ADDIS ABEBA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

MORPHOLOGICAL, CYTOGENETIC AND ESTERASE ISOZYME VARIATION OF ROOT-KNOT NEMATODES FROM ETHIOPIA

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology

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May, 1998

Acknowledgment

I would like to extend my gratitude to Dr. Kifle Dagne, for his interest in the project, unreserved encouragement and follow-up of the research work and critical review of the manuscript as well as an excellent photographic work..

I thank, Ato Gashaw Mammo for allowing me access to his electrophoretic facilities; Dr. Eyualem Abebe and Prof. K.R. Barker for providing pertinent literature and sharing useful ideas; Dr. Legesse Negash for providing me space in his greenhouse; W/o Alemnesh Lemma for handling the nematode culture at PPRC, Ambo, in my absence. My thanks are also due to my parents who covered the costs of key electrophoresis reagents, and for their support and encouragement.

The Institute of Agricultural Research (IAR) is thanked for sponsoring my study and also for covering the costs of the field trips and thesis write-up. My thanks also goes to Swedish Agency for Research Cooperation with Developing Countries (SAREC) for covering the costs of the project through the School of Graduate Studies.

I also thank all those who helped me with one or another aspect of the research work. Last but not least, I thank my wife, W/o Yemisrach Tsegaye, for her help and patience.

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Abstract

Fifty-five populations of root-knot nematodes were established from individual egg masses collected from different parts of the country. Perineal pattern, stylet length, chromosome number and esterase isozyme phenotypes of these populations were investigated. Three species were identified: *Meloidogyne incognita* (58.2%), *M. javanica* (16.4%) and *M. ethiopica* (25.4%) based mainly on perineal pattern and esterase isozyme phenotypes. Populations of *M. incognita.* exhibited extensive variation in perineal pattern, whereas less variability was observed in populations of *M. javanica* and *M. ethiopica.* Stylet length of populations of *M. javanica* and *M. ethiopica* obtained from enset was found to be significantly larger ($P = 0.05$) than the other populations. This is thought to be a possible form of an adaptation.

Chromosome number was counted in the germinal zone of the ovary. High intra-specific and intra-population variability was observed in populations of *M. incognita* and *M. ethiopica.* In both species diploid, 36-38 and hypotriploid, 40-46 chromosome numbers were counted. *M. javanica* did not show much variability with a hypotriploid chromosome number of 40-46.

Four esterase isozyme phenotypes were identified in the populations of the three species. *M. incognita* and *M. javanica* populations had the I1 and J3 phenotypes, respectively, while *M. ethiopica* populations had two phenotypes, E3 and E2. E3 was found to be species-specific and potentially useful for diagnosis. Due to its high incidence, *M. ethiopica* is considered to be an important parasite.

1. Introduction

Plant-parasitic nematodes are one of the important plant pathogens. According to Agrios (1988), they stand fourth next to fungi, bacteria and viruses. They are widely distributed and attack almost all kinds of plants. Sasser (1987) ranks *Meloidogyne* (Goeldei) the first among ten most important genera of plant-parasitic nematodes.

From the world collection of 850 *Meloidogyne* populations, 97% were identified as belonging to only four species (Sasser, 1979b; 1980). The four commonly occurring species are: *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. *M. incognita* is the most dominant, accounting for approximately 64% of the populations. *M. javanica* accounts for 28% while *M. arenaria* and *M. hapla* together accounts for 5% of all populations.

Root-knot nematodes (*Meloidogyne* spp.) have a worldwide distribution, attacking many economically important crops (Sasser, 1979b; 1980). Wherever they are found serious losses result due to yield reduction, often related to the biological stress placed on the crop plant following infection. Crop plants manifest this stress in the form of suppressed root and shoot growth, discoloration of foliage, small size and poor quality of product and yield (Mai, 1985).

Important requirements for *Meloidogyne* to survive, infect and cause disease are; a suitable host plant, adequate moisture and favorable temperature for the host and for the development of the nematode (De Guiran and Ritter, 1979). In the presence of suitable hosts, nematode population densities remain high and hence, damage can be severe. In addition, interaction with other soil-borne pathogenic microorganisms sometimes enhances root destruction. For example, *M. incognita* infection predisposes the host plant to *Fusarium oxysporium* infection and also shows a synergistic action (Abawi and Barker, 1984). In general, the magnitude of damage is influenced by the nematode population density, soil type, and other biotic and abiotic factors which place the plant under stress (Shane and Barker, 1986; Barker and Noe, 1987).

The situation of plant-parasitic nematodes in Ethiopia is not well known. Stewart and Dagnachew (1967) first reported large galls of root-knot nematodes on some vegetables. Later, a more detailed survey of plant-parasitic nematodes revealed the presence of dozens of nematode species infecting several crops (O'Bannon, 1975). According to O'Bannon (1975), out of one hundred sixty nine samples collected from fifty-three major crops and potentially important plants from various agroecological zones, twenty-one genera of plant-parasitic nematodes were detected (Table 1). Among these, four genera were found to have the highest frequency.

Table 1. Nematode genera and percentage of occurrence.

Number	Nematode genera	% frequency of occurrence
1	*Aphelenchoids*	0.33
2	*Criconema*	1.30
3	*Ditylenchus*	0.66
4	*Helicotylenchus*	17.98
5	*Hemicycliophora*	1.63
6	*Heterodera* (larvae)	1.63
7	*Hoplolaimus*	0.98
8	*Macropostonia*	1.63
9	*Meloidogyne*	21.57
10	*Paratylenchus*	0.98
11	*Paratylenchoides*	0.66
12	*Pratylenchus*	18.96
13	*Radopholus*	1.63
14	*Rotylenchulus*	14.05
15	*Scutelonema*	1.30
16	*Trichodorus*	1.96
17	*Trophurus*	0.98
18	*Tylenchorhynchus*	4.90
19	*Tylenchulus*	1.96
20	*Tylenchus*	4.25
21	*Xiphinema*	0.66

The root-knot nematodes, *Meloidogyne* spp. were the most predominant species found associated with about 40% of the crops sampled (O'Bannon, 1975). Three species of this genus, namely, *M. incognita*, *M. javanica* and *M. ethiopica* were identified from the country. According to this survey, *Pratylenchus* spp., *Hemicotylenchus* spp. and *Rotylenchulus* spp. occurred in 34, 32 and 25% of the crops sampled, respectively. *Radopholus similis*, a serious pest of banana, and *Tylenchulus semipenetrans,* citrus and grape parasite, were found at lower frequencies but are potentially serious parasites. In subsequent years, other reports showed that root-knot nematodes are widely distributed in many parts of the country (Terefe Deyessa, 1986; Mehariew Genet, 1993). The problem of root-knot nematodes in horticultural crops, particularly vegetables, was also well-documented (Tsedeke Abate, 1985; Heusler and Ayele Onke, 1987).

Average annual yield loss on the world's major crops due to plant-parasitic nematodes is about 12.3% (Sasser, 1987). In terms of value, on 21 crops of which 15 are 'life sustaining', annual losses due to nematodes were estimated to be 77 billion dollars world wide. According to Sasser (1987), the real figure, when all the crops are considered, probably exceeds 100 billion dollars annually. Tropical countries had the majority of this loss annually. In Ethiopia, the loss due to nematodes is not actually known. The only yield loss study made, so far, indicated 55% and 46% loss in dry matter in Kenaf (*Hibiscus canabinus*) at Didessa and Melkawerer, respectively (IAR, 1985; 1986).

Species identification of nematodes, particularly the genus *Meloidogyne,* is difficult. This is due to a relatively small differentiation between species due to recent origin. There is a high intra-specific variation in many of their characters (Jepson, 1987; Bernard, 1989) possibly due to mutation event. Variability has been widely recognized in *Meloidogyne* spp. with morphometric, physiological, and cytogenetic characters (Netscher and Taylor, 1979). Hence, they are said to be a complex group of organisms morphologically and in all other aspects of their biology.

The complexity of the genus is even greater when some characters are looked at in more detail. There are different ploidy levels in some species. For instance *M. hapla* has diploid populations with n = 17, 16, 15 (2n = 34, 32, 30) and a triploid 3n = 45, 48 chromosomes (Triantaphyllou, 1966). Besides, the naming of chromosomal races, subspecies, varieties and host races have been established (Bernard, 1989).

Accurate identification of nematode species is highly required for nematological research, quarantine enforcement for regulatory purposes, and nematode management that does not rely on nematicides. Particularly, it becomes inevitable when control is planned using rotation of susceptible and resistant cultivars, and when more than one crop is involved (Sasser and Kirby, 1979). For species having a host race, like *M. incognita* and *M. arenaria,* identification to species level in itself is not sufficient. The existence of host races limit the use of resistant cultivars without local screening programs (Sasser, 1979a; Sasser *et al.*, 1982).

Investigation of proteins and various isozymes showed the presence of high genetic variability in the genus *Meloidogyne* (Dalmasso and Berge', 1978; Esbenshade and Triantaphyllou, 1985b). Among several isozymes studied, non-specific esterases were found to be useful to supplement morphological characters for the identification of species (Esbenshade and Triantaphyllou, 1985a). Besides, it also gives information on the phylogenetic relationships of the species.

No attempt has been made so far to characterize the morphological, particularly perineal pattern, cytogenetic or esterase isozyme phenotype variations among populations of *Meloidogyne* species in Ethiopia. The International *Meloidogyne* Project (IMP), which collected and characterized *Meloidogyne* populations from around the world, did not include Ethiopia (Saka, 1985). This could be due to lack of awareness of the nematode problem in the country and absence of a cooperator. Moreover, species identification was only done by O'Bannon (1975) and little effort was made later.

The objectives of this study were:

1. To identify isolates of root-knot nematode species from different areas of the country.

2. To characterize the cytogenetic variability of the isolates.

3. To characterize the non-specific esterase isozyme phenotype of the isolates.

4. To assess the morphological variability of the isolates in perineal pattern.

5. To assess the stylet length variation of the isolates.

6. To assess the taxonomic status of root-knot nematode populations.

2. Literature review

2.1. Plant-parasitic nematodes

Plant-parasitic nematodes are grouped into two Orders in the Phylum Nematoda. The majority are classified in the Order Thylenchida and the remaining three genera, known to be vectors of viruses, in the Order Dorylaimida (Jones and Jones, 1977; Dropkin, 1980). The majority of the plant-parasitic nematodes are ectoparasites and migratory endoparasites. These groups of nematodes are slenderical and vermiform. Sedentary endoparasites evolved to a more peered and sachet forms. The females when mature, remains fixed at one position while the male moves here and there in search of food. In this group, which includes root-knot nematodes, females are considered as parasitic and males as non-parasitic.

According to Eisenback and Triantaphyllou (1991), until 1989, the genus *Meloidogyne* had 61 identified species and two subspecies. Four species, *M. incognita, M. javanica, M. arenaria,* and *M. hapla*, are major pests with worldwide distribution and wide host range. Other seven species are important but they have a limited distribution and host range. Several other species attack few crops in specific agricultural regions and the rest are specialized to only one or few hosts and are highly localized (Sasser, 1979b; 1980; Jepson, 1987).

Most plant-parasitic nematodes infect root systems, while some species affect the above-ground plant parts. No specific above-ground symptoms are produced by root-knot nematodes.

Even below-ground symptoms are not always obvious. It is only the common species, *M. incognita, M. arenaria, M. javanica* and *M. hapla,* which produce more or less consistently large and obvious galling on their hosts (Jepson, 1987).

2.2. Morphological variability

Most of the morphological characters used in identification of *Meloidogyne* spp. exhibit large intra-specific variation. For characters to be useful in taxonomy they must have at least small variation within individual species and large range over the whole genus (Jepson, 1987). Concerning *Meloidogyne* females, the most important characters for identification are, stylet length, stylet shape and perineal pattern (Hirschmann, 1985; Jepson, 1987). The morphology of the perineal pattern is considered to be a highly valuable character for differentiating the most common species, although it is variable among populations and individuals within the same species (Eisenback, 1985b; Jepson, 1987). Diagnostic characters of perineal patterns of taxonomic importance include: dorsal arch, lateral field, striae, tail terminus and phasmids (Fig. 1).

In addition to qualitative description, Grimaldi De Zio *et al.* (1979) has attempted to describe perineal patterns of different populations of *M. incognita* with quantitative measurements. Three ratios with some consistency were obtained. However, it needed further tests on as much populations and species as possible to confirm for use in further diagnostic work.

Some of the variability is genetically controlled and some are as a result of external factors. Triantaphyllou and Sasser (1960) have shown that perineal patterns have a regular cuticular striae and additional foldings of the outer cuticular layer that could change from individual to individual within the same species. The change in general body shape also brings changes to the pattern as the female matures. This explains for some of the observed variability.

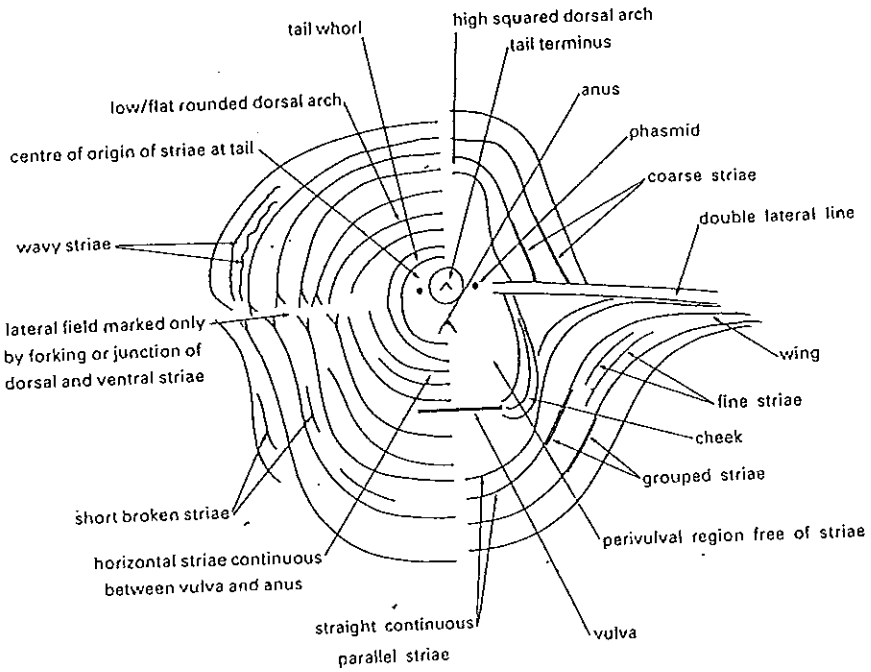


Fig. 1. Basic morphology of the perineal pattern of *Meloidogyne* females (Jepson, 1987).

Female stylet shape and length are useful diagnostic characters. For all *Meloidogyne* species the range of female stylet length is from 10 - 23 μm (Jepson, 1987). Majority of the species has a stylet length of 14 - 16 μm. Hence, the measurements of most species overlap with high intra-specific variation. For instance, different populations of *M. javanica* had a mean of 17, 16 and 15 μm length having a range of 14 - 18 μm (Jepson, 1987).

It is due to such extensive morphological variability that other less variable and accurate diagnostic characters are often required. In order to be meaningful in taxonomy, any other character, biochemical or molecular, should agree with the morphological distinction of the species (Triantaphyllou and Hirschmann, 1980).

2.3. Cytogenetics of root-knot nematodes

2.3.1. Modes of reproduction

Root-knot nematodes have undergone an extensive cytogenetic diversity. The main characteristic feature is the establishment of meiotic and mitotic parthenogenesis in association with various degrees of polyploidy and aneuploidy (Triantaphyllou, 1979; 1985a).

Three modes of reproduction are known in the genus *Meloidogyne*. These are: cross-fertilization (apomixis), facultative meiotic parthenogenesis (automictic) and obligatory mitotic parthenogenesis (apomictic) (Triantaphyllou, 1979). According to Triantaphyllou (1985a), out of the twenty-three cytogenetically described species, only six, namely, *M. kikuyensis, M. spartinae, M. carolinensis, M. megatyla, M. microtyla* and *M. subacrita*, appear to reproduce exclusively by cross-fertilization (Table 2). Another seven species reproduce by facultative meiotic parthenogenesis. When males are present they inseminate the female and proceed to normal cross-fertilization. Whereas in conditions where males are rare or absent, females produce eggs which, during maturation, undergo meiosis that results in the reduction of

chromosome number. Later, the somatic chromosome number will be re-established through fusion of polar nucleus with egg pronucleus (Triantaphyllou, 1979).

The apomictic group comprises the three agronomically important parasitic species: *M. incognita, M. javanica*, and *M. arenaria*. Females of this group produce oocytes, which do not undergo meiosis. At the end of the maturation period, the eggs will have the somatic chromosome number and proceed to cleavage without the need to be fertilized. When males are present and inseminate the females, the sperm do not participate in actual fertilization but rather dissolve in the cytoplasm (Triantaphyllou, 1962; 1979).

Apomixis excludes genetic recombination, but has got quite an advantage over the other forms of reproduction. The main advantage is that it supports extensive heterozygosity. Any gene mutation and most chromosomal mutations that may occur tend to accumulate in the genome making individual nematode heterozygous for many loci. The other advantage is that a population consisting entirely of females can give rise to progeny (Triantaphyllou, 1960; 1979). The extensive geographical distribution and host range of these species is attributed to this mode of reproduction and to their polyploid nature.

2.3.2. Polyploidy and aneuploidy (numerical variation)

Cytological studies of root-knot nematodes have focused mainly on the determination of

chromosome numbers during gametogenesis and early cleavage. This is due primarily to the extremely small size of their chromosomes (Triantaphyllou, 1962; 1981). The largest chromosome in *M. incognita* is 3μm and the smallest is about 0.5μm 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, 1962). Otherwise, with the feulgen staining method, the size of the chromosomes is very small and thus counting of the chromosomes is rather difficult.

Karyotypes of *Meloidogyne* spp. are not properly established. Attempts made to classify the chromosomes of *M. incognita* into groups, based on their size, did not give consistent results in different populations. According to Triantaphyllou (1981), this 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 - 1.0μm) 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).

