Polyploidy in meiotic parthenogenetic populations of *Meloidogyne hapla* and a mechanism of conversion to diploidy

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SUMMARY

A tetraploid isolate (n = 34) was obtained from a diploid (n = 17) population of *M. hapla*. Tetraploid females produced, by meiotic parthenogenesis, tetraploid (78%) and diploid (22%) progeny. Tetraploid progeny derived from oocytes in which the products of the second maturation division fused to form an egg pronucleus with 68 chromosomes. Diploid progeny developed from tetraploid oocytes, following the expulsion of the second polar nucleus as a polar body and the formation of an egg pronucleus with 34 chromosomes. The tetraploid isolate was maintained successfully on tomato for fifteen generations by establishment of new cultures after each generation, with selected egg masses produced by tetraploid females serving as inoculum. Without such selection, no tetraploids could be detected in two cultures after five generations of propagation. Tetraploidy was detected also in another three among twenty meiotic populations of *M. hapla* from sixteen countries surveyed. The frequent occurrence of polyploids in *M. hapla*, and the fact that the great majority of nematodes have a haploid number of less than nine chromosomes, allows one to speculate that the entire genus *Meloidogyne* derived following polyploidization that possibly occurred during a parthenogenetic phase of its evolution. Subsequently, forms, such as *M. carolinensis*, *M. microtyla*, *M. megatyla* and others, converted to amphimixis and became highly host specific ; others, such as the four major species of root-knot nematodes, continued evolving within the parthenogenetic phase, maintained a wider host range and underwent an extensive cytogenetic diversification involving aneuploidy and higher degrees of ploidy.

RÉSUMÉ

La polyploïdie chez les populations de Meloidogyne hapla *à parthénogenèse meiotique et le mécanisme de la conversion à la diploïdie*

Une souche tétraploïde (n = 34) a été obtenue à partir d'une population diploïde (n = 17) de *M. hapla*. Par parthénogenèse meïotique, les femelles tétraploïdes ont produit une descendance à 78% tétraploïde et à 22% diploïde. La descendance tétraploïde provient d'oocytes dans lesquels les produits de la seconde division de maturation ont fusionné pour former un pronucleus à 68 chromosomes. La descendance diploïde s'est développée à partir d'oocytes tétraploïdes après l'expulsion du second noyau dans le globule polaire et la formation d'un pronucleus à 34 chromosomes. La souche tétraploïde a été maintenue avec succès sur tomate pendant quinze générations en faisant une nouvelle culture après chaque génération, à l'aide de masses d'œufs sélectionnées produites par des femelles tétraploïdes. En l'absence d'une telle sélection, aucun tétraploïde n'a pu être détecté dans deux élevages après cinq générations. La tétraploïdie a également été observée chez trois autres populations de *M. hapla* sur les vingt populations étudiées provenant de seize pays. L'apparition fréquente de polyploïdes chez *M. hapla* et le fait que la grande majorité des nématodes ont un nombre haploïde de chromosomes inférieur à neuf permettent de supposer que le genre *Meloidogyne* a été formé par une polyploïdisation qui peut avoir eu lieu pendant une phase parthénogénétique de son évolution. Par la suite, des formes telles que *M. carolinensis*, *M. microtyla*, *M. megatyla* et autres sont retournés à l'amphimixie et ont acquis une haute spécificité parasitaire, alors que d'autres, telles les quatre principales espèces, restaient à la phase parthénogénétique, gardaient une gamme d'hôtes très étendue et subissaient une large diversification cytogénétique comprenant l'aneuploïdie et des degrés élevés de ploïdie.

