Gametogenesis and the Chromosomes of Two Root-Knot Nematodes, *Meloidogyne graminicola* and *M. naasi*[1]

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Abstract: Oogenesis and spermatogenesis were studied in populations of *M. graminicola* and *M. naasi* from which the species were originally described. Maturation of oocytes and spermatocytes in both species was by normal meiosis. The haploid chromosome number determined during the first and second maturation divisions was n = 18 with no variation. The somatic chromosome number determined in early cleavage divisions and, to a limited extent, in oogonial divisions was 2n = 36. Reproduction was regularly by meiotic parthenogenesis in both species. Re-establishment of the somatic chromosome number in mature oocytes, apparently took place through fusion of the second polar nucleus with the egg pronucleus. Occasional reproduction by cross-fertilization was demonstrated in *M. graminicola* and it is suspected in *M. naasi* in cultures with abundant males. Phylogenetic relationships in the family Heteroderidae are discussed in the light of the new cytological information. The peculiar behavior of nucleoli persisting during metaphase, anaphase and telophase of cleavage divisions is reported.

Cytological studies with various species of *Meloidogyne* Goeldi and *Heterodera* A. Schmidt in the last few years (7) have provided valuable information with regard to the phylogeny within the family Heteroderidae (Filipjev). Studies of various *Heterodera* species have indicated that the basic chromosome number in this genus is x = 9 and that parthenogenetic species with approximately 27 chromosomes are triploids. Studies in the genus *Meloidogyne* have revealed that populations of *M. hapla* Chitwood (race A) which undergo meiosis during maturation of the oocytes have a chromosome number of n = 15 to 17, whereas, species that reproduce by mitotic parthenogenesis have a somatic chromosome number of 2n = 31 or higher. Thus, a population of an undescribed *Meloidogyne* species studied recently has 2n = 31 chromosomes, *M. arenaria* (Neal) Chitwood has 2n = 36 or 2n = 51 to 54 (5), *M. hapla* (race B) has 2n = 45 (6), *M. javanica* (Treub) Chitwood has 2n = 43 to 48 (4), and *M. incognita* (Kofoid and White) Chitwood has 2n = 41 to 44 (unpublished information). Interpretation of the cytological situation in the genus *Meloidogyne* is difficult and several alternative hypotheses have been proposed. According to one hypothesis, the basic chromosome number in *Meloidogyne* is the same as in *Heterodera,* i.e. x = 9, and all *Meloidogyne* species, studied thus far, are polyploid or aneuploid derivatives of polyploids. *M. hapla* with n = 15, 16 and 17 chromosomes is recognized as representing a series of hypotetraploid forms, probably derived from tetraploid ancestors with n = 18 chromosomes. However, no *Meloidogyne* species with n = 18 chromosomes had been found at the time this hypothesis was formulated. Subsequent cytological studies with numerous populations of various *Meloidogyne* species revealed that *M. graminicola* Golden and Birchfield, and *M. naasi* Franklin, have indeed 18 chromosomes. The cytological observations with these two species are presented and evaluated in this article.

Received for publication 8 July 1968.

[1] Paper number 2676 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina. This study was supported by a grant of the National Science Foundation (GB-7214).

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MATERIALS AND METHODS

The population of *M. graminicola* used in this study was a greenhouse culture of the original species described by Birchfield and Golden (2). *M. naasi* (1) was furnished by Dr. Mary Franklin of Rothamsted

