Further observations on chromosomal processes during oogenesis of *Meloidogyne hapla* (Nematoda)

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SUMMARY

Oogenesis of two populations (A and B) of *Meloidogyne hapla* was studied using the Giemsa C banding technique. Population A underwent a normal meiotic process and had 2 n = 30 somatic chromosomes while population B showed a single maturation division of equational type and 2 n somatic karyotype = 45. In both cases, the Giemsa C technique provided good figures, even of the "pseudointerphase" or "diffuse stage" which takes place during oocyte growth and which previously has been designed by this term because the poor staining of chromosomes when Schiff or orcein techniques were used. In population A, this phase corresponded to diplotene. In population A 30 chromosomes could be distinguished during oogonial mitosis, but only nine or ten pairs were observed during diplotene. At diakinesis fifteen bivalents were present. Maturation was normal : the first polar body with fifteen chromosomes was rejected and a second mitotic division followed. The nine or ten pairs observed during meiotic prophase were thought to be relictual chromosome feature and the higher number observed in the other stages could be the result of some chromosome breaks. The variation of the chromosome number previously signalized for *M. hapla* (n = 14-15-16-17) would be related to this phenomenon. Population B possessed 45 chromosomes. At the early prophase of the single maturation division, chromosomes were not paired, contrary to population A. At the end of the prophase some of them assembled forming large chiasmatic or aggregate chromosomes ; chromatin reduction did not take place at the end of the division.

RÉSUMÉ

Nouvelles observations sur les processus chromosomiques pendant l'ovogénèse de Meloidogyne hapla *(Nematoda)*

L'ovogenèse de deux populations A et B de *Meloigogyne hapla* a été étudiée en utilisant une technique de Giemsa C banding. La population A subit une méiose normale et présente 2 n = 30 chromosomes, tandis que la population B n'a qu'une seule division de maturation de type équationnel et 2 n = 45. Dans les deux cas la technique employée a donné d'excellentes figures, même pour la pseudointerphase ou stade diffus qui prend place pendant la croissance de l'ovocyte et qui a été ainsi nommée antérieurement à cause de la non colorabilité de la chromatine par les techniques traditionnelles au Schiff ou à l'orcéine. Cette phase correspond pour la population A au diplotène. Chez cette population A on observe 30 chromosomes pendant la mitose oogoniale, mais seulement neuf ou dix paires sont observées pendant le diplotène alors qu'on compte quinze bivalents à la diacinèse. La maturation est normale : le premier globule polaire avec quinze chromosomes est rejeté et la seconde division mitotique suit. Les neuf ou dix paires observées pendant la prophase méiotique traduisent peut-être un aspect relictuel des chromosomes, le nombre plus élevé observé dans les autres stades pouvant résulter de fragmentations stabilisées. Les variations du nombre de chromosomes antérieurement signalées pour *M. hapla* (n = 14-15-16-17) pourraient être liées à ce phénomène. La population B possède 45 chromosomes. Au début de la prophase de l'unique division de maturation, les chromosomes ne s'apparient pas, contrairement à la population A. A la fin de la prophase quelques-uns sont assemblés, formant des agrégats ; la réduction chromatique ne se produit pas à la fin de la division.

Gametogenesis and karyotypes of nematodes belonging to the genus *Meloidogyne* were first studied using orcein as a stain (Triantaphyllou, 1963, 1966). It was demonstrated that *M. hapla* reproduced either by facultative meiotic parthenogenesis, or by obligatory "mitotic" partheno-

