Oogenesis and the Chromosomes of the Parthenogenetic Root-knot Nematode Meloidogyne incognita[1]

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Abstract: 220 populations of *Meloidogyne incognita* and related forms from 46 countries reproduced by mitotic parthenogenesis (apomixis). Determination of somatic chromosome numbers from oogonia and oocytes revealed the existence of a predominant, possibly triploid race A with $3n = 40$ to 46 and a rare, diploid race B with $2n = 32$ to 36 chromosomes. There is no correlation between cytological races and the four recognized host races of this species. The characteristic behavior of prophase I chromosomes of maturing oocytes, which results in a prolonged prophase stage, is a unifying feature of all forms of *M. incognita* and supports monophyletic evolution, distinct from that of other *Meloidogyne* species. Extensive chromosomal polymorphism detected among populations can be helpful in elucidating the cytological pathway of evolution of the species. *Key words:* cytology, reproduction, polyploidy, polymorphism, races, evolution.

Meloidogyne incognita Chitwood is the most important species of root-knot nematodes severely affecting crop production in most countries of the world, especially in tropical, subtropical, and temperate regions (5,11,12,13). Cytological and cytogenetic studies of this species have been conducted in our laboratory for the last 15 years, but the results have been inconclusive and have been reported only as preliminary observations or unpublished data (17,18). Cytological methodology employed in the study of other species of root-knot nematodes (14,15, 16) has not been satisfactory for *M. incognita.* However, recent modifications of cytological procedures have permitted relatively accurate chromosome counts in many populations. An effort was made, therefore, to complete these studies and assess the cytogenetic relationship of *M. incognita* to the other major species of root-knot nematodes; i.e., *M. hapla, M. arenaria,* and *M. javanica,* whose cytogenetic status is known (2,3,10,14,15,16,18).

The study was facilitated by the collec-

tion of a large number of populations of *M. incognita* from tropical and subtropical regions of the world representing a wide variety of climatic and soil habitats to which this species appears to be well adapted. The nematode populations were provided by about 80 nematologists from 46 countries participating in a cooperative international program for the study of root-knot nematodes. The program is supported by the U.S. Agency for International Development (AID) and is administered by the North Carolina State University.

The binomen *Meloidogyne incognita* in this study is inclusive of all *Meloidogyne* forms that have been referred to in the literature as *M. incognita, M. incognita incognita, M. incognita acrita, M. acrita, M. incognita wartellei* and *M. grahami* (1,6,7, 19). Population "282 – S.C., USA," which before its description as *M. grahami* was regarded as a race of *M. incognita* (9), was included in this study together with the other forms of *M. incognita* because of its cytogenetic similarity with them. Population "450 – La., USA" is the original population from which *M. incognita wartellei* was described (6).

MATERIALS AND METHODS

The 220 nematode populations of the present study originated from various parts of the world (Table 1). Identification as to

species was based primarily on morphology of the cuticular pattern of the perineal region of adult females, on differential host response tests, and occasionally on morphology of second stage juveniles and adult males and females. Greenhouse cultures were established on tomato (cv. Rutgers) using as inoculum egg masses recovered from infected roots of a variety of crop and some noncrop plants on which the field populations occurred. Adult females used for cytological study were obtained from 40–50-day-old greenhouse cultures maintained at 22–28 C. Five females with egg masses containing about 150 eggs each were transferred into a drop of 0.9% NaCl solution containing 0.5% colcemid on a microscope glass slide. The cuticle of each female

Table 1. Populations of *M. incognita* classified according to host race, cytological race, and geographical origin.