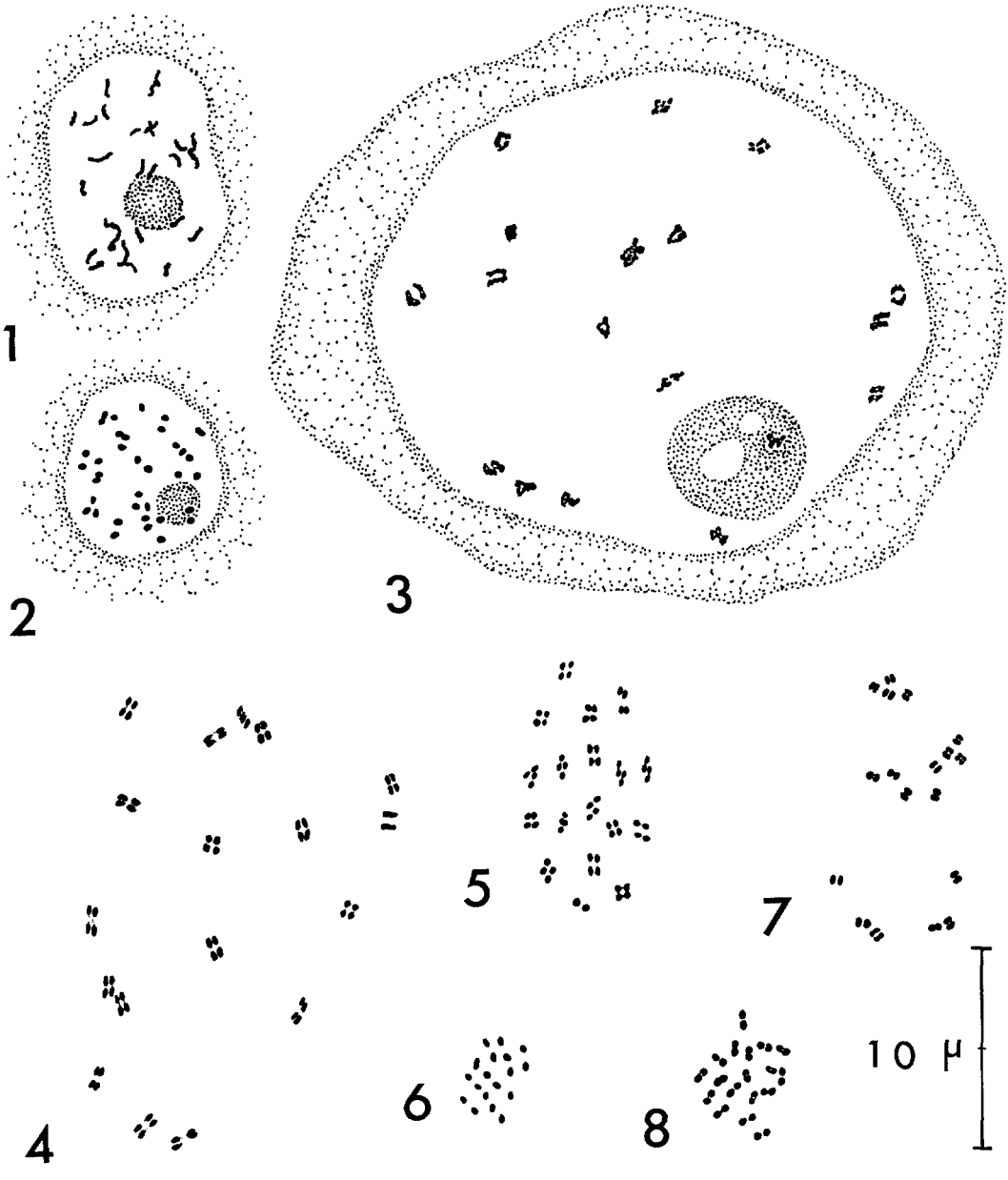

FIG. 1–8. Camera lucida drawings illustrating gametogenesis in *M. graminicola* and *M. naasi*: 1 and 2. Respectively, early and late prophases of oogonial divisions of *M. graminicola* showing comparative chromosome morphology; 3. Advanced diakinesis in an oocyte of *M. graminicola*. In reality the chromosomes are arranged close to the periphery of the nucleus; 4 and 5. Prometaphase 1 chromosomes of *M. graminicola* and *M. naasi* respectively, showing details of their morphology; 6. The 18 telophase 1 chromosomes that form the first polar nucleus in *M. naasi* oocytes; 7 and 8. Prometaphase chromosomes in primary spermatocytes of *M. graminicola* and *M. naasi*, respectively.