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genesis. In populations undergoing meiosis, somatic chromosomes varied in number : 2 n = 30, 32 or 34. In mitotic populations, 45 chromosomes were visible in female gamete and a single non reducing division took place. Two other species, *M. naasi* and *M. graminicola* which were found to have 2 n = 36 in oogonia and n = 18 chromosomes at diakinesis, reproduced by facultative parthenogenesis, while some other species like *M. arenaria, M. javanica* or *M. incognita* reproduced by obligatory parthenogenesis and possessed high and variable chromosome numbers (Triantaphyllou, 1969). On the other hand the basic number of *Heterodera*, a closely related genus, was n = 9. Recently two hypothesis have been formulated by Triantaphyllou (1966, 1969) : *i*) meiotic *M. hapla* (like most meiotic species of *Meloidogyne*) is diploid. The basic number n = 18 is sometimes altered by aneuploidy ; *ii*) the species is tetraploid, possibly *Meloidogyne* and *Heterodera* have a common ancestor ; individuals with 45 chromosomes are pentaploid. Yet molecular data (Dalmasso & Bergé, 1978) were in favour of hypothesis that the meiotic form of *M. hapla* is diploid. Recently synaptonemal complexes and recombination nodules were demonstrated during the early prophase in meiotic populations of *M. hapla* while they were not present in mitotic population (Goldstein & Triantaphyllou, 1978).

The present study was an attempt to obtain new informations on the reproduction and chromosomes changes in this species by means of the C Giemsa technique.

Materials and methods

Two populations of *M. hapla* were grown on tomato cv. Marmande in a greenhouse at 20° : population A from Le Beausset (France) had a meiotic mode of reproduction with n = 15 chromosomes in the post meiotic oocytes ; population B from Saulcy (France) was mitotic with 2 n = 45. Chromosomes were stained using the Feulgen technique.

Giemsa C banding technique was adapted for nematode gonads and oocytes from Summer's technique (1972) for staining the centromeric heterochromatin. Gonads of *Meloidogyne* from adult female were dissected and soaked in a hypotonic solution of sodium citrate for 15 min. (0.85%) in conic glass Eppendorfs. The material was centrifugated at 200 g for 10 min. and the supernatant gently removed subsequently. Fixative (ethanol, acetic acid : 3/1) was added and the tube placed at 4° for 3 h. The material was centrifugated again at 200 g for 10 min. and the supernatant removed. Gonads and cells were suspended in the fixative and dropped on a cold glass slide previously covered by a film of water. Entire gonads were also directly treated with a hypotonic solution and fixative on slides conserved in a moist chamber between each step. The slides were air dried, soaked in 5% barium hydroxide at 50° for 15 min. and washed in water. Then they were incubated in 2 × ssc (0.3 M sodium chloride : 0.03 M sodium citrate) at 60° for 1 h. The slides were rinsed and stained in Giemsa (4% in pH 6.8 buffer) for 20-30 min. and mounted after dehydratation in Canada balsam or DPX.

Results

Aspects of the meiosis in population A (from Le Beausset)

Oogonia divided normally (Fig. 1 a, b), 30 chromosomes (Fig. 1 c) could be distinguished. Meiosis was preceded by a brief interphase (Fig. 1 d) as in *Ascaris* (Bogdanov, 1977). During zygotene chromatin was concentrated at the proximity of the nuclear enveloppe showing characteristic "bouquet" conformations. In the subsequent pachytene chromosomes appeared paired. Curiously the number of chromatic filaments (9 or 10) was less than expected from observations on oogonia (Fig. 1 e). Then during the long period of growth of the oocyte I, a large nucleus was present. The chromatin was not very poorly colored by Schiff or orcein techniques at this stage, called pseudointerphase in nematodes (Nigon, 1949) or diffuse stage in other organisms (Wilson, 1925 ; Klasterska, 1977). The Giemsa C technique allowed a better coloration of this stage which corresponded to the diplotene (Fig. 1 f, g, h). It is characterized