There is an extensive variation in chromosome number between and within species of *Meloidogyne*, particularly among the agronomically important species (Table 2). Marias *et al.* (1991) also found inter-specific and intra-specific differences in chromosome numbers from various populations of six South African *Meloidogyne* species. Amphimictic and facultative parthenogenetic species undergo meiosis during maturation of the gametocyte and have n = 18 chromosomes. Deviations from this number are found in the amphimictic *M. microtyla*, n = 19 and in the populations of facultative parthenogenetic species, *M. chitwoodi*, n = 14 - 18 and *M. hapla* with n = 17, 16, 15 and 14 chromosomes. *M. kikuyensis* and *M. spartinae* deviate substantially from the rest of the root-knot nematodes by having a haploid complement of only seven chromosomes (Eisenback and Triantaphyllou, 1991). There are no known polyploid forms among amphimictic or facultative parthenogenetic *Meloidogyne* species, except for an egg mass isolate of *M. hapla* with n = 28, 34 chromosomes (Triantaphyllou, 1966; 1984).

Several attempts were made to establish the basic chromosome number of the genus and to establish the evolutionary relationship of the *Meloidogyne* 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).

Table 2. Summary of cytogenetic information related to root-knot nematodes.

Meloidogyne spp.	Popln Studied	Countries of Origin (Number)	Chromosome Number		Mode of Reproduction
			n	2n	
M. Kikuyensis	1	1	7		
M. spartinae	4	1	7		
M. carolinensis	2	1	18		
M. megatyla	1	1	18		Amphimixis
M. microtyla	2	1	18-19		
M. subacrita	1	1	18		
M. exigua	6	1	18		
M. graminicola	1	1	18		Facultative
M. graminis	10	5	18		
M. nassi	1	1	18		Meiotic
M. ottersoni	1	1	18		
M. chitwoodi	4	3	14-18		Parthenogenesis
M. hapla (race A)	48	24	13-17		
Polyploid	1	1	28		
Polyploid	2	2	34		
M. arenaria	18	13		30-38	
	34	21		40-48	
	68	32		51-56	
M. cruciani	1	1		42-44	
M. enterolobii	1	1		~46	Obligatory
M. hapla	6	3		30-32	
	11	8		43-48	
M. hispanica	4	4		33-36	
M. incognita	6	6		32-38	Mitotic
	215	64		41-46	
M. javanica	126	45		42-48	
M. microcephala	3	2		36-38	
M. oryzae	2	1		51-55	Parthenogenesis
M. platani	1	1		42-44	
M. quericiana	1	1		30-32	

Total 584

Source: (Triantaphyllou, 1985; updated 1991), Esbenshade and Triantaphyllou, 1991.

Triantaphyllou (1979) and Triantaphyllou and Hirschmann, (1980) have proposed an explanation for cytogenetic evolution involving four major steps. The first step, represents the evolution of meiotic parthenogenesis from amphimixis; the second involves changes in the karyotype, which resulted in the reduction of the haploid chromosome number; the third step includes the evolution of mitotic parthenogenesis. The fourth step includes the establishment of polyploidy in mitotic parthenogenetic forms. In each step an increased level of parasitism is associated. Particularly the second and fourth step gave rise to the most successful forms of root-knot nematodes of agricultural importance.

M. hapla has a peculiar cytologic character. In addition to different ploidy levels and two modes of reproduction (Table 2), Triantaphyllou (1984) has found a tetraploid isolate (n = 34) from a diploid n = 17 population. This tetraploid progeny produced, by meiotic parthenogenesis, a tetraploid and a diploid progenies in the proportions of 78% and 22%, respectively. In continuous culture, however, the tetraploid isolate could only be maintained by an artificial selection. Without such selection, no tetraploid could be detected in cultures after five generations of propagation. Triantaphyllou (1984) has strongly speculated that the genus *Meloidogyne* has arisen following a cycle of polyplodization and parthenogenetic phase.

Triantaphyllou (1991) also found tetraploid (n = 34 and 28) isolate of *M. hapla* which could be maintained, without an artificial selection, for only 2 years. This is due to conversion of the polyploids to diploids at a rate of 2.5%, and a low competitive ability of the polyploid to that of the diploid. Crossing of diploid and tetraploid isolates produced few triploid females that produced few egg masses that failed to produce an infection and gave only a small number of

viable juveniles. This indicated a partial reproductive isolation. Based on this observation, henceforth, he postulated that the present amphimictic forms of *Meloidogyne* with n = 18 chromosomes may represent a tetraploid rather than a haploid as a result of polyplodization of earlier forms with n = 9 chromosomes (Triantaphyllou, 1984). This observation revived the previously agreed upon question on the basic chromosome number of the genus and the possible pathway of parthenogenetic evolution, about which so far no conclusive statement has been made. Although it was not reviewed, the two amphimictic species, *M. kikuyensis* and *M. spartinae,* with n = 7 supported this statement.

Another unique feature of *M. hapla* is the occurrence of hermaphroditism both in diploid and polyploid isolates (Triantaphyllou, 1993). Once initiated, spermatogenesis continues for the whole life span of the female while oogenesis continues in the normal fashion. But the sperm does not take part in actual reproduction. This is because the sperm does not pass to the spermatheca, or the eggshell thickens prior to the maturation of the sperm. Hence, it is called non-functional hermaphroditism. Such hermaphroditic females give rise to ca. 50% hermaphrodite and 50% normal female. Similarly, the normal females of hermaphroditic isolates give the same proportion of progeny. Crossing studies done strongly suggested cytoplasmic rather than nuclear inheritance (Triantaphyllou, 1993).

Sex determination in the genus is not yet established. No sex chromosome has been distinguished in any species of *Meloidogyne* (Triantaphyllou, 1966; 1979). Several factors play a role in the sexual differentiation and inter-sexes in the genus. If conditions are unfavorable for the development of the nematode either due to shortage of food, overcrowding, or harsh

environment, most of the young females at second stage juvenile develop into males (Triantaphyllou, 1960). Normal male and male inter-sexes, having one testis and two testis, respectively, will be developed. In favorable conditions, in contrast, most or all of the juveniles develop into female with two ovaries.

2.3.3: Cytotaxonomy

Cytological information can assist in the identification of the most common species of root-knot nematodes (Triantaphyllou, 1979; Sasser, 1980). The most important cytogenetic features of taxonomic importance, particularly for species identification, are mode of reproduction and chromosome number (Triantaphyllou, 1985a). 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 (Triantaphyllou, 1979; 1985a).

The chromosome behavior 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. Reasonable counts are only possible in very few oocytes at the end of the uterus at metaphase I. Since they are apomictic species, the chromosome number is the same as that of somatic or oogonial cells.

Two groups with distinct chromosome numbers have been recognized in *M. incognita*. Populations with 32 to 38 chromosomes are considered to be diploid (n = 16 - 18) and those with more than 41 chromosomes are assumed to be triploid or derivatives of triploid (hypotriploid) (Triantaphyllou, 1981; 1985a). They are not considered a chromosomal race because of absence of a distinct gap between them. More than 95% of the populations studied have 2n = 42 - 44 chromosomes (Table 2).

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). There are no chromosomal races. Determination of the chromosome number is difficult in oogonial cells because metaphase cells with chromosomes aligned at equatorial plate are very rare. Precise counts are possible almost only in oocytes that have passed to uterus.

Cytologically, multiplication of oogonia and maturation of oocyte of *M. arenaria* have the same pattern as that of the two previous species (Triantaphyllou, 1963; 1985a). With regard to chromosome number, however, the species is a complex one. The most common is a triploid form with more than 50 chromosomes and this is considered as typical of the species (Table 2). Other forms are diploid 2n = 30 - 38, and hypotriploid with 2n = 40 - 48 chromosomes. These forms are considered as variants of the species and are sometimes assigned as chromosomal races temporarily (Triantaphyllou, 1985a).

M. hapla is the most diversified species of the root-knot nematodes (Triantaphyllou, 1966; 1984; 1991; 1993). Cytogenetically, the most distinguishable race of the species is race A, which exhibits bivalents (tetrads) in advanced oocytes. Except for some populations of *M. chitwoodi* that have n = 18 chromosomes, race A can be readily identified by its being facultative meiotic pathenogenetic. Race B of *M. hapla* with 2n = 30 - 32 chromosomes is similar to *M. arenaria* and thus not easily distinguishable. Similarly, other race B populations with 2n = 43 - 48 chromosomes are indistinguishable from *M. arenaria* and *M. javanica* which have the same range of chromosome number (Table 2).

Not much is said about the less common, minor *Meloidogyne* species. This is because they are encountered much less frequently and are less important as plant pathogens worldwide (Sasser, 1980; 1985; Jepson, 1987; Sasser and Carter, 1985). 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 (Triantaphyllou, 1969; 1985a).

2.4. Protein and enzyme polymorphism

The presence of high variability in morphological character (perineal pattern) and the occurrence of physiological races create difficulty in the taxonomy of the genus *Meloidogyne*.

quality and quantity of proteins and enzymes are also affected by environment. According to Hussey (1985), a good chemical taxonomic character should; (i) remain constant within samples being studied, (ii) be unresponsive to environmental influence, and (iii) correlate with existing classifications based on other characters.

Polyacrylamide gel electrophoresis (PAGE) of total proteins provides information which is helpful in characterizing and elucidating the relationships among nematode species (Hussey, 1979). Among the four common species, unique non-enzymatic protein profiles having taxonomic values were found. However, the presence of large number of bands creates difficulty in the interpretation. Furthermore, the need for large number of females to get good result reduces its potential as a biochemical tool. This difficulty can be resolved by running large gels and different fractions of proteins. The sensitivity of PAGE is greatly enhanced when specific enzymatic proteins are localized in the gel through specific activity stain.

Isozymes are enzymes catalyzing the same reaction, but differing in a measurable property such as electrophoretic mobility. Isozyme technique is much more advantageous because it is more sensitive and one can examine single individuals in-groups such as root-knot nematodes that have large females.

Extensive characterizations of isozymes have been carried out in root-knot nematode species (Dalmasso and Berge', 1978; 1979; 1983; Esbenshade and Triantaphyllou, 1985a; 1985b). Analysis of a number of different isozymes in individual root-knot females by PAGE has

shown that characteristic isozyme patterns can be used to reliably identify species. About 30 enzymes have been detected and studied in various *Meloidogyne* species (Esbenshade and Triantaphyllou, 1985b). Among these, only few of them have been investigated extensively and assessed for their usefulness as taxonomic characters (Dalmasso and Berge', 1978; Esbenshade and Triantaphyllou, 1985a). Esbenshade and Triantaphyllou (1985a) examined 291 populations comprising 16 species of *Meloidogyne* originating from 65 countries. They found that four enzyme systems showed species specific phenotypic patterns. These are: esterase, malate dehydrogenase (MDH), superoxide dismutase (SOD) and glutamate oxaloacetate transaminase (GOT) (Esbenshade and Triantaphyllou, 1985a).

2.4.1. Esterase isozyme

Esterases are enzymes that hydrolyze esters of short chain fatty acids. They were first described in *Meloidogyne* by Bird (1966). The esterase activity in whole mounted nematode was found concentrated in the amphidial pouch (buchal region) in all stages and at the base of spicules in males.

Esbenshade and Triantaphyllou (1986) have characterized the esterases in the genus *Meloidogyne* thoroughly. Two fractions of esterases were found through PAGE. The cathodal fraction, which did not enter into the 7% acrylamide gel, was found to be acetylcholinesterase (Esbenshade and Triantaphyllou, 1986). The esterases in the anodal region of the gel were similar to each other, in six populations studied, in their molecular weight, substrate or

inhibitor specificity. Thus, they are classified as carboxylesterases because of their sensitivity to the organophosphate inhibitors, and their reaction with specific substrates. The differences in relative mobility among the esterases are said to be due primarily to differences in total net charge since there is no difference in molecular weight. According to their activity esterases are classified in to two, b and β. b-esterases hydrolyze both α and β-napthylacetate whereas, β-esterases are active only on α-napthylacetate (Dalmasso and Berge', 1978). Esterases in *Meloidogyne* are believed to have an active role in the nematodes' sensory systems in the same way that acetylcholinesterase is associated with nervous system (Bird, 1966; Esbenshade and Triantaphyllou, 1986).

A number of investigators have observed that esterase isozyme patterns are different for each of the major root-knot nematode species and thus are particularly useful as taxonomic character (Dalmasso and Berge', 1978; Esbenshade and Triantaphyllou, 1985a; 1985b). According to Esbenshade and Triantaphyllou (1985a), a single esterase phenotype J3 (javanica 3 band) was observed in all 46 populations of *M. javanica*. This was not found in any other species or population. This indicated that the esterase pattern is a very reliable character for identification of *M. javanica*. Similarly, 99% of the populations of *M. incognita* and 94% of *M. hapla* had esterase phenotypes characteristic for these species; i.e. I1 (incognita 1 band) and H1 (hapla 1 band), respectively. *M. arenaria* was not firmly associated with any particular esterase phenotype. Nonetheless, 84% of the populations identified as *M. arenaria* had one of the three esterase phenotypes designated as A1, A2 and A3 (arenaria 1, 2 and 3 band, respectively). Cenis *et al.* (1992) obtained a similar result, using different populations and shows the consistency of esterase isozyme analysis as a taxonomic character. Similarly, Souza *et al.*

(1994) have identified *Meloidogyne* species with the help of esterase isozyme phenotype together with a differential host test.

Protein polymorphisms inherent in *Meloidogyne* spp. (Dalmasso and Berge', 1978; Esbenshade and Triantaphyllou, 1985a) have shown that there is a permanent heterozygosity occurring frequently in apomictic species than in the related diploid species (Dalmasso and Berge', 1983). This heterozygosity, according to Dalmasso and Berge' (1983), might have originated when mitotic parthenogenesis was established.

Intra-specific polymorphism is not very extensive in *Meloidogyne* (Dalmasso and Berge' 1978; Esbenshade and Triantaphyllou, 1985a). The three esterase phenotypes of *M. arenaria* do not correspond with either cytogenetic or host race definitions (Esbenshade and Triantaphyllou, 1985b).

Meloidogyne species, being parthenogenetic organisms, create a dilemma to systematic. Taylor and Netscher (1979) argue that the concept of 'species' does not work in the case of parthenogenetic populations, and maintain that each *Meloidogyne* population should be taken as an entity. Similarly, they proposed a monophyletic evolution in which today's species come from one ancestral stock. However, cytogenetic and electrophoretic results do not agree with this hypothesis. Dalmasso and Berge' (1983) and Hyman and Powers (1991) argue that *Meloidogyne* species are discrete and recognizable. The different clones have originated from several diploid ancestors through hybridization. Accordingly, the suggested pathway of

polyphyletic evolution explains that the facultative meiotic parthenogenetic forms gave rise to obligatory mitotically parthenogenetic nematodes.

Isozyme study has become a reliable tool of the taxonomy although there are some limitations. Hyman (1996), in his article, has indicated that if molecules are to be of importance in taxonomy, they must agree with morphology and the descriptive literature of taxonomy. Hyman and Powers (1991) and Caswell-Chen *et al.* (1993) also agree that *Meloidogyne* spp., being partly parthenogenetic, need their systematics examined critically. However, evolutionary patterns and their rates at molecular, molecular and phenotypic level could be quite different.

The important limitations of isozyme analysis in the identification of *Meloidogyne* spp. are that; enzyme expression may vary relative to environment and nematode life stage, single juvenile does not contain enough enzyme to allow its identification, and it cannot allow successful identification of intra-specific variation within populations or isolates (Caswell-Chen *et al.*, 1993).

Eventhough there are some drawbacks and skepticism, esterase isozyme analysis has been shown to be more reliable in the identification of major *Meloidogyne* species. Cenis *et al.* (1992) have recommended esterase isozyme approach as most economical, workable in short time, and having experimental simplicity. In addition, esterase analysis is widely used to check the purity of isolates from cross contamination with other isolates or populations (Esbenshade

and Triantaphyllou, 1985a; 1985b). This procedure might be very useful in the genus *Meloidogyne*, where many populations exhibit atypical taxonomic characters in the perineal patterns, host ranges and karyotype (Dalmasso and Berge', 1978; Hyman and Powers, 1991; Caswell-Chen *et al.*, 1993).

3. Materials and methods

3.1. Sample collection

Root-knot nematode-infected plants were collected from different parts of the country. The sample area included both rainfall and irrigated fields in wet and dry seasons (Table 2). Nematode infection was checked at the spot through root-galling index (0 - 5 scale). The sampled plants were put in plastic bags and taken to the laboratory along with field data.

3.2. Establishment of *Meloidogyne* populations

Three to five galls with well-developed large egg masses were selected from each nematode-infected plant root. The galls were teased with a knife and forceps and the egg masses were taken by pincet and transferred to 1 % saline solution (NaCl) in microcentrifuge tubes. Each tube was labeled to designate the population. The isolated egg masses were then placed beneath the roots of two weeks old susceptible tomato (cv. Rutgers) planted into a 15 cm diameter foam pot filled with a steam sterilized 1:1 mix of sand and soil. The plants were watered properly and maintained in the greenhouse for 50-60 days. The nematode populations were maintained by continuous re-inoculation of the egg masses into tomato seedlings. To avoid cross contamination of populations, all materials used and benches were cleaned with NaOCl solution (commercial bleaching agent).

3.3. Collection of nematodes from infected roots

50-60 days after inoculation the plants were removed by inverting the pot and while holding the plant the soil were removed carefully. The roots were washed free of soil with running tap water. The rootlets with conspicuous galls were removed with the help of forceps and placed under a stereomicroscope (Fig. 2).

Several egg masses were removed from the roots by pincet and placed into 1% saline solution in microcentrifuge tubes until inoculation. Mature female nematodes were taken out carefully dissecting the roots with the help of a knife using a stereomicroscope with 10x magnification. It was then kept in 0.5% saline solution for a while until smeared for cytogenetic analysis or directly for isozyme study.

3.4. Preparation of perineal pattern

Perineal pattern was prepared according to Hartman and Sasser (1985). Egg laying female nematodes collected in 0.5% saline were transferred into 45% lactic acid in a cavity microslide. The head region was cut off at the neck under stereomicroscope and the body content was removed by pressing the body and was left in the acid for five days. After five days they were removed from lactic acid and placed on a clean plastic Petridish. The cuticle was cut crosssectionally and then trimmed into a square, leaving the perineal pattern at the

center. All the debris and the remaining tissues were removed by placing into a drop of lactic acid with very fine needle.

The perineal patterns were then transferred to a drop of glycerin on a clean microscope slide. The patterns were aligned so that the interior surface is placed against the slide and is pressed. A coverslip was finally placed and the slide was sealed with nail varnish. At least ten perineal patterns were prepared for each population.

