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A REVIEW ON CYTOGENETICAL STATUS OF PLANT-PARASITIC ROOTKNOT NEMATODE (*Meloidogyne* spp).

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Abstracts: **Root-knot nematodes (RKN), (*Meloidogyne* spp.) are obligate endoparasites of more than 3000 species of plants, which results in $80 billion worth of economic loss every year, worldwide. Considerable variation in ability to break crop resistance and to reproduce on different crop species is observed both between and within *Meloidogyne*. The main feature of plant-parasitic root-knot nematode is its potential host range encompassing more than 3,000 plant species. The adaptation of root-knot nematodes to its various environments of plant hosts raises questions about genome plasticity leading to genetic variation and adaptive evolution. It has been proposed that epigenetic as well as cytogenetic variation mechanisms might in part be responsible for the generation of phenotypic variants that provide material for rapid adaptation of more than 3000 plant host environments. As the results, *Meloidogyne species* constitutes a unique model system to study the links between variation in genome structure, mode of reproduction, and adaptation to environment and hosts, in relation with parasitic success. Controlling of obligate plant parasitic-root-knot nematode is generally difficult and also with the banning of nematicides chemicals that control nematode, because of adverse environmental impacts, it is imperative that new safe disease control strategies for nematode management should be developed and implemented. In order to develop new and environmentally safe disease control strategies, it is essential to understand the molecular, cellular and genetics basis of how RKN interact, evolve and adapt various host plants. Thus, in this review paper, therefore, we have conducted extensive literature reviews to identify the cytogenetic status (classical cytogenetics-molecular cytogenetics) of root-knot nematodes (*Meloidogyne spp*.). Specifically, the status of the studied areas of cytogenetic in RKN as well as the pattern of cytogenetic variation and evolution were summarized.**

Keywords: **Cytogenetics, Cytotaxonomy, Evolution, Karyotype, Meloidogyne spp, Rootknot nematodes.**

1. INTRODUCTION

Root-knot nematodes (RKN), (*Meloidogyne* spp.) are obligate, sedentary endo-parasites of more than 3000 species of plants. The root-knot nematodes (genus Meloidogyne) are among the world's most destructive crop pests, causing global agricultural losses close to $80 billion annually (Moens, *et al*., 2009). The genus *Meloidogyne* contains over 90 described species and each of these species typically has an extremely broad host range as many as 3000 plant species (Trudgill and Blok, 2001). During parasitism, RKNs engage in prolonged and intimate relationships with their host plants (up to six weeks) often involving complex morphological and physiological alterations of host cell into specialized cell called giants (**Figure:1**). The giant cells are essential for successful parasitism because this unique feeding structure provides nutrition to develop second stage juvenile (J2) in to adult (Abed, *et al*., 2003).

Controlling of obligate plant parasitic-root-knot nematode is generally difficult and also with the banning of nematicides chemicals that control nematode, because of adverse environmental impacts, it is imperative that new safe disease control strategies for nematode management should be developed and implemented. In order to develop new and safe disease control strategies, it is essential to understand the molecular, cellular and genetic mechanisms of how RKN interact with

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diverse host environments. Considerable variation in ability to break crop resistance and to reproduce on different crop species is observed both between and within species (Williamson and Roberts, 2009). The main feature of RKN is its potential host range encompassing more than 3,000 plant species. The adaptation of RKN species to its environment raises questions about genome plasticity leading to genetic variation and adaptive evolution. It has been proposed that epigenetic as well as cytogenetic variation mechanisms might in part be responsible for the generation of phenotypic variants that provide material for rapid adaptation of more than 3000 plant host environments (Trudgill and Blok, 2001). Thus, RKN species constitutes a unique model system to study the links between variation in genome structure, mode of reproduction, and adaptation to environment and host, in relation with parasitic success. Hence, in this review paper, therefore, we have conducted extensive literature reviews to identify the cytogenetic status (from classical cytogenetic to molecular cytogenetics) of root-knot nematodes (*Meloidogyne spp.*). Specifically, the status of the studied areas of cytogenetic in RKN as well as the pattern of cytogenetic variation and evolution were summarized.