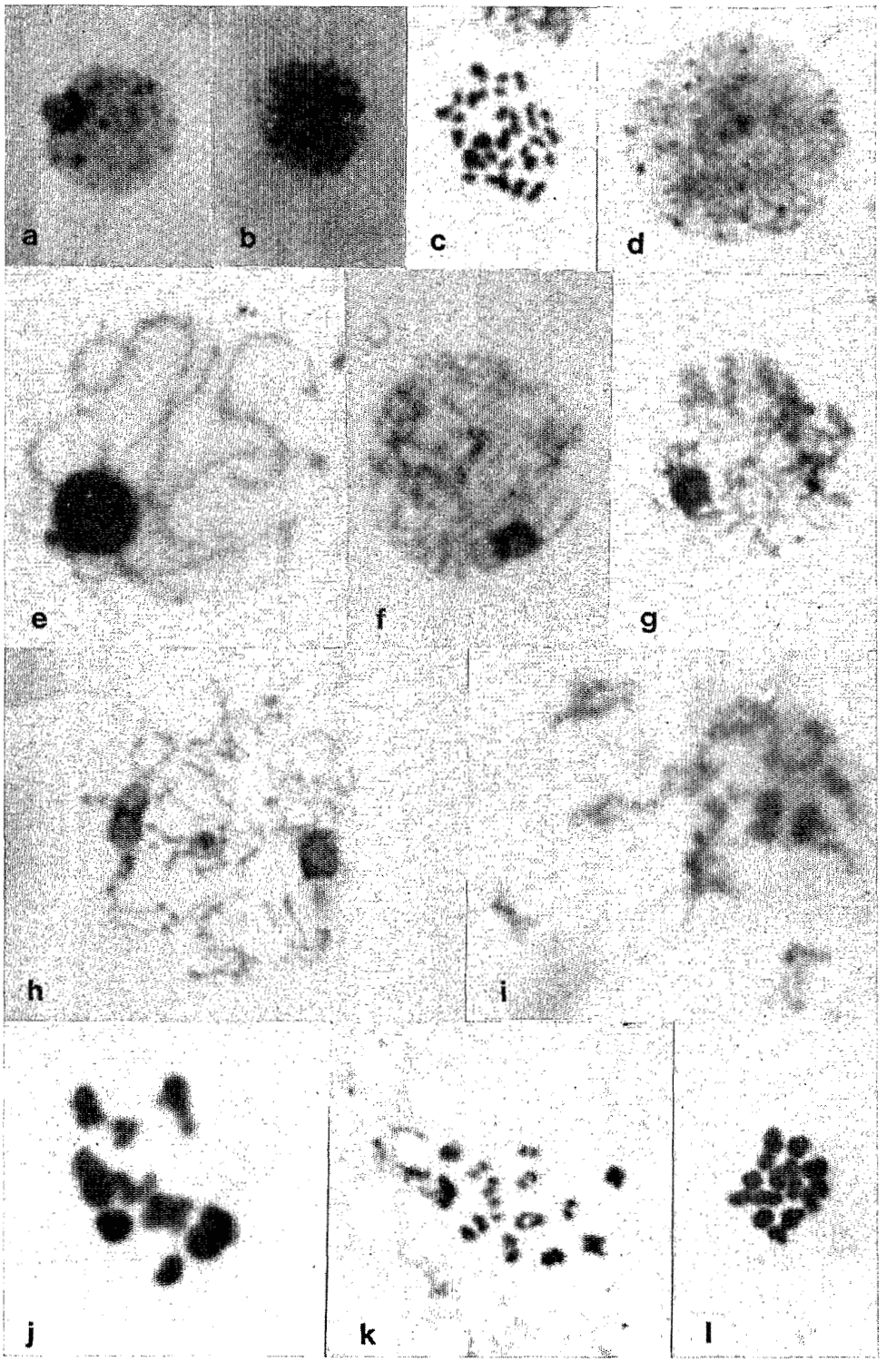

Fig. 1. a, h : Oogenesis in *Meloidogyne hapla* meiotic form (× 3200) ; a, b : Oogonial interphase ; c : Oogonial metaphase (30 chromosomes) ; d : Late premeiotic interphase ; e : Pachytene ; f, g, h, i : 9-10 pairs with centromeres, diplotene sequences ; j : 9 pairs clearly visible ; k : Early metaphase of meiosis (15 bivalents) ; l : Typic metaphase of meiosis showing 15 chromosomes.

by many nucleolus-like vesicles probably resulting from intense RNA synthesis. Chromatic filaments were double ; later during diplotene, the chromatic filaments spiralised (Fig. 1 i). Centromeres, chiasmata became discernible, multiple regions of heterochromatin were seen. Chromosome pairs were very long and still numbered 9-10. Subsequently chromosomes contracted and became dense (Fig. 1 j). Schiff and orcein colorations did not stain chromosomes, therefore their low number had escaped attention. Following this stage the nuclear membrane disappeared, and at diakinesis 15 bivalents were distinctly visible (Fig. 1 k, l). During anaphase I, bivalents separated and two sets each of 15 chromosomes could be counted. That is the reduced number of chromosomes observed during oogonial division.

