Gametogenesis and Reproduction of Meloidogyne graminis and M. ottersoni (Nematoda: Heteroderidae)[1]

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Abstract: Oogenesis and spermatogenesis of seven populations of *Meloidogyne graminis* and one population of *M. ottersoni* (formerly *Hypsoperine* spp.) were of the meiotic type. When males were abundant, reproduction was by amphimixis. In most greenhouse cultures, however, males were rare and reproduction was by meiotic parthenogenesis. *M. graminis* and *M. ottersoni* are closely related to each other and to *M. graminicola* and *M. naasi*, but differ in some respect from other *Meloidogyne* species. It is suggested that these four species be treated together as a group of species, either in the genus *Meloidogyne* or in the genus *Hypsoperine*. *Key Words:* Parthenogenesis, polyploidy, chromosomes, phylogeny, *Hypsoperine*.

Cytological studies of various *Meloidogyne* species have been helpful in elucidating the biological status of the genus and in clarifying phylogenetic relationships among species (6). It has been realized that the establishment of various types of parthenogenesis, combined with changes of the chromosomal complement (polyploidy, aneuploidy) has played an important role in the evolution of these organisms. Furthermore, it has been recognized that the relationships among these parasites will be better understood as cytological information about additional member species becomes available. The present study attempts to compare cytologically two closely related species, which were formerly in the genus *Hypsoperine*, with other members of *Meloidogyne*.

MATERIALS AND METHODS

Seven populations of *M. graminis* and one

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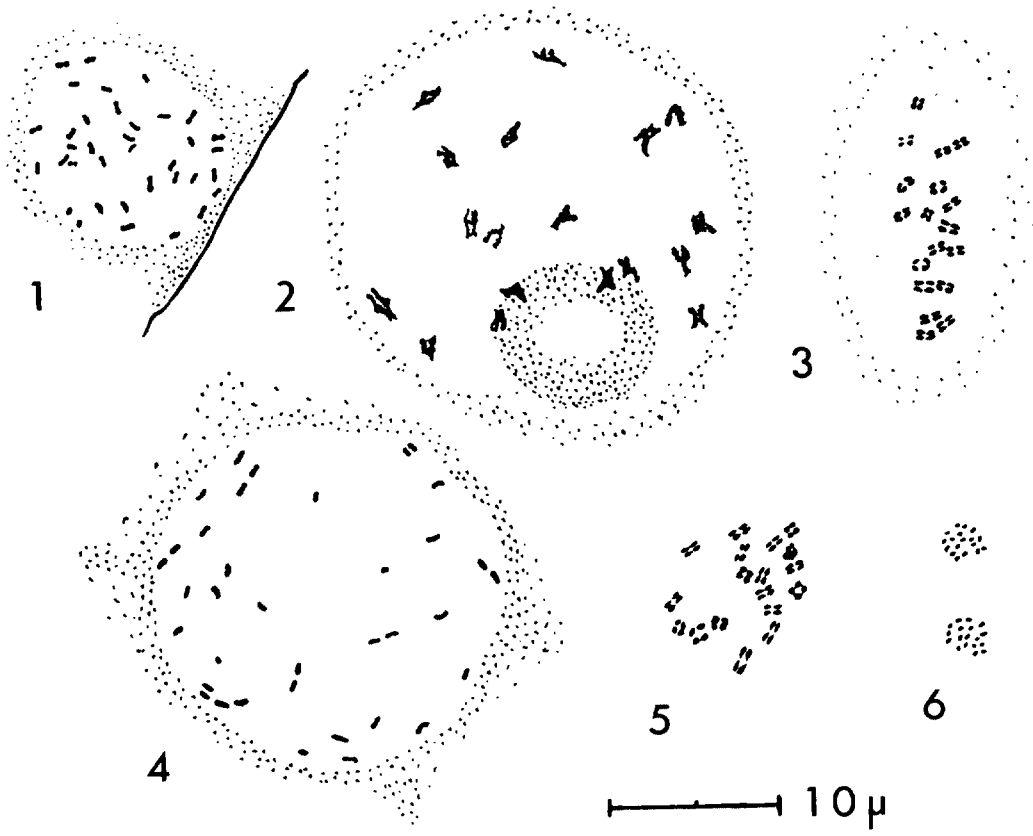