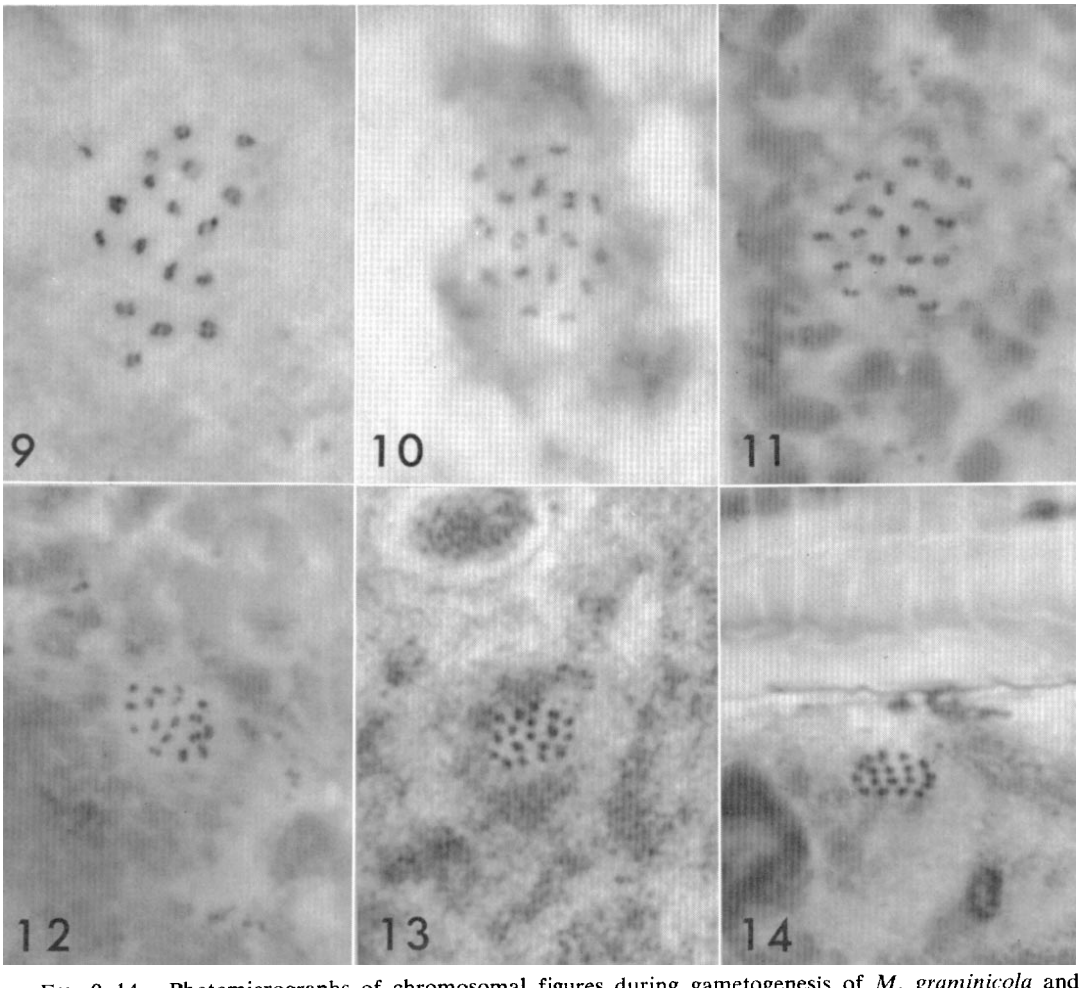

FIG. 9–14. Photomicrographs of chromosomal figures during gametogenesis of *M. graminicola* and *M. naasi*: 9 and 10. Prometaphase chromosomes in primary oocytes of *M. graminicola* and *M. naasi*, respectively; 11. The 18 metaphase I chromosomes in an oocyte of *M. naasi*; 12. Early telophase I figure in an oocyte of *M. naasi*; 13 and 14. Telophase I or metaphase II figures during spermatogenesis of *M. graminicola*. × 2850.

Experimental Station, England. Both nematode species were propagated in the greenhouse; *M. graminicola* on the grass *Echinochloa colonum* L. and *M. naasi* on barley (*Hordeum vulgare* L.). Young female and male nematodes were obtained from 45 to 60 day-old greenhouse cultures and processed for cytological study as described earlier (8) for *Anguina tritici* (Steinbuch) Chitwood. The eggs, however, were subjected to more severe treatment with sodium hypochlorite (30 min in 4.5% solution) than in the case of *Anguina tritici*.