Geographical origin	Host race*			
	Race 1	Race 2	Race 3	Race 4
	Cytological race A (3n = 40-46 chromosomes)			
North America	4-N.C., USA†	19-N.C., USA	73-Fla., USA	282-S.C., USA
	22-N.C., USA	79-Ohio, USA	108-Ala., USA	401-N.C., USA
	53-Ark., USA	477-N.C., USA	246-N.C., USA	
	68; 71-N.C., USA		257-Fla., USA	
	78-Ga., USA		285-Tenn., USA	
	83-N.C., USA		299-Ari., USA	
	183-N.M., USA		403; 418-N.C., USA	
	198-N.C., USA		450-La., USA	
	424-N.C., USA		472-N.C., USA	
	488-Ga., USA		484; 485-Ga., USA	
	498-N.C., USA		509-La., USA	
	E463; E488-Bermuda		E456; E458-Tx., USA	
Central America	360; 390-Panama	340-Guatemala	416-El Salvador	110-Guatemala
	415; 417; 470-El Sal.	E27-El Salvador	E26-El Salvador	
	E2-Guadeloupe	E31-Panama	E264-Mexico	
	E25-El Salvador	E34-Panama	E683-Mexico	
	E36; E38-Panama	E42-Jamaica		
	E40-Jamaica	E179-Puerto Rico		
	E48-Guadeloupe	E313-Trinidad		
	E176-Puerto Rico			
	E256-Costa Rica			
	E263-Mexico			
	E269-Trinidad			
	E324-El Salvador			
South America	182; 189-Peru	302-Colombia	178-Peru	E103-Peru
	301; 312-Colombia	391-Colombia	265-Peru	E237-Ecuador
	325-Peru	429-Surinam	344-Peru	E783-Uruguay
	338; 339-Colombia	475-Ecuador	E68-Uruguay	
	343-Peru	E166-Brazil	E105-Uruguay	
	366; 370; 406-Brazil	E219-Colombia	E186-Brazil	
	430-Surinam	E239-Colombia	E253-Brazil	
	E52-Argentina	E281-Colombia	E565-Peru	
	E104-Peru	E330-Chile	E573-Chile	
	E136-Colombia	E431-Brazil	E578-Peru	
	E165-Brazil	E438-Brazil		
	E193-Surinam	E536-Ecuador		
	E199-Brazil			
	E502-Peru			
Europe	89; 90; 91-Belgium	92-Belgium		
	462-Switzerland	130; 131; 132-Greece		
	E406-Greece	332-Germany		

Table 1. (Continued)

Geographical origin	Race 1	Race 2	Race 3	Race 4
	Cytological race B (2n = 32-36 chromosomes)			
Africa	163; 187-Africa‡ 194; 203; 211-Africa 225; 226-Africa 371; 374-Nigeria E89; E90-Ghana E139; E140-Senegal E150-Nigeria E155; E156-Nigeria E169; E170; E171-Ghana E182; E183; E202-Ghana E228; E236-Egypt E316-Rhodesia E543; E563-Ivory Coast E687-Tanzania	157-Africa 162-Africa 271-Nigeria 373-Nigeria 377-Nigeria E152-Nigeria E246-Nigeria E562-Ivory Coast	159-Ivory Coast E184-Ghana E185-Ghana E201-Ghana E233-Egypt	270-Nigeria E88-Ghana
Near East	E96-Sri Lanka E270; E271-Pakistan E359-Yemen E639; E647; E649-India E651; E652-India E792-Iran E653; E675-India	E100-Sri Lanka E506-Saudi Arabia E671-India	E124-Yemen E672-India	E437-Sri Lanka
Far East	121; 127-Taiwan 167-Japan 190-Taiwan 378 to 381-Philipp. 388; 490-Philippines E10; E11-Taiwan E12; E14-Taiwan E23-Korea E74-Fiji Islands E159; E160-Thailand E215-Philippines E221; E484-Australia E590; E593-Philippines M38-Philippines	355-Fiji Islands E7-Taiwan E158-Thailand E209-Philippines E290-Taiwan E389-Fiji Islands M23-Malaysia	161-Philippines 493-Philippines M33-Indonesia	
North America	E487-Bermuda			63-Tenn., USA
Central America		117-Guatemala E301-Puerto Rico		
South America			E337-Argentina E586-Argentina	
Europe		103-Belgium	188-Belgium	

*Host races 1-4 are characterized by their ability to reproduce on cotton (cv. Deltapine 16) and tobacco (cv. NC 95) as follows: race 1, cotton —, tobacco —; race 2, cotton —, tobacco +; race 3, cotton +, tobacco —; race 4, cotton +, tobacco + (13). Tests on host specificity have been done in the laboratory of J. N. Sasser, Department of Plant Pathology, North Carolina State University.

†Populations are designated by a code number, or a letter-number combination, and the origin. The same designations are used for cross-reference purposes in all publications of the International *Meloidogyne* project. "E" numbers indicate populations for which a great deal of ecological data is also available.

