

Cytology of parthenogenesis of five *Meloidogyne* species

Johan G. VAN DER BEEK*, Jan A. LOS* and Laas P. PIJNACKER**

* Research Institute of Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands, and ** Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.

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Summary – Oogenesis was investigated using a fluorescent staining technique with Hoechst 33258 in several isolates of facultative meiotic parthenogenetic *Meloidogyne hapla* race A, *M. chitwoodi*, *M. fallax* – and obligate ameiotic (mitotic) parthenogenetic – *M. hapla* race B, *M. javanica* – and isolate Xa of an unidentified species. Without fertilisation, the somatic chromosome number was restored by fusion of pronucleus and second polar body in *M. hapla* race A, *M. chitwoodi*, and *M. fallax* isolates. In oocytes of isolate Xa, a second meiotic division occurred in a few cases while in all other cases the somatic chromosome number was restored by duplication of the chromosomes of the egg nucleus after the first division. In one mono-female line of Xa, embryonic development occurred within the body of the female and was genetically controlled. In *M. hapla* race B and *M. javanica*, maturation of the oocytes consisted of one mitotic division. This study confirmed that a large variation of the mode of reproduction exists in *Meloidogyne* spp., which may be a reason for their world-wide success. In one *M. hapla* race A isolate, only 12 % of the oocytes of inseminated females contained sperm, and prophase I instead of prometaphase I oocytes were found posterior to the spermatheca. © Orstom/Elsevier, Paris.

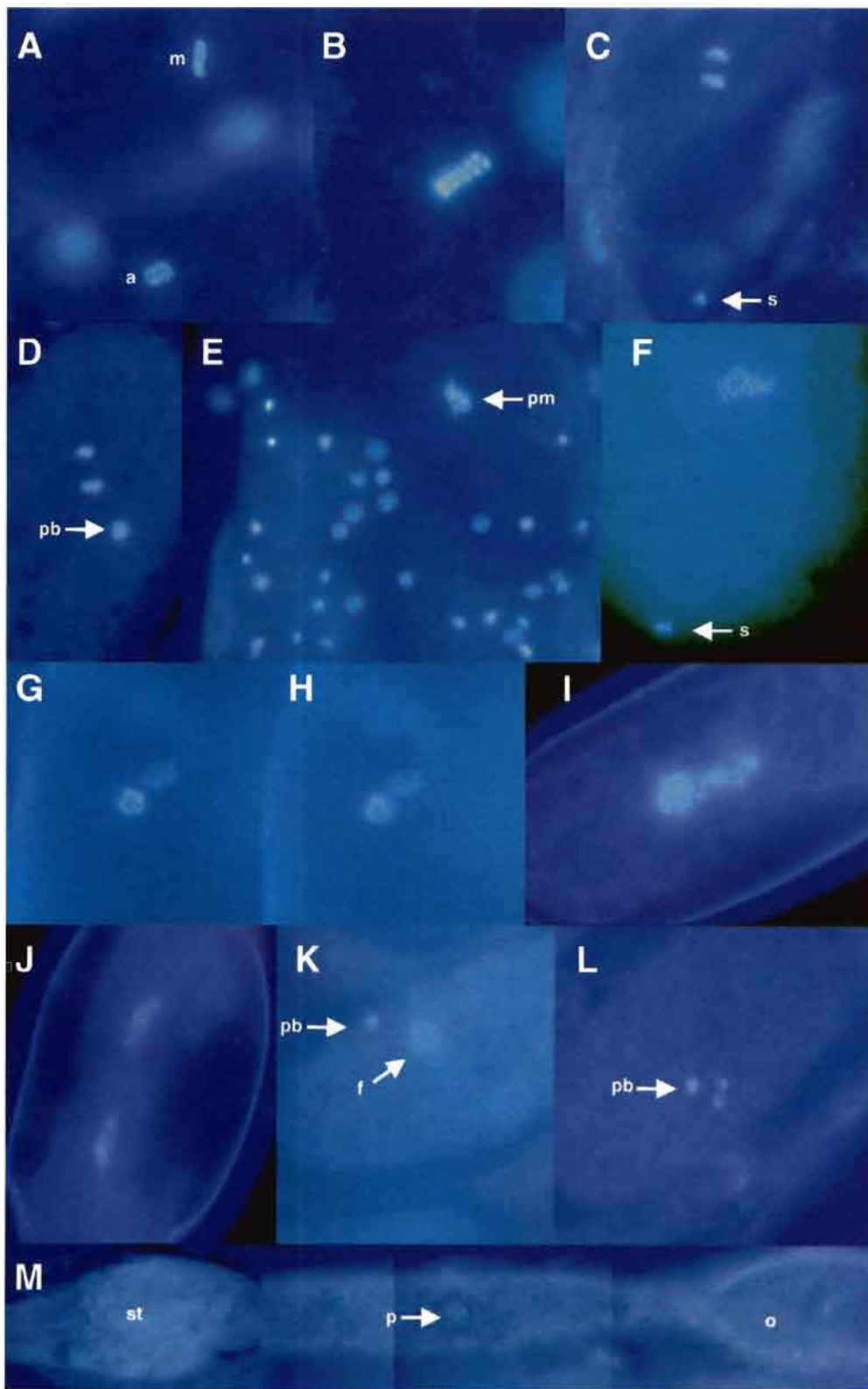
Résumé – Étude cytologique de la parthénogenèse chez cinq espèces de *Meloidogyne* – La présente étude a porté sur l'ovogenèse de plusieurs isolats à parthénogenèse méiotique facultative – *Meloidogyne hapla* race A, *M. chitwoodi*, *M. fallax* – et à parthénogenèse améiotique (mitotique) – *M. hapla* race B, *M. javanica* et l'isolat Xa appartenant à une espèce non identifiée – et ce au moyen d'une technique de coloration fluorescente au Hoechst 33258. En l'absence de fécondation, le nombre de chromosomes somatiques est rétabli par fusion du pronucleus et du second corps polaire chez les isolats de *M. hapla* race A, de *M. chitwoodi* et de *M. fallax*. Dans les oocytes de l'isolat Xa une deuxième division méiotique, exceptionnelle, se produit ; dans tous les autres cas la réduction chromosomique est compensée par la duplication des chromosomes dans le noyau de l'œuf après la première division. Chez l'une des lignées mono-femelles de l'isolat Xa le développement embryonnaire, génétiquement contrôlé, se produit à l'intérieur de la femelle. Chez *M. hapla* race B et *M. javanica* la maturation des oocytes consiste en une division mitotique. Cette étude confirme la grande variabilité des modes de reproduction des espèces de *Meloidogyne* ce qui peut expliquer leur succès mondial. Dans un isolat de *M. hapla* race A, seuls 12 % des oocytes des femelles fécondées contiennent des spermatozoïdes et ce sont les oocytes en prophase I - au lieu de la prometaphase I - qui sont observés en position postérieure à la spermatheque. © Orstom/Elsevier, Paris.