Meloidogyne hapla is a species complex that includes two cytogenetic races, recognized on the basis of the pattern of maturation of oocytes. Race A undergoes meiosis and exhibits bivalent chromosomes in the haploid number at metaphase of the first maturation division. Race B does not undergo meiosis, and shows the somatic number of univalent chromosomes at metaphase of the single maturation division

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(Triantaphyllou, 1966). All populations of race A, studied thus far, have somatic chromosome numbers varying from 28 to 34 (n = 14 to 17) and are considered to be diploid or aneuploid derived from ancestral forms with n = 18 (Triantaphyllou & Hirschmann, 1980). Some populations of race B are diploid with 2n = 30 to 32 chromosomes, but most populations are polyploid or polyploid derivatives with 2n = 45 to 48.

Occasionally, giant second-stage (polyploid ?) juveniles are observed among the normal-size juveniles of many *Meloidogyne* and some *Heterodera* species (Triantaphyllou & Hirschmann, 1978). The nature of these giant juveniles is not known, but they are suspected to develop from giant eggs, commonly present in egg masses of these species. Cytological observations of *M. incognita* and *M. arenaria* have revealed that giant eggs result from unequal cytokineses during the last oogonial divisions, which give rise to large and small primary oocytes. Giant juveniles, hatched from giant eggs in all these cases, usually develop to normal diploid females that produce juveniles of normal size. An exception was observed in a population of *M. hapla* from South Korea in which most of the juveniles that hatched from certain egg masses were of large size. Preliminary cytological observations of females developing from such large-size juveniles indicated a higher haploid chromosome number, compared to numbers encountered earlier in this population or in other populations of *M. hapla*. The results of a more comprehensive cytogenetic study establishing the polyploid nature of this population are reported in this article. Furthermore, an extensive survey involving about twenty populations of *M. hapla* from many parts of the world was conducted to determine how common polyploidy is in meiotic populations of *M. hapla* and what its evolutionary significance is. Karyotype analysis of the same tetraploid population by electron microscopy has already been reported (Goldstein & Triantaphyllou, 1981).

Materials and methods

Meloidogyne hapla of the present study (population E24 of the International *Meloidogyne* Project collection), originated from South Korea and was received in 1978 on infected roots of *Arctium* sp. (edible burduck). It was identified to species on the basis of perineal pattern morphology, host range, esterase pattern and cytogenetic characteristics. The haploid chromosome number was 17, and maturation of oocytes was by meiosis (race A). Reproduction was primarily by meiotic parthenogenesis and occasionally by amphimixis. The population was maintained as a greenhouse culture on Rutgers tomato at 23 to 28°.

The polyploid isolate (E24P) was established by inoculating a tomato seedling with about 200 large-size juveniles obtained from a single egg mass of population E24. The progeny of the polyploid isolate were studied cytologically, and the egg masses from three polyploid females were used to inoculate individual tomato seedlings. Similar egg-mass isolations were made from polyploid progeny for four consecutive generations, and the percentage of polyploid or diploid progeny of a given polyploid female was determined.

For the cytological study, egg-laying females were processed and stained with propionic orcein as described earlier (Triantaphyllou, 1979).

The ability of the polyploid to maintain itself in culture was determined by propagation of the juveniles produced by two polyploid females for two generations on tomato. The degree of ploidy of 50 females was then determined cytologically. Further, the same cultures were maintained in the greenhouse for an additional nine months. They were reestablished on tomatoes every three months using as inoculum 5 000 juveniles extracted from infected roots. The ploidy state of 200 females was redetermined at the end of this nine-month period.

Results

Cytological observations

Cytological study of 40 females of population E24 revealed that all had 17 bivalent chromosomes at metaphase of the 1st maturation division of the oocytes (Fig. 1A). A second maturation division took place and the second polar nucleus fused with the egg pronucleus to reestablish the diploid chromosomal complement. The diploid number of 34 was verified in several eggs during the first and second cleavage divisions as well as in oogonial cells of the ovaries of many females (Fig. 2 A).

Three satisfactory chromosomal figures were found during the cytological study of the female whose oversized juveniles were used to initiate the polyploid isolate E24P. One oogonial metaphase figure had 68 compact, rod-shaped chromosomes (Fig. 2 B), whereas, two metaphase I figures in oocytes had 34 bivalent chromosomes (Fig. 1 B). No sperm was present either in the spermatothecae or inside the oocytes, suggesting parthenogenetic reproduction.