2. CYTOGENETICAL STATUS OF PLANT-PARASITIC ROOT-KNOT NEMATODE (*MELOIDOGYNE* SPECIES)

Root-knot nematodes (RKN), *Meloidogyne* spp., belong to the order Tylenchida. These small round worms (typically from 300 µm to 2 mm for vermiform juveniles and pyriform females, live in soils and are obligate and sedentary endoparasites of plant roots. They harbour at their anterior end a hollow, protrusible stylet, which they use to both inject secretions into and withdraw nutrients from the infected root cells. They have evolved very sophisticated interactions with their host (Abad, *et al.*, 2003). On the bases of cytogenetic studies of about 600 populations (representing 24 species) and in collaboration with the International *Meloidogyne* Project, Triantaphyllou (1985) was able to demonstrate that root-knot nematodes have undergone extensive cytogenetic diversification, probably unparalleled by that of any other animal group. Triantaphyllou concluded that characteristic features are the establishment of meiotic and mitotic parthenogenesis in association with various degrees of polyploidy and aneuploidy (Triantaphyllou, 1985a).

Mode of Reproduction and Ploidy Level of Root-knot Nematodes

Root-knot nematodes have undergone extensive cytogenetic diversification. According Triantaphyllou, 1985, the diverse cytogenetic features that RKN exhibited included, the meiotic and mitotic parthenogenesis in association with various degrees of polyploidy and aneuploidy. Obligatory cross-fertilization also occurs in some diploid and polyploid forms (e.g. *M. kikuyensis* and *M. megatyla*), whereas facultative meiotic (automixis) (e.g. *M. exigua*, *M. chitwoodi* and *M. graminicola*) and obligatory mitotic parthenogenesis (apomixis) (e.g. *M. incognita*, *M. enterolobii* and *M. oryzae*) prevail in most polyploid and aneuploid forms (**Table 1**). The trend from amphimictic reproduction to apomixis is generally associated with shorter life cycles, higher reproductive rates and increasing pathogens (Triantaphyllou, 1985b).

Table 1: Mode of reproduction and ploidy level of root-knot nematodes, *Meloidogyne spp.*

Meloidogyne **Species**	**Mode of Reproduction**	**Predicted Ploidy level**	**References**
M. arenaria	Obligate	Diploid	Triantaphyllou, 1963, Janati, *et al.*, 1982, Goldstein and Triantaphyllou (1982) Marais and Kruger, 1991
	Mitotic parthenogenesis	(hypo)triploid	
M. carolinensis	Amphimixis	Diploid	
M. incognita	Obligate	Diploid	Janati, *et al.*, 1982, Marais and Kruger, 1991
	Mitotic parthenogenesis	(hypo)triploid	
M. hapla	Facultative	Diploid (aneuploid?)	Triantaphyllou, 1966, Dalmasso and Bergé, 1975, Marais and Kruger, 1991.
	Meiotic parthenogenesis	Tetraploid	Triantaphyllou, 1984, 1991
		Tetraploid	Triantaphyllou,1984, 1991, Van der Beek, *et al.*, 1998
M. hapla	Obligate	Diploid	Triantaphyllou and Hirschmann, 1980, Marais and Kruger, 1991.
	Mitotic parthenogenesis	(hypo)triploid	Dalmasso and Bergé, 1975, Triantaphyllou, 1966.