OBSERVATIONS

OOGENESIS: The reproductive system of *M. graminicola* and *M. naasi* is morphologically similar to that of *M. javanica* (4). Oogenesis in these two species follows the same pattern observed in populations of *M.*

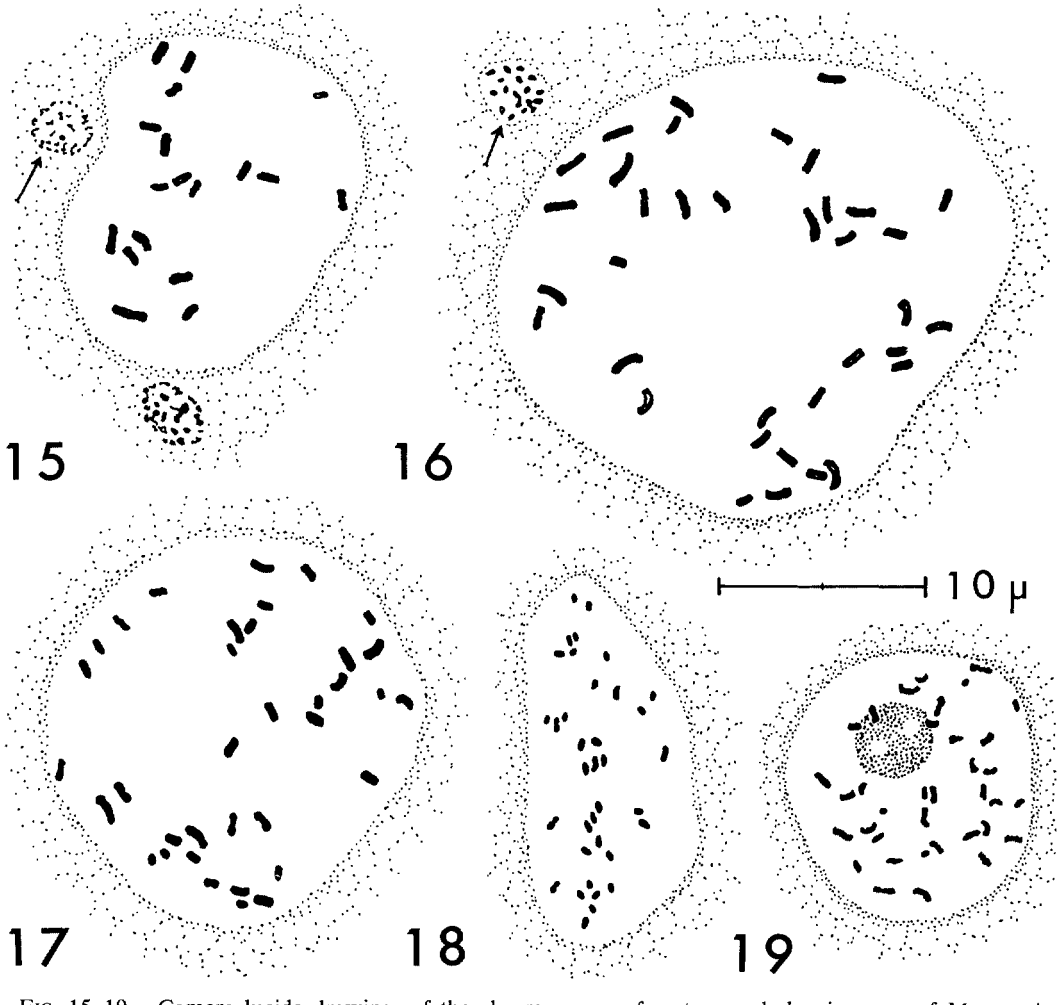

FIG. 15–19. Camera lucida drawings of the chromosomes of mature and cleaving eggs of *M. graminicola* (15, 16, 17, 19) and *M. naasi* (18): 15. Egg pronucleus shortly after its formation, with 18 chromosomes. The second polar nucleus (arrow) is closely associated with the egg pronucleus. The first polar nucleus is also visible; 16. Advanced egg pronucleus with 36 chromosomes. The first polar nucleus is still visible (arrow). The second polar nucleus probably fused with the egg pronucleus and contributed its chromosomes for re-establishment of the somatic number; 17. Late prophase figure during the second cleavage division with 36 chromosomes; 18. Prometaphase chromosomes in a nucleus from a 12-celled egg of *M. naasi*; 19. Prophase figure from a 16-celled egg of *M. graminicola* showing the 36 chromosomes and a large nucleolus.

hapla (race A) that undergo meiosis (6). Oogonial divisions take place in the germinal zone of the ovaries of young females. The chromosomes appear as elongated threads at early prophase (Fig. 1), but become contracted and assume the shape of small spheres at prometaphase (Fig. 2). Only one definite chromosomal count was made at this stage in *M. graminicola* and the chromosome number was found to be $2n = 36$. In three other oogonial prometaphases the number was approximately 36. More than

ten figures of oogonial divisions were scored in *M. naasi* and had approximately 36 chromosomes.