‡Populations designated as "Africa" have orignated from countries of Africa that are located west of Niger and Nigeria.

was cut in the neck region with a sharp knife to facilitate colcemid contact with the gonads. The slide was then placed in a moisture chamber at 25 C. After 3–4 h the colcemid solution was drawn off with a fine pipette or filter paper and the females were smeared on the slide to spread the gonads. Further treatment with HCl, fixation, and staining with propionic orcein were carried out as described earlier (18). Pretreatment with colcemid was necessary to relax the spindle fibers and allow the chromosomes of primary oocytes to spread over a larger area.

Slides without colcemid treatment were also prepared from each nematode population to study oogonial divisions and also to serve as control for the study of the normal process of maturation of oocytes and the behavior of the chromosomes during late prophase.

Cleavage divisions were studied in smears of eggs obtained from egg masses and processed for staining in the same manner as slides of females without colcemid treatment.

OBSERVATIONS

Oogenesis: The reproductive system of *M. incognita* females is similar to that of *M. javanica* (14). Many oogonial divisions occur in the apical, oogonial region of each of the two ovaries of young, egg-producing females (Fig. 1). Prophase and metaphase figures of oogonial divisions, although not completely satisfactory for precise determination of chromosome numbers, are the best for approximate counts (Figs. 2–8). The chromosomes are usually well spread and discrete, but often two or more of them may be overlapping, making precise counting difficult.

Chromosomes vary in length from 0.5 to 3 μm in late prophase or metaphase figures (Figs. 2–8, 19–22) and can often be classified roughly into two or three groups according to size. However, there appears to be extensive variation in the relative degree of condensation of individual chromosomes from one figure to another, and this variation makes identification of individual chromosomes extremely difficult, except for the very large chromosomes of some popu-

lations. Furthermore, no homologies among chromosomes could be established with any degree of certainty.

The somatic chromosome number of each population was estimated from counts of 10–15 oogonial metaphases from an equal number of females. Each estimate may involve an error of ± 1 chromosome. This amount of variation was usually encountered even in single egg-mass cultures of many populations and was attributed to technical difficulties rather than to actual variation in chromosome number. In spite of the above difficulties, the 220 populations could be classified into two chromosomal forms which for convenience are considered and referred to as cytological races: race A (possibly triploid) with somatic chromosome numbers varying from 40 to 46, and race B (diploid) with 32 to 36 chromosomes. About 96% of the populations studied were race A and only 4% were race B (Table 1). About 80% of the populations of race A had 2n = 43–45 chromosomes and 75% of the populations of race B had 2n = 34–36 chromosomes. Only population "63 – Tenn., USA," classified as race B in Table 1, had 2n = 36–38 chromosomes and, therefore, tends to bridge the two races.