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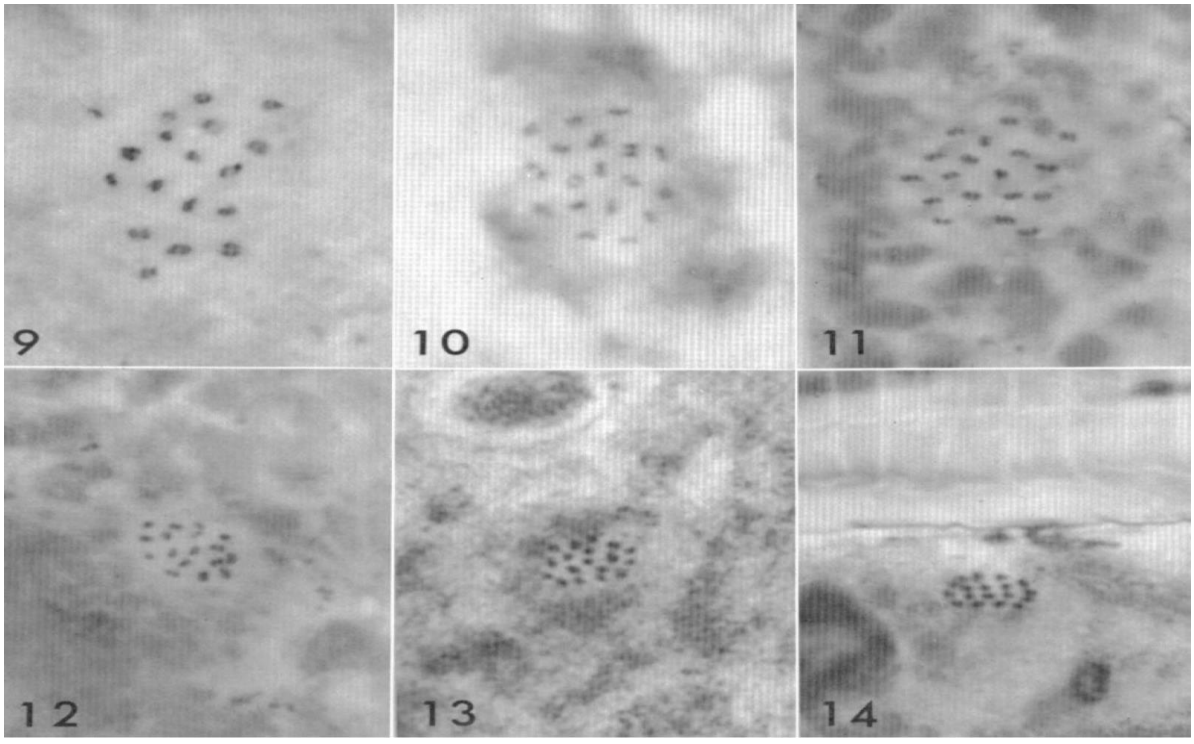
Karyotype Study of Root-knot Nematodes Species (*Meliodogyne Spp*.)

The main studied cytogenetic features of plant-parasitic root-knot nematode that have undergone an extensive diversity are chromosome number with various degrees of polyploidy and aneuploidy results from meiotic and mitotic parthenogenesis. Cytological studies of root-knot nematodes have mainly focused on the determination of chromosome numbers during gametogenesis and early cleavage, since the size of chromosomes are extremely small. (Triantaphyllou, 1981).

Chromosome Number of Root-Knot Nematode (*Meioidogyne Species*)

Root-knot nematodes (RKNs) (*Meloidogyne spp*.) are obligate endoparasites of majority of conomically importance crops. Root-knot Nematodes (*Genus Meloidogyne*) contain more than 300 species. They exhibit a wide continuum of variation in their reproductive strategies, ranging from amphimixis to obligatory mitotic parthenogenesis. The chromosome number of RKN is quite variable between and within species of *Meloidogyne*, even populations of the same parthenogenetic species may have different number of chromosomes (**Table 2).** For example, various members of *M. arenaria* species contained 36, 51, 53 or 54 chromosomes. Similarly, among 220 isolates of *M. incognita* species, majority of the isolates contain primarily 40–46 chromosome number whereas, some isolates had 32–36 chromosomes and one female had 88 chromosomes number (Triantaphyllou, 1966, 1981). The majority of 29 members of facultative meiotic parthenogenic of *M. hapla* had 15-17 haploid chromosome number, whereas three presumably polyploidy mitotic pathenogens possessing 45 chromosomes (Triantaphyllou, 1966). Other author reported that some facultative meotic parthenogenic strains of *M. hapla* had *n* = 16 (Liu and Williamson, 2006).