In the following generation, some of the progeny of the original polyploid female were polyploid with n = 34 (Fig. 1 C, D), and others were diploid with n = 17 chromosomes. These results suggested that a conversion from polyploidy to diploidy was taking

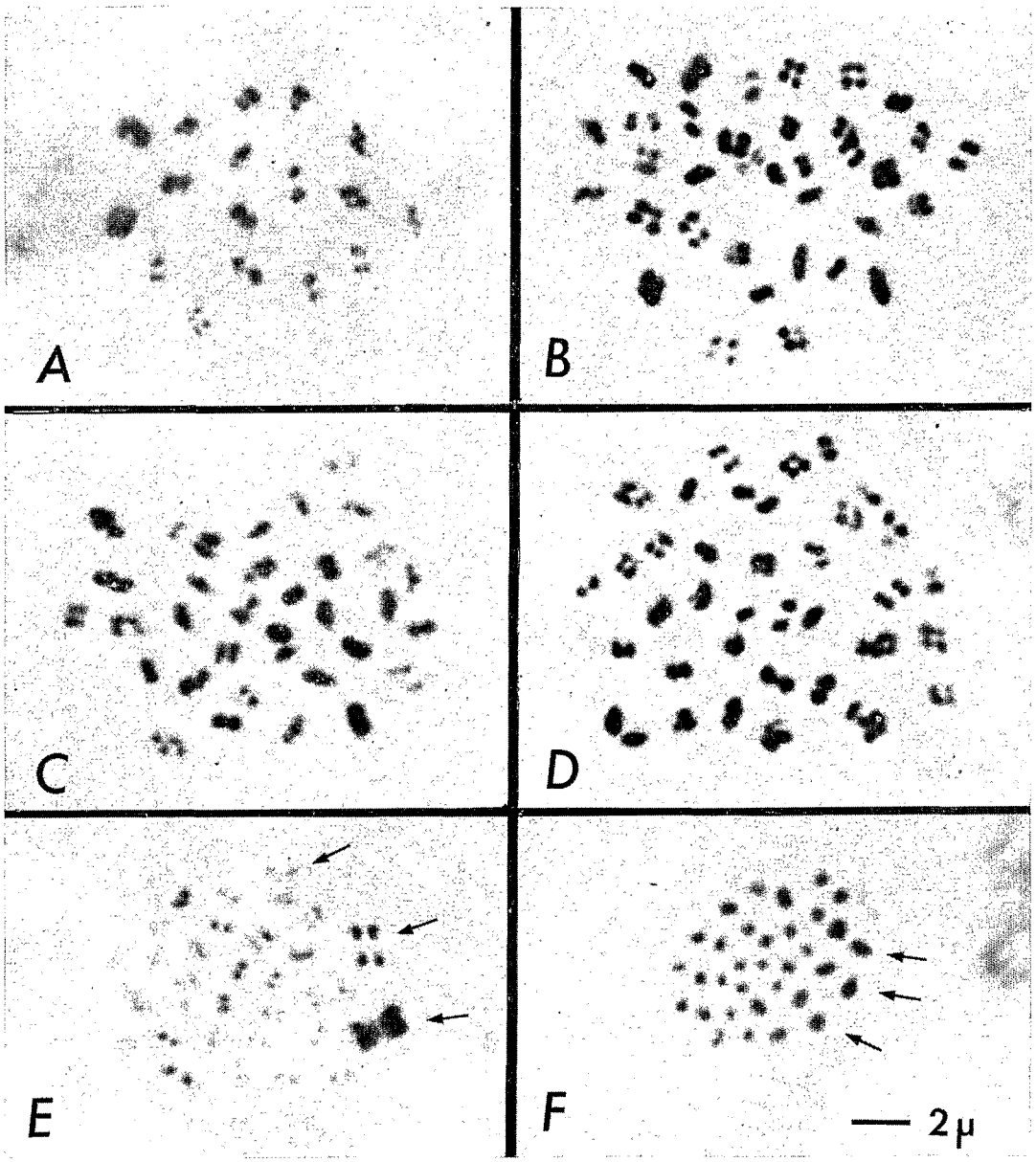

Fig. 1. Photomicrographs of the chromosomes in maturing oocytes of diploid (A) and tetraploid (B-F) *M. hapla*. A : The 17 bivalents of the diploid at metaphase I. B-D : The 34 bivalents of the tetraploid at metaphase I. E-F : Metaphase, or early anaphase-II chromosomes in the tetraploid. The arrows point to six large chromosomes which are arranged in three pairs, presumably representing pairs of homologues. Apparently, homologous chromosomes of the two haploid sets that constitute the tetraploid form are attracted to one another and are closely associated during the meiotic process, although they do not undergo synapsis and do not form quadrivalents.

place. The cytological mechanism involved in this conversion was studied in the next generation by following the process of oocyte maturation after metaphase I. The first maturation division was normal in all oocytes of a polyploid female and resulted in the formation of the first polar body (Fig. 3 A). A second maturation division also took place, but maturation of the oocytes followed two different pathways after telophase II :

