Morphological Variation of Root-Knot Nematode Populations from Ethiopia

Wondirad Mandefro
Plant Protection Research Center, EARO P.O. Box 37, Ambo, Ethiopia

Kifle Dagne
Department of Biology, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia.

Abstract

Fifty populations of root-knot nematodes were established from individual egg masses collected from different parts of the country. Perineal pattern morphology and stylet length were investigated. Three species were identified: *Meloidogyne incognita* (58%), *M. javanica* (18%) and *M. ethiopica* (24%). 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.

Introduction

Root-knot nematodes, *Meloidogyne* spp., are one of the most important parasites of economically important crops in most parts of the world (Sasser 1980, 1987). Until 1989, the genus *Meloidogyne* had 61 identified species and two subspecies (Eisenback &Triantaphyllou 1991). Four species, *M. incognita, M. javanica, M. arenaria*, and *M. hapla*, are major pests with worldwide distribution and wide host range. In Ethiopia three species: *M. incognita, M. javanica* and *M. ethiopica*, were identified (O'Bannon 1975).

Identification of nematode species is highly required for nematological research, quarantine enforcement, and nematode management that does not rely on nematicides. However, it is difficult particularly in the genus *Meloidogyne,* due to a relatively small difference between species. In addition, there is a high intra-specific variation in many of their characters (Jepson 1987, Bernard 1989).

Most of the morphological characters used in identification of *Meloidogyne* spp. exhibit large intra-specific variation. The most important characters for identification of females 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 highly variable (Eisenback 1985, Jepson 1987). Female stylet shape and length are also useful diagnostic characters with high intra-specific variation.

No attempts have been made, so far, to characterize the morphological variability of perineal pattern and stylet length of 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 due to lack of awareness of the nematode problem in the country and absence of a cooperator (Saka 1985). Moreover, species identification was made only by O'Bannon (1975), and little effort was made subsequently. Hence, this study was made to identify and assess the morphological variability of perineal patterns and stylet length variation of root-knot nematode species and pOpulations from different parts of the country.

Materials and Methods

Sample Collection

Root-knot nematode-infected plants were collected from different parts of the country. The sample area included both rainfed and irrigated fields in wet and dry seasons. Nematode infection was checked at the spot and plants were put in plastic bags and taken to the laboratory along with field data.

Establishing *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 to snatch egg masses and each were put in 1 % saline solution (NaCl) in microcentrifuge tubes. The isolated egg masses were then each inoculated into 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 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).

Perineal Pattern

Perineal patterns were prepared according to Hartman & Sasser (1985). At least ten perineal patterns were prepared for each population. The patterns were examined under a compound microscope (40x). From each population Camera Lucida drawings were made of the typical pattern describing the species and those that are not specific.

Stylet Length

Adult female stylet was prepared by cutting the head region and stained with propionic orcein stain. On the average, seven stylets were measured for each of *Meloidogyne* population. For measurements of populations of *M. javanica* and *M. ethiopica*, the data was subjected to ANOVA and statistical test was made with Multiple Range Test using MSTAT-C.

Results

About 150 *Meloidogyne* populations were initially established from individual egg masses. However, only 50 populations were considered for analysis due to loss of most of the populations as a result of wilting and death of tomatoes during the study period. The area of sample collection was presented in a map (Fig. 1).

The populations belong to three species: *M. incognita* 58% (29), *M. javanica* 18% (9) and *M. ethiopica* 24% (12) (Table. 4).

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 accepted in perineal patterns.

In *M. incognita,* typical pattern had the distinguishing feature of high and squared dorsal arch (Fig. 2). 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 2, 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. 3). 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 patterns of populations of *M. ethiopica* were not much variable (Fig. 4). Striae were fine and paired, and lateral lines were indistinct. The dorsal striae patterns were low and rounded arch and the phasmids were prominent but were with about the same distance apart to the ends of vulva. Population Sd-t-423 (Fig. 6, c), 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.