Figures :1 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.

Data emerged from six South African *Meloidogyne* species showed the existence of the inter-specific and intra-specific variation in chromosome number among six Meloidogyne species (Marias and Kruger, 1991). Accordingly, Amphimictic and facultative parthenogenetic species have n = 18 chromosomes. In contrast, facultative parthenogenetic species, *M. chitwoodi* and *M. hapla* had chromosome number, n = 14 – 18 and n = 17, 16, 15 & 14 chromosomes respectively. The chromosomes numbers of two other *Meloidogyne* species, *M. Idkuyensis* and *M. spartinae* are unique by having a haploid complement of only seven chromosomes (Eisenback and Triantaphyllou, 1991).

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RKN are also highly variable with respect to their chromosomal complement. Several attempts were made to establish the basic chromosome number of the genus and to establish the evolutionary relationship of the *Meioidogyne* species on the basis of cytogenetics. Earlier studies postulated that the basic chromosome number of the genus is nine, and the facultative parthenogenetic populations are tetraploid, whereas the obligatory parthenogenetic populations are pentaploid (Triantaphyllou, 1963, 1966). Subsequent studies, however, showed that the basic chromosome number of the genus is n = 18 (Triantaphyllou, 1979; 1981; Triantaphyllou and Hirschmann, 1980). The two amphimictic species with a haploid chromosome number of seven are not considered in this analysis (Eisenback and Triantaphyllou, 1991). RKN are also highly variable with respect to their chromosomal complement. It is generally admitted that the haploid number of the genus is *n*=18, but most populations have somatic chromosome numbers ranging from 30 to 50, and thus are thought to be either diploids or triploids (Triantaphyllou, 1985a). In fact, somatic chromosome numbers that are perfect multiple of 18 are not frequently observed, implying that there has been extensive aneuploidy or polysomy and structural rearrangements such as deletions, duplications, and translocations. These events may have been frequent, in part because, like most nematodes, RKN have a diffuse centromere lacking localized kinetochore activity (Triantaphyllou, 1983). Amphimictic RKN species are exclusively diploid, while diploid, triploid and rare tetraploid forms are encountered within parthenogenetic species (**Table: 2**). As an example, most populations of *M. incognita*, the most prevalent apomictic RKN species, are considered to be (hypo)triploid, with a set of 3*n*=40–48 chromosomes, although diploid populations with chromosome numbers ranging from 2*n*=30 to 39 are not so infrequent (Janati, *et al*, 1982; Marais and Kruger, 1991).

Table 2: Typical cytogenetic variation of root-knot nematodes (*Meloidogyne species*).

Meloidogyne **Species**	**Mode of Reproduction**	**Somatic Chromosome Number**	**Predicted Ploidy level**	**Pattern Frequency**	**References**
M. arenaria	Obligate	35–38	Diploid	+	Triantaphyllou, 1963, Janati, *et al*., 1982, Goldstein and Triantaphyllou (1982)
»	Mitotic parthenogenesis	40–48	(hypo)triploid	+	
		50–56	Triploid	+++	
M. carolinensis	Amphimixis	36	Diploid	+++	
M. incognita	Obligate	30–39	Diploid	+	Janati, *et al*., 1982, Marais and Kruger, 1991
»	Mitotic parthenogenesis	40–48	(hypo)triploid	+++	
M. hapla	Facultative	26–36	Diploid (aneuploid?)	+++	Triantaphyllou, 1966, Dalmasso and Bergé, 1975, Marais and Kruger, 1991.
»	Meiotic parthenogenesis	56	Tetraploid	+	Triantaphyllou, 1984, 1991
		68	Tetraploid	+	Triantaphyllou,1984, 1991, Van der Beek, *et al*., 1998
M. hapla	Obligate	30–37	Diploid	+	Triantaphyllou and Hirschmann, 1980
»	Mitotic parthenogenesis	42–48	(hypo)triploid	+++	Dalmasso and Bergé, 1975, Triantaphyllou, 1966.

The prevalence of each chromosomal pattern in the natural populations studied: +++, prevalent form; +, rare form.