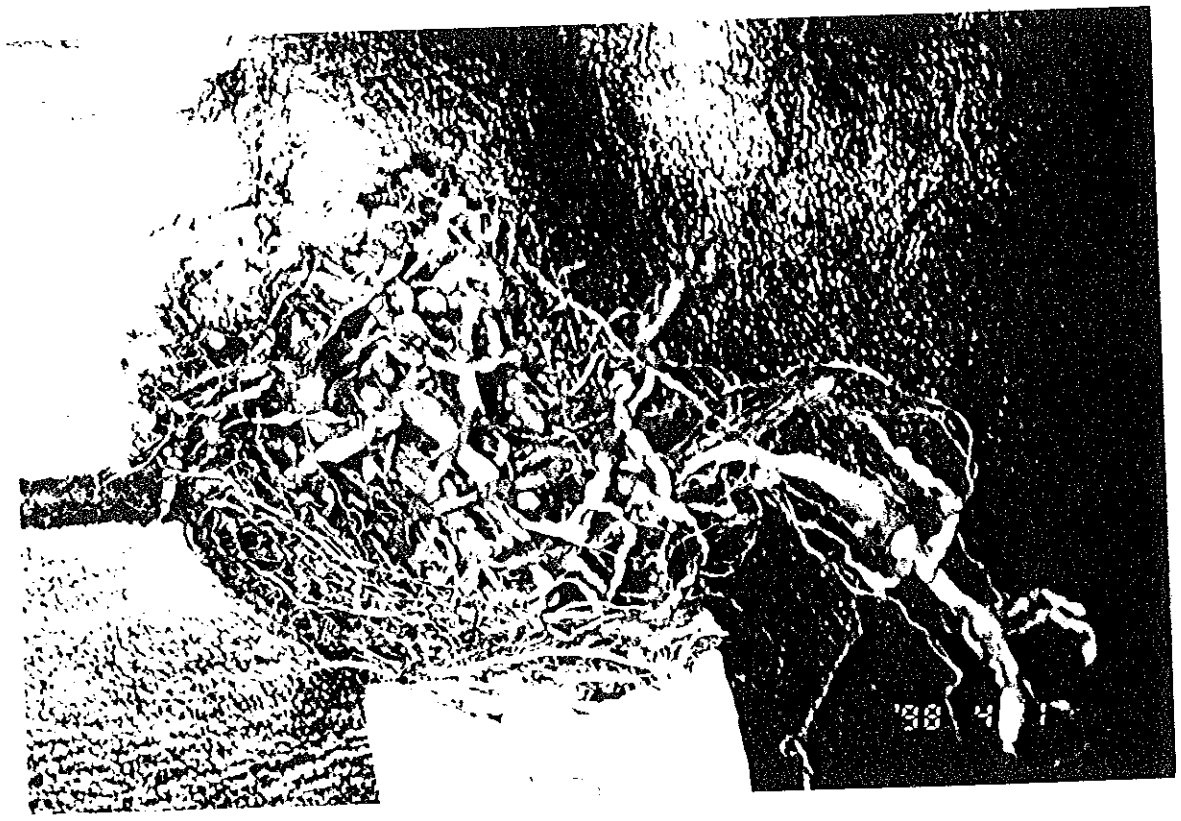


Fig. 2 A photograph of tomato roots (cv. Rutgers) infected with *M. incognita*.

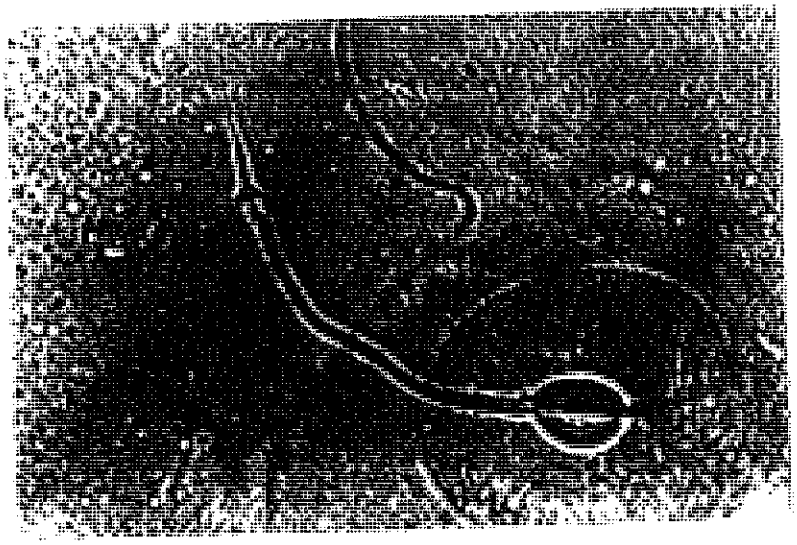
Note :- glandular structures are root galls

3.5. Examination of perineal pattern

The perineal patterns were examined under a compound microscope (40x). The main features of the pattern that help to distinguish the species were examined. From each population Camera Lucida drawings were made at 100x objective and 13x tube and photograph was taken with a photographic attachment microscope with 40x objective and 2.5x tube (Nikon Labophot 2 microscope, Nikon 35DX Camera) of the typical pattern describing the species and those that are not specific. The drawings were copied into tracing paper and photocopied reducing the size while the photographs were processed in the dark room of the Department of Biology.

3.6. Measurement of stylet

Adult female stylet was prepared while smearing for chromosome staining. The head region, after cutting, was left at the edge and the slide was stained. The stylet length was measured using an ocular micrometer at 100x objective. Total length, i.e., from the tip to end of the knob was taken in micrometer (μm) (Fig. 3). On the average, seven stylets were measured for each of *Meloidogyne* population. For populations of *M. javanica* and *M. ethiopica*, statistical test was done with ANOVA and then Multiple Range test, to check weather there was a statistically significant difference in mean stylet length between different populations.



25μm

Fig. 3 A photograph showing female stylet of *M. javanica*.

a, stylet knob; b, stylet length; c, esophageal lumen lining; and d, median bulb.

3.7. Cytogenetic study

3.7.1. Preparation of propionic-orcein stain

Stain solution were prepared according to Triantaphyllou (1979; 1985b). 2 gm orcein (natural) was added to 100 ml of 45% glacial propionic acid in a flask. A condenser, circulating cold water, was adapted over the flask and the content was boiled for about 45 minutes. The vapor condensed and returned back to the flask and maintained the concentration of the acid more or less constant. When the stain cooled, it was filtered and kept in an airtight dropping bottle.

3.7.2. Preparation of smear and chromosome staining

Egg laying females with white egg masses were transferred to a clean microscope slide with minimum saline solution. A single nematode was moved to the center at a time and the neck was cut off using a sharp knife. The body content was smeared uniformly to a length of about 1cm while pressing the body with fine forceps. Five to seven females were smeared on each slide and the cuticles were either transferred to lactic acid for perineal pattern study or discarded. The slides were labeled and the upper side marked.

The smeared slide was immersed into 1N HCl for 6 minutes in a Petridish. In order to avoid loss of material, the slide was placed on glass rods in inverted position covered by the acid solution. Then it was taken out, wiped, and dried with tissue paper while leaving the smeared material wet. The slide was then put into freshly prepared fixative of absolute ethyl alcohol and glacial acetic acid (3:1).

After 30 minutes, the slide was removed from the fixative solution, wiped, and dried. A few drops of propionic-orcein stain were added to cover the smeared material and left for 25 minutes. The slide was put on a level surface in a plastic Petridish to avoid sliding of the stain and drying. After 25 minutes, the stain solution was drained off and the slide was immersed into 45% propionic acid for about 3 seconds to remove the excess stain. A coverslip was wetted with 45% propionic acid and placed on the smeared material. After the excess acid has been dried the slide was sealed with molten paraffin wax.

3.7.3. Cytogenetic examination

Stained and sealed slides were scanned under a compound microscope at 10x objective for female ovary, and 100x objectives for countable prometaphase or metaphase chromosomes. All chromosome counts were made through 15x eyepiece at a total magnification of 1500x. Good metaphase chromosomes in polar view, where the chromosomes are aligned equatorially, were photographed.

3.8. Isozyme study

3.8.1. Protein extraction

Young females that have deposited small, white egg masses were teased from the tomato roots and placed in a drop of extraction medium containing 20% glycerol and 2% Triton X-100. With the help of a fine pipette three nematodes were transferred into an extraction tube made from a microhaematocrite tube previously washed with acetone, broken into two, and heat sealed at one end. The nematodes were macerated in the extraction tube with a small glass pestle made of glass rod.

The nematodes were macerated in a minimal amount of liquid, and then additional glycerol-Triton solution was added to bring the total volume to about 10 μl. The macerates were frozen

immediately after extraction in a freezer (-15°C) and kept there until electrophoresis. Since the populations were single egg mass isolates, i.e., homogenous, three females were drawn from each isolate to constitute individual electrophoresis sample.

3.8.2. Preparation of gel

The following stock solutions were made (Esbenshade and Triantaphyllou, 1985c).

Solution A

1N HCl ~ 20 ml

Tris, 18.3 g

TEMED, 0.23 ml

Adjust to pH 8.9 with 1N HCl

Water to 100 ml

Solution B

1N HCl ~ 48 ml

Tris 5.98 g

TEMED, 0.46 ml

Adjust to pH 8.6 with 1N HCl

Water to 100 ml

Solution C

Acrylamide, 28.0 g

BIS, 0.735 g

Water to 100 ml

Solution D

Acrylamide, 10.0 g

BIS, 2.5 g

Water to 100 ml

Solution E

Solution F

Riboflavin, 4 mg

Water to 100 ml

Bridge-buffer stock solution

Tris, 6.0 g

Glycine, 28.8 g

Adjust to pH 8.3

Water to 1 liter

Dilute 1 part with 9 parts

of water before use

Sucrose, 40.0 g

Water to 100 ml

Solution G

Ammonium persulfate, 0.5 g

Water to 100 ml

Bromophenol-blue dye

Bromophenol blue, 1 mg

Bridge buffer, 1 ml

Stock solutions were kept at 4°C until needed. Prior to gel preparation, stock solutions were warmed to room temperature. The stacking and separation gels were prepared according to the following proportion.

Separation gel (7%)

1 part solution A

2 parts solution C

1 part water

Stacking gel (4%)

1 part solution B

2 parts solution D

1 part solution E

4 parts solution G

4 parts solution F

pH 8.9

pH 6.7

3.8.3. Casting of the gel

A minigel apparatus (Biorad mini protean II) and an electric source (Biorad 500/200) were used. Glass plates were with a dimension of (w, 11x L, 7 cm) and spacer 1 mm thick with 10 teeth and had a dimension of bottom 3mm and 13mm length. For comparison of the result to previous studies we used a minigel, however larger gels would separate the bands widely to see the difference clearly.

The separation gel was prepared first. The stock solutions were mixed in the proper proportion, but without the ammonium persulfate (APS) solution. The mixture was vacuumed for 5 minutes and then APS solution was slowly added and mixed. The liquid gel was then poured slowly into a properly fixed cassette. A layer of water was added on top of the gel and left for 30 minutes.

After the gel polymerized, the water was poured and dried with filter paper. The stacking gel

was prepared from stock solutions as indicated above and vacuumed. While preparing the stacking gel, a comb was inserted at the top of the cassette leaving 5mm distance from the surface of separation gel. The stacking gel was added by means of a Pasteur pipette and left to polymerize. After 30 minutes the comb was pulled up gently and all the wells cleaned of the loose gel with sample pipette. The cassette was placed in the electrophoresis tank and the upper and lower tanks were filled with bridge buffer.

3.8.4. Electrophoresis

Shortly before electrophoresis, the frozen samples were thawed and centrifuged at 13,000 g for 15 minutes at room temperature in a microhaematocrit centrifuge. After centrifugation each tube was broken just below the upper layer formed by the lipid portion of the extract. The clear aqueous phase was then used directly for electrophoresis. The samples were transferred into a sample well in the stacking gel by a sample pipette. After all the samples were loaded, a drop of bromophenol-blue dye solution was added into the buffer and an electric current was turned on. The electric potential of 80 volt was used for the first 30 minutes to allow stratification of the proteins. After 30 minutes, it was increased to 200 volts for the remainder of the separation period of about 30 minutes. When the bromophenol-blue dye was about to reach the bottom of the gel, the current was turned off and the cassette was removed from the tank. The gel was removed from the cassette, rinsed several times with distilled water, and put immediately into the stain solution after labeling by cuttings at the edge.

3.8.5. Enzyme staining

Enzyme-stain solution was prepared as follows (Esbenshade and Triantaphyllou, 1985c).

Potassium phosphate buffer 0.1 m pH 7.2, 100 ml

EDTA, 30 mg

Fast Blue RR salt, 60 mg

α-napthylacetate, 40 mg

The α-napthylacetate was dissolved in 2 ml acetone and added to the buffer drop-wise while stirring. The solution was filtered to remove insoluble material.

Stain solution was finally poured into the gel and placed into an incubator in the dark at 37°C. The stain solution was agitated every 10 minutes for about 1-hour. Then the stain solution was poured off and the gel rinsed with several changes of distilled water. The gel was finally stored in a 7% acetic acid solution in a Petridish. Enzyme phenotypes were recorded and the gels were photographed. Relative migration (Rm) in percentage was measured and calculated for each band from the photographs.

3.9. Principal Component (PCA) and Dendrogam analysis

45 populations of the three species in this study were analyzed with Principal Component

4. Result

About 150 *Meloidogyne* populations were initially established from individual egg masses. However, only 55 populations were considered for analysis due to loss of the most of the populations as a result of wilting and death of tomatoes during the study period. Other populations, where complete data were not collected, were also excluded from the study. Designation of populations was made according to the order of sample collection. Root-knot index was scored for each sampled plants to show the level of nematode infection (Table 3). Area of sample collection was presented in a map (Fig. 4).

The established 55 *Meloidogyne* populations were identified to belong to three species, based primarily, on perineal pattern morphology and esterase isozyme phenotype. The species identified are; *M. incognita* 58.2% (32), *M. ethiopica* 25.4% (14) and *M. javanica* 16.4% (9) (Table. 6). For an easy and logical sequence, comparative description and characterization of the populations were made, both in the results and discussion parts of the thesis, after categorizing the populations into their respective species.

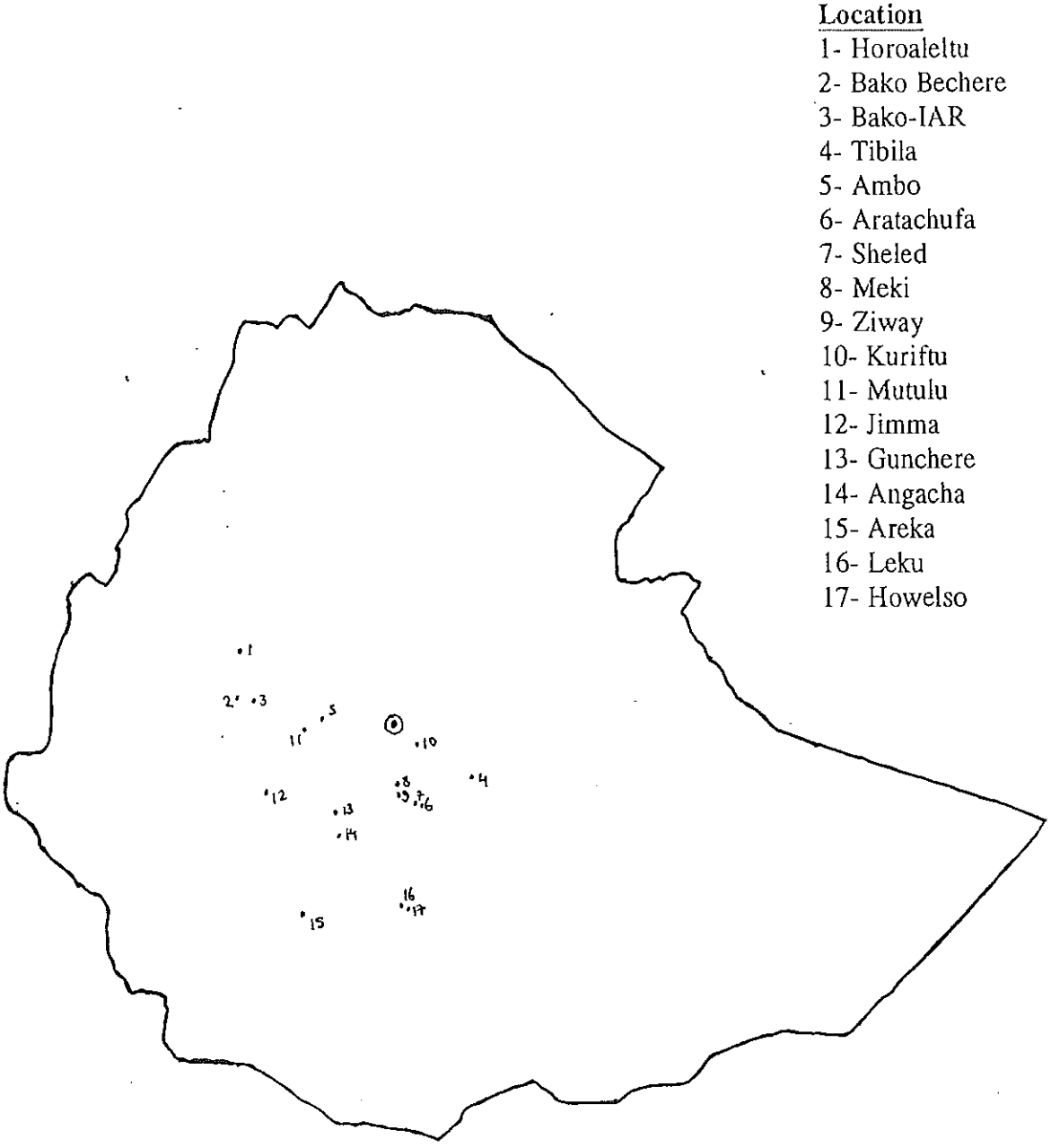


Fig. 4 Map showing areas of sample collection

Table 3. Locality of collection, host plant and root-knot index of the 55 nematode populations used in the present study

No.	Popln	Locality	Crop	RKNI[a]
	251	Horoaleltu	Tobacco	5
2	266	Bako,Bechere	Pepper	2
3	268	Bako IAR	Pepper	2
4	270	Bako IAR	Pepper	2
5	271	Bako IAR	Pepper	2
6	326	Tibila	Tomato	3
7	327	Tibila	Tomato	3
8	328	Tibila	Tomato	3
9	330	Tibila	Tomato	3
10	335	--*	--*	--*
11	355	Ambo	-*	-*
12	401	Aratachufa	Pepper	2
13	402	Aratachufa	Pepper	2
14	404	Aratachufa	Pepper	2
15	405	Aratachufa	Pepper	2
16	406	Aratachufa	Pepper	2
17	408	Aratachufa	Pepper	2
18	409	Aratachufa	Pepper	2
19	481	Aratachufa	Tomato	3
20	482	Aratachufa	Tomato	3
21	483	Aratachufa	Tomato	3
22	484	Aratachufa	Tomato	3
23	485	Aratachufa	Tomato	3
24	423	Sheled	Tomato	3
25	427	Sheled	Tomato	3
26	433	Meki	Snapbean	5
27	435	Meki	Snapbean	5
28	437	Meki	Snapbean	5
29	441	Ziway	Tomato	3
30	442	Ziway	Tomato	3
31	443	Ziway	Tomato	3
32	444	Ziway	Tomato	3
33	445	Ziway	Tomato	3
34	451	Kuriftu	Tomato	5
35	453	Kuriftu	Tomato	5
36	468	Mutulu	Tomato	3
37	601	Jimma	Beat root	4
38	603	Jimma	Beat root	4
39	604	Jimma	Beat root	4
40	605	Jimma	Cabbage	4
41	606	Jimma	Cabbage	4
42	607	Jimma	Cabbage	4
43	803	Gunchere	Enset	2
44	805	Gunchere	Enset	2
45	806	Gunchere	Enset	2
46	807	Gunchere	Enset	2
47	901	Angacha	Enset	2
48	912	Areka 01	Enset	3
49	931	Areka	Enset	2
50	920	Leku	Enset	2
51	921	Leku	Enset	2
52	922	Leku	Enset	2
53	923	Leku	Enset	2
54	942	Howelso	Enset	2
55	943	Howelso	Enset	2

RKNI - root-knot nematode index

* - nematode culture maintained in greenhouse

4.1. Morphology of perineal pattern

Perineal patterns of all populations were examined as a basis for species identification. Most of the populations had species-specific character that can easily be identified, whereas some populations had characters that overlap a species boundary. Populations of *M. incognita* and *M. javanica* had the most diverse perineal patterns. *M. ethiopica* did not show significant variation, beyond the variation that is naturally present in perineal patterns.