1) The second polar nucleus, with 34 rod-shaped chromosomes, was not extruded as a polar body. It remained in the cytoplasm and, eventually, fused with the egg telophase-II plate, which also had 34 rod-shaped chromosomes. Thus, the egg pronucleus was formed by the fusion of the products of the second maturation division (Fig. 3 B). Later, oocytes proceeded with the first cleavage division during which 68 chromosomes could be observed in a few cases.

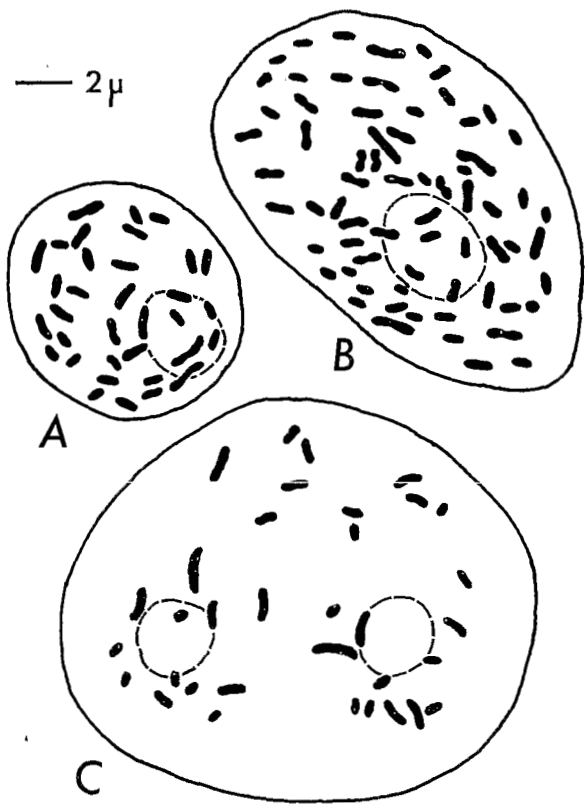

Fig. 2. Camera lucida drawings of the prophase chromosomes of diploid and tetraploid *M. hapla*. A : Nucleus of an oogonium at prophase of a mitotic division from a diploid female with 2n = 34 chromosomes. B : Nucleus of an oogonium at prophase from a tetraploid female with 4n = 68 chromosomes. C : Nucleus at prophase of 1st cleavage in a diploid egg (2n = 34) produced by a tetraploid female (4n = 68). There are two nucleoli, each one surrounded by a group of 17 chromosomes, presumably corresponding to the haploid sets of chromosomes from which the tetraploid derived. Segregation of the two sets, usually, is not perfect.

