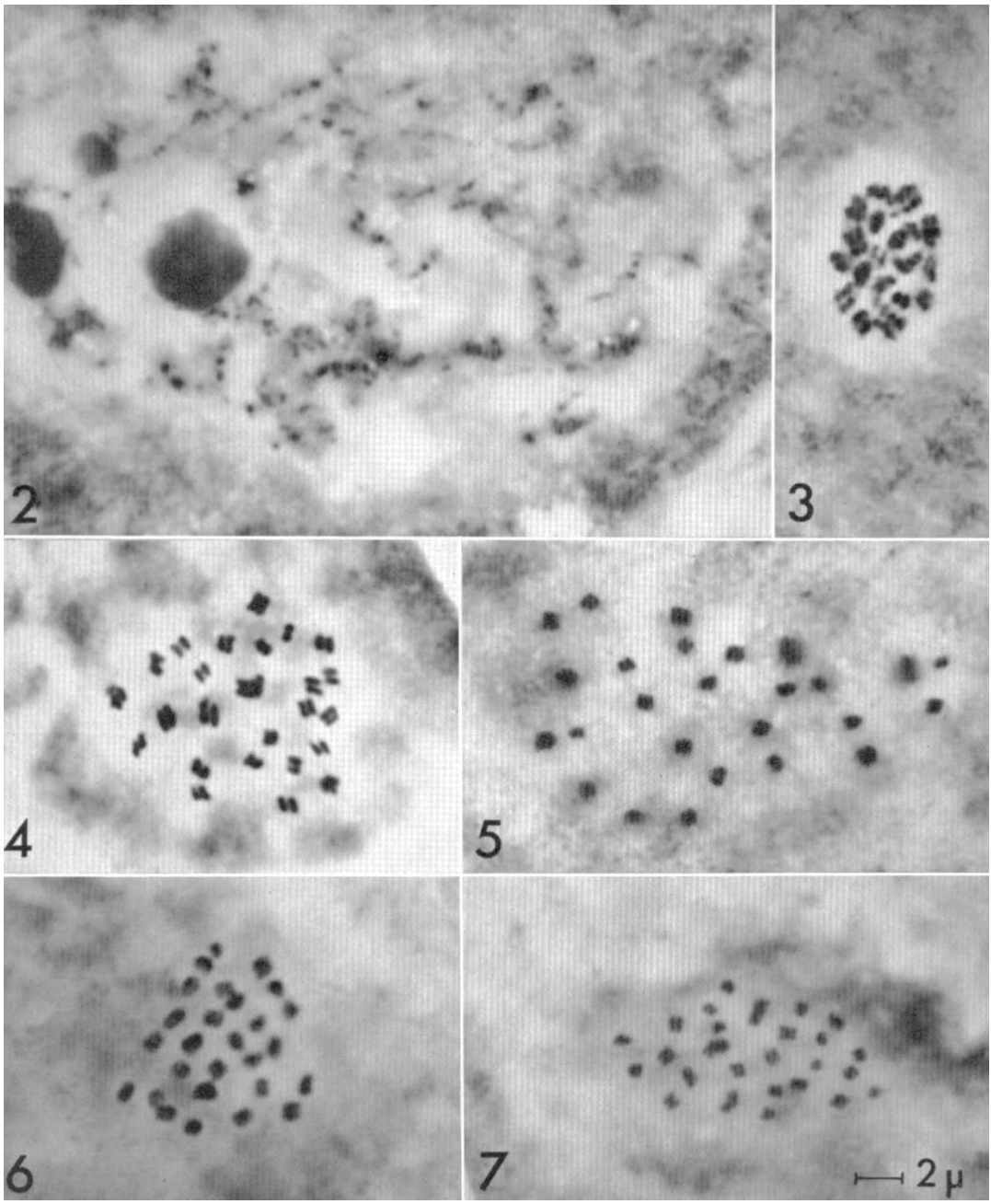

FIG. 2–7. Photomicrographs of chromosomal figures of *M. floridensis* during maturation of the oocytes: **2.** The nucleus of an advanced oocyte with three nucleoli and the chromatin forming long, knotted threads; **3.** The chromosomes of a fully grown oocyte at late prophase; **4–5.** Prometaphase figures from population 21-NC with 26 chromosomes; **6–7.** Prometaphase figures from populations 250-NC and 264-Fla, respectively, with 27 chromosomes. Magnification of Figs. 2–6 same as Fig. 7.