Chromosomes Size of Root-knot Nematodes (*Meloidogyne spp.*)

Cytogenetic data of root-knot nematode emerged from several previous studies have focused mainly on identification of chromosome number during gametogenesis & early cleavage along with mode of reproduction. This is due primarily to the extremely small size of their chromosomes (Triantaphyllou, 1962). The largest chromosome in *M. incognita* is 3~um and the smallest is about 0.5~um in length (Triantaphyllou, 1981). Such chromosome size is obtained with propionic orcein method which increases their actual size due to swelling effect of the propionic acid (Triantaphyllou, 1981). Study conducted by Mandefro and Dagne, in 1998, in Ethiopia showed the largest chromosome in M. *incognita* was 1.6um and the smallest 0.3um long. The chromosome size of *M. incognita* populations did not show much variation. Most populations had a more or less equal chromosome size (O.4um). In all populations of *M. incognita* only 3 and 4 large chromosomes were found (1.5u). *M. javanica* populations, similarly did not show high polymorphism. The largest

chromosome was 1.6um and the smallest 0.3um long. Chromosomes of *M. ethiopica* populations were similar in size to that of *M. javanica.* Populations of *M. incognita, M. javanica* and *M. ethiopica* were not polymorphic in chromosomal size (Mandefro and Dagne, 1998 unpublished).

In general, attempts made to identify individual chromosome of RKN, based on size did not give consistent results in different populations. It was proposed that this inconsistence result is due to the extensive variation in the relative condensation of chromosomes, which makes identification of individual chromosomes highly difficult. Prometaphase chromosomes of *M. incognita* show considerable polymorphism with regard to relative size (Triantaphyllou, 1981). Some populations have small chromosomes (0.4 - 1.0 um) of uniform size. In some populations, however, several chromosomes are found distinctly larger than the rest, the larger ones being 3 to 4 times the size of the smaller chromosomes. Relative to other species, pronounced chromosomal variation is found in this species (Triantaphyllou, 1985a). Unlike Triantaphyllou (1981), *M. incognita* populations of from Ethiopia (Mandefro and Dagne, 1998) did not show extensive chromosomal polymorphism. Similarly, *M. javanica* and *M. ethiopica* populations did not show such variability. *Meloidogyne* populations show very low chromosomal size polymorphism. Although some variation in chromosome size within the chromosomal complement of, each species exists, differences are not very extensive.

Karyotype Evolution of Root-Knot Nematode (*Meioidogyne Spp.*)

Various interpretations of the karyotypic relationships within and between genera of the family ***Meioidogyne*** have been expressed (Triantaphyllou, 1970). The relationship of the karyotypes of the various genera, however, is more difficult to understand. Since Meloidogyne (n=l8) has twice as many chromosomes as Heterodera (n=9). The 1:2 numerical relationship of the basic chromosome numbers of the genera Heterodera (n=9) and Meloidogyne (n=18) has favored the hypothesis that the Meloidogyne karyotype evolved from Heterodera karyotype by polyploidizadon (tetraploid state Heterodera karyotype). Meloidogyne chromosomes, however, are significantly smaller than Heterodera chromosomes. This suggests that Meloidogyne karyotype may have been derived from the Heterodera karyotype by chromosomal fragmentation or other methods of chromosome number increase, rather than by polyploidizadon. Alternatively, it can be assumed that Meloidogyne karyotype is the ancestral one, and that the Heterodera karyotype has evolved from it through centric fusions or other mechanisms of chromosome number reduction. This view is partially supported by the trend for chromosome number reduction that is evident within the genus Meloidogyne. Thus, *M. hapla* populations with n=17, 16 or 15 are regarded to have been evolved from other Meloidogyne forms with n=l8. Still, there are several objections to the hypothesis that Heterodera karyotype evolved from the Meloidogyne karyotype (Lapp and Triantaphyllou, 1972). In general, therefore, the karyotypic relationships of the genera Heterodera and Meloidogyne are still not understood. The difficulty of establishing a definite relationship between the karyotypes of these two genera may actually indicate the lack of a close relationship between them (Triantaphyllou, 1970). Within the genus Meloidogyne, the two M. hapla populations that undergo meiosis (n=15 and 17) appear to have the same total DNA content. This means that whatever the pathway of derivation of these forms has been, it involved a rearrangement of the same genetic material rather than addition or elimination of chromosomes. The population with 45 chromosomes which undergoes no meiosis, and which has been considered to be a triploid, has slightly more DNA per nucleus but the value is not proportional to its chromosome number. Therefore, if this population is indeed a triploid, some reduction of DNA must have occurred following polyploidization (Lapp and Triantaphyllou, 1972).