Following the last oogonial division in the germinal zone of the ovary, the oogonial cells enter a short zone of the ovary which, based on the heavy chromatin concentration in the nuclei, has been characterized as zone of synapsis in other root-knot nematodes (17). The behavior of the chromatin in such early oocytes of *M. incognita* is the same as described for *M. hapla* (16). However, electron microscopy observations of thin sections of oocytes corresponding to a pachytene stage have revealed no synaptonemal complexes in *M. incognita* (8). Apparently, no synapsis of homologous chromosomes takes place in oocytes of *M. incognita*. All other stages of maturation of oocytes of *M. incognita* are similar to those of *M. arenaria* (15) and *M. javanica* (14) until the oocytes reach the oviduct-spermatotheca region. From there on, the process of maturation is different because of the characteristic behavior of the chromosomes of *M. incognita*. Thus, shortly before the oocytes enter the oviduct, the chromosomes condense and become distinct, each consisting of two chro-
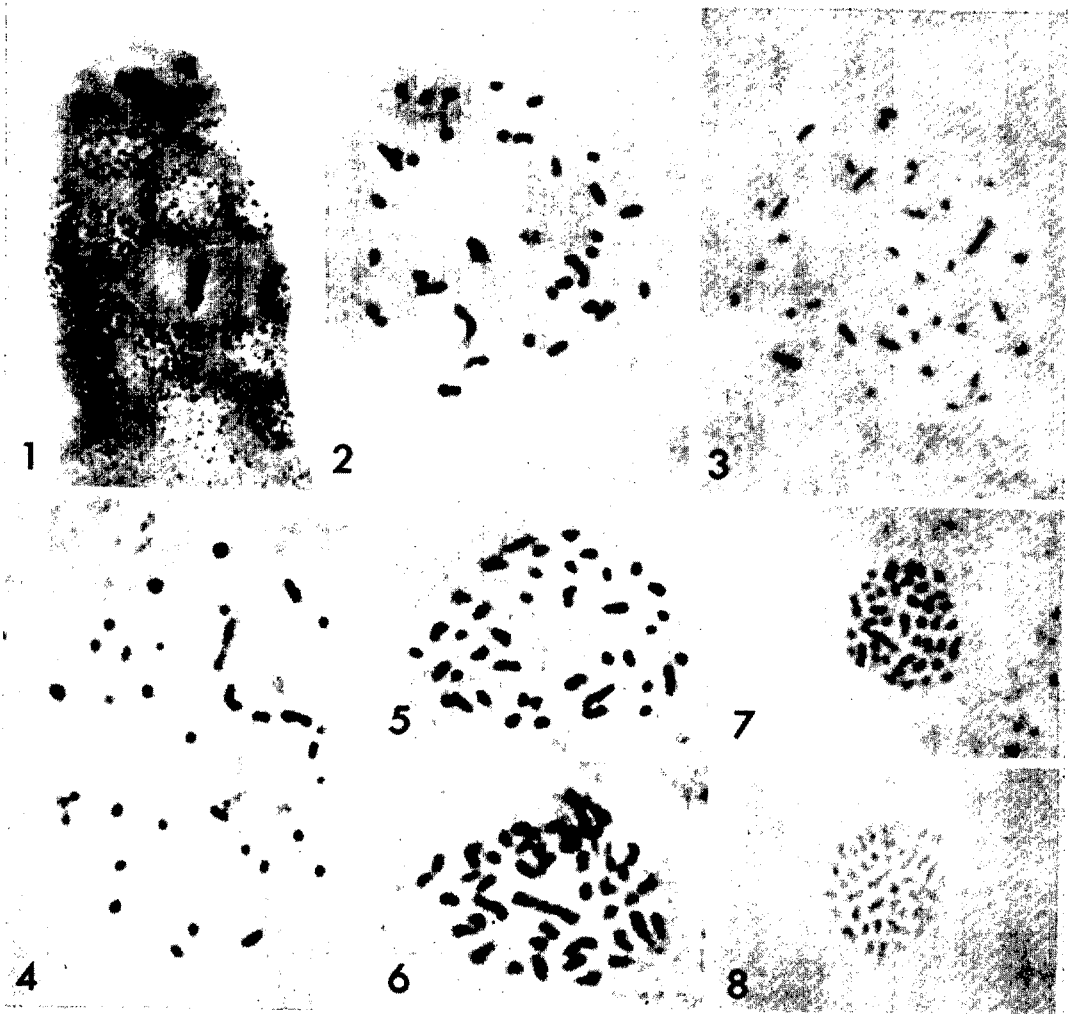
Figs. 1–8. Oogonial divisions in the germinal zone of the ovaries of various populations of *M. incognita.* 1) Apical part of ovary with distinct cap cell (epithelial), many oogonia at interphase and one oogonium at metaphase. 2–4) Oogonia at late prophase with chromosomes widely spread. 5–6) Early metaphase figures. 7–8) Metaphase figures in polar view. Fig. 1, × 1250, Figs. 2–8, × 2700.

matids that lie parallel to each other (Fig. 10). Most chromosomes, however, are arranged close to each other and cannot be counted precisely even in the most favorable figures. When the oocytes pass into the uterus, the chromosomes condense further, becoming spherical with no distinct chromatids, and are characteristically crowded in a small, oval or spherical area of about 10–15-μm diameter (Figs. 11–12). Counting of the chromosomes at this stage is impossible. This arrangement persists until the oocytes have migrated into the posterior half of the uterus where they advance to metaphase of the first maturation division (Figs. 9,13,14). Only 1–3 of the approxi-

mately 5–15 oocytes present in the uterus of a female are at metaphase. Even at metaphase, the chromosomes continue to be arranged close to each other and counting is very difficult (Fig. 14).