In *M. incognita,* typical pattern had the distinguishing feature of high and squared dorsal arch (Fig. 5 and 6). They had various types of striae: smooth, fine, coarse, wavy, zigzag and often paired. The phasmids were not prominent and were about the same distance to the ends of the vulva. There was no lateral line to separate the dorsal and ventral striae. The perivulval area was generally free of striae but in some patterns few lines of striae were present.

Figure 5, a and b show typical patterns; and c and d show some of the diversity in perineal patterns of *M. incognita*. The presence of a faint lateral line and low to moderately high squarish dorsal arch, are somewhat confusing with the perineal pattern of *M. javanica*. The phasmids in some patterns were prominent and slightly widely spaced than the ends of the vulva.

In *M. javanica,* the species-specific double incisor of lateral line was present in all the populations (Fig. 7 and 8). The dorsal arch was low and the phasmids were either equally or widely spaced than the ends of vulva. In some patterns of most of the populations, a wing pattern was present which is not common in *M. javanica*. Generally, the patterns of *M.*

javanica were easily distinguished from that of the other species.

The perineal pattern descriptions of the populations of *M. ethiopica* in this study were similar to the description given in Jepson (1987) (Fig. 9 and 10). Striae were fine and paired, and lateral lines were indistinct. The dorsal striae patterns were low, and rounded arch, similar to description of *M. arenaria* (Eisenback, 1985b; Jepson, 1987), and the phasmids were prominent but were with about the same distance apart to the ends of vulva. Population 423 (Fig. 9, c and 10, d), unlike the rest, had high squarish dorsal arch similar to that of *M. incognita*. In this population, the striae, lateral field and other characters, however, were similar to that of the rest of *M. ethiopica* populations.

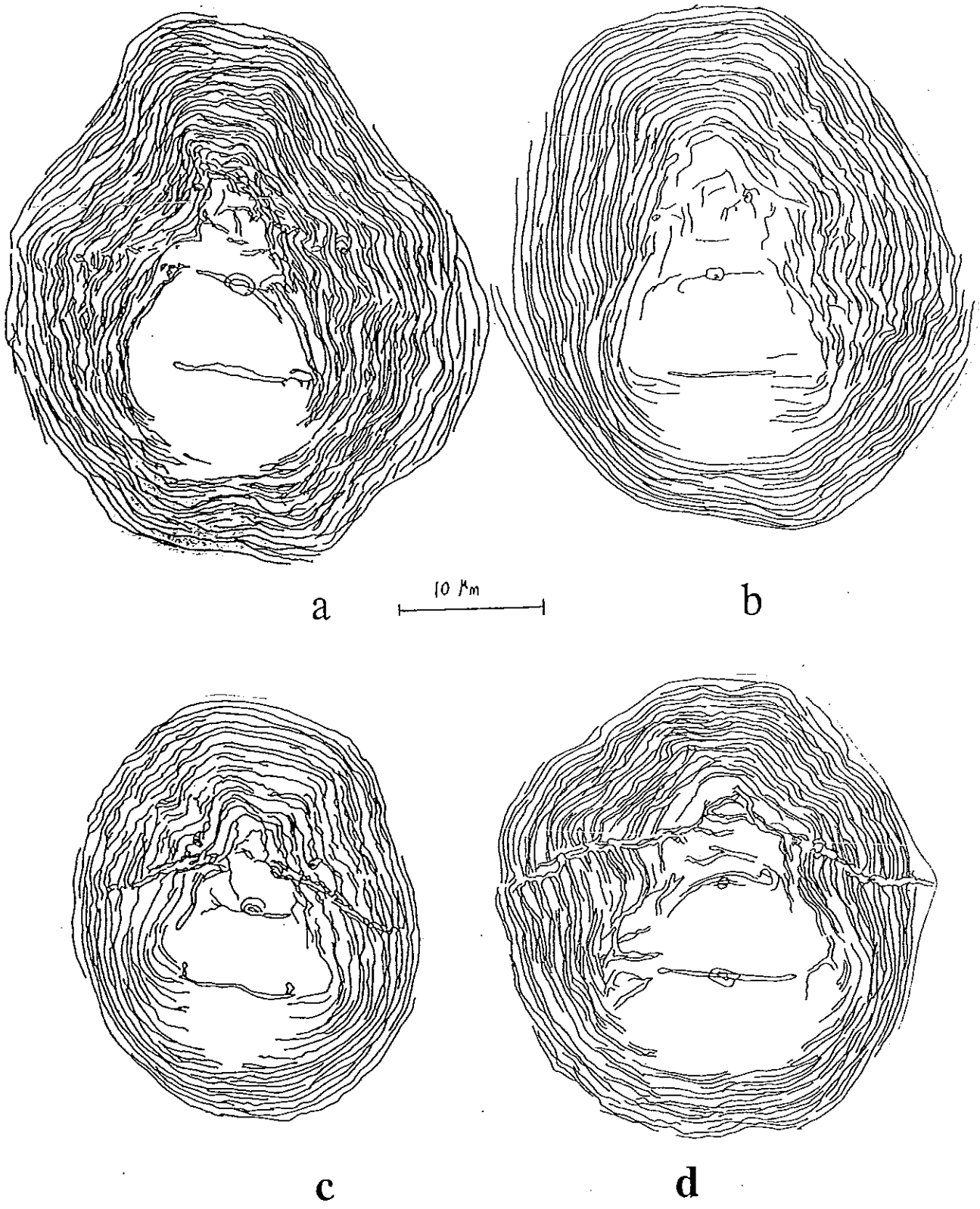


Fig. 5. Camera lucida drawings showing variation in perineal pattern of *M. incognita*. a and b: typical patterns; c and d: atypical patterns of the species.

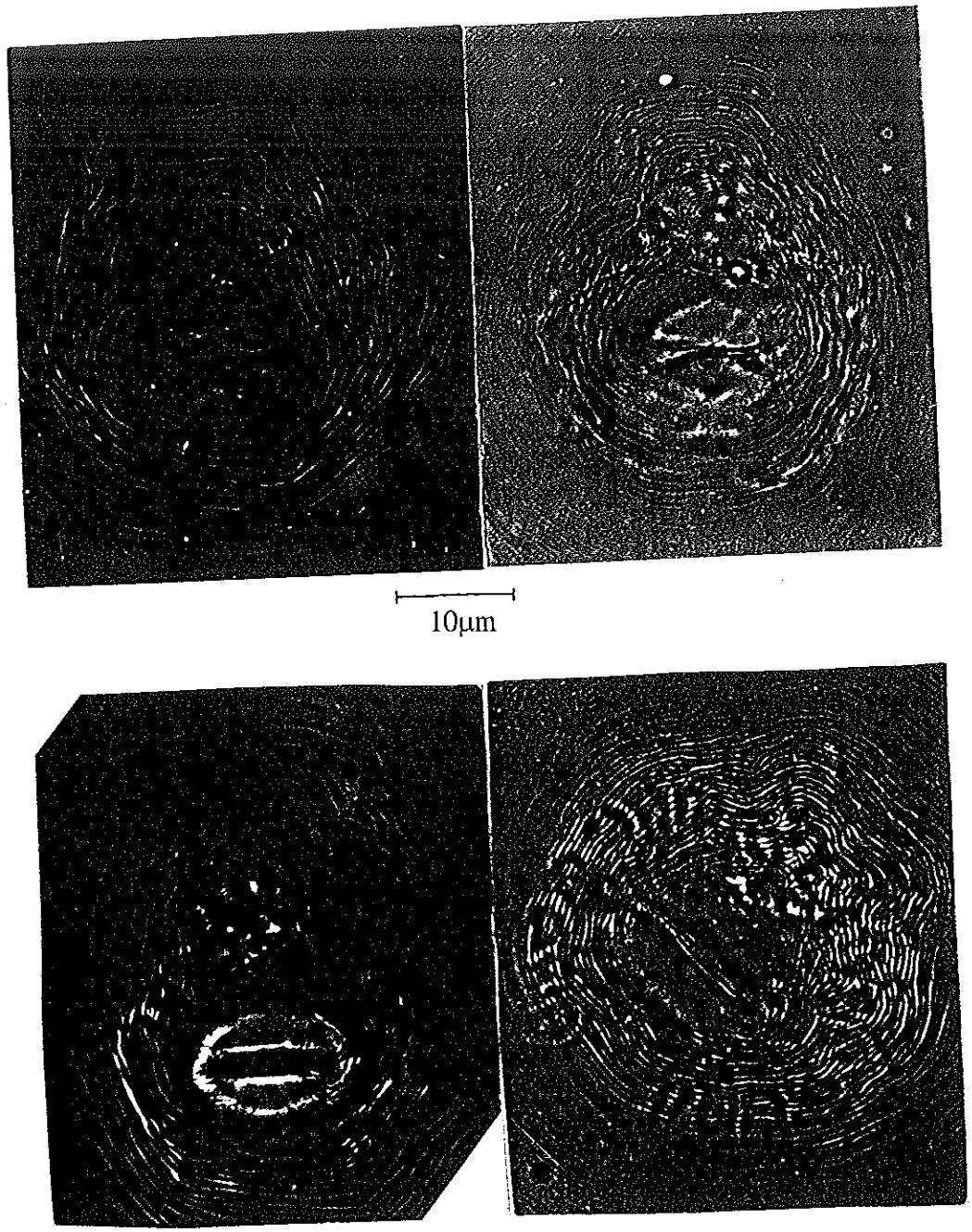


Fig. 6. Photographs of perineal patterns of *M. incognita*.

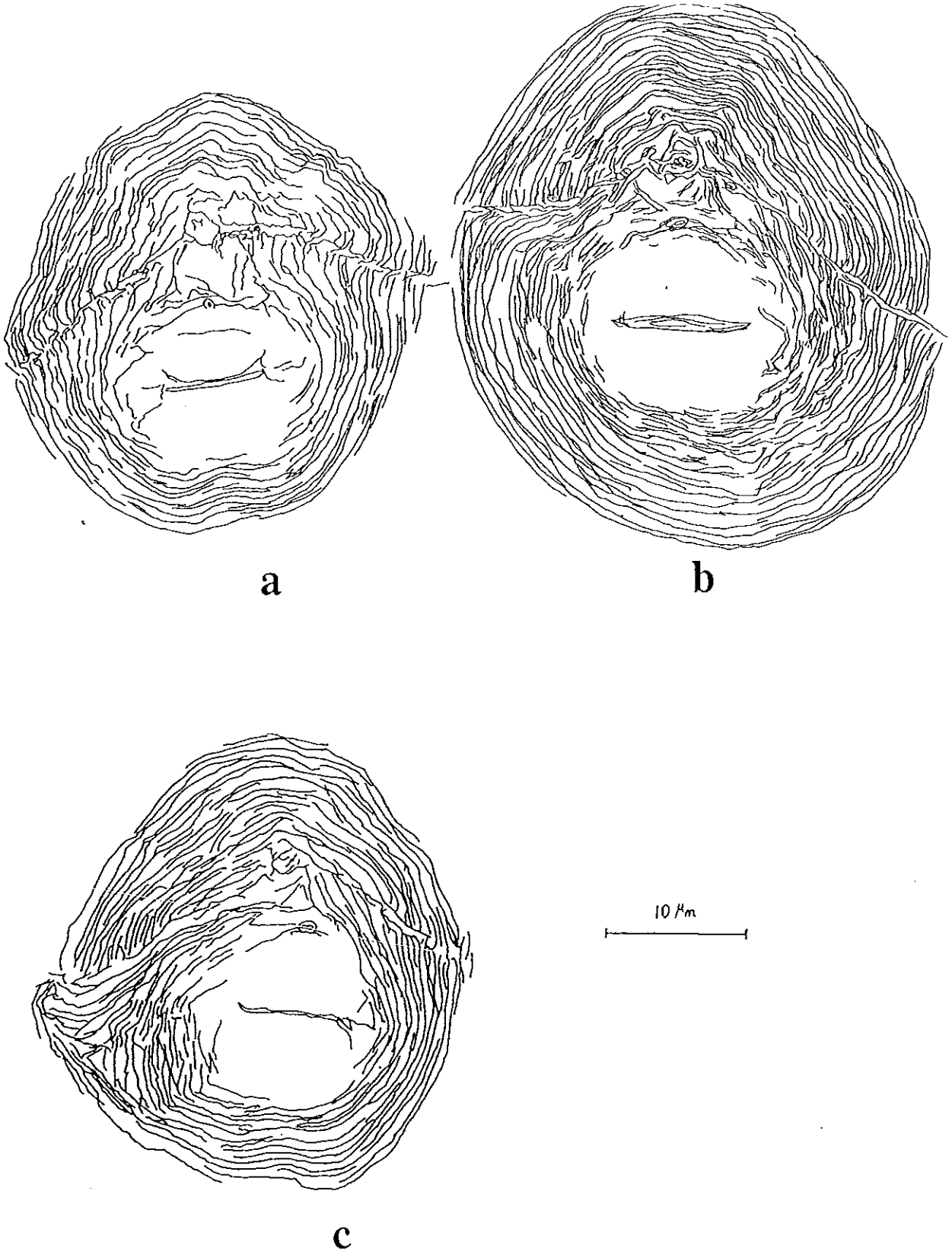
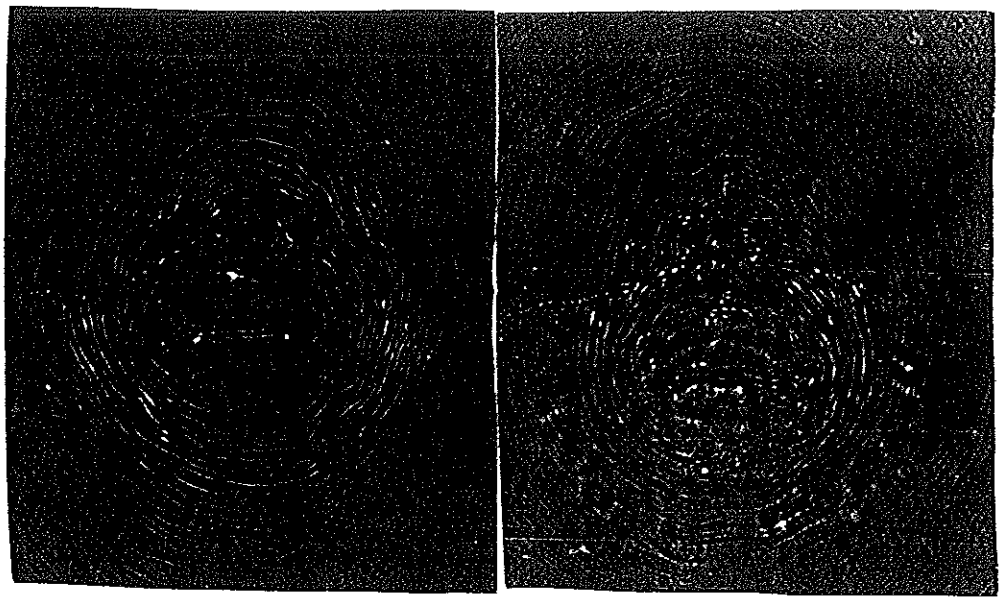


Fig. 7. Camera lucida drawings showing variation in perineal pattern of *M. javanica*. a and b: typical patterns; c: atypical pattern of the species.



10μm

Fig. 8. Photographs of perineal patterns of *M. javanica*.