Cytogenetic and Parthenogenesis Evolution of Root-Knot Nematode (*Meloidogyne spp.*)

The evolution of the genus, especially in relation to the mode of reproduction as well as the evolution of parthenogenesis recently attracted many researchers. Besides the practical aspects of the use of cytological characteristics for taxonomic purposes, cytogenetic information has helped to elucidate more fully the phyletic relationship and evolution of root-knot nematodes. Various major evolutionary cytogenetic steps have been revealed. These include cytogenetic aspects of nematode evolution speculated by Triantaphyllou (1982), the evolution of meiotic and mitotic parthenogenesis from cross-fertilization and the establishment of polyploidy and aneuploidy (Triantaphyllou, 1982). Polyploid parthenogenetic forms are responsible for more than 85% of the damage to agricultural crops caused by root-knot nematodes. Apparently, mitotic parthenogenesis combined with polyploidy and aneuploidies have given to the root-knot nematodes advantageous adaptations toward successful plant parasitism and have extended their ecological niche. He regarded the obligate amphimictic species (e.g. *M. megatyla*, *M. microtyla* and*M. carolinensis*) with *n* = 18 or 19 as the current species most closely related to the ancestral predecessors of *Meloidogyne* spp. He also speculated that the low chromosomal numbers

in most other nematodes (generally *n* = 4–12) (Coghlan, 2005) offered support for a polyploidy origin of nearly all of the species of *Meloidogyne*. At that time *M. spartinae* was regarded as being in a now-defunct closely related genus, *Hypsoperine*, but its low chromosomal complement (*n* = 7) was regarded as additional evidence for a condition of tetraploidy in the many species of *Meloidogyne* with *n* = 14–18. Plantard, *et al*. (2007) consider that the *n* = 7 chromosome number found in only a few species of *Meloidogyne* is a derived character from species with *n* = 13–19. Triantaphyllou (1985) regarded parthenogenetic species with 30–38 chromosomes as diploids, having arisen from diploid amphmictic species with *n* ~18, and species with *c*. 54 chromosomes as being triploids produced by the fusion of the chromosomal complements of diploid and haploid forms. The previously discussed existence of naturally occurring polyploid individuals in diploid populations provides additional support for polyploidy as a force in evolution, with aneuploidy or chromosomal fragmentation further modifying the chromosomal complement. Triantaphyllou (1985) pointed out that as most species of *Meloidogyne* reproduce by mitotic parthenogenesis and have variable chromosome numbers, their status as distinct species may be unclear.