More reliable chromosome counts were made from colcemid-treated material in which chromosomes were spread in a relatively large area. Early prometaphase chromosomes appeared as dyads with two chromatids lying parallel to each other (Figs. 15–18). In the best preparations such chromosomes appeared similar to those of other mitotic parthenogenetic species of *Meloidogyne,* but usually they were not well stained and not arranged on a plane.
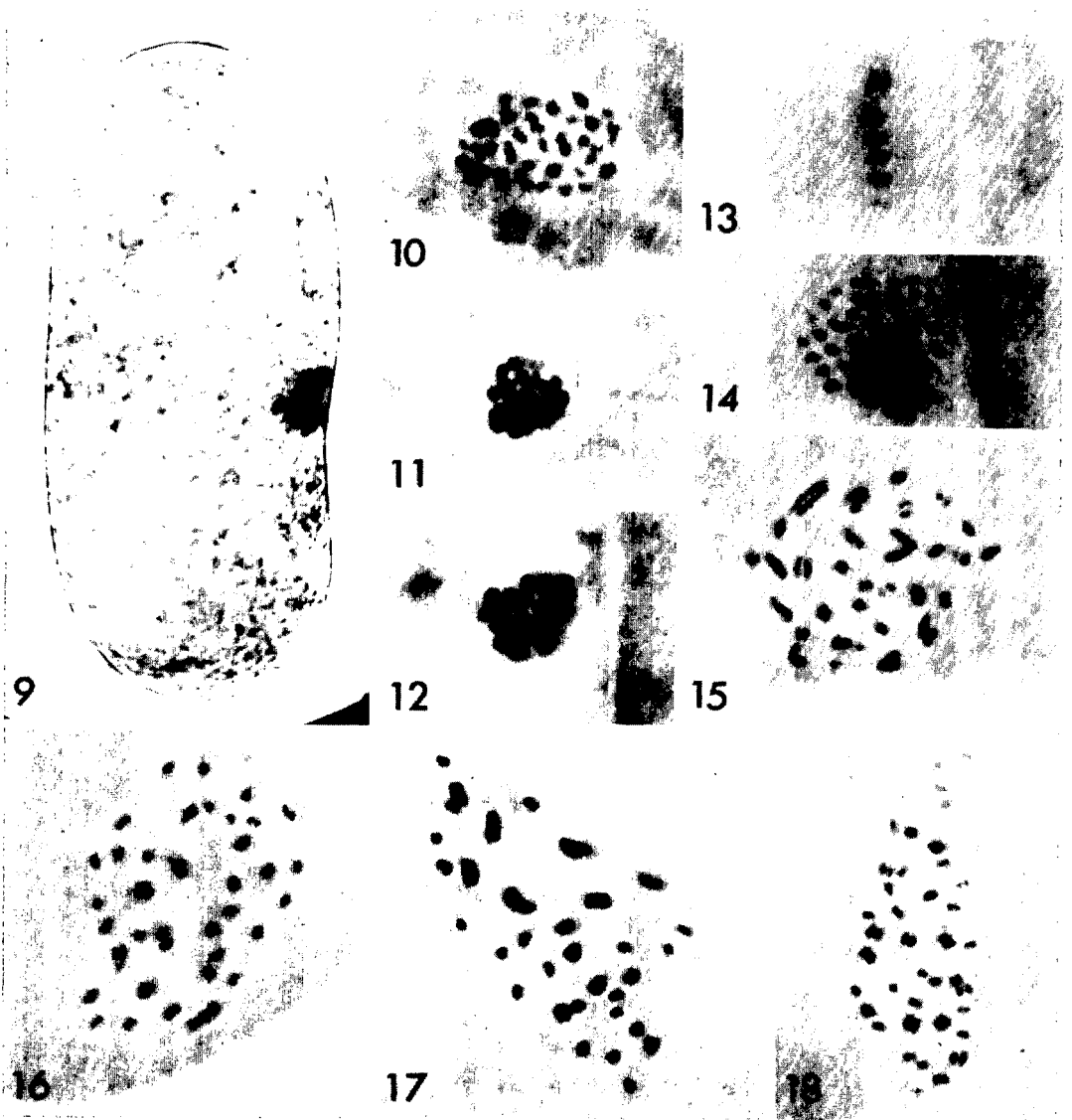
Figs. 9–18. Maturation of oocytes of *M. incognita.* 9) Oocyte at metaphase I. 10) The prophase chromosomes of an oocyte approaching the oviduct-spermatotheca region. 11–12) Two prophase figures of oocytes located in the anterior half of the uterus illustrating the characteristic behavior of the chromosomes of *M. incognita* (for explanations see text). 13–14) Metaphase figures of oocytes located in the posterior half of the uterus. 15–18) Prophase figures of oocytes in the same stage as oocytes of figures 11 and 12, but following treatment with colcemid. The chromosomes are spread over a large area and their chromatids are distinct. Fig. 9, × 1000; Figs. 10–18, × 2700.