FIG. 1-6. Camera lucida drawings of *Meloidogyne graminis* and *M. ottersoni* chromosomes during oogenesis (1-4) and spermatogenesis (5, 6): **1.** Prometaphase of an oogonial division of *M. graminis* (population 193-Md.) with 36 univalent chromosomes; **2.** Late diakinesis of an oocyte of *M. graminis* (255-NC) with 18 bivalents; **3.** Metaphase I of an oocyte of *M. ottersoni* with 18 bivalents; **4.** Prophase of the first cleavage division of *M. ottersoni* with 36 chromosomes. The homologous chromosomes often appear to be closely associated with each other – arrangement in pairs; **5.** Prometaphase in a primary spermatocyte of *M. graminis* (97-Fla.) with 18 bivalents; **6.** Anaphase in a secondary spermatocyte of *M. graminis* (97-Fla.).

population of *M. ottersoni* were studied. Populations 97-Fla. of *M. graminis* from St. Augustine grass, *Stenotaphrum secundatum* (Walt.) and 237-Wisc. of *M. ottersoni* from canary grass, *Phalaris arundinacea* (L.) represent the original populations from which these species were described (3, 4). *M. graminis* populations 193-Md. from Maryland, and 262-Ark. from Arkansas were obtained from Zoysia grass, *Zoysia japonica* Steud., whereas populations 260-Va., 262-Ga., 254-Tenn. and 255-NC from Virginia, Georgia, Tennessee and North Carolina, respectively, were from Bermuda grass, *Cynodon dactylon* L. All populations were propagated in a 23-28 C greenhouse on their respective hosts for 4-8 years, and were studied cytologically shortly after they were obtained and at two-year intervals thereafter. Egg-laying females and young males from 45-day-old greenhouse cultures were processed for cytological studies as described earlier (7).

OBSERVATIONS

Oogenesis: In all populations of *M. graminis* and *M. ottersoni*, oogenesis follows exactly the same pattern as described for *M. graminicola* and *M. naasi* (5). The somatic number of 36 chromosomes was observed in numerous oogonial divisions occurring in the germinal zone of the ovary (Fig. 1). Eighteen bivalent chromosomes were counted in at least 20 oocytes of each population at late prophase and prometaphase of the first maturation division (Fig. 2, 3, 7). The chromosomes are

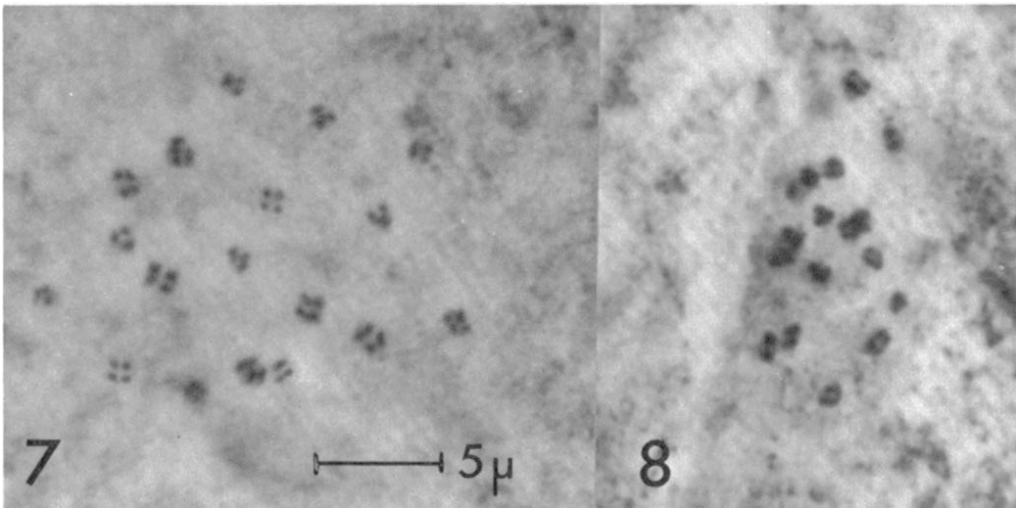

FIG. 7-8. Photomicrographs of chromosomal figures during gametogenesis of *M. graminis*: 7. Prometaphase in a primary oocyte with 18 bivalent chromosomes, each consisting of four distinct chromatids; 8. Late prophase in a primary spermatocyte with 17 chromosomes visible.

indistinguishable from those of *M. graminicola* and *M. naasi* (5). Following anaphase I, one polar nucleus is formed, and the second maturation division follows. The behavior of the chromosomes after metaphase II, and the way by which reestablishment of the somatic chromosome number occurs in maturing oocytes without the presence of sperm nuclei could not be ascertained. The somatic number of 36 chromosomes was observed in blastomeres of several eggs during early cleavage (Fig. 4).