Considering, the origin and evolution of parthenogenesis, It seems like they appear to have reproduced exclusively asexually for a long stretch of evolutionary time, the apomictic RKN species have been considered as one of the putative 'ancient asexual scandals' (Judson and Normark, 1996). Indeed, although the calibration of dates used indirect evidence, the divergence of the parthenogenetic RKN species from the amphimictic meiotic ones has been estimated to have occurred about 43 Myr ago (Esbenshade and Triantaphyllou, 1987) and might be far older (Hugall, *et al*., 1997). However, no definitive evidence of asexuality has been provided for these nematodes (such as the Meselson effect described in Judson and Normark, 1996). Molecular studies have nevertheless confirmed that the apomictic RKN species share a common lineage, and that they diverged early from meiotic species (Castagnone-Sereno, *et al*., 1993b, Baum, *et al*., 1994). There are several ways in which parthenogenetic lineages could arise (Simon, *et al*., 2003). In the case of RKN, no fossil records are available, and the ancestors of the genus are unknown. However, based on cytogenetic (Triantaphyllou, 1985) and isoenzyme data (Dalmasso and Bergé, 1983, Esbenshade and Triantaphyllou, 1987), the following assumptions are currently widely accepted: (1) the ancestral RKN were amphimictic animals, and the rare amphimictic species encountered today (eg *M. carolinensis*, *M. megatyla*) are considered as their closest relatives; (2) parthenogenetic species evolved from amphimictic species; (3) obligatory parthenogenetic (mitotic) species evolved from facultative parthenogenetic (meiotic) species, following suppression of meiosis during oocyte maturation (**Figure 4:**). Hybridization is a major route to parthenogenesis in animals, and may be implicated in the RKN. Polyploidization probably occurred by either intra- or inter-specific hybridization (ie fertilization of an unreduced diploid oocyte by an haploid spermatozoon). Since functional, parthenogenetically produced males may be present in populations under poor environmental conditions, they could be involved in such exceptional fertilization events. For example, *M. javanica* is suspected to be a triploid interspecific hybrid species (Dalmasso and Bergé, 1983). In the same line, a possible reticulate hybrid origin of apomictic RKN has been hypothesized as the result of combinations of closely related females with more diverse parental lineages (Hugall, *et al.*, 1999). So far, no evidence has been provided for alternative hypotheses about the origin of parthenogenesis in RKN, such as spontaneous origin by mutation in genes involved in the production of sexual forms, or due to infection by microorganisms such as *Wolbachia*. However, we cannot definitely exclude the possibility that there have been several different routes to apomixis in RKN species, as examplified by the insects *Otiorhynchus scaber* and *Rhopalosiphum padi* (Delmotte, *et al*., 2003)

Cytotaxonomy of Root-Knot Nematodes

About 500 populations of root-knot nematodes from the International Meloidogyne Project (IMP) collection have been examined with regard to mode of reproduction, chromosomal complement, and process of maturation of oocytes and spermatocytes. Such characters have proven to be very helpful and reliable in the taxonomic characterization of many species. The most important cytogenetic features of taxonomic importance, particularly for species identification, are mode of reproduction and chromosomal complement. Other cytogenetic characters of less significance are chromosome size and general morphology, chromosome behavior during maturation of oocytes and amount of DNA per haploid and diploid nuclei, process of maturation of oocytes and spermatocytes (Triantaphyllou, 1979, 1985a).

For example, *M. incognita* populations reproduce exclusively by mitotic parthenogenesis. There are two chromosomal forms within this species. One form has 2n=32-36 chromosomes and is considered to be diploid; the other form has

2n=41-46 chromosomes and probably represents a triploid. All populations of *M. incognita* have a unique cytological feature that separates them from populations of all other species of Metoidogyne: oocytes of *M. incognita* are at prophase as they pass through the spermatotheca and remain in this stage until they have migrated to the posterior part of the uterus, when they suddenly advance to metaphase. During all this prolonged period of prophase, the chromosomes are bunched close to each other and cannot be seen individually or counted. Contrary to this situation, oocytes of all the other *Meloidogyne species* pass through the spermatotheca into the uterus. Furthermore, the chromosomes are spread in a large area, are discrete, and can be counted fairly easily. These chromosome behaviors in the first maturation division in *M. incognita* is highly characteristic of the species (Triantaphyllou, 1981). The chromosomes are crowded together in a small spherical area and are not discrete. Reliable counting of chromosomes at this stage is very difficult if not impossible. Furthermore, the prophase is much more extended in time. In *M. javanica* there are no distinct cytological phenomena other than chromosome number. Chromosome number varies from 2n = 42 - 48, but most populations have 2n = 45 – 46 (Triantaphyllou, 1962, 1985a). At metaphase of the single maturation division" the chromosomes of M. *javanica* are univalent (dyads), spread in a large metaphase plate and can be counted more easily than those of any other species. Usually, two to four oocytes located in the uterus close to the spermatotheca are at metaphase and can be studied. All other oocytes in the uterus have advanced to anaphase and telophase and are of limited value for cytological study.