A second maturation division took immediatly place afterwards. If sperm occurred, two polar bodies were emitted, if not, the diploidy was restored by fusion of the second polar body with the female pronucleus.

ASPECTS OF THE MATURATION OF OOCYTES IN POPULATION B (from Saulcy)

45 chromosomes were counted in the oogonial zone with difficulty due to the small size of cells (Fig. 2 a). At the beginning of the maturation, chromosomes became filamentous forming a discrete network ; later chromosomes shortened and thickened (Fig. 2 b). Generally pairing was not observed excepted in a small region but we suspected this to be fortuitous. Then multiple centromeric heterochomatin centers appeared along chromosome filaments, no coupling of chromosomes was observed at this stage which corresponded to the period of strong growth of the cells (diffuse chromatin stage according classical Feulgen and orcein techniques) (Fig. 2 c). The number of chromosomes appeared unchanged, this confirms that no pairing took place during zygotene. Later, the chromatic material condensed. Punctiform elements aligned forming composive chromosomes (Fig. 2 d). Finally about ten to fifteen masses with different size were visible at the end of this special prophase (Fig. 2 e). At early metaphase disjonction occurred and 45 chromosomes were observed which gave rise to two anaphase sets each with 45 chromosomes. As reported by Triantaphyllou (1966) a single equational division occurred.

Discussion

Refering to Triantaphyllou's hypothesis, meiotic forms of *M. hapla* may be either tetraploid or diploid. Recently, this author inclined for the second possibility considering 45 chromosomes populations as triploid. The basic number of the genus *Meloidogyne* would be n = 18, changes in chromosome number in *M. hapla* (n = 15, 16 and 17) would have resulted from aneuploidy.

The Giemsa technique applied to a meiotic and a mitotic population of *M. hapla*, supplied additional information as it was possible to stain chromosomes during the diffuse stage of the prophase in both populations. Observations could also be made in the zone of the oocytes. Counting of chromosomes at diplotene in population A showed a abnormally low number of pairs. The eventuality of some supernumerary element (trisomy) seems to be dismissed for only couple have been observed. Another explanation would attribute to chromosome breaks the higher numbers in metaphases I. Meiotic prophase chromosomes should be considered as a relic chromosome entity. This would not be so surprizing for similar breaks have been reported in *Ascaris* but in somatic cells during embryogenesis. Chromosome breaks may be correlated with polycentromeric structures of diffuse centromeres, already reported in nematodes by Boveri (1888) and several authors. Thus chromosome numbers in *Meloidogyne* species may result on the first place from breaks which would have given a new basic number n = 18 and secondly from polyploidy and in some cases, from loss of chromosomes (aneuploidy). In reality these losses would only concerne pieces of ancestral chromosomes. In population B, chromatic figures tended to confirm that normal pairing does not occur. The number of chromosomes remained unchanged all long the prophase of the single division. Yet, just before metaphase, we have observed some apparently disordered assemblage of these chromosomes. This is a fugacious event and rapidly