Female stylet length measurements were taken for 50 populations of *Meloidogyne* spp. (Table 1,2 and 3). Populations of *M. incognita* had a mean stylet length of 15.47 ±0.29µm (Table 1). Only two populations, Ti-t-330 and Ar-e-931, had stylet length of greater than 16µm, with 16.11 ±0.17µm and 16.22 ±0.35µm, respectively. The smallest measurement was taken from population Ti-t-427 with a length of 14.8µm. 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 14.8µm and 16.22 ±0.35µm, respectively.

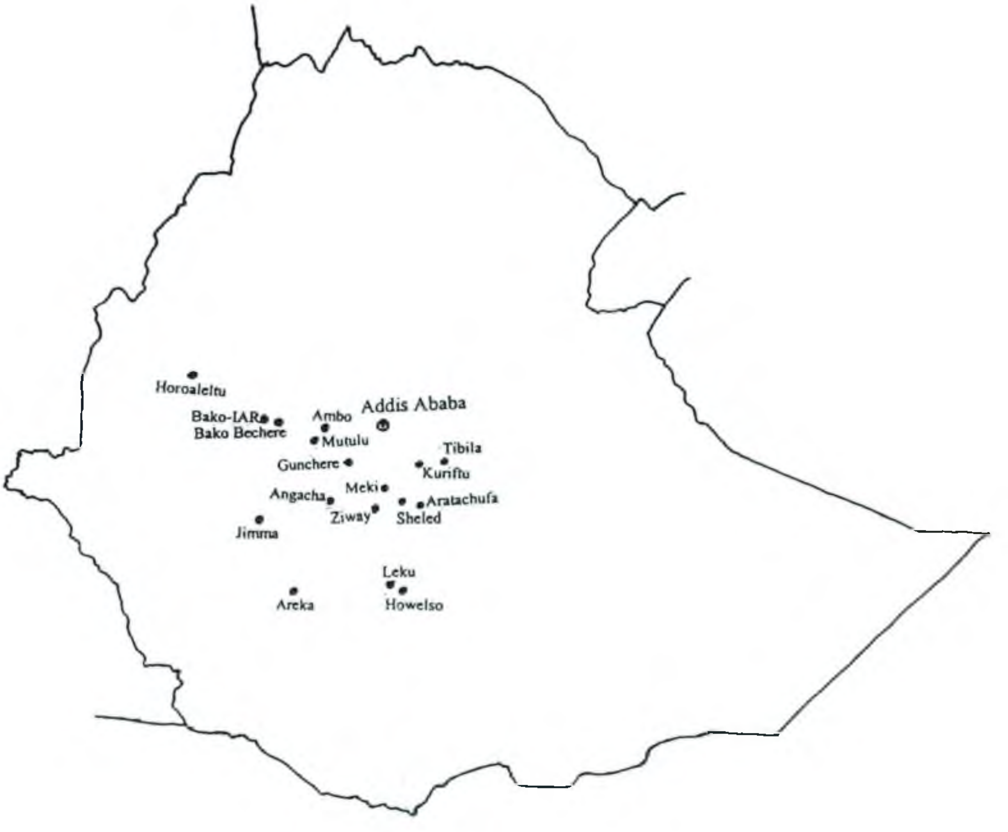

Fig. 1 **Sample collection areas**

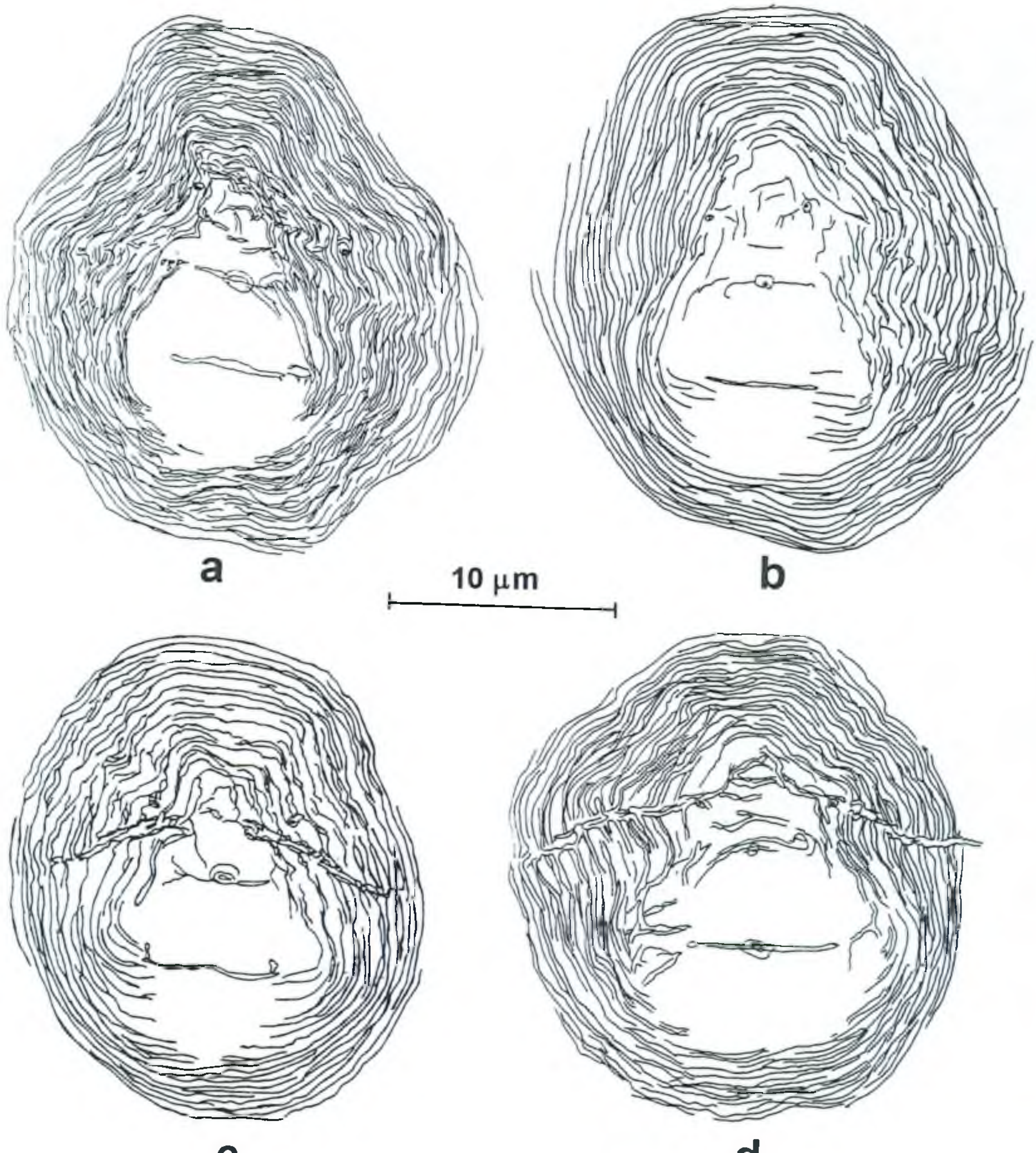

Fig. 2. *Camera lucida* drawings showing variation in perineal pattern of *M. incognita* . a and b: typical patterns; c and d: atypical patterns of the species.