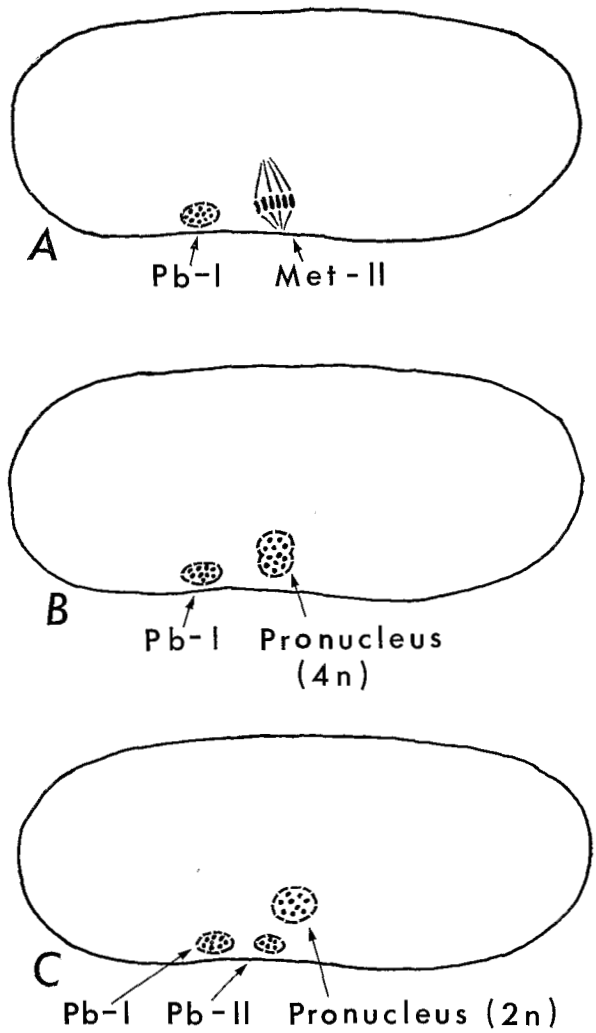

Fig. 3. Two pathways of maturation of oocytes of tetraploid *M. hapla*, leading to the production of tetraploid and diploid eggs (diagrammatic). A : Oocyte at metaphase II with one polar body already formed. All oocytes of tetraploid females advance to this stage. B : Tetraploid eggs are produced when the products of the second maturation division are enclosed in one pronucleus which, thus, has 68 chromosomes (34 from each telophase-II plate). C : Diploid eggs are produced when the second maturation division results in the formation of a second polar body and an egg pronucleus. The latter has 34 chromosomes.

Apparently this process of maturation maintains the tetraploid state.

2) The second polar nucleus was extruded from the cytoplasm as a polar body (Fig. 3 C). Consequently, the oocyte was left with the 34 telophase-II chromosomes which eventually were included in the egg pronucleus. Soon after the formation of a pronucleus the egg proceeded with the first cleavage. This process of maturation reduced the somatic chromosome number to one half of the original number and, thus, converted tetraploid oocytes to diploid eggs.

At metaphase II of tetraploid oocytes, morphologically similar chromosomes (homologues ?) were positioned close to each other. This orderly arrangement was particularly obvious among the three or four larger pairs of chromosomes which were distinct from the rest of the chromosomes (Figs. 1 E, F ; arrows). In some cases, there was even evidence of physical contact between these chromosomes.

In many diploid eggs (2n = 34) produced by tetraploid females, the chromosomes at prophase of the first cleavage tended to become arranged in two groups, each one with 17 chromosomes and one nucleolus (Fig. 2 C). In females developing from such eggs, and generally in diploid females derived from tetraploid parents, pairing of the chromosomes was always normal, resulting in the formation of 17 bivalent configurations at metaphase I. The cytological significance of these observations is discussed later.

Morphometrics

The tetraploid isolate was characterized by larger measurements of five characters of second stage juveniles (Tab. 1). The most useful morphometric character for distinguishing the isolates under the stereoscope was body length, with differences up to 20%. Females of the two isolates produced approximately the same number of eggs on tomato within 45 days from inoculation.