Spermatogenesis: The process of sperm formation appears to be normal, consisting of two maturation divisions in three populations of *M. graminis* studied. Several prometaphase I figures were observed in young males of populations 97-Fla., 255-NC and 262-Ark., and all had 18 bivalent chromosomes (Fig. 5, 8). A second maturation division followed immediately after the first. The haploid number of 18 chromosomes was determined in five secondary spermatocytes at metaphase or anaphase from population 97-Fla. (Fig. 6).

Mode of reproduction: Population 97-Fla. of *M. graminis* reproduced by cross-fertilization in greenhouse cultures from 1961 to 1966. A sperm nucleus was observed in all maturing eggs and fusion of sperm and egg pronuclei was observed very frequently. No single-larva isolates were made during that period, however, to determine whether reproduction by cross-fertilization was obligatory or not. In the summer of 1966 several egg-laying females were observed with no sperm in the spermatotheca and with oocytes developing normally in the absence of a sperm nucleus. Reproduction was by meiotic parthenogenesis. This type of reproduction has been confirmed in population 97-Fla. on several occasions since 1966, but as late as 1971, most individuals of this population reproduced by cross-fertilization. Many males were always present in cultures of this population.

All other populations studied reproduced primarily by meiotic parthenogenesis and occasionally by cross-fertilization. The male to female ratio in the latter populations was always very low and appeared to increase only slightly in older cultures where the nematodes were more crowded. No attempt was made, however, to study experimentally the mechanism of sex determination or sex expression in these populations.

DISCUSSION AND CONCLUSIONS

Oogenesis and spermatogenesis of *M. graminis* and *M. ottersoni* are normal, of the meiotic type. Reproduction is by amphimixis when males are present, and by meiotic parthenogenesis when males are absent. Previous studies had shown that single-larva isolates from two populations of *M. graminis*

from Virginia and Maryland could produce progeny without insemination (9).

M. graminis and *M. ottersoni* appear to be very closely related cytologically by having indistinguishable chromosomal complements of n=18. These two species are also similar cytologically to the previously-studied *M. graminicola* and *M. naasi* (5). Furthermore, all four species have similar host ranges. They are primarily parasites of various members of the plant family Gramineae and cannot parasitize many of the common hosts of most other *Meloidogyne* species such as tomato, tobacco, cucumber, etc. Recent studies (2) revealed that hypodermal chord nuclei of preparasitic, second-stage larvae of *M. graminis, M. graminicola* and *M. ottersoni* contain approximately the same amount of DNA which is significantly smaller than that of other *Meloidogyne* species, such as *M. incognita, M. arenaria, M. javanica* and *M. hapla. M. naasi* was not included in these studies but the similarity of its karyotype with that of *M. graminicola* suggests that this species may also have similar DNA content. All this, then, suggests a close phylogenetic relationship among these four species.

M. graminis and *M. ottersoni* were originally placed in the genus *Hypsoperine* which was later synonymized with *Meloidogyne* (8). Golden (1), however, is still of the opinion that *Hypsoperine* is a valid genus and, therefore, the above species should be regarded as *Hypsoperine graminis* and *H. ottersoni*. The present study indicates a close phylogenetic relationship of *M. graminis, M. ottersoni, M. graminicola* and *M. naasi*. It is also apparent that these four species are in some respect different from the other well-known species of *Meloidogyne*. It is suggested, therefore, that these four species be treated as a homogeneous group in the genus *Meloidogyne* or in the genus *Hypsoperine*, but not separately in two different genera. Additional morphological and physiological studies will be helpful in clarifying the relationships within this group of nematodes.

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