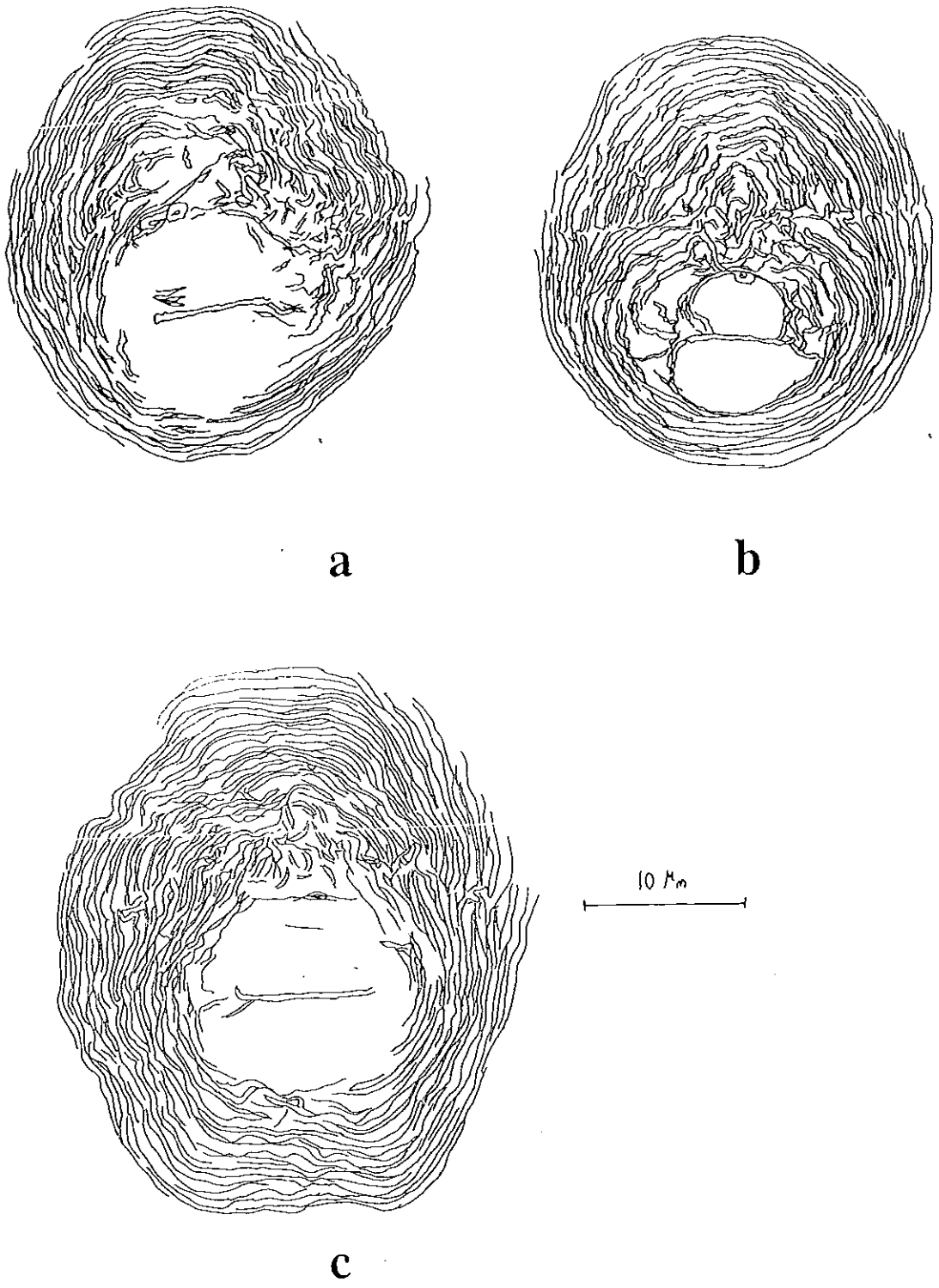
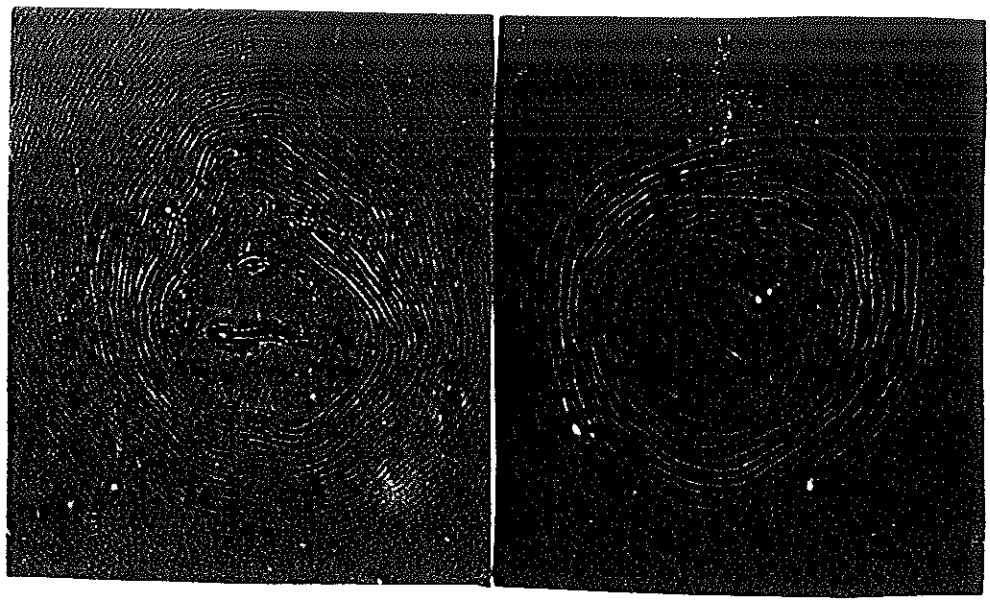


Fig. 9. Camera lucida drawings showing variation in perineal patterns of *M. ethiopica*. a and b: typical pattern (arenaria type); c: incognita type pattern.



10μm

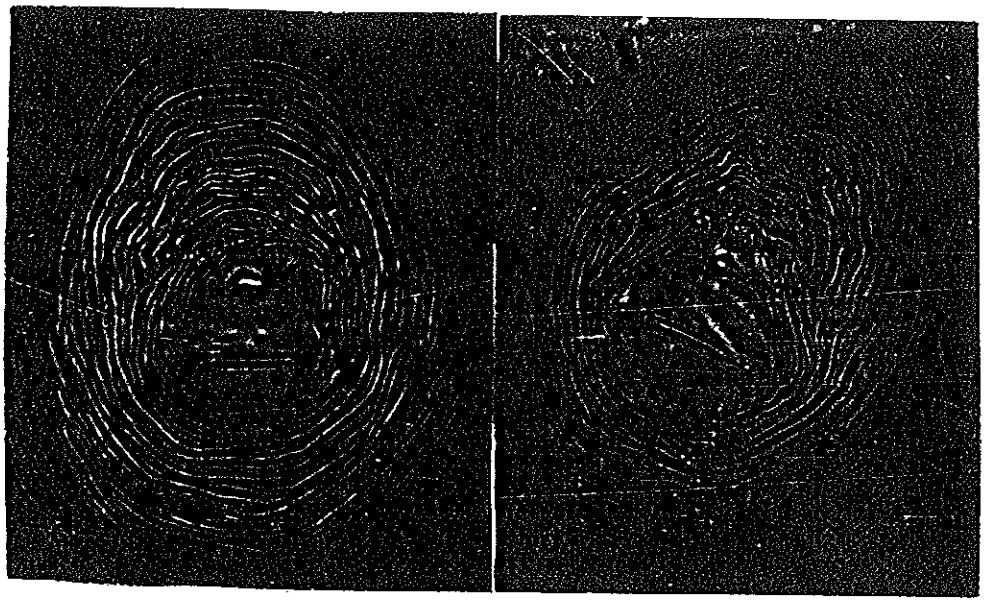


Fig. 10. Photograph of perineal patterns of *M. ethiopica*.
a and b, arenaria type; c and d, incognita type

4.2. Stylet length

Female stylet length measurements were taken for 50 populations of *Meloidogyne* spp. (Table 4,5 and 6). Means of stylet length and standard error were given in micrometer (μm). Populations of *M. incognita* had a mean stylet length of 15.47 ±0.29 μm. Only two populations, 330 and 931, had stylet length of greater than 16 μm, with 16.11 ±0.17 and 16.22 ±0.35 μm, respectively. The smallest measurement was taken from population 427 with a length of 14.8 μm. Due to shortage of sample, however, only one measurement was taken from this population. The minimum and maximum mean stylet lengths for populations of *M. incognita* were 14.8 and 16.22 ±0.35 μm, respectively. According to Jepson (1987) the range of stylet length for species of *M. incognita* was between 13 - 16 μm. In some populations it could also reach upto 18.0 μm.

Populations of *M. javanica* and *M. ethiopica* had a highly variable stylet length (Table 5 and 6). The smallest and largest mean stylet lengths in *M. javanica* were 14.55 ±0.2 and 17.64 ±0.07 μm for populations 268 and 806, respectively. The established range of mean stylet length for this species was 14 - 18 μm. All populations from enset had a stylet length of more than 16 μm, whereas the rest had less than 16.0 μm. Statistical test with multiple range test indicated that, there was a statistically significant difference ($P = 0.05$) in mean stylet length between populations isolated from enset and the rest.

The highest and lowest mean stylet lengths for populations of *M. ethiopica* were 13.91 ±0.13μm and 15.96 ±0.35 and 15.96 ±0.14 μm for populations 423 and both 805 and 907, respectively (Table 6). The majority of the populations, however, had a stylet length between

14.0 and 15.9 μm. From the descriptive literature of Jepson (1987). *M. ethiopica* had a stylet length of 11 - 15 μm. Like *M. javanica*, *M. ethiopica* populations isolated from enset had an average stylet length of 15.77 ±0.13 μm, which was statistically significantly (P = 0.05) longer than that of the rest of the populations which had a mean stylet length of 14.32 ±0.26 μm.

Higher intra-population variation in stylet length was observed in most of the populations. The highest variation was observed in population 483 with a SE of ± 0.55 μm.

4.3. Cytological study

Chromosome number was counted in the dividing cells of germinal zone of the ovary, just below the apical cells. In an average ten cells with discrete chromosomes were counted per population. Oocytes located in the anterior part of the uterus were found to be unsuitable for counting because chromosomes of the oocytes were lightly stained and in all cases equatorially aligned, which were difficult if not impossible, for counting.

All countings were made using prometaphase and metaphase chromosomes. Most of the oogonial cells were at prophase and in most of the examinations very few or sometimes no metaphase chromosomes were found per slide. Metaphase chromosomes were consisting of unpaired individual chromosomes that represent the somatic chromosome numbers. In the process of oogenesis the oogonium undergoes mitotic division and produces eggs with unreduced chromosome number. There was no pairing of homologous chromosomes.

Table 4. Chromosome number, isozyme phenotype and stylet length of populations of *M. incognita* by locality and host plant

Popln No.	Locality	Host plant	Chromosome No.	Isozyme[a] phenotype	Stylet length (μm) (mean)
266	Bako bechere	Pepper	40-44	I1	15.37±0.18
270	Bako IAR	Pepper	38-40	I1	15.75±0.00
271	Bako IAR	Pepper	40-42	I1	NA
327	Tibila	Tomato	41-42	NA	15.37±0.19
328	Tibila	Tomato	38-42	I1	15.32±0.15
330	Tibila	Tomato	41-44	I1	16.11±0.17
335	-*	-*	NA	I1	NA
355	Ambo	-*	41-44	I1	15.07±0.23
401	Aratachufa	Pepper	41-42	I1	15.33±0.17
402	Aratachufa	Pepper	40-42	NA	15.52±0.16
404	Aratachufa	Pepper	41-42	I1**	15.06±0.20
405	Aratachufa	Pepper	40-42	I1	15.90±0.14
406	Aratachufa	Pepper	40-42	I1	15.63±0.21
408	Aratachufa	Pepper	36	I1	15.10±0.16
409	Aratachufa	Pepper	43	I1**	15.00±0.00
481	Aratachufa	Tomato	41-42	I1	15.49±0.23
482	Aratachufa	Tomato	41-42	I1	15.23±0.29
483	Aratachufa	Tomato	NA	I1	15.36±0.55
484	Aratachufa	Tomato	42	I1**	15.43±0.37
485	Aratachufa	Tomato	41-42	I1**	15.23±0.40
427	Sheled	Tomato	42-44	I1	14.8±0.00
441	Ziway	Tomato	42	NA	NA
442	Ziway	Tomato	37-42	NA	15.54±0.25
443	Ziway	Tomato	41-42	I1	15.19±0.23
444	Ziway	Tomato	42	NA	15.28±0.04
445	Ziway	Tomato	42	I1	15.80±0.39
451	Kuriftu	Tomato	40-42	I1	15.81±0.28
453	Kuriftu	Tomato	40-42	I1	15.23±0.15
912	Areka 01	Enset	42	NA	15.78±0.16
931	Areka	Enset	44-46	I1**	16.22±0.35
942	Howelso	Enset	42	I1	15.92±0.23
943	Howelso	Enset	42	I1	15.83±0.37

a for explanation of isozyme refer Figs. 13 and 14
* greenhouse culture
** with strong second band
NA not analyzed

Table 5. Chromosome number, isozyme phenotype and stylet length variation among populations of *M. javanica* by locality and host plant.

Population No.	Locality	Host plant	Chromosome No.	Isozyme phenotype [a]	Stylet length (μm) (mean)
251	Horoaleltu	Tobacco	42-44	J3	15.90 ± 0.24 bc[b]
268	Bako IAR	Pepper	41-42	J3	14.55 ± 0.26 a
326	Tibila	Tomato	40-43	J3	15.13 ± 0.27 ab
806	Gunchere	Enset	44	J3	17.64 ± 0.07 c
901	Angacha	Enset	42	NA	16.20 ± 0.27 c
920	Leku	Enset	46	NA	16.10 ± 0.28 bc
921	Leku	Enset	46	NA	16.80 ± 0.35 c
922	Leku	Enset	44	NA	16.17 ± 0.33 c
923	Leku	Enset	44-46	J3	16.23 ± 0.24 c

NA not analysed
a for explanation of isozyme refer Figs. 13 and 14
b Similar letter indicates no significant difference at P = 0.05 with Multiple Range Test (MRT)

Table 6. Chromosome number, isozyme phenotype and stylet length variation among populations of *M. ethiopica* by locality and host plant.

Population No.	Locality	Host plant	Chromosome No.	Isozyme phenotype[a]	Stylet length (μm) (mean)
423	Sheled	Tomato	40-42	E3	13.91 ± 0.13 a
433	Meki	Snap bean	42	E3	14.28 ± 0.28 ab
435	Meki	Snap bean	40-42	E3	14.62 ± 0.12 ab
437	Meki	Snap bean	NA	E3	NA
468	Mutulu	Tomato	NA	E3	14.90 ± 0.31 bc
601	Jimma	Beetroot	36	NA	NA
603	Jimma	Beetroot	NA	NA	14.04 ± 0.11 ab
604	Jimma	Beetroot	41	NA	14.56 ± 0.28 ab
605	Jimma	Cabbage	NA	E3	14.15 ± 0.15 ab
606	Jimma	Cabbage	38	E3	14.55 ± 0.21 ab
607	Jimma	Cabbage	40	NA	13.91 ± 0.18 ab
803	Gunchere	Enset	43-44	2 (3)	15.40 ± 0.37 cd
805	Gunchere	Enset	44	NA	15.96 ± 0.35 d
807	Gunchere	Enset	NA	NA	15.96 ± 0.14 d

NA not analysed
a for explanation of isozyme refer Figs. 13 and 14
b Similar letter indicates no significant difference at P = 0.05 with MRT.

Thirty populations of *M. incognita* were cytologically studied in order to determine the variation in chromosome number (Table 4). Prometaphase chromosomes of *M. incognita* were very small and highly crowded (Fig. 11, a and b). Therefore, precise counting was only possible in some cells. Due to this difficulty, in the majority of the cases, counting was made with ± 1 chromosome. In few cases, prometaphase and metaphase chromosomes were found in polar view and were photographed.

Most of the populations of *M. incognita* had chromosome number of 40 - 44 (Table 4). Only population 408 had a chromosome number of 36. Some populations had chromosome number with wide range that crossed the border of diploid and hypotriploid. Populations 270, 328 and 442 had chromosome numbers within the ranges of 38 - 40, 38 - 42 and 37 - 42, respectively. Higher chromosome numbers of up to 44 were counted in four populations: 266, 330, 355, and 427. Among the populations of *M. incognita* cytologically investigated, population 931 was the only one with chromosome number above 44, which ranged 44 - 46 chromosomes.

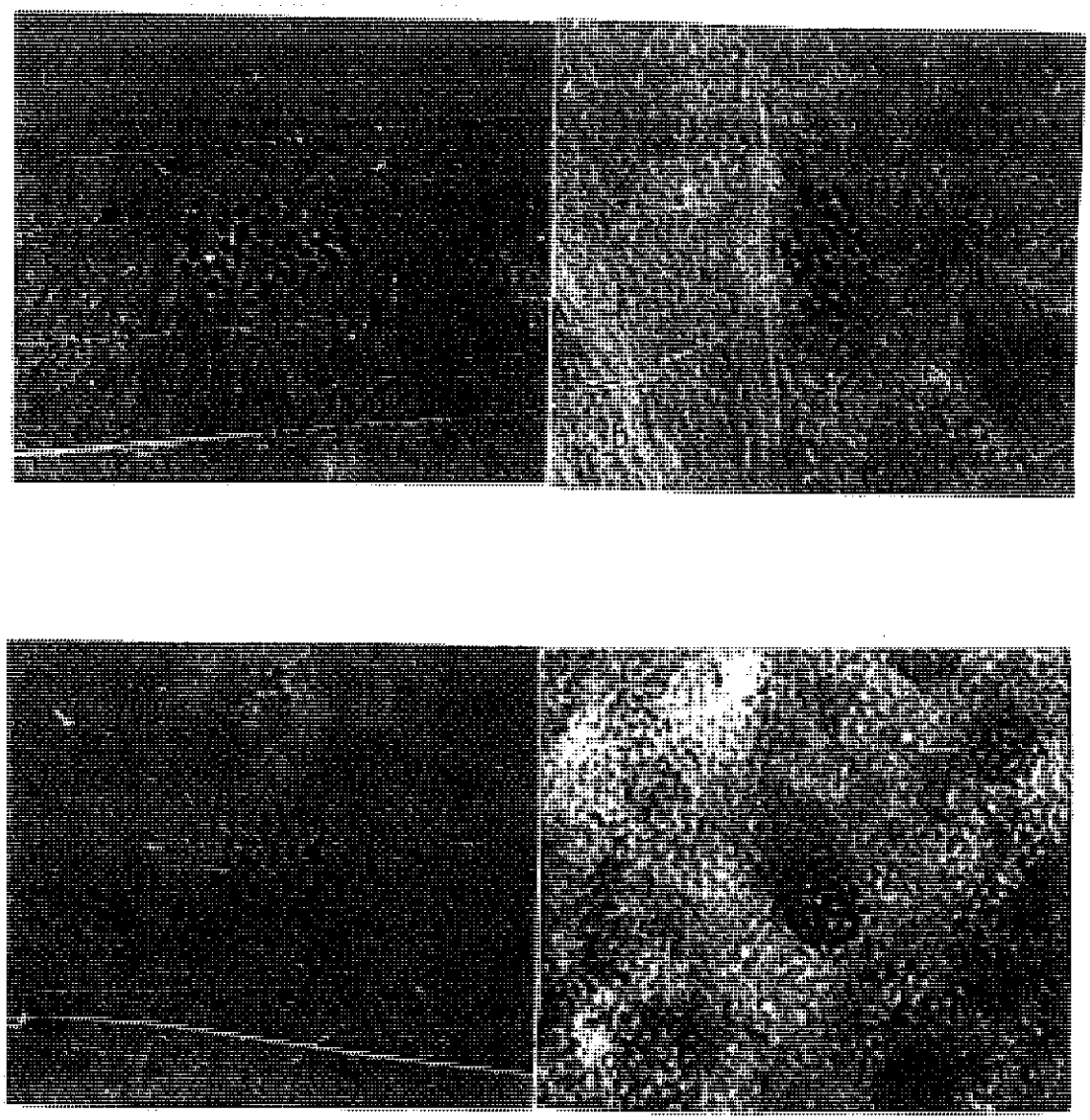
Cytologically, *M. javanica* chromosomes were similar to that of *M. incognita*. Only mitotic division takes place in the ovary, and thus the somatic chromosome number is represented. Unlike *M. incognita*, the chromosomes of *M. javanica* were more discrete and better spread at prometaphase and early metaphase stages and thus counting was more accurate (Fig. 12).