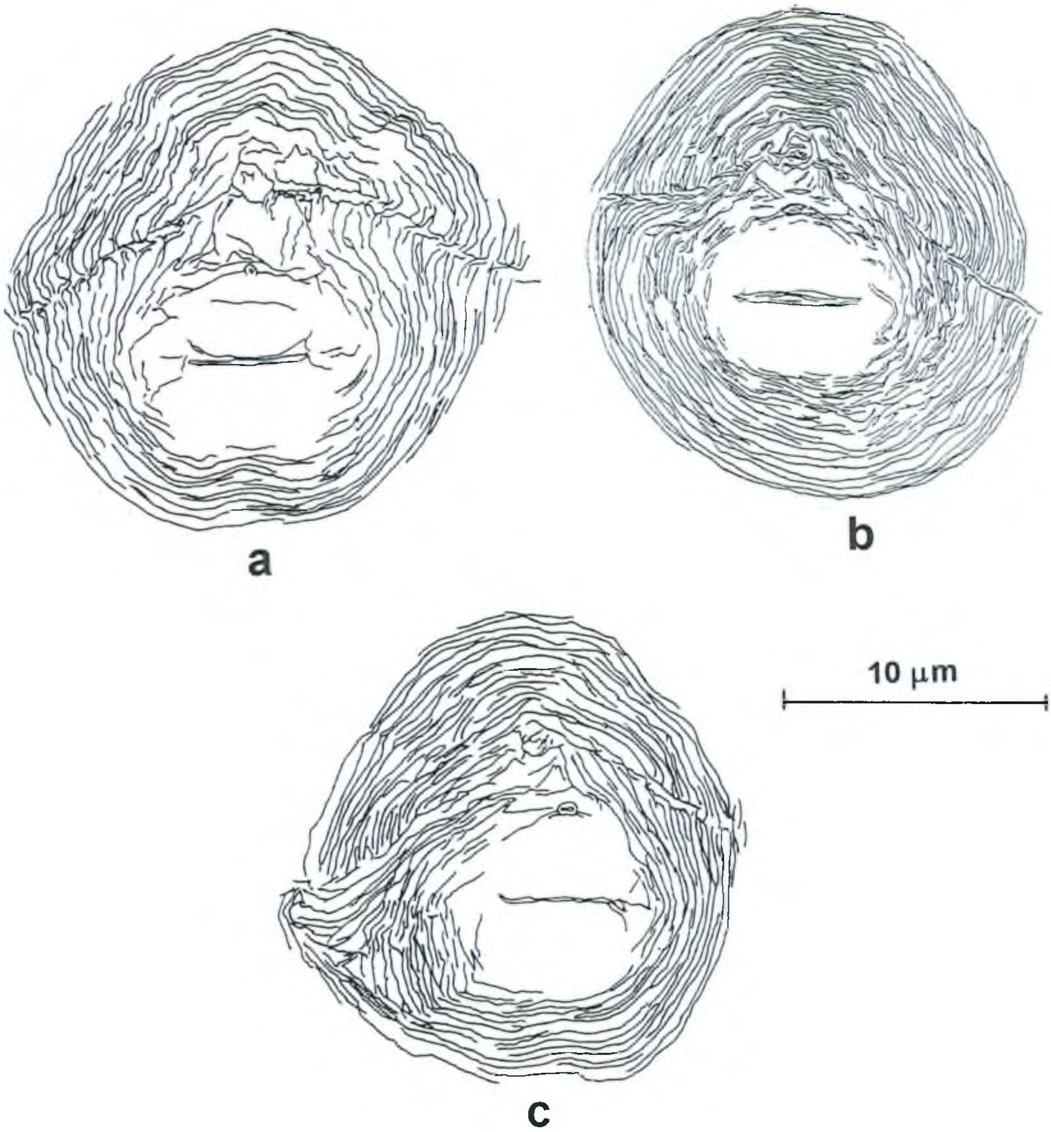

Fig. 3 Camera lucida drawings showing variation in perineal pattern of *M. javanica*. a and b: typical patterns; c: atypical pattern of the species.