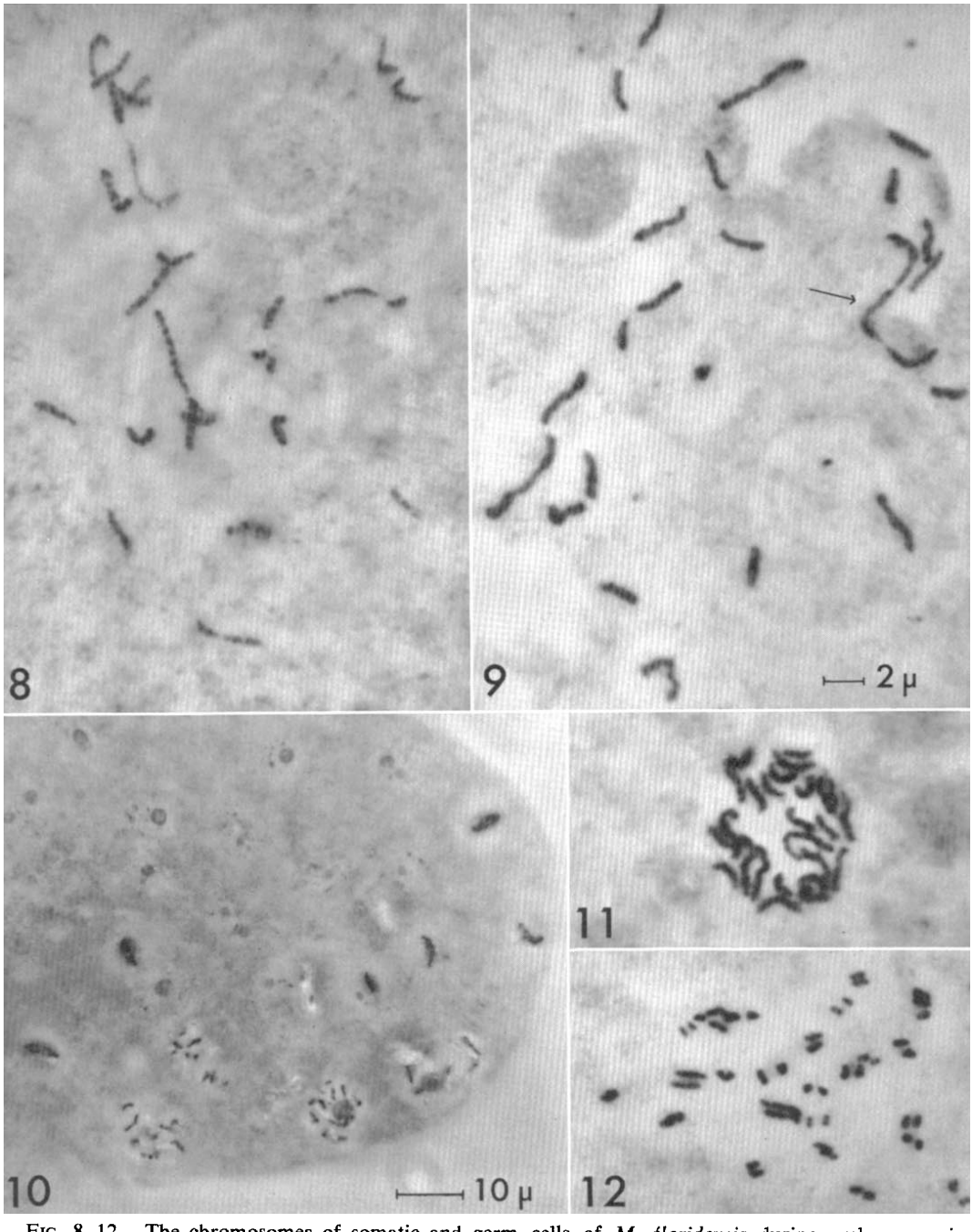

FIG. 8–12. The chromosomes of somatic and germ cells of *M. floridensis* during embryogenesis: **8–9.** The chromosomes of early and midprophase figures, respectively, of somatic (mesodermal or endodermal) cells. Chromosome No. 1 identified in Fig. 9 (arrow); **10.** The posterior half of an egg at the gastrula stage showing divisions of somatic cells in the ventral-posterior side; **11.** The metaphase chromosomes of a somatic cell at the blastula stage. The chromosomes are still like single threads; **12.**

that lie parallel to each other. Occasionally some chromosomes appear to be tetrapartite and resemble bivalent chromosomes. Apparently this is due to a constriction that appears in the middle of each chromatid of such chromosomes. The chromosome number of 2n = 26 was determined in more than 30 oocytes from population 21-NC. Seven good chromosomal figures were scored from populations 250-NC and 10 from population 264-Fla and all had 2n = 27 chromosomes. Shortly after metaphase I, permeability of the oocytes to the fixative and stain decreased considerably; for this reason the remainder of the process of maturation was not studied in detail. Occasional observations, however, indicated that with the completion of the first maturation division, the egg pronucleus is formed and the first cleavage division follows. Males were not observed in any of the cultures used for the cytological work, and no sperm were detected in the spermatotheca of the females or in the maturing oocytes.[3] Development of the eggs, therefore, was by mitotic parthenogenesis.

THE CHROMOSOMES OF SOMATIC AND GERM-LINE CELLS OF THE EMBRYO: The only cells in the nematode embryo that could be utilized in cytological studies were those occupying the posterior ventral part of the embryo at the 14 to 100-cell stage of development (Fig. 10). According to the embryological work of Pai (3) conducted with *Turbatrix aceti* Muller, and on the basis of their position in the embryo, these cells would correspond roughly with the mesodermal and endodermal cells derived from the EMST cell and with the P_3 or P_4 cells of the germline. All these cells divided almost simultaneously during the blastula stage and contain large, clearly distinct chromosomes. The ectodermal cells that occupy the dorsal, anterior and lateral surfaces of the embryo are small cells, and their chromosomes are not discernible during division.