Out of nine populations of *M. javanica* examined, seven had chromosome numbers of 42 - 46 (Table 5). Populations 268 and 326 had 41 - 42 and 40 - 43 chromosomes, respectively. No new or unusual chromosomal forms were encountered in populations of *M. javanica*.

M. ethiopica populations exhibited the most diverse chromosome numbers (Table 6). Out of 14 populations of the species in this study, nine have been analyzed for chromosome number. The range of chromosome number for the species was between 36 and 44. Small chromosome numbers were counted in populations 601 and 606, with 36 and 38 chromosomes, respectively. Populations 803 and 805 from Gunchere (Gurage) had 43 - 44 chromosomes while the rest of the populations had chromosome numbers between 40 and 42.

Among the four localities sampled, *M. ethiopica* populations from Jimma had diverse chromosome number. Each of the four populations from this locality had different chromosome numbers. The other populations, however, had similar chromosome number with a narrow range.

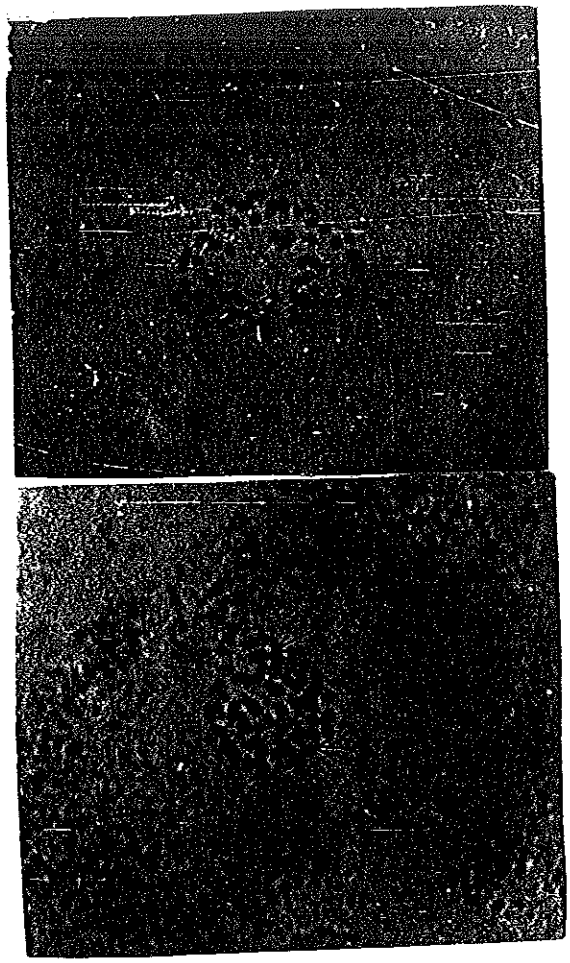
Concerning the chromosome size, *M. incognita* populations did not show much variation (Fig. 11). Most populations had a more or less equal chromosome size (0.4μm). In all populations of *M. incognita* only 3 and 4 large chromosomes were found (1.5μm). *M. javanica* populations, similarly did not show high polymorphism (Fig. 12). The largest chromosome was 1.6μm and the smallest 0.3μm long. Chromosomes of *M. ethiopica* populations were similar in size to that of *M. javanica*.



10μm

Fig. 11 Photographs of *M. incognita* chromosomes.

Population: a, 427; b, 442, c and d, 402. Arrows indicate large chromosomes



10μm

Fig. 12 Photographs of *M. javanica* chromosomes.

Populations: a, 326 and b, 920. Arrows indicate large chromosomes

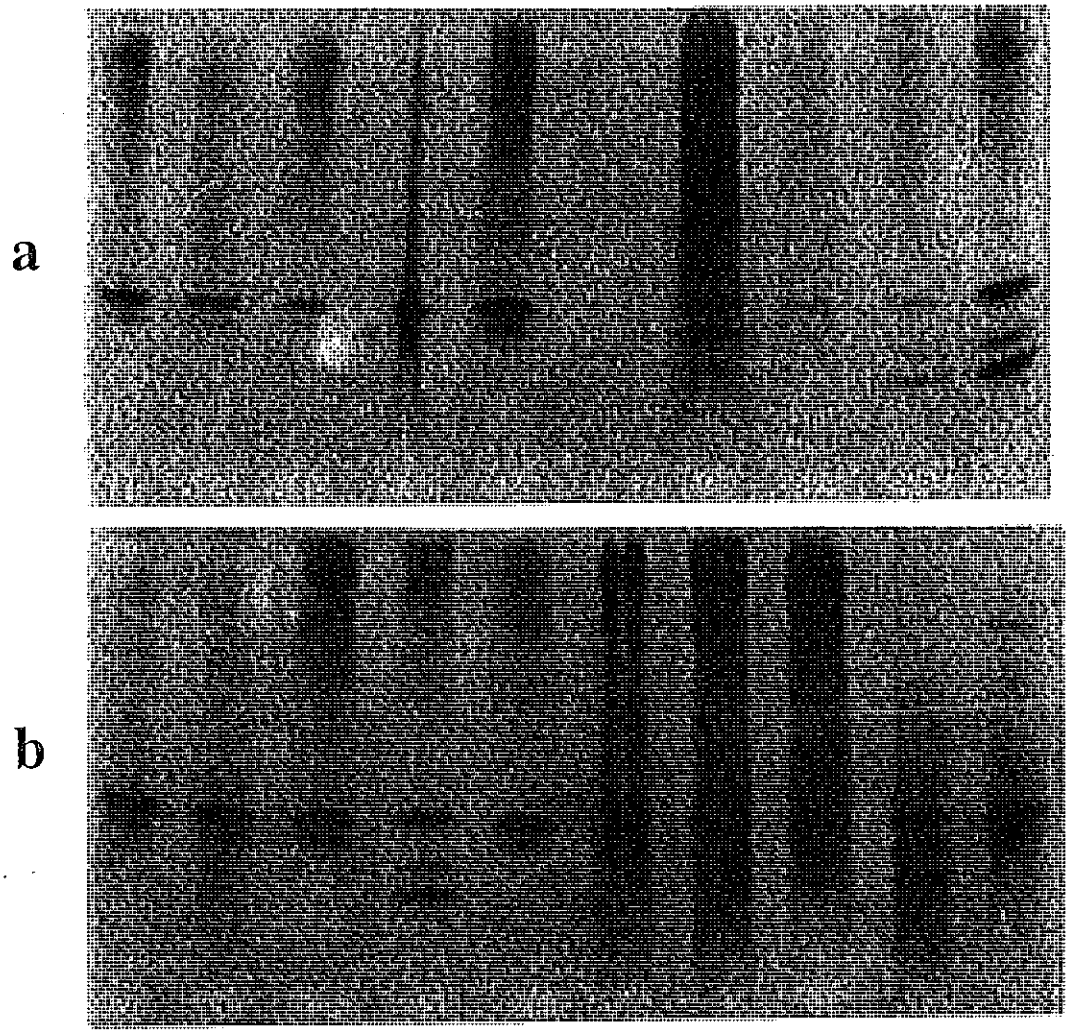
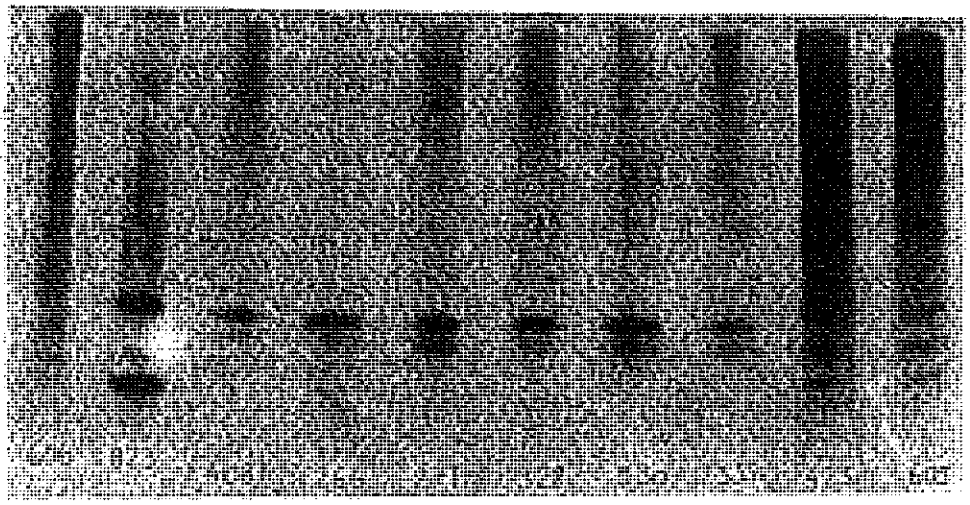


Fig. 13. Photograph of 1-mm thick polyacrylamide gel slab showing the different esterase phenotypes of *Meloidogyne* spp. (bottom number indicates the population)

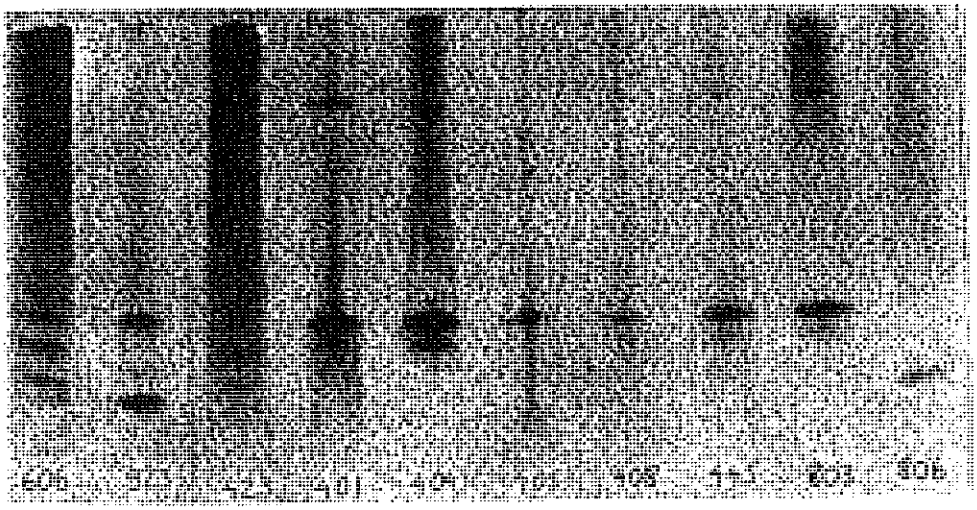
a: lanes 1-5 *M. incognita,* lanes 6,7 *M. ethiopica,* and lanes 8,9 *M. javanica.*

b: lanes 1-3,5,10 *M. incognita*, lane 4 *M. javanica,* and lane 6-9 *M. ethiopica.*

c

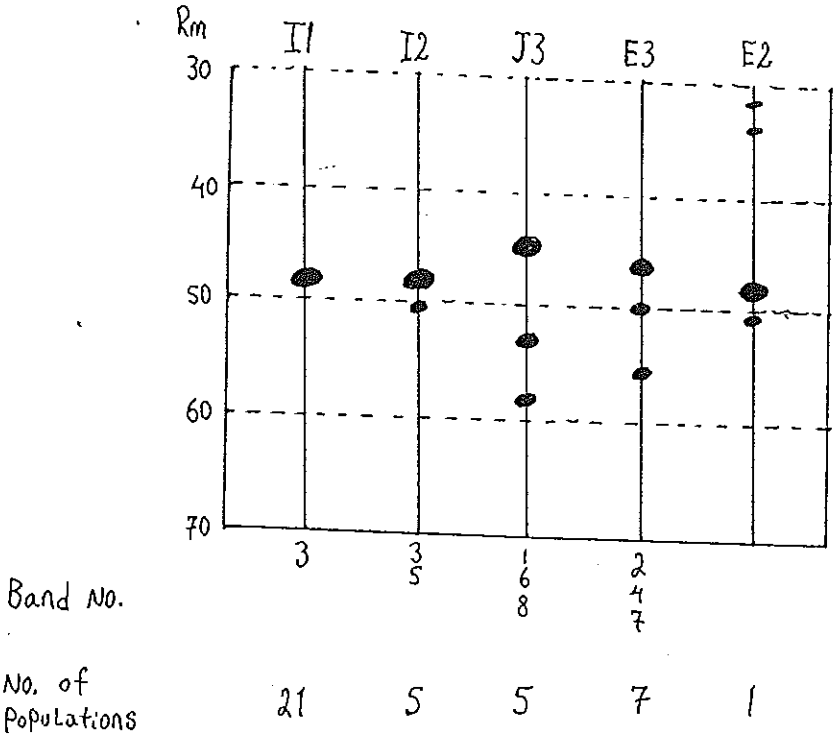


d



c: lanes 3-7 *M. incognita,* lanes 1,9,10 *M. ethiopica* and lane 2 *M. javanica.*

d: lanes 4-8 *M. incognita,* lanes 1,3,9 *M. ethiopica* and lanes 2,10 *M. javanica.*



Band No.	Rm (x100)	Designation of Migration speed
1	45.0	Slow
2	46.0	
3	48.0	
4	50.0	Medium
5	51.0	
6	53.0	
7	55.9	
8	58.0	Fast

Fig. 14. Esterase phenotypes observed in 39 populations of three *Meloidogyne* spp. Each phenotype is designated by a letter suggestive of the species and number of bands and the rate of migration of the band.

Two isozyme phenotypes were observed in populations of *M. ethiopica* (Fig. 13). Among the eight populations investigated, the most prominent phenotype was found to be a three band designated as E3 with Rm of 45.9, 50, and 55.9 (Fig. 14). No significant variation was observed in the phenotypes among different populations.

In population 803, however, two bands similar to I2 of *M. incognita* were encountered in all the cases. The main esterase bands had Rm values of 47 and 51, whereas in the cathodal fraction near the origin two minor bands were observed (Fig. 13d lane 9). Generally, cathodal fractions of esterase isozyme were not considered in the analysis. According to all other criteria, the isolate was identified as *M. ethiopica,* although the esterase isozyme phenotype was that of *M. incognita*.

In all the populations of *M. ethiopica* with E3 phenotype a deep staining smear was found without a defined band (Fig. 13). Such phenomenon was not observed in other populations. In some gels the smear goes in front of the band reaching Rm of greater than 65.

In this study, the 55 populations of *Meloidogyne* spp. investigated were identified to belong to three species on the basis of perineal pattern morphology, esterase phenotype and chromosome numbers. Arranged in order of their agricultural significance, the three species are *M. incognita* 58.2% (32), *M. javanica* 16.4% (9) and *M. ethiopica* 25.4% (14) (Table 7). The importance and significance of the first two species as parasites are very well known, whereas the third species is not considered as important parasite as the other two.

Table 7. Number and percentage of populations of *Meloidogyne* spp.

Species	No. of locations	No. of crops isolated from	No. of populations	Percentage
M. incognita	12	3	32	58.2
M. javanica	6	4	9	16.4
M. ethiopica	5	5	14	25.4
Total			55	100.0

4.5. PCA and Dendrogram analysis

The cluster of principal component analysis (PCA) is presented in Fig. 15. Three patterns of variations are obtained which include the three species with some outlying populations. Out of nine populations of *M. javanica*, seven are grouped together and out of seven populations of *M. ethiopica* five of them are grouped together with one *M. incognita* population. Similarly, one of 27 populations of *M. incognita*, 25 have same characteristic grouping. However, the distance of the PCA indicated in both axes was very small.

Hierarchical dendrogram of the populations was presented in Fig. 16. Five clusters of dendrogram were recognized. The first group had one distinct population of *M. incognita* with lowest chromosome number of 36. The rest four groups separate populations of *M. incognita* were found scattered in all groupings.

The first comprises of five populations of *M. incognita* and one population of *M. incognita* with a higher chromosome number of 44 and longer stylet length. The second group consists

of two *M. ethiopica* and three *M. incognita* populations which they share lower chromosome number of 38-40. The third group had two *M. javanica*, four *M. ethiopica* and 20 *M. incognita* populations. All populations in this group had chromosome number of 41-42 and an average stylet length for respective species.

The last group had seven populations: two *M. javanica*, two *M. ethiopica* and three *M. incognita* populations. Populations of the three species do not share similar character. *M. javanica* populations had chromosome number of 42 and an average stylet length and *M. ethiopica* populations had chromosome number of 43-44 and longer stylet length. Whereas, *M. incognita* populations had a chromosome number of 42.5-43 with an average stylet length. The distance given for the dendrogram clusters are at 0.1.