Conversion frequency of polyploids to diploids

Of the 28 females that developed from the original polyploid isolate E24P, twenty-two were tetraploid, and six were diploid (Tab. 2). These initial data suggested that conversion to diploidy does occur. For assessment of the rate of conversion, egg masses from tetraploid females were used as inoculum in the establishment of new isolates. Such egg-mass isolates were examined cytologically 45 days later to determine what proportion of the female progeny were diploid. Similar egg-mass isolations from polyploid females were continued for four consecutive generations (Tab. 2). The rate of conversion from polyploidy to diploidy varied from 10 to 40%, with an average of approximately 22%. This means that under the greenhouse conditions of these tests, about 78% of the progeny of polyploid females maintained their polyploid nature.

Ability of tetraploids to maintain themselves in cultures in the absence of artificial selection

When all egg masses produced by the progeny of two tetraploid females were used as inoculum in establishing new cultures on tomatoes, about 80% of the females recovered from these cultures 70 days later were diploid. The excessively high proportion of diploids in the second generation may have been due primarily to the progeny of the first-generation diploid females.

The ability of the tetraploids to maintain themselves in culture, in the absence of any directed selection, was tested by continued maintenance of the greenhouse cultures for another nine months. No tetraploid females were found among the 200 females studied cytologically at the end of that period. Apparently, the conversion from polyploidy to diploidy decreased the proportion of polyploids to an undetectable level and/or the ability of the tetraploids to compete with the diploids was diminished.

Table 1

Egg counts and morphometrics of second-stage juveniles of diploid and tetraploid Meloidogyne hapla. [a]

Character	*Diploid isolate*	*Tetraploid isolate*
1. Body length (µm)	405.4 ± 13.41[b]	510.4 ± 15.89
2. Tail length	51.1 ± 7.31	68.0 ± 3.40
3. Tail terminus length	12.9 ± 0.72	15.1 ± 0.85
4. Distance of dorsal esophageal gland orifice from stylet	3.6 ± 0.32	4.1 ± 0.50
5. Head end to base of stylet knobs	14.7 ± 0.21	17.0 ± 0.25
6. Number of eggs per egg mass	456.0 ± 52.00	420.0 ± 65.00

[a] Data based on measurements of 25 second stage juveniles. [b] Mean ± standard error of the mean.

Table 2

Frequency of conversion of *Meloidogyne hapla* from polyploidy to diploidy

Generation	*Tetraploid isolate*	*Number of female progeny studied*	*Number of tetraploids*	*Number of diploids*	*% diploids*
1st	E24P	28	22	6	21
2nd	E24P-5	20	18	2	10
2nd	E24P-8	20	17	3	15
2nd	E24P-12	10	9	1	10
3rd	E24P-5-9	20	17	3	15
3rd	E24P-8-15	20	12	8	40
3rd	E24P-12-3	20	16	4	20
4th	E24P-5-9-16	30	18	12	40
4th	E24P-12-3-12	30	24	6	20
Total		198	153	45	
Average					22

FREQUENCY OF POLYPLOIDS IN POPULATIONS OF *M. hapla* WORLD-WIDE

A cytological survey of twenty additional meiotic populations of *M. hapla* from sixteen countries revealed the existence of polyploidy in three populations. A total of 1 200 female nematodes were studied.

Population E289 from Taiwan had n = 14 chromosomes, whereas an egg-mass isolate derived from it had large-size juveniles and a haploid chromosomal complement of approximately 28. This polyploid isolate was converted to diploidy (no polyploid females could be found) after two generations of propagation without selection on tomato. Some second-stage juveniles of this isolate had malformed tails, suggesting developmental anomalies, possibly due to polyploidy.

A single tetraploid female with n = 34 chromosomes was observed also in population 86-VA from Virginia, USA, which normally had n = 17 chromosomes.

Another tetraploid female with n = 28 chromosomes was detected in population E284 from Holland. This population normally had a haploid complement of 14 chromosomes.