At early prophase the chromosomes are long with many chromomeres visible along their length (Fig. 8). As they contract they take a more definite shape, but most of them cannot be identified individually (Fig. 9). The No. 1 and No. 2 chromosome, as these were characterized in oogonial divisions, could be recognized in some nuclei. They are the largest chromosomes and have two constrictions each in the same relative position as in oogonial cells. No. 3 and No. 4 chromosomes which exhibit one satellite each could also be recognized in some nuclei.

At metaphase the chromosomes are arranged close to each other and are still undivided, like the metaphase chromosomes of oogonial divisions (Fig. 11). The chromatids of each chromosome become visually distinguishable only at early anaphase as they separate and migrate toward the opposite poles. An exception to this behavior was observed in the chromosomes of one cell from each of three embryos at the late blastula stage. The chromosomes of this single cell were double stranded even during the early prophase and appeared similar to the chromosomes of oocytes during the first maturation division. At prometaphase these chromosomes clearly consisted of two chromatids arranged parallel to each other (Fig. 12).

[3] Numerous males can be obtained by incubating heavily-infected roots in moist chambers for a period of 10 to 30 days. Second-stage larvae maintained in tap water at room temperature undergo three molts and develop into small sized adult males. Studies on the mechanism of sex differentiation were conducted recently and will be presented in a separate paper.

←

The prometaphase chromosomes of what has been interpreted as a "germ-line cell" from an egg at the gastrula stage. The chromosomes consist of two chromatids each and resemble those of maturing oocytes. Magnification of Figs. 8, 11 and 12 same as Fig. 9.

It is suspected that this cell is the P_3 or P_4 cell of the germ line from which the germ cell or cells of the germinal primordium of the larva will be derived eventually (see discussion).

Conclusions and Discussion

The present cytological study demonstrated that *M. floridensis* has $2n = 26$ and 27 chromosomes and reproduces by mitotic parthenogenesis. Earlier cytological work established that the closely related genus *Heterodera* has a basic chromosome number of $x = 9$, and that most of its members are amphimictic (5). If the same basic chromosome number is assumed for the genus *Meloidodera*, then *M. floridensis* can be regarded as a triploid parthenogenetic form derived from an amphimictic ancestor with $n = 9$ chromosomes.

Since no synapsis takes place during oogenesis of *M. floridensis*, no homologies among chromosomes can be established with certainty. The chromosomes that occasionally can be identified morphologically do not fall into groups of three as would be expected in a newly established triploid organism. They can more easily be classified into pairs, but still the chromosomes of each pair are not identical. This great deviation of the karyotype from the expected suggests that mitotic parthenogenesis was established quite early in the phylogenetic development of *M. floridensis*.

In the blastula stage, the prometaphase chromosomes of one blastomere consisted of two distinct chromatids each, whereas, the chromosomes of all other dividing cells were single-stranded until late metaphase. It is suspected that this special cell is the P_3 or P_4 cell of the germ line. If this is true, *M. floridensis* will be the second case, besides various ascarids, in which the chromosomes of the germ-line cells behave differently than the chromosomes of the somatic cells of the embryo. In *Parascaris equorum* and other ascarids the somatic cells undergo chromosome fragmentation and chromatin diminution so that at metaphase of each division they have many small chromosomes. In contrast to this, the cells of the germ line (P_2, P_3 or P_4) maintain the original small number of large chromosomes observed at metaphase of the first cleavage. Although much effort was made to verify this assumption through study of thousands of eggs at the blastula-gastrula stage, technical difficulties associated with staining and also the low frequency of occurrence of cells with double chromosomes did not permit further elucidation.

Literature Cited

1. Chitwood, B. G., C. I. Hannon, and R. P. Esser. 1956. A new nematode genus, *Meloidodera*, linking the genera *Heterodera* and *Meloidogyne*. Phytopathology 46:264–266.
2. Colbran, R. C. 1966. Studies of plant and soil nematodes. 12. The eucalypt cystoid nematode *Cryphodera eucalypti* n. g., n. sp. (Nematoda: Heteroderidae), a parasite of eucalypts in Queensland. Queensland J. Agr. Anim. Sci. 23:41–47.
3. Pai, S. 1928. Die Phasen des Lebenscyclus der *Anguillula aceti* Ehrbg. und ihre experimentell-morphologische Beeinflussung. Z. wiss. Zool. 131:293–344.
4. Triantaphyllou, A. C. 1963 Polyploidy and parthenogenesis in the root-knot nematode *Meloidogyne arenaria*. J. Morphol. 113:489–499.
5. Triantaphyllou, A. C. 1970. Cytogenetic aspects of evolution of the family Heteroderidae. J. Nematol. 2:26–32.
6. Triantaphyllou, A. C. and H. Hirschmann. 1966. Gametogenesis and reproduction in the wheat nematode, *Anguina tritici*. Nematologica 12:437–442.