Considerable polymorphism was observed among populations with regard to relative size of prometaphase chromosomes. Some populations had chromosomes of small (0.4–1.0 μm) uniform size (Figs. 27–28). In some populations, however, several chromosomes were distinctly larger than the rest. Examples of the most commonly observed variation is given in Figs. 23–26, where populations with 3–5 dis-

tinctly larger and 2–4 intermediate size chromosomes are illustrated.

The chromosome number determined in prometaphase and metaphase figures of colcemid-treated oocytes was approximately the same as that of oogonial divisions and represented the unreduced, or somatic, number. Such chromosome counts were more precise and constant within a given population than counts made from oogonial di-
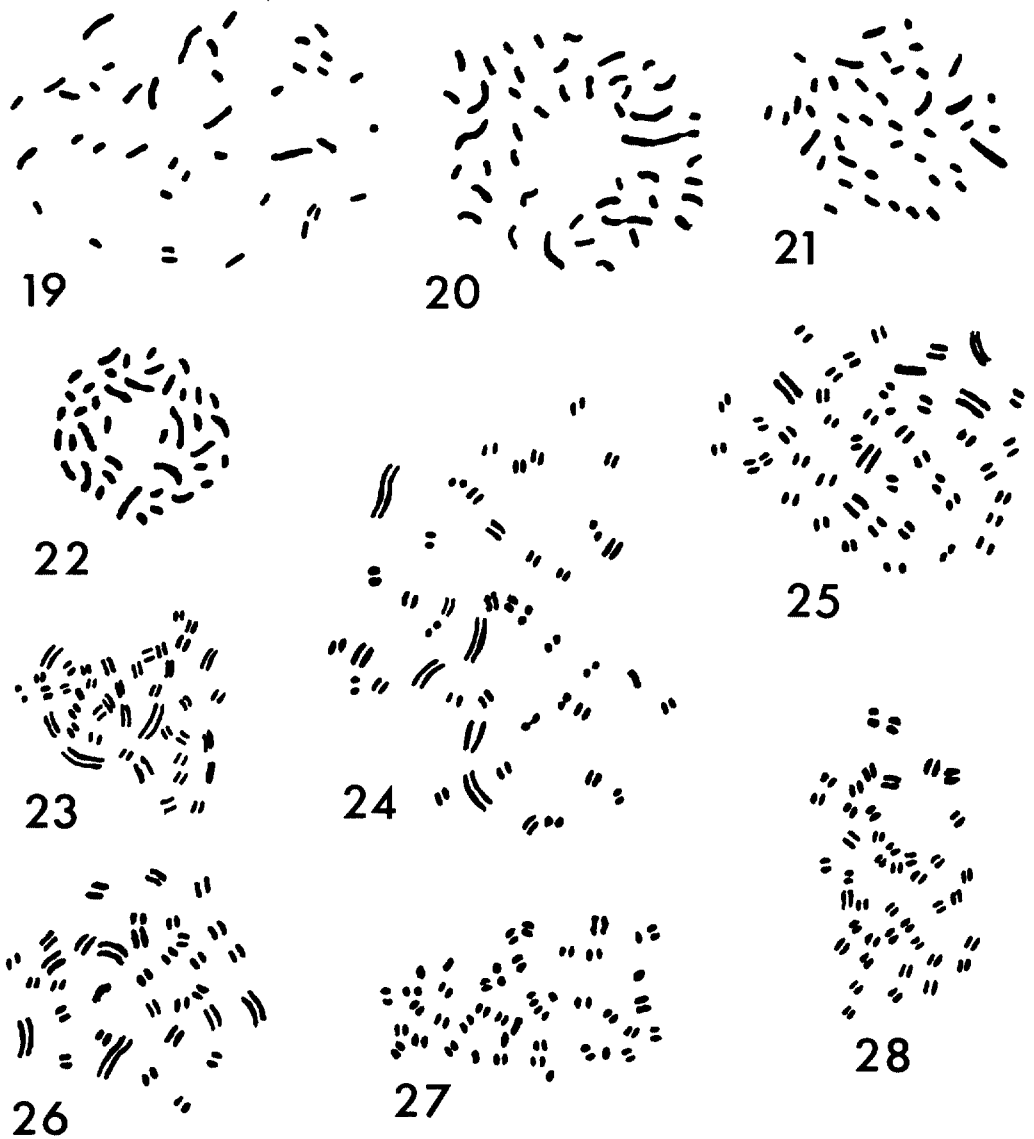
Figs. 19–28. Camera lucida drawings of prophase and early metaphase figures of various populations of *M. incognita* illustrating some of the chromosomal polymorphism encountered in this species. 19–22) Oogonial divisions. 23–28) Prophase figures in primary oocytes following treatment with colcemid. × 2700.