Table 8. Populations used in PCA and dendrogram analysis

No.	Population	Species
1	251	*M. javanica*
2	266	*M. incognita*
3	268	*M. javanica*
4	270	*M. incognita*
5	326	*M. javanica*
6	327	*M. incognita*
7	328	*M. incognita*
8	330	*M. incognita*
9	355	*M. incognita*
10	401	*M. incognita*
11	402	*M. incognita*
12	404	*M. incognita*
13	405	*M. incognita*
14	406	*M. incognita*
15	408	*M. incognita*
16	409	*M. incognita*
17	481	*M. incognita*
18	482	*M. incognita*
19	484	*M. incognita*
20	485	*M. incognita*
21	423	*M. ethiopica*
22	427	*M. incognita*
23	433	*M. ethiopica*
24	435	*M. ethiopica*
25	442	*M. incognita*
26	443	*M. incognita*
27	444	*M. incognita*
28	445	*M. incognita*
29	451	*M. incognita*
30	453	*M. incognita*
31	604	*M. ethiopica*
32	606	*M. ethiopica*
33	607	*M. ethiopica*
34	803	*M. ethiopica*
35	805	*M. ethiopica*
36	806	*M. javanica*
37	901	*M. javanica*
38	912	*M. incognita*
39	931	*M. incognita*
40	920	*M. javanica*
41	921	*M. javanica*
42	922	*M. javanica*
43	923	*M. javanica*
44	942	*M. incognita*
45	943	*M. incognita*

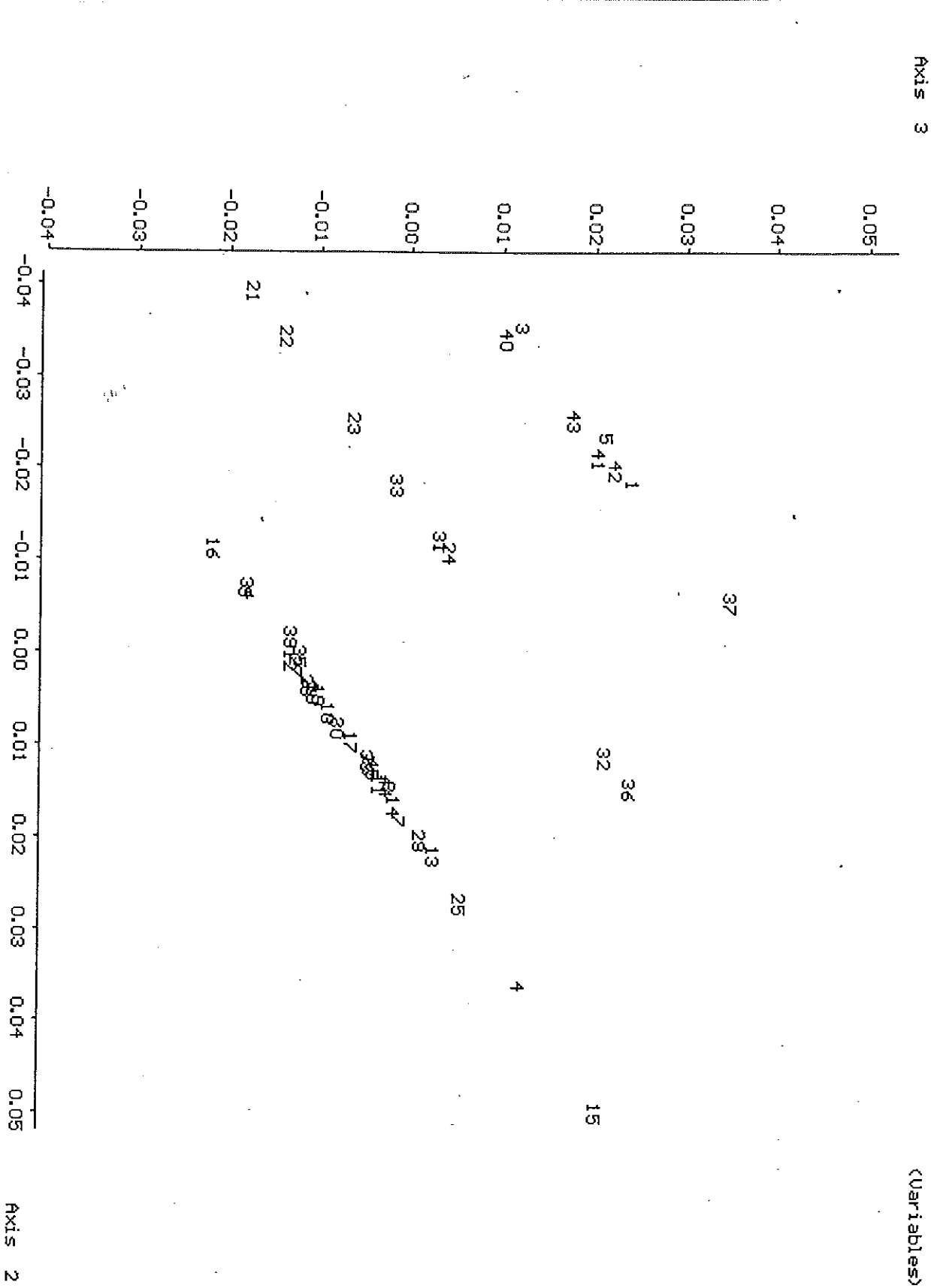


Fig. 15. Clusters of 45 *Meloidogyne* populations resultedfrom PCA.

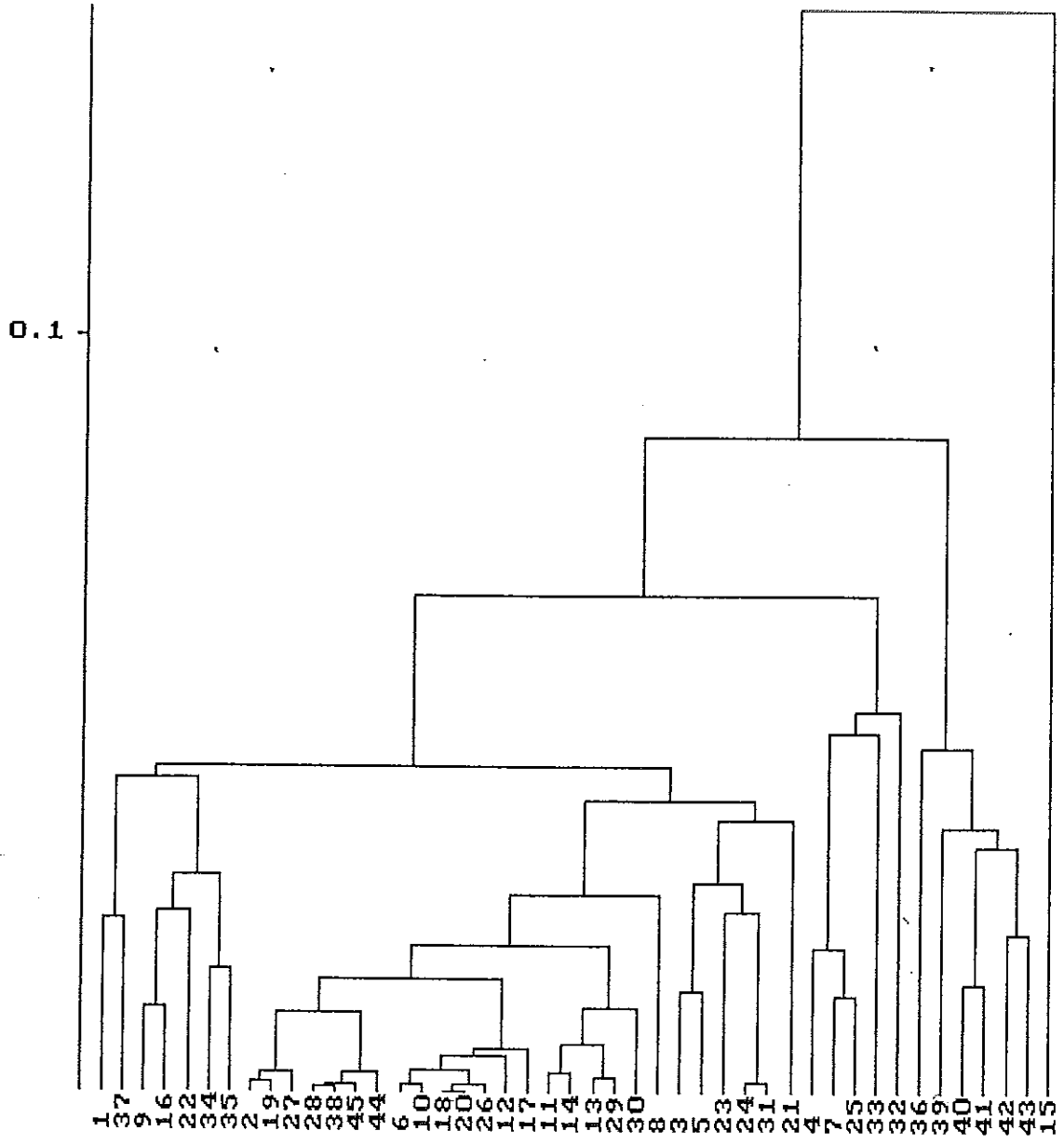


Fig. 16. Hierarchial dendrogram of 45 *Meloidogyne* populations

5. Discussion

Populations of *M. incognita*, *M. javanica* and *M. ethiopica* were found to be highly polymorphic in perineal pattern morphology. Particularly some populations of *M. incognita* proved to be difficult to identify on the perineal pattern alone. Due to such variability, in the past *M. incognita* and *M. javanica* were used to have ten and seven synonyms, respectively (Eisenback and Triantaphyllou, 1991).

Some of the variability is genetically controlled and some are the result of external environmental factors. (Triantaphyllou and Sasser, 1960). The extensive distribution of the species could also contribute to the observed variability. Particularly, *M. incognita* and *M. javanica* have a worldwide distribution, from tropics to sub-temperate regions, with very extensive host range (Sasser, 1987; Netscher and Sikora, 1990). Parthenogenetic mode of reproduction may also contribute to the polymorphism through extensive heterozygosity. Due to limited capacity of migration as well, populations remained isolated which through time could bring permanent differences (Triantaphyllou, 1979).

Although highly variable, several authors agree on the importance of perineal pattern as a basic taxonomic character. Hirschmann (1985) indicated that although perineal patterns vary within species and populations, basic species characteristics do not change significantly. Further he noted that in no case do pattern characteristics change from one species to another. Various authors also agree on this matter (Jepson, 1987; Eisenback and Triantaphyllou, 1991). The most important point in identification using perineal pattern morphology is, therefore, familiarity with the basic characters and some experience.

Female stylet length was found to be variable among populations of the three species. Unlike that of the perineal pattern, the variation of stylet length in *M. javanica* and *M. ethiopica* was higher than in *M. incognita*. The minimum and maximum stylet lengths found in this study, however, were within the known range according to Jepson (1987).

Eisenback (1985a) and Hirschmann (1985) indicated that stylet lengths of females do not vary significantly within populations. Those measurements were mostly done with scanning electron microscope (SEM). Some of the intra-population variation observed in this study could, therefore, be ascribed to the preparation technique used and examination with the light microscopy.

The longer stylet length observed in two populations of *M. incognita* did not show similarity except that both do have a chromosome number of above 42. Since other populations, with lower chromosome number, also share similar character, no reasonable conclusion could be drawn.

In contrast, *M. javanica* and *M. ethiopica* populations isolated from enset had stylet lengths that are statistically significantly longer compared to other populations, which is quite uncommon. Stylet length of a species is somehow instructive in terms of its ecology. Jepson (1987) has shown that 75% of species with stylet length less than 14 μm parasitize graminae or cyperaceae, and 71% of species with stylet length greater than 16 μm primarily parasitize woody hosts. In 14 - 16 μm range species may parasitize several hosts without such distinction.

Populations of the two species that infect enset may possibly show a form of an adaptation. Eisenback (1985b) explained that stylets are used in two ways, that is, to penetrate tissues and cells and as a passage for nematode secretions and food. For roots like that of enset, where the primary roots have an internal diameter of about 5 mm, nematodes with longer stylets would have an advantage.

Hence, the longer stylets possessed by populations of *M. javanica* and *M. ethiopica* could be an adaptation to successfully parasitize this and possibly other hosts. This observation was not made earlier within species of the genus *Meloidogyne*. However, it needs further study to find weather such an adaptation was permanent or temporary, and weather this is also true for other populations and species.

Cytological observation showed that all populations of the three species in this study are reproduced by obligatory mitotic parthenogenesis. This was due to the presence of only mitotic division in the maturing oocyte and no pairing of homologous chromosomes in the entire oogenesis (Triantaphyllou, 1966; 1979). Species of *M. ethiopica*, not characterized earlier, did not show peculiar feature which is different from the other well-characterized species.

Out of 30 *M. incognita* populations investigated, 26 had chromosome number of 40 - 46. Hence, the majority of the populations were hypotriploids. This chromosomal form was the most dominant and widespread form of the species world-wide (Triantaphyllou, 1985a). Only population 408 was found to be a diploid, with 36 chromosome, which is a rare chromosomal form. Three other populations, 270, 328 and 442 consisted of individuals with the diploid and hypotriploid chromosome numbers. Such observations were not reported before

(Triantaphyllou, 1985a; Eisenback and Triantaphyllou, 1991). Triantaphyllou (1981; 1985a) did not consider the diploid and hypotriploid as separate chromosomal races due to absence of clear demarcation between the two. Due to the fact that aneuploidy and different chromosomal structural changes were involved to give rise to chromosomal polymorphisms, the present observation with intermediate chromosome number might exist naturally.

Chromosome number of populations of *M. incognita* from different localities did not show much variation. However, populations from Bako-IAR, Tibila and Aratachufa showed more polymorphism than the rest. Compared to other localities, large number of populations were studied from these localities that could have increased the chance of getting high variation.

Most populations of *M. javanica* had chromosome number of 42 - 46. This number, according to Triantaphyllou (1962; 1985a), is within the range of the established chromosome number for the species. However, populations 268 and 326 with chromosome numbers of 40 and 41, respectively, probably indicate the presence of lower chromosome number below 42. According to Eisenback and Triantaphyllou (1991), all chromosome numbers between 40 - 50 are considered as hypotriploid. Hence, this chromosomal form can be considered as additional to hypotriploid populations of *M. javanica*. There could also be a possibility that the observed variability either attributed to error in counting instead of aneuploidy or chromosomal structural changes (Triantaphyllou, 1979).

As far as literatures are concerned, cytogenetically, *M. ethiopica* was investigated for the first time in this study. Reproduction was found to be by obligatory mitotic parthenogenesis. Prometaphase and metaphase chromosomes of *M. ethiopica* were similar in size and arrangement to that of *M. javanica* than to that of *M. incognita*. There is high variability in chromosome number between populations of the species. Like *M. incognita,* it has two chromosomal forms: diploid form, with 36 - 38 chromosomes, and hypotriploid form with 40 - 44 chromosomes.

Despite the differences in perineal pattern, cytogenetically the existence of two chromosomal forms with equal ploidy level makes *M. ethiopica* very closely related to *M. incognita,* which is the highly successful parasitic species of the genus. Reproduction through mitotic parthenogenesis could also have contributed in similar way through increasing of heterozygosity.

Populations of *M. ethiopica* from Gunchere had chromosome number of 43 - 44. This population also had a relatively longer stylet than the rest of the populations. Unlike in other species, it is only in *M. ethiopica* that populations from enset had higher chromosome number. This observation, although not studied, might show a possible form of aneuploidy operating in the adaptation of these populations in the host plant of enset.

Populations of *M. incognita, M. javanica* and *M. ethiopica* were not polymorphic in chromosomal size. Unlike Triantaphyllou (1981), *M. incognita* populations of this study did

not show extensive chromosomal polymorphism. The extensive chromosomal polymorphism are the result of various chromosomal structural changes (Triantaphyllou and Hirschmann, 1980). Similarly, *M. javanica* and *M. ethiopica* populations did not show such variability. Hence, *Meloidogyne* populations in this study showed only extensive numerical variation with very low chromosomal size polymorphism.

Esterase isozyme phenotypes proved to be highly species-specific (Esbenshade and Triantaphyllou, 1985a; 1985b; Cenis *et al.*, 1992). All populations of *M. incognita* exhibited the most prominent I1 band with similar Rm values. Despite high variation in perineal patterns, the species can be identified with esterase phenotype with high accuracy. For instance, of the 291 *Meloidogyne* populations investigated by Esbenshade and Triantaphyllou (1985a), *M. incognita* populations were identified with 98% accuracy with the esterase isozyme alone. Cenis *et al.* (1992) have also showed that Spanish populations of *M. incognita* had a similar isozyme pattern.

The second band, observed in some populations of *M. incognita,* were also detected in earlier studies (Esbenshade and Triantaphyllou, 1985a; 1986). However, due to high closeness to the major band, weak intensity and inconsistency within the same population, it was not considered in the analysis. Hence in the present study no new form of esterase phenotype were found in *M. incognita*. However, this can be resolved by running larger gels.

Only one esterase phenotype was found in all the populations of *M. javanica* investigated in the present study. The phenotype designated as J3 was highly distinct. Due to its highly characteristic nature, Esbenshade and Triantaphyllou (1985b) have recommended its use as an internal reference to compare other phenotypes within the gel. In this study as well, the presence of J3, in most gels, made comparison easier.

According to literature, esterase isozyme of *M. ethiopica* was characterized for the first time in the present study. Phenotype designated as E3 was found to be highly characteristic of the species. In this study, 87% of the populations had this form. The deep stained smear observed in phenotype E3 was not observed in populations of other species. Due to its large gel coverage from cathodal (near sample well) to anodal (downward) regions of the lane, it was difficult to designate the smear as esterase. On the other hand, it was clear that it could not be acetylcholinesterase, because it cannot enter into 7% separating gel due to its high molecular weight (Esbenshade and Triantaphyllou, 1986). Hence the smear could be a form of esterase that reacted with the substrate α-napthylacetate.

The rare form of the esterase phenotype of *M. ethiopica* had similar Rm values to I1 of *M. incognita*. The only difference between the two esterase phenotypes was the presence of two cathodal bands in the former. Due to lack of sample, however, only one population was characterized in esterase isozyme from the same location. Hence, study of more populations may reveal this and possibly other new forms of esterase isozyme phenotype.