Key-words : meiosis, *Meloidogyne arenaria*, *M. fallax*, *M. hapla*, *M. javanica*, parthenogenesis, root-knot nematode.

Root-knot nematodes (*Meloidogyne* spp.) are widely distributed plant pathogens with a very wide host-range. They cause more economic damage to food crops than any other group of plant-parasitic nematodes (Lawrence Apple, 1985). Several species reproduce by either facultative meiotic or obligate ameiotic (or mitotic) parthenogenesis. The apomicts are geographically more widespread, ecologically less restricted, and more polyphagous than their amphimictic relatives (Triantaphyllou, 1985a), a phenomenon also described in other animals (Schultz, 1977; Lokki, 1983). Besides parthenogenesis, these nematodes have undergone extensive cytogenetic diversification, with chromosome numbers varying from $2n = 14$ in the amphimictic *Meloidogyne spartinae* Whitehead, 1968, to $2n = 51-56$ in the apomictic *M. arenaria* Chitwood, 1949 (Triantaphyllou, 1985a; Eisenback

& Hirschmann-Triantaphyllou, 1991). Males play a facultative role in reproduction of meiotic parthenogenetic populations. In ameiotic populations, males may be produced, but their function is still unclear.

An in-depth knowledge of cytogenetics is a prerequisite for studying the genetic variation in parthenogenetic *Meloidogyne* spp. The oogenesis of ameiotic *M. arenaria*, *M. hapla* race B Chitwood, 1949, *M. incognita* Chitwood, 1949, and *M. javanica* Chitwood, 1949 and of meiotic *M. hapla* race A has been studied earlier (Triantaphyllou, 1962, 1963, 1966, 1981; Dalmasso, 1973; Dalmasso & Bergé, 1975). We have used Hoechst 33258 and fluorescent UV microscopy to investigate the oogenesis of several *Meloidogyne* populations. The results are presented and their relevance to the reproduction of *Meloidogyne* spp. is discussed.



phase nuclei resulting from the second maturation division fuse in the centre of the egg after oviposition.

M. chitwoodi isolates Cb, Cj, Ck, Co, and Caq and *M. fallax* isolate Fb have a similar oogenesis, with or without sperm, which also demonstrates a facultative and meiotic parthenogenetic type of reproduction.