visions. Still, an exact chromosome number could be determined definitively in only 30% of the populations, whereas a range of ±1 chromosome was accepted as a more realistic estimate of the chromosome number of the rest of the populations.

Following metaphase, the first maturation division of oocytes was completed with anaphase and telophase occurring rapidly, while the oocytes were still in the posterior part of the uterus. One polar nucleus and the egg nucleus were formed. A second maturation division was never observed.

Instead, the egg nucleus proceeded with the first cleavage division which, most often, occurred after egg deposition.

Numerous spermatozoa were observed in the spermatothecae of females associated with males and one sperm nucleus was seen inside one end of most oocytes present in the uteri of such females. The sperm nucleus did not form a true pronucleus and did not fuse with the egg nucleus. It remained unchanged in the oocyte until completion of the maturation division and became diffuse and degenerated in the cyto-

plasm during or after the first cleavage division. Therefore, no true fertilization occurred and reproduction was by mitotic parthenogenesis (apomixis).

The chromosome number was determined for a small number of populations from metaphase figures of the first or second cleavage division and corresponded to the number determined from oogonia and oocytes. In one female of population "E506–Saudia Arabia," 88 chromosomes were counted at metaphase of the second cleavage division; i.e., twice the chromosome number observed in oogonia and oocytes of other females of the same population. This is the only case of higher degree of ploidy observed in *M. incognita.*

DISCUSSION AND CONCLUSIONS

The present study confirms earlier tentative conclusions (18) that *M. incognita* is a conglomerate of a large number of obligatorily parthenogenetic populations, cytogenetically closely related to each other and constituting a biological unit (species) distinct in many respects from other species of root-knot nematodes.

All 220 populations reproduced exclusively by mitotic parthenogenesis. Although two chromosomal forms could be recognized, one with 32–36 and another with 40–46 chromosomes, those forms do not correspond to any of the subspecies or other forms of *M. incognita* recognized by some taxonomists. Also, there seems to be no correlation between the two cytological races and the four recognized host races (Table 1). Furthermore, preliminary comparative studies, not included in this paper, failed to consistently differentiate the two cytological races morphologically.

The peculiar behavior of prophase chromosomes during maturation of oocytes is the most striking cytological feature that differentiates *M. incognita* from all other *Meloidogyne* species. In other species, oocytes advance to metaphase as soon as they pass from the spermatotheca into the uterus, and their prometaphase and metaphase chromosomes are well separated from each other and can be counted relatively easily. Contrary to this, the prophase chromosomes of maturing oocytes of *M. incog-*

nita are packed together in a small area. They remain in this stage till the oocytes pass to the posterior part of the long uterus and then suddenly advance to metaphase. Most of the oocytes present in the uterus of *M. incognita,* therefore, are in prophase with the chromosomes crowded in a small area near the surface of the oocytes. This characteristic chromosomal behavior is a unifying feature for members of the *M. incognita* complex and appears to have phylogenetic significance. It indicates monophyletic evolution for *M. incognita* distinct from all other known lines of cytological evolution in the genus *Meloidogyne.*

The chromosomal polymorphism observed among populations, especially of race A, suggests an extensive karyotypic evolution in these parthenogenetic organisms. I have not attempted to group the populations according to the morphology of their chromosomal complement. This will be done in conjunction with a precise determination of the amount of DNA per nucleus and may help elucidate the evolutionary relationship of the various cytological forms of *M. incognita.*