Discussion

Polyploidy is common among mitotically parthenogenetic species of *Meloidogyne* and other animals but it is quite rare among amphimictic animals including nematodes (Lokki & Saura, 1979 ; Mayr, 1970 ; Triantaphyllou & Hirschmann, 1980 ; White, 1978). A viable tetraploid form of *Caenorhabditis elegans* has been obtained following heat treatment of the diploid hermaphroditic form (Nigon, 1951 ; Madl & Herman, 1979), and a tetraploid isolate has been obtained from an amphimictic population of *Heterodera glycines* (Triantaphyllou & Riggs, 1979). Polyploidy is suspected to have played an important rôle in the cytogenetic evolution of some amphimictic groups of nematodes, especially among some animal- and plant-parasitic forms (Triantaphyllou, 1983).

Imbalance in the sex-determining mechanism is an acceptable explanation of the rarity of polyploidy in amphimictic animals, although many other factors may also be involved (Schultz, 1979 ; Triantaphyllou & Riggs, 1979 ; White, 1978). If establishment of polyploidy is hindered by the disturbance it causes in sex expression and, consequently, in impairing reproduction, it should be less inhibited in meiotically parthenogenetic organisms in which males are not essential for reproduction. In fact, Mayr (1970) has suggested that if polyploidy really exists in some amphimictic animals, it may have been established during a parthenogenetic stage in their evolution. It is of interest, therefore, to analyze polyploidy as it occurs in a meiotically parthenogenetic organism, such as *M. hapla*.

The present extensive cytological survey demonstrated that polyploidy does exist in meiotic popula-

tions of *M. hapla*. Three polyploid females were found among 1 200 females studied. At this low frequency of occurrence, investigating the cytological mechanism by which polyploidy is established has been difficult. It is very likely that conversion occurs during the last oogonial division, which may involve preparation for division and doubling of the chromosomes, but no actual cytokinesis. Such a duplication has not been directly observed in *M. hapla*, but oogonia located close to the end of the germinal zone often have distinct chromosomes, as if they were in the process of division. Still, no divisions have been observed in this zone. Most oogonial divisions in these nematodes occur in oogonia located close to the apex of the ovaries.

The present study revealed that conversion of polyploids to diploids is of major importance and accounts mostly for the sparsity of polyploids in *M. hapla*. Another reason may be that polyploids are at a disadvantage when in competition with diploids within a population, but this aspect was not studied here. Conversion to diploidy apparently results from the occasional expulsion of the second polar nucleus as a polar body and, therefore, its failure to fuse with the egg pronucleus at the end of the second maturation division. The phenomenon of conversion has not been observed previously in nematodes and does not occur in oocytes of the diploid *M. hapla* in which the second polar nucleus is extruded as a polar body only in oocytes that have been penetrated by a spermatozoon. The entrance of a spermatozoon in such cases guarantees reestablishment of the somatic chromosome number through fertilization. In the absence of a sperm nucleus, however, the second polar nucleus always remains in the cytoplasm and eventually fuses with the egg pronucleus to reestablish the somatic chromosome number (Triantaphyllou, 1966).

During prophase of the first cleavage of diploid eggs produced by tetraploid females, the 34 chromosomes are often arranged in two groups of 17, each one with its own nucleolus. Undoubtedly, the two groups correspond to the two telophase II sets of chromosomes of the maturing oocyte and, consequently, to the two haploid sets of the tetraploid female. These two sets of chromosomes must be completely homologous as evidenced by their regular pairing during meiosis of diploid females in subsequent generations. Consequently, the tetraploid can be viewed as representing an autotetraploid in spite of the complete lack of multivalent associations, as explained in the next paragraph.