The process of synapsis and growth of the oocytes in both species followed the same pattern observed in *M. hapla* (6). Half-grown oocytes, located in the posterior end of the ovaries close to the oviducts, are at advanced diakinesis. The chromosomes are distinct and usually are distributed near the periphery of the large nucleus (Fig. 3). One large nucleolus is also visible.

The best stage for observing and counting the chromosomes is prometaphase of the first maturation division. It is encountered in oocytes that pass from the spermatotheca into the uterus. Eighteen bivalent chromosomes were observed in at least 40 prometaphase or metaphase figures from each of the two species studied. Camera lucida drawings of the prometaphase chromosomes of each species showing details of their structure are presented in Figs. 4 and 5. Photomicrographs of similar prometaphase figures are shown in Figs. 9 and 10. The prometaphase chromosomes of both these species are very similar and are also indistinguishable from those of *M. hapla*. In all three species they are quadripartite in nature (tetrads); each one of the four components represents a single chromatid. At metaphase I they are highly contracted and appear as in Fig. 11. At telophase I, the chromosomes of each telophase plate are bipartite (dyads, Fig. 12). The telophase chromosomes that will form the first polar nucleus maintain their stainability by contracting further (Fig. 6), and usually remain visible until the egg advances to the first or second cleavage division (Fig. 22—arrow). No polar body appears to be extruded from the egg cytoplasm, although in most cases the first polar nucleus is located very close to the periphery of the egg. The telophase I chromosomes of the egg enter the second maturation division directly, i.e., without intermediate interphase or prophase stages. Following completion of the second maturation division, the egg chromosomes become diffuse and disappear, whereas, the second polar nucleus remains faintly visible and usually stays closely associated with the forming egg pronucleus. At a slightly more advanced stage, the stainability of the entire egg is reduced considerably, probably because the permeability of the egg membranes to the fixative and stain is diminished. Consequently, the polar nuclei cannot always be seen and, the second polar nucleus, in particular, may disappear completely. The egg pronucleus at this stage is very distinct and appears as a clear spherical area surrounded by condensed cytoplasm (Fig. 20). Shortly afterwards the chromosomes reappear in the egg pronucleus (Figs. 15, 21). The haploid number of 18 chromosomes was present in approximately one-half of the egg pronuclei scored. In the other half, the diploid number of 36 chromosomes was observed (Figs. 16, 22). The eggs with the diploid number probably represent a more advanced stage of development, although this could not be substantiated by other cytological or morphological characteristics. How the diploid chromosome number was established within the formerly haploid nuclei is not yet clear