Chromosomal structural changes such as translocations, chromosome fragmentation, and fusions may have been responsible for some of the observed chromosomal polymorphism and the variation in chromosome numbers present within each chromosomal race. Aneuploidy (loss or gain of whole chromosomes) may also have contributed to the variation in chromosome numbers within each race and may also be responsible for the cytological evolution of race A. Thus race A may have evolved from the diploid race B as a cytological form, trisomic or polysomic for a number of chromosomes (possibly 4–10). It may be equivalent to a triploid for about one-half of its chromosomes and diploid for the rest. Such a pathway of evolution, however, does not explain the chromosomal polymorphism observed in race A. An extensive karyotypic evolution leading to chromosomal polymorphism would not normally be expected in a mitotically parthenogenetic organism. Therefore, one could assume that much of the observed chromosomal polymorphism was established at an early period when these nematodes were undergoing meiosis

and possibly reproduced by amphimixis or faculative parthenogenesis. This view supports a different pathway of evolution according to which race A evolved as a triploid following hybridization and the subsequent establishment of apomictic reproduction. This is further explained in the last paragraph of the present discussion.

Alternatively, the observed variation in chromosome numbers can be attributed to simple breakages of certain long chromosomes without reassociation of the fragments. The chromosome fragments would not be lost in subsequent generations because of the holocentric nature of the chromosomes of these nematodes, and individuals with such chromosome fragments would give rise to clones with increased chromosome numbers. Such chromosomal breakages undoubtedly have occurred, especially following the establishment of mitotic parthenogenesis, and have contributed to the variation in chromosome numbers within each chromosomal form. It is doubtful, however, that they are solely responsible for the cytological evolution of race A. If this were the case, populations of race A with 40–46 chromosomes would tend to have fewer large chromosomes than populations of race B, and this apparently is not true. Also the two chromosomal forms would have the same amount of DNA per nucleus, and this again is not true (unpublished data). For this reason I have considered race A as a triploid derivative rather than a diploid derivative. Biochemical data involving the electrophoretic patterns for certain enzymes of *Meloidogyne* have been interpreted as supporting diploidy for *M. incognita* (4). Still, these interpretations, as well as interpretations about the exact or presumed genetic basis of enzyme polymorphism in the genus *Meloidogyne* (assignment of loci and alleles per locus), have not been confirmed genetically and are highly theoretical.

The cytogenetic relationships among species of *Meloidogyne* have been discussed earlier (18). Briefly, the basic chromosomal complement of the genus has been assumed to have n = 18 chromosomes as in *M. graminicola, M. naasi, M. graminis, M. ottersoni, M. exigua,* and other amphimictic or facultatively parthenogenetic species.

This number has been reduced to n = 17, 16, 15, and 14 in facultatively parthenogenetic populations of *M. hapla.* Furthermore, mitotic parthenogenetic populations of *M. hapla* with 2n = 30, 3n = 45, and 3n = 48 chromosomes and possibly diploid or triploid populations of *M. arenaria* with 36 and 54 chromosomes, respectively, have been assumed to have evolved from ancestral meiotic forms of *M. hapla.* The cytogenetic relationship of *M. incognita* and *M. javanica* to other members of the genus is not understood. Nevertheless, in the light of the information of the present study, the cytogenetic status of *M. incognita* can be reassessed. On the assumption that the basic number for the genus is n = 18, the 32–36 chromosomes of *M. incognita* race B could represent a diploid or various aneuploid forms derived from diploids with n = 18 chromosomes. However, the 40–46 chromosomes of the predominant race A of *M. incognita* are too many for a diploid and too few for a triploid. To explain this deviation one may assume that race A represents a group of triploids derived from meiotic relatives with reduced basic chromosome numbers; i.e. with n = 14 or 15, as assumed earlier, for triploid apomictic populations of *M. hapla* (18). Unfortunately, meiotic forms that could be regarded as progenitors of *M. incognita* have not been found among the 500 populations of root-knot nematodes studied thus far. Therefore, the origin of *M. incognita* remains unresolved. It can only be stated that *M. incognita* may have followed an evolution analogous to that of *M. hapla* originating from a meiotic stock slightly different from that of *M. hapla* or from the same ancestral stock from which *M. hapla* has evolved but at a much earlier evolutionary period.

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