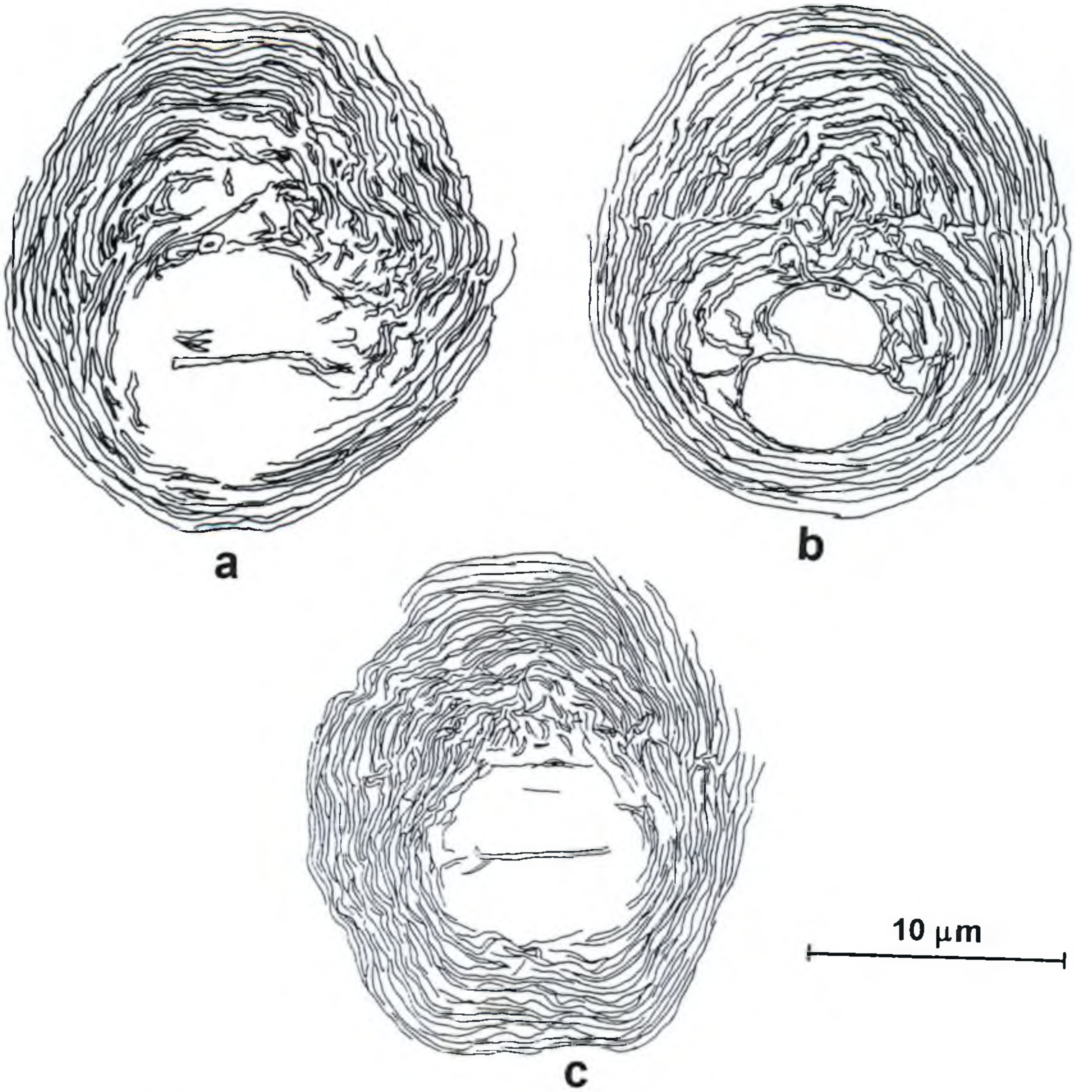

Fig. 4 *Camera lucida* **drawings showing variation in perineal patterns of** ***M. ethiopica*****. a and b: typical pattern (arenaria type); c: incognita type pattern.**