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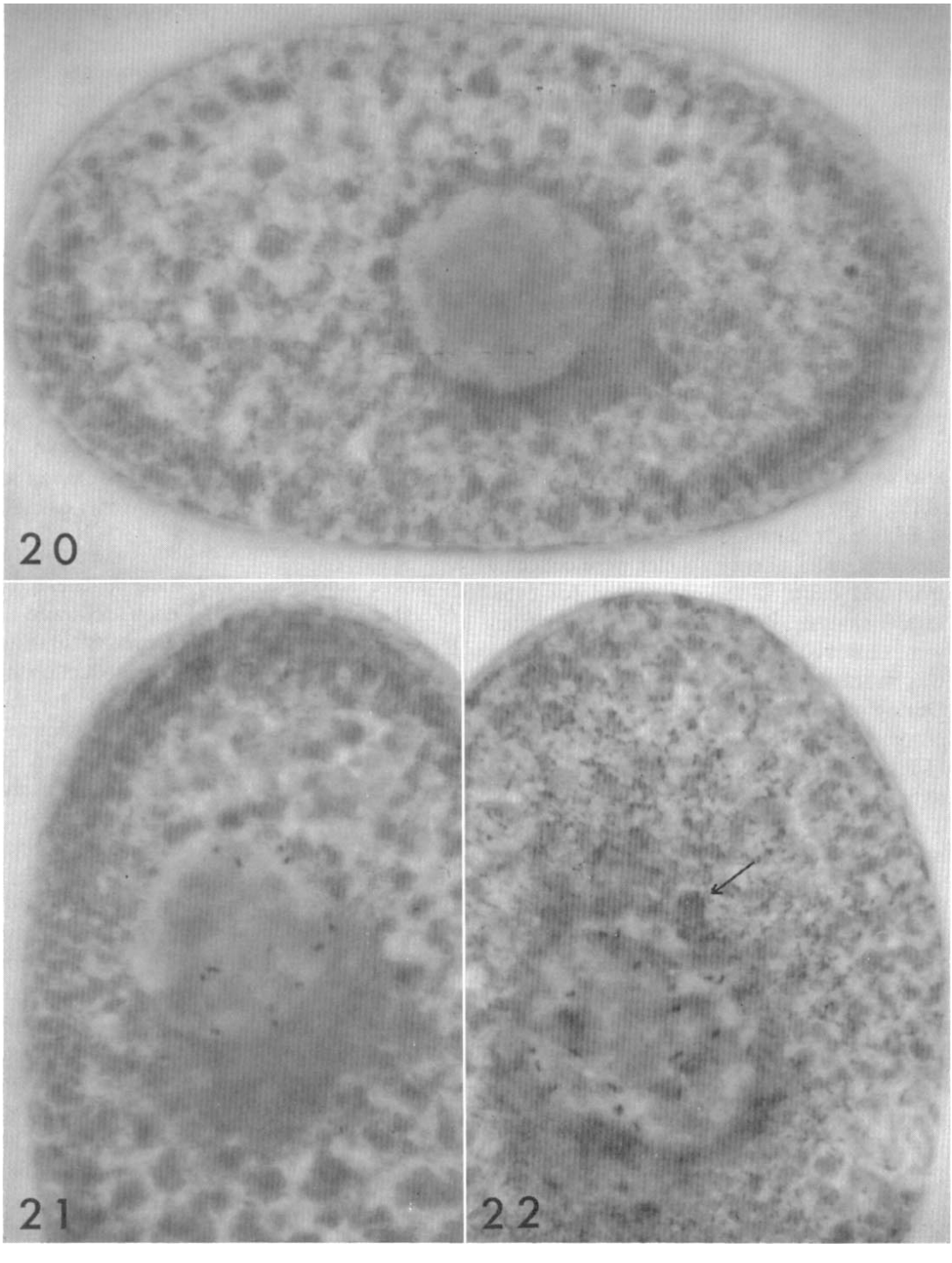
FIG. 20–22. Photomicrographs of mature eggs of *M. graminicola* illustrating the various steps in the process of re-establishment of the somatic chromosome number: 20. Egg with the egg pronucleus just formed. No chromosomes visible in egg pronucleus; 21. Slightly advanced egg with 18 chromosomes visible in the egg pronucleus; 22. A more advanced egg with 36 chromosomes in the egg pronucleus. The additional set of 18 chromosomes is believed to have been contributed by the second polar nucleus. The first polar nucleus is also visible (arrow). × 2400.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(see discussion). Completion of the first cleavage division and subsequent cleavages were normal, although the following peculiarity was noticed. The nucleolus present in prophase nuclei of the first and subsequent cleavage divisions often persisted during metaphase and anaphase (Figs. 23, 24—arrows), and was located outside the metaphase plate and approximately in the same plane with the metaphase chromosomes. During cytokinesis it was included in one of the blastomeres. It is not certain whether this peculiarity, which has been encountered in some other animals (3), has been reported previously for nematodes.

Precise chromosome counts were made also in prophase nuclei of eggs which were in the 2- to 16-cell stage (Figs. 17, 18, 19).

SPERMATOGENESIS: Approximately one hundred males of each species were collected from old cultures and were used for cytological analysis. No spermatogonial divisions were observed. Maturation of spermatocytes was normal, consisting of two divisions. During prometaphase or metaphase I, 18 chromosomes were observed in both species (Figs. 7, 8). The chromosomes were relatively more contracted than those of oocytes. The second maturation division took place immediately after the first. Chromosomal figures at late telophase I or metaphase II were the best for chromosome counts. They were oriented usually in polar view and the chromosomes were highly contracted and well separated from each other (Figs. 13, 14). Eighteen chromosomes were counted in all such figures.