Multiple bands of *M. ethiopica* indicated that there is permanent heterozygosity and polyploidy. Based on the total number of different bands for five enzyme systems of most important species, Dalmasso and Berge` (1978; 1983) have shown that, *M. javanica* and *M. arenaria* were more closely related to each other than to *M. incognita*. This was in good agreement with classification based on chromosome number and also agrees with host race (Hyman and Powers, 1991). Hence, *M. ethiopica* is similar to *M. arenaria* race B in having diploid forms and similar to *M. javanica* in having hypotriploid forms. Furthermore, its possession of multiple allelic forms makes it more related to those two species than to *M. incognita* and *M. hapla*.

The distance that resulted from principal component analysis was very small, hence, the groups are not well discriminated. However, three patterns of variations were observed that did not delimit the three species into an orderly established classification.

Similarly, the clusters of the dendrograms did not group the species properly. Instead it grouped populations of the three species together since they have similar characteristics. There is an overlapping of characters between species. Therefore, the present numerical treatment of *Meloidogyne* populations did not allow discrete grouping of the three species.

M. incognita was found to be the most encountered species followed by *M. ethiopica* and *M. javanica*. The high incidence of *M. incognita* was in agreement with its world-wide distribution (Sasser, 1987; Sasser and Carter, 1985). In tropical soils, the economically

important species after *M. incognita* are *M. javanica*, *M. arenaria* and *M. hapla* (Sasser, 1979b, Eisenback and Triantaphyllou, 1991).. According to Netscher and Sikora (1990) and Shepherd and Barker (1990), the importance and distribution of *M. ethiopica* is very low, and it is only known infecting very few plants in tropical and subtropical regions. Sasser (1979b) ranked *M. ethiopica* ninth in the region Africa

In Ethiopia, *M. ethiopica* was identified from Awassa and Tendaho infecting lettuce, soybean, cotton and some weed species (O'Bannon, 1975). There was no comprehensive list of host range for *M. ethiopica*. It was only known to infect plants in different orders of four families (Jepson, 1987; Eisenback and Triantaphyllou, 1991). Interestingly, in this study, *M. ethiopica* was isolated from more host plants than the other two species. The presence of diverse cytological and morphological forms in this study indicated the potential of the species as serious parasite of several economically important plants, particularly vegetables and enset.

5. Conclusion

Three species of root-knot nematodes were identified from different agroecological areas of the country in the following order of occurrence: *Meloidogyne incognita*, *M. ethiopica* and *M. javanica*. Morphology of perineal patterns and stylet length were highly polymorphic. Stylet length of populations isolated from enset was longer which is thought to be an adaptation.

Cytological study revealed that all the three populations reproduce by mitotic parthenogenesis. Chromosomes of *M. incognita* and *M. ethiopica* showed high numeric variability compared to *M. javanica*. Chromosomal size variation was low among populations of the three species.

Esterase isozyme was found to be very useful in species identification. It gives a discrete phenotypes, therefore, identification is more simple and accurate. Esterase phenotypes of *M. ethiopica*, particularly, the three band designated as E3, was found to be peculiar to the species.

Electrophoretic and cytologic observations indicated that, *M. ethiopica* was more related to *M. arenaria* and *M. javanica* than *M. incognita* and *M. hapla*. The high incidence of *M. ethiopica* coupled with the presence of more than one chromosomal form and isozyme phenotypes, indicated that the species is potentially serious parasite.

6. References

Abawi, G.S. and Barker, K.R. (1984). Effects of cultivar, soil temperature and levels of *Meloidogyne incognita* in root necrosis and *Fusarium* wilt of tomatoes. *Phytopath.* **74:** 433 - 438.

Agrios, G.N. (1988). *Plant pathology*. Third edition. Acadamic press. pp. 487.

Barker, K.R. and Noe, J.P. (1987). Establishing and using threshold population levels. **In:** *Vistas on Nematology,* pp. 75 - 81, (Veech, J.A. and Dickson, D.W., eds). Society of Nematologists INC, Hyatsville, USA.

Bernard, E.C. (1989). Variation among root-knot nematodes (*Meloidogyne* spp.). **In:** *Variability and population dynamics of root-knot and cyst nematodes in the southern region of the United States,* pp. 17 - 21. Southern Cooperative series Bulletin no. 336.

Bird, A.F. (1966). Esterases in the genus *Meloidogyne*. *Nematologica* **12**: 359 - 361.

Caswell-Chen, E.P., Williamson, V.M. and Westerdahl, B.B. (1993). Applied biotechnology in Nematology. Viewpoint. *Supp. J. Nematol.* **25:** 719 - 730.

Cenis, J.L., Opperman, C.H. and Triantaphyllou, A.C. (1992). Cytogenetic, enzymatic, and restriction fragment length polymorphism variation of *Meloidogyne* spp. from Spain. *Phytopath.* **82**: 527 - 531.

Dalmasso, A. and Berge`, J. B. (1978). Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp.: Application to the taxonomy of *Meloidogyne*. *J. Nematol.* **10**: 323 - 332.

Dalmasso, A. and Berge`, J.B. (1979). Genetic approach to the taxonomy of *Meloidogyne* species. **In:** *Root-knot nematodes (Meloidogyne species) systematics, biology and control,* pp. 111 - 114, (Lamberti, F. and Taylor, C.E., eds). Academic Press.

Dalmasso, A. and Berge`, J.B. (1983). Enzyme polymorphism and the concept of parthenogenetic species, exemplified by *Meloidogyne*. **In:** *Concepts in nematode systematics,* pp. 187 - 196, (Stone, A. R., Platt, H. M., and Khalil, L.F., eds). Acadmic Press, New York.

De Guiran, G. and Ritter, M. (1979). Life cycle of *Meloidogyne* species and factors influencing their development. **In:** *Root-knot nematodes (Meloidogyne species) systematics, biology and control*, pp. 173 - 191, (Lamberti, F. and Taylor, C.E., eds). Academic Press.

Dropkin, V.H. (1980). *Introduction to plant nematology*. A Wiley-Interscience publication. John Wiley & sons. 348 pp.

Eisenback, J.D. (1985a). Detailed morphology and anatomy of second-stage juveniles, males and females of the genus *Meloidogyne* (Root-knot nematodes). **In:** *An advanced Treatise on Meloidogyne Vol. I. Biology and control,* pp. 47 - 77, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dept. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Eisenback, J.D. (1985b). Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). **In:** *An advanced Treatise on Meloidogyne. Vol. I. Biology and control,* pp. 95-112, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dept. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Eisenback, J.D. and Triantaphyllou, H.H. (1991). Root-knot nematodes: *Meloidogyne* species and races. **In:** *Manual of Agricultural Nematology,* pp. 191 - 274, (Nickle, W.R., ed). Marcel Dekker, INC. New York.

Esbenshade, P.R. and Triantaphyllou, A.C. (1985a). Use of enzyme phenotypes for identification of *Meloidogyne* species. *J. Nematol.* **17**: 6 - 20.

Esbenshade, P.R. and Triantaphyllou, A.C. (1985b). Identification of major *Meloidogyne* species employing enzyme phenotypes as differentiating characters. **In:** *An advanced Treatise on Meloidogyne. Vol. I. Biology and control,* pp. 135-140, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dept. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Esbenshade, P.R. and Triantaphyllou, A.C. (1985c). Electrophoretic methods for the study of root-knot nematode enzymes. **In:** *An advanced Treatise on Meloidogyne. Vol. II. Methodology,* pp. 115 - 126, (Barker, K.R., Carter, C.C. and Sasser, J.N., eds). A co-operative publication. Dep. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Esbenshade, P.R. and Triantaphyllou, A.C. (1986). Partial characterization of Esterase in *Meloidogyne* (Nematoda). *Comp. Biochem. Physiol.* **83B:** 31 - 38.

Grimaldi De Zio, S., Padula, L., Lamberti, F., De Lucia Morone, M.R. and Gallo D'Adobo, M. (1979). Observations on the variability of biometrical characters in the perineal region of *Meloidogyne incognita*. **In:** *Root-knot nematodes (Meloidogyne species) systematics, biology and control,* pp. 55 - 58, (Lamberti, F. and Taylor, C.E., eds). Academic Press.

Hartman, K.M. and Sasser, J.N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. **In:** *An advanced Treatise on Meloidogyne. Vol. II. Methodology*, pp. 69 - 77, (Barker, K.R., Carter, C.C. and Sasser, J.N., eds). A co-operative publication. Dep. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Heusler, P. and Ayele Onke (1987). Development of tomato production in Ethiopia. **In:** *Proceeding of first Ethiopian horticultural workshop*, pp. 147 - 152, (Godfrey - SamAgrey, W. and Bereke Tsehayi Tuku, eds). Feb, 20 - 22, 1985. ILCA, Addis Ababa, Ethiopia.

Hirschmann, H. (1985). The genus *Meloidogyne* and morphological characters differentiating its species. **In:** *An advanced Treatise on Meloidogyne. Vol. I. Biology and control,* pp. 79 - 93, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dept. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Hussey, R. S. (1979). Biochemical systematics of nematodes – a review. *Helminto. Abstr.* **(B) 48**: 141 – 148.

Hussey, R.S. (1985). Biochemistry as a tool in identification and its probable usefulness in understanding the nature of parasitism. **In:** *An advanced Treatise on Meloidogyne. Vol. I. Biology and control,* pp. 127 - 133, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dept. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Hyman, B.C. (1996). Molecular systematics and population biology of phytonematodes: Some unifying principles. *Fundam. Appl. Nematol.* **19:** 309 - 313.

Hyman, B.C. and Powers, T.O. (1991). Integration of molecular data with systematics of plant-parasitic nematodes. *Ann. Rev. Phytopathol.* **29**: 89 - 107.

IAR. (1985). Crop protection department progress report for the period 1980/81 - 1982/83. pp. 149-154.

IAR. (1986). Crop protection department progress report for the period 1983/84. pp. 108-110.

Jepson, S.B. (1987). *Identification of root-knot nematodes (Meloidogyne species)*. CAB International. 265 pp.

Jones, F.G.W. and Jones, M.G. (1977). *Pests of field crops*. Edward Arnold Ltd. Second edition. pp. 228 - 289.

Mai, W.F. (1985). Plant-parasitic nematodes: their threat to agriculture. **In:** *An advanced Treatise on Meloidogyne. Vol. I. Biology and Control,* pp. 11-17, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dep. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Marias, M., Kruger, Jc.DeW. and Kruger, DeW.Jc. (1991). Cytogenetics of some South African root-knot nematodes (Heteroderidae : Nematoda). *Phytophylac.* **23**: 265 - 272.

Mehariew Genet. (1993). Plant-parasitic nematodes situation in Ethiopia: preliminary result. **In:** *Proceedings of the joint conference: EPC and CEE*, p.57. 5-6 March, 1992. Addis Ababa, Ethiopia. Crop Protection Society of Ethiopia (CPSE).

Netscher, C. and Taylor, D.P. (1979). Physiologic variation with the genus *Meloidogyne* and its implication on integrated control. **In:** *Root-knot nematodes (Meloidogyne species) systematics, biology and control,* pp. 269 - 294, (Lamberti, F. and Taylor, C.E., eds). Academic Press.

Netscher, C. and Sikora, R.A. (1990). Nematode parasites of vegetables. **In:** *Nematodes in tropical and sub-tropical countries,* pp. 237 - 283, (Luc, M., Sikora, R.A. and Bridge, J., eds). CAB International.

O'Bannon, J.H. (1975). Report of nematode survey in Ethiopia. FAO, Rome. 29 pp.

Saka, V.W. (1985). *Meloidogyne* spp. Research in Region V of the International *Meloidogyne* Project (IMP). In: *An advanced Treatise on Meloidogyne. Vol. I. Biology and Control,* pp. 361-368, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dep. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Sasser, J.N. (1979a). Pathogenecity, host ranges and variability in *Meloidogyne* species. **In:** *Root-knot nematodes (Meloidogyne species) systematics, biology and control,* pp. 257 - 268, (Lamberti, F. and Taylor, C.E., eds). Academic Press.

Sasser, J.N. (1979b). Economic importance of *Meloidogyne* in Tropical countries. **In:** *Root-knot nematodes (Meloidogyne species) systematics, biology and control,* pp. 359 - 374, (Lamberti, F. and Taylor, C.E., eds). Academic Press.

Sasser, J.N. (1980). Root-knot nematodes: a global menace to crop production. *Plant Disease* **64:** 36 – 41.

Sasser, J.N. (1987). A perspective of nematode problems worldwide. **In:** *Nematodes parasitic to cereals and legumes in temperate semi-arid regions,* pp. 1 - 12, (Saxena, M.C., Sikora, R.A. and Srivastava, J.P., eds). Proceedings of a workshop held in Larnaca, Cyprus, 1 - 5 March, 1987. ICARDA.

Sasser, J.N. and Kirby, M.F. (1979). *Crop cultivars resistant to root-knot nematodes, Meloidogyne species (with information on seed sources).* International *Meloidogyne* project. A co-operative publication. Dep. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C. 49 pp.

Sasser, J.N. and Carter, C.C. (1985). Overview of the International *Meloidogyne* Project, 1975-1984. **In:** *An advanced Treatise on Meloidogyne. Vol. I. Biology and Control,* pp. 19-24, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dep. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C

Sasser, J.N., Carter, C.C. and Taylor, A.L. (1982). *A guide to the development of a plant nematology programme.* A co-operative publication. Dep. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C. 21 pp.

Shane, W.W. and Barker, K.R. (1986). Effects of temperature plant age, soil texture and *Meloidogyne incognita* on early growth of soybean. *J. Nematol.* **18:** 320 - 327.

Shepherd, J.A. and Barker, K.R. (1990). Nematode parasites of tobacco. **In:** *Nematodes in tropical and sub-tropical countries,* pp. 493 - 517, (Luc, M., Sikora, R.A. and Bridge, J., eds). CAB International.

Souza, R.M., Dolinski, C.M. and Huang, S.P. (1994). Survey of *Meloidogyne* species in native Cerrado of Districto Federal, Brazil. *Fitopath. Brasil.* **19:** 463 - 465.

Stewart, R.B. and Dagnachew Yirgou (1967). *Index of plant diseases in Ethiopia.* Experiment station Bulletin No. 30, College of Agriculture, Haile Sellasie I University. Debrezeit, Ethiopia.

Taylor, D.P. and Netscher, C. (1979). A suggested model to visualize variability of *Meloidogyne* populations. **In:** *Root-knot nematodes (Meloidogyne species) systematics, biology and control,* pp. 311 - 316, (Lamberti, F. and Taylor, C.E., eds). Academic Press.

Terefe Deyessa (1986). Plant pathology activities and major constraints encountered. **In:** *Proceedings of the 11th annual meeting of EPC,* pp. 13 - 17. 6-7 February, 1986. Holetta, Ethiopia.

Triantaphyllou, A. C. (1960). Sex determination in *Meloidogyne incognita*, Chitwood, 1949 and intersexuality in *M. javanica* (Treub, 1885) Chitwood, 1949. *Ann. Inst. Phytopath. Benaki,* **3:** 12 - 31.

Triantaphyllou, A. C. (1962). Oogenesis in the root-knot nematode (*Meloidogyne javanica*).

Nematologica **7:** 105 - 113.

Triantaphyllou, A. C. (1963). Polyploidy and parthenogenesis in the root-knot nematode *Meloidogyne arenaria*. *J. Morphol.* **113**: 489 – 499.

Triantaphyllou, A. C. (1966). Polyploidy and reproductive patterns in the root-knot nematode *Meloidogyne hapla*. *J. Morphol.* **118**: 403 – 413.

Triantaphyllou, A. C. (1969). Gametogenesis and the chromosomes of two root-knot nematodes, *Meloidogyne graminicola* and *M. naasi*. *J. Nematol.* **1**: 62 – 71.

Triantaphyllou, A.C. (1979). Cytogenetics of root-knot nematodes **In:** *Root-knot nematodes (Meloidogyne species) systematics, biology and control,* pp. 85-110, (Lamberti, F. and Taylor, C.E., eds). Academic Press.

Triantaphyllou, A. C. (1981). Oogenesis and the chromosomes of the parthenogenetic root-knot nematode *Meloidogyne incognita*. *J. Nematol.* **13**: 95 – 104.

Triantaphyllou, A. C. (1984). Polyploidy in meiotic parthenogenetic populations of *Meloidogyne hapla* and a mechanism of conversion to diploidy. *Rev. Nematol.* **7:** 65 – 72.

Triantaphyllou, A.C. (1985a). Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. **In:** *An advanced Treatise on Meloidogyne. Vol. I. Biology and control,* pp. 113-126, (Sasser, J.N. and Carter, C.C., eds). A co-operative publication. Dept. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Triantaphyllou, A.C. (1985b). Cytological methods for the study of oogenesis and reproduction of root-knot nematodes. **In:** *An advanced Treatise on Meloidogyne. Vol. II. Methodology*, pp. 107 - 114, (Barker, K.R., Carter, C.C. and Sasser, J.N., eds). A co-operative publication. Dept. Plant Path., North Carolina S.U. and USAID. Raleigh, N.C.

Triantaphyllou, A. C. (1991). Further studies on the role of polyploidy in the evolution of *Meloidogyne*. *J. Nematol.* **23:** 249 – 253.

Triantaphyllou, A. C. (1993). Hermaphroditism in *Meloidogyne hapla*. *J. Nematol.* **25:** 15 – 26.

Triantaphyllou, A.C. and Sasser, J.N. (1960). Variation in perineal patterns and host specificity of *Meloidogyne incognita*. *Pythopath.* **50:** 724 – 735.

Triantaphyllou, A.C. and Hirschmann, H. (1980). Cytogenetics and morphology in relation to evolution and speciation of plant-parasitic nematodes. *Ann. Rev. Pythopath.* **18:** 333 - 359.

Tsedeke Abate (1985). *Review of Crop Protection Research in Ethiopia*. Proceedings of the First Ethiopian Crop Protection Symposium. 4-7 February, 1985. Addis Ababa, Ethiopia.

Williamson, V.M. (1991). Molecular techniques for nematode species identification. **In:** *Mannual of Agricultural Nematology,* pp. 107 – 123, (Nickle, W.R., ed). Marcel Dekker, INC. New York.