Populations of *M. javanica* and *M. ethiopica* had a highly variable stylet length (Table 2 and 3). The smallest and largest mean stylet lengths in *M. javanica* were 14.55 ±0.2µm and 17.64 ±0.07µm for populations Bi-p-268 and Gu-e-806, respectively (Table 2). The established range of mean stylet length for this species was 14µm -18µm. All populations from 'Enset' (*Enset ventricosum*) had a stylet length of more than 16µm, whereas the rest had less than 16.0µm. 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 ±0.35µm and 15.96 ±0.14µm for populations Sd-t-423 and both Gu-e-805 and Gu-e-807, respectively (Table 3). The majority of the populations, however, had a stylet length between 14.0µm and 15.9µm. Like *M. javanica*, *M. ethiopica* populations isolated from 'Enset' had an average stylet length of 15.77 ±0.13µm, 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 ±0.26µm.

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

Table 1. Stylet length of populations of *M. incognita* by locality and host plant.

Popln No.	Locality	Host plant	Stylet length (µm) (mean)*
Bb-p-266	Bako bechere	Pepper	15.37±0.18
Bi-p-270	Bako IAR	Pepper	15.75±0.00
Ti-t-327	Tibila	Tomato	15.37±0.19
Ti-t-328	Tibila	Tomato	15.32±0.15
Ti-t-330	Tibila	Tomato	16.11±0.17
Am-c-355	Ambo, PPRC	Carrot	15.07±0.23
Ac-p-401	Aratachufa	Pepper	15.33±0.17
Ac-p-402	Aratachufa	Pepper	15.52±0.16
Ac-p-404	Aratachufa	Pepper	15.06±0.20
Ac-p-405	Aratachufa	Pepper	15.90±0.14
Ac-p-406	Aratachufa	Pepper	15.63±0.21
Ac-p-408	Aratachufa	Pepper	15.10±0.16
Ac-p-409	Aratachufa	Pepper	15.00±0.00
Ac-p-481	Aratachufa	Tomato	15.49±0.23
Ac-p-482	Aratachufa	Tomato	15.23±0.29
Ac-p-483	Aratachufa	Tomato	15.36±0.55
Ac-p-484	Aratachufa	Tomato	15.43±0.37
Ac-p-485	Aratachufa	Tomato	15.23±0.40
Sd-t-427	Sheled	Tomato	14.80±0.00
Zi-t-442	Ziway	Tomato	15.54±0.25
Zi-t-443	Ziway	Tomato	15.19±0.23
Zi-t-444	Ziway	Tomato	15.28±0.04
Zi-t-445	Ziway	Tomato	15.80±0.39
Ku-t-451	Kuriftu	Tomato	15.81±0.28
Ku-t-453	Kuriftu	Tomato	15.23±0.15
Ar-e-912	Areka 01	'Enset'	15.78±0.16
Ar-e-931	Areka	'Enset'	16.22±0.35
Hs-e-942	Howelso	'Enset'	15.92±0.23
Hs-e-943	Howelso	'Enset'	15.83±0.37

* *Values are means in µm and ± standard error*

Table 2. Stylet length of populations of *M. javanica* by locality and host plant.

Population No.	Locality	Host plant	Stylet length (μm) (mean) *
Ha-tb-251	Horoaleltu	Tobacco	15.90 ± 0.24 bc**
Bi-p-268	Bako IAR	Pepper	14.55 ± 0.26 a
Ti-t-326	Tibila	Tomato	15.13 ± 0.27 ab
Gu-e-806	Gunchere	'Enset'	17.64 ± 0.07 c
Ag-e-901	Angacha	'Enset'	16.20 ± 0.27 c
Lk-e-920	Leku	'Enset'	16.10 ± 0.28 bc
Lk-e-921	Leku	'Enset'	16.80 ± 0.35 c
Lk-e-922	Leku	'Enset'	16.17 ± 0.33 c
Lk-e-923	Leku	'Enset'	16.23 ± 0.24 c