MODE OF REPRODUCTION: Males were rare in cultures of these species, and sperm was observed in the spermatothecae of females on very few occasions. Among the several thousand advanced oocytes or uncleaved eggs examined, only four contained a sperm nucleus, which was still located at one pole of the egg. No sperm pronuclei were seen in any egg. In order to increase the probability of ascertaining the role of the sperm in reproduction, only eggs from females that were associated with males were used in later studies. It was found that nearly all of these eggs were developing parthenogenetically as described earlier. However, of approximately 600 uncleaved eggs of *M. graminicola* in which an egg pronucleus was observed, three eggs had a sperm pronucleus. In two of them the sperm and egg pronuclei were in the process of fusing into a zygote nucleus, and in the other the pronuclei were still separated. This demonstrates that cross-fertilization does occur in *M. graminicola,* although rarely. A similar study with eggs of *M. naasi* failed to demonstrate cross-fertilization in this species.

DISCUSSION

These studies showed that oogenesis in *M. graminicola* and *M. naasi* followed a normal pattern. It included two maturation divisions which resulted in the reduction of the somatic chromosome number to the haploid condition (n = 18), and the formation of two polar nuclei. Reproduction in both species was by meiotic parthenogenesis, but in addition, it was demonstrated that cross-fertilization occurs occasionally in *M. graminicola.* It is suspected that cross-fertilization takes place sporadically also in *M. naasi* but this was not demonstrated in the limited amount of material available for study.

The method by which the somatic chromosome number was re-established in parthenogenetically developing eggs is not known. When the egg pronucleus was formed, no chromosomes were discernible in it. The second polar nucleus at that time was either closely associated with the egg pronucleus or it had disappeared completely. In more advanced eggs two situations were encountered with respect to the chromosome num-