No multivalents were observed during prophase of the first maturation division in tetraploid *M. hapla*, although homologous, or possibly homeologous bivalents tended to associate closely with each other. The same conclusion was reached by the analysis of its pachytene karyotype when 34 simple synaptonemal complexes, corresponding to bivalent associations of chromosomes, were observed (Goldstein & Triantaphyllou, 1971). Similar observations were made earlier in a polyploid form of *H. glycines* (Goldstein & Triantaphyllou, 1980). Apparently, something inhibits the formation of multivalents in polyploid forms of these nematodes. This property would be expected to facilitate the establishment of polyploidy by eliminating the cytological difficulties associated with segregation of multivalent chromosomes.

Since direct evolution to polyploidy in amphimictic animals is difficult, if not impossible, evolution to polyploidy during a parthenogenetic phase and subsequent conversion to amphimixis may be an attractive assumption, expressed already by White (1978) and by Astaurov (1969). This assumption is also supported by the present study. Once polyploidy is established in a meiotically parthenogenetic population, it can be maintened without difficulty by meiotic parthenogenesis, in a similar manner as described for *M. hapla*. However, in the presence of males, which occur occasionally in such populations, fertilization with sperm from tetraploid males could also maintain the tetraploid state of tetraploid oocytes. Such a process of facultative amphimixis could eventually evolve into obligatory amphimixis, thus establishing polyploid (diplodized) amphimictic forms. It is suspected that this has been the mechanism by which *Meloidogyne* species with 18 chromosomes have been established, *i.e.* as polyploid forms from ancestors which had about one half as many chromosomes. Such an assumption becomes quite reasonable considering that the basic chromosomal complement of all nonspecialized nematodes contains about 6, and definitely less than 9, small-size chromosomes. Similarly, the existence of *Meloidogyne (Hypsoperine) spartinae* with a haploid number of 7, small-size chromosomes, strongly supports this assumption. Furthermore, Janati and Dalmasso (1981) observed 9 to 10 pairs of chromosomes during a prediakinetic stage in an *M. hapla* population which had 15 bivalent chromosomes at metaphase I. This peculiarity can be explained if one assumes that *M. hapla* has evolved as a polyploid and, therefore, its chromosomal complement consists of two sets of homologous chromosomes that, through time, have become homeologous. Accordingly, some of the observed 9 to 10 chromosome pairs may have not been simple pairs but chromosomal configurations, each one formed by the close association of two homeologous chromosome pairs. Such an affinity and close association of homologous or homeologous bivalent chromosomes is common in polyploid organisms and was observed in the tetraploid *M. hapla* of the

present study (see previous paragraph). This interpretation is also supported by observations that only simple synaptonemal complexes, equal in number to the haploid chromosome number, were present in pachytene nuclei of a population of *M. hapla* (Goldstein & Triantaphyllou, 1978).

The above discussion suggests that the increased haploid chromosome number (n = 18) of the genus *Meloidogyne* represents a derived condition, presumably a state of polyploidy. Indeed, contrary to the views held earlier, amphimictic species within the genus *Meloidogyne*, with n = 18 chromosomes, are highly specialized parasites and should not be considered as ancestral forms. *M. carolinensis* and *M. megatyla* infect only blueberry and pine, respectively, whereas *M. microtyla* infects primarily cereal crops. Conversely, the meiotically parthenogenetic *M. hapla* has a wide host range and, in this respect, may be considered as a more primitive or ancestral form. The evolutionary relationships of root-knot nematodes, therefore, may need to be reconsidered in the light of the present investigation.

One may speculate that the genus *Meloidogyne* has arisen following a cycle of polyploidization and subsequent diploidization of the genome during a parthenogenetic phase. From that point on, part of the genus, exemplified by *M. carolinensis*, *M. megatyla* and *M. microtyla*, returned to obligatory amphimixis and followed an adaptation toward a more specialized type of parasitism. The rest of the genus continued evolving within the parthenogenetic phase, with less intensive host specialization, but with extensive cytogenetic diversification involving aneuploidy and higher degrees of ploidy. This group is represented by the most common species of root-knot nematodes, namely *M. incognita*, *M. arenaria*, *M. javanica* and numerous other parthenogenetic forms, hitherto unrecognized taxonomically.

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