M. hapla are cytologically the most diversified species of RKN, belonging to two distinct cytogenetic races (A and B). Race A, the more common race, reproduces by cross-fertilization when males are present in a population and by meiotic parthenogenesis when males are absent or rare. The haploid chromosome number varies from n= 14-17. Pairing of homologous chromosomes has been verified by electron microscopy studies of the synaptonemal complexes formed during pachytene in oocytes and spermatocytes. Synaptonemal complexes and recombination nodules have been observed in all populations of race A. Race B populations reproduce exclusively by mitotic parthenogenesis. Some of them are diploid with 2n = 30-31, but most are triploid with 3n =43-48 chromosomes. No synaptonemal complexes occur in oocytes of Race B . Though the two races differ in their process of oocyte maturation, the development and morphology of the spermatogonial cells are quite similar. Populations of race A are readily identifit:d cytogenetically by the presence of 14 to 17 bivalent chromosomes (tetrads) at metaphase of the first maturation division of oocytes. None of the other three major species form bivalent chromosomes. However, it is not possible to distinguish race B of M. hapla from other species by this taxonomic character. Race B populations have univalent chromosomes (dyads) similar in morphology and behavior to those of M. arenaria and M. javanica. Also there is an overlap in chromosome number between M. hapla (race B) and M. javanica. Although more than 17 species have been investigated cytogenetically with chromosome number and other characters, cytogenetic features of taxonomic importance are not much justified for these species (TriantaphyIlou,1969, 1985a).

3. SUMMARY AND CONCLUSIONS

Root-knot nematodes (RKN), *Meloidogyne spp*., belong to the order Tylenchida. RKN have undergone extensive cytogenetic diversification in chromosome number and mode of reproduction. They exhibit a wide continuum of variation in their reproductive strategies, ranging from amphimixis to obligatory mitotic parthenogenesis. Majority of *Meloidogyne* species are apomictic. Some species can reproduce by automictic. Very few RKN species, *M. carolinensis, M. megatyla, M. microtyla, M. pini* are reproduced by amphimixis. Sex determination in the genus is not yet established. Environmental factors play a role in the sexual differentiation and inter-sexes in the genus. The trend from amphimictic reproduction to apomixis is generally associated with shorter life cycles, higher reproductive rates and increasing pathogens. The association between mode of reproduction and ploidy level were observed in RKN species. For, example, obligatory cross-fertilization occurs in some diploid and polyploidy, whereas automixis and prevail in most polyploid and aneuploid forms.

RKN are also highly variable with respect to their chromosomal complement, even populations of the same parthenogenetic species may have different number of chromosomes. The basic chromosome number of the genus is n = 18. Chromosome size of RKN ranged from 0.4 - 1.6 um. Although some variation in chromosome size within the chromosomal complement of, each species exists, differences are not very extensive. Extensive variation in chromosome number and mode of reproduction are important in cytotaxonomy. It has been proposed that *Meloidogyne karyotype* evolved from *Heterodera karyotype* by polyploidizadon since the ratio of basic chromosome number of *Heterodera* (n=9) to *Meloidogyne* (n=18) are **1:2**. Alternatively, RKN karyotype may have been derived from the *Heterodera karyotype* by

chromosomal fragmentation since its chromosome is much smaller than Heterodera. Based on cytogenetic and isoenzyme data the following assumptions are currently widely accepted: (1) the ancestral RKN were amphimictic animals, and the rare amphimictic species encountered today (eg *M. carolinensis*, (*M. megatyla*) are considered as their closest relatives; (2) parthenogenetic species evolved from amphimictic species; (3) obligatory parthenogenetic (mitotic) species evolved from facultative parthenogenetic (meiotic) species, following suppression of meiosis during oocyte maturation. In conclusion, data reviewed in this paper show that karyotypes of *Meloidogyne spp*., are not properly established.

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Conflict of Interest:

The authors declare there is no potential conflict of interest.

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