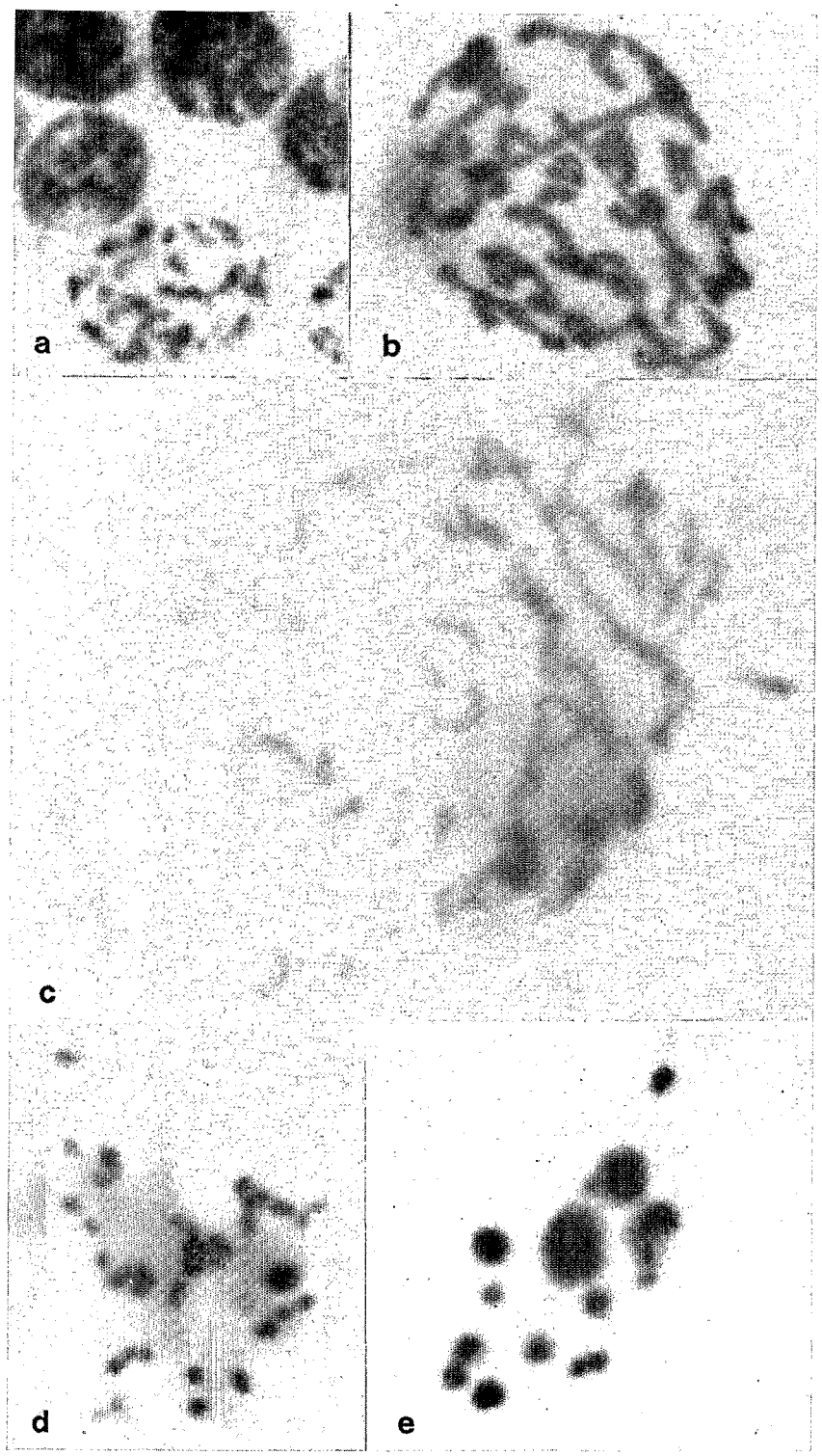

Fig. 2. a, e : Oogenesis in *M. hapla* "mitotic" form (× 3200) ; a : Oogonial nuclei ; b : Early prophase ; c : Prophase ; d, e : Late prophase of the single maturation division showing composite elements and then the only 12 to 15 chromatin blocks visible.

45 units were again observed at the metaphase. It could be possible that some chromatid exchange occurred during this short period.

Further ultramicroscopic investigations are needed to answer this question. What is most probable is that the 45 chromosomes represent a triploid state as demonstrated by DNA dosage (Lapp & Triantaphyllou, 1972).

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Accepté pour publication le 13 mars 1981.

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