In the seventeen inseminated females of *M. hapla* isolate He, 33 of the 379 oocytes studied did not contain sperm, ranging from 50 to 100 % in individual females. These oocytes were observed in the uterus directly posterior to the spermatheca. They were often found to be in late prophase I whereas they should have been at least in metaphase I (Fig. 1M). Typically, sperm was observed not only in the spermatheca but also in the lumen of that region of the uterus.

OOGENESIS IN *M. HAPLA* RACE B AND *M. JAVANICA*

Oogenesis of *M. hapla* race B (Fig. 2) and *M. javanica* isolates was also identical to what was described by Triantaphyllou (1962). Only univalents were observed at the end of prophase I and during prometaphase I, which indicates absence of chiasmata formation and of genetic cross-over. The single maturation division is mitotic and produces two sets of chromosomes, each with the somatic number of one-chromatid chromosomes (Fig. 1G, H). The orientation and position of the spindle is as described above. One set of chromosomes migrates towards the cell surface, where it disintegrates as the first polar body after oviposition. The other set takes an interphase structure (Fig. 1I), then starts embryonic development after deposition (Fig. 1J). In inseminated females, a spermatozoon enters the metaphase I oocyte as described above, but it does not interfere with the maturation of the oocyte and probably degenerates without any migration (Fig. 1F). This demonstrates that obligate ameiotic parthenogenesis occurs also in the Dutch *M. hapla* race B populations and in the Chinese *M. javanica*.

OOGENESIS IN ISOLATE XA

In isolate Xa, oogenesis follows a different pattern (Fig. 2). Bivalents are formed and, at anaphase I, the

reduced number of two-chromatid elements, oriented parallel to the spindle, goes to each pole. At telophase, one set of elements takes a flocculated appearance, while the other set remains contracted and becomes the first polar body, which moves towards the surface (Fig. 1K), then degenerates after oviposition.

The flocculated set enters mitosis of the first cleavage division early after oviposition. Females may become inseminated by males and the spermatozoon enters the oocyte normally when in prometaphase I. However, in inseminated females no spermatozoon could be found in oocytes in stages beyond metaphase I.

In three oocytes out of more than 100, a second maturation division was observed similar to that in *M. hapla* race A (Fig. 1L). It concerned two oocytes from an inseminated female and one oocyte from a non-inseminated female, neither of them containing a spermatozoon. This type of meiosis occurred rarely in two subsequent parthenogenetic generations of mono-female lines in which parthenogenetic reproduction could be inferred from the production of offspring by single females.

Although cleavage divisions normally start after oviposition, all eggs of one female started embryonic development within the body of this female. Apparently, all the parthenogenetic mono-female progeny of this female shared this trait.

Discussion

These studies on meiotic divisions of various isolates of meiotic parthenogenetic *M. hapla* race A and of ameiotic parthenogenetic *M. hapla* race B and *M. javanica* confirm earlier work on the cytology and oogenesis of these *Meloidogyne* spp. (Triantaphyllou, 1962, 1963, 1966, 1981; Dalmasso, 1973; Dalmasso & Bergé, 1975). Oogenesis of *M. chitwoodi*, *M. fallax*, and, in a few cases, isolate Xa, seemed to be similar to that of the other investigated meiotic parthenogenetic species.

The various meiotic systems occur in populations of seven species from various sites world-wide (Eisen-

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Fig. 1. Oogenesis in *Meloidogyne* spp. A: Metaphase I (m) and early anaphase I (a) in *M. hapla* race A isolate H_i; B: Metaphase I in *M. hapla* race A isolate H_j; C: Early telophase I with penetrated sperm (s) in *M. hapla* race A isolate H_j; D: Telophase II with the first polar body (pb), in *M. chitwoodi* isolate C_z; E: Prometaphase I (pm) in *M. hapla* race B isolate H_h, passing through the spermatheca in which sperm is visible; F: Metaphase I in *M. hapla* race B isolate H_h with penetrated sperm (s); G, H: Interkinesis in *M. hapla* race B isolate H_h using different focusses; I: Egg outside the female body just prior to first cleavage division in *M. hapla* race B isolate H_h; J: First cleavage division in *M. hapla* race B isolate H_h; K: Oocyte after the first maturation division in isolate Xa of unidentified *Meloidogyne* sp., showing first polar body (pb) and the second set chromosomes in flocculated appearance (f); L: A rare late anaphase II in isolate Xa of unknown *Meloidogyne* sp. (pb = first polar body); M: Spermatheca and the adjacent anterior part of the uterus in *M. hapla* race A isolate He showing successively a spermatheca (st) filled with sperm, two oocytes in prophase I, of which one clearly visible (p) and an oocyte in metaphase I (o) without sperm in the oocytes (A 100× objective was used for all illustrations, except for B: 200× objective.)