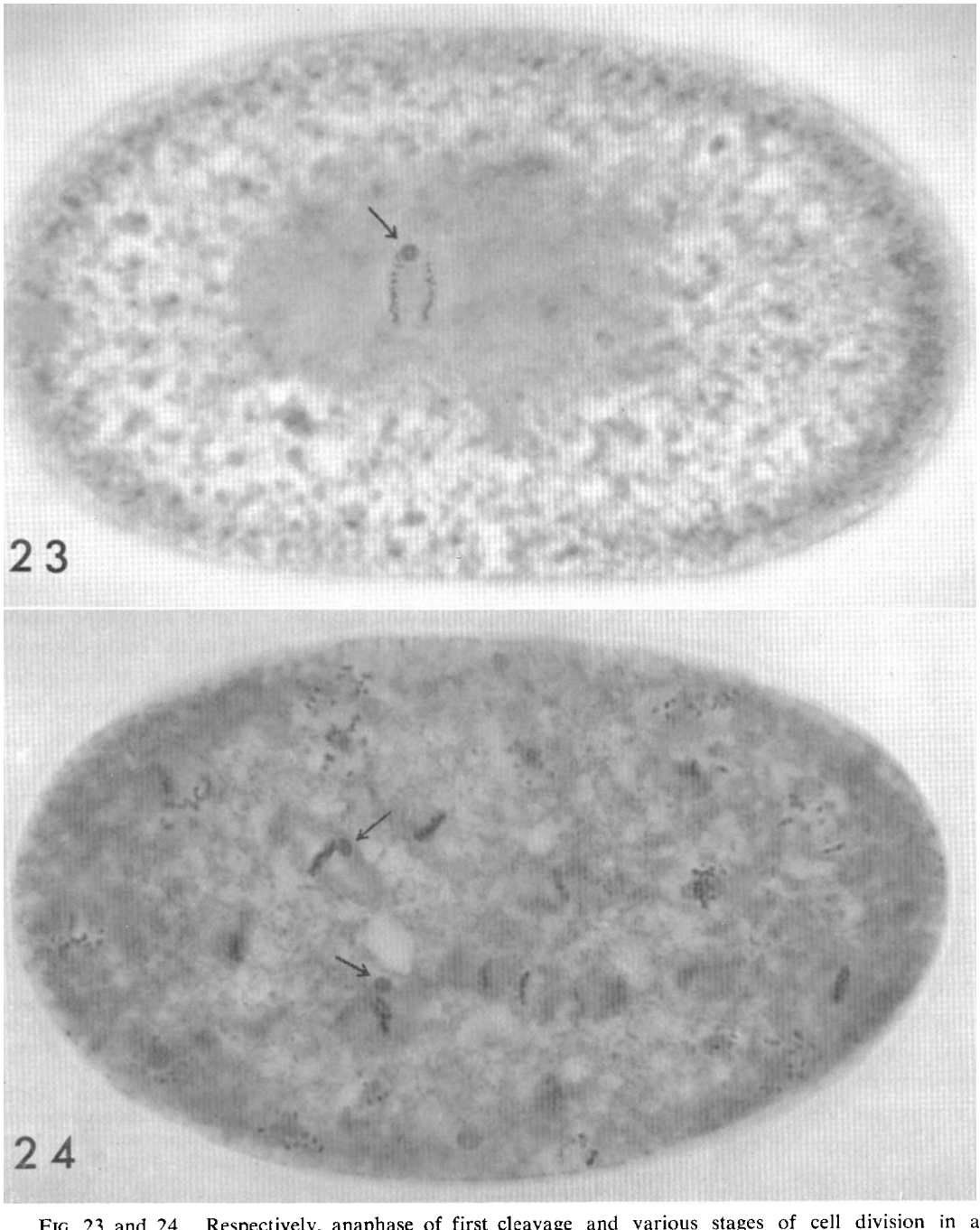

FIG. 23 and 24. Respectively, anaphase of first cleavage and various stages of cell division in a multi-celled egg of *M. graminicola*. The arrows point to the nucleoli which persist during metaphase and later stages of cell division. × 2400.

ber. Some eggs had the haploid number of 18 chromosomes, others had the diploid number of 36 chromosomes. Since it was not possible to determine the relative state of development of the two types of eggs, it may be assumed that the egg pronucleus originally had the haploid number of chromosomes and that the diploid number was re-established later. How this was accomplished is not clear. It is possible that the chromosomes of the egg pronucleus divided once before the first cleavage took place. However, nothing was observed that could support or even suggest the occurrence of endomitosis in the egg pronucleus. It is more likely that the second polar nucleus fused with the egg pronucleus. Actual fusion was never observed, probably because the stainability of the polar nucleus after the formation of the egg pronucleus was very poor. It was observed, however, that the egg pronucleus was vaguely subdivided in some cases into a larger and a smaller portion, each containing approximately the haploid number of chromosomes. This observation tends to support the assumption that fusion of the polar nucleus with the egg pronucleus took place.

The discovery of these two species with n = 18 chromosomes undoubtedly strengthens our previously expressed view concerning polyploidy in the genus *Meloidogyne*. According to this view, the basic chromosome number of the ancestor of these organisms may have been x = 9 as represented by the majority of the species of *Heterodera*. The various species of *Meloidogyne*, including *M. hapla*, probably represent polyploids, or aneuploid forms derived from polyploid ones. This discovery, however, does not exclude the possibility that the common ancestor of these organisms had a chromosome number of x = 18. It is possible that the n = 9 condition was derived from n = 18 through centric fusions or other processes of chromosomal number reduction. However, it will be difficult to justify why the reduced chromosome number became stabilized at exactly one half the original number rather than some other number. Populations of *M. hapla* with a chromosome number smaller than 18 (n = 17, 16 and 15) probably have evolved from forms with n = 18 chromosomes. *M. hapla* then shows the trend of chromosomal number reduction that may have played a role in karyotype evolution in this group of organisms. Also, the various chromosomal forms of higher degrees of ploidy encountered in mitotically parthenogenetic species of *Meloidogyne* may have been derived through fusion of gametes of reduced, unreduced or modified chromosome numbers (7).

Until more evidence becomes available regarding the real course of karyotype evolution, we may regard 18 as the basic chromosome number for the genus *Meloidogyne* and 9 for the genus *Heterodera*, as was proposed earlier (7), without reference to the true relationship between the karyotypes of the two genera. Comparative DNA measurements now underway in our laboratory with various members of the two genera should assist in interpreting karyotype relationships between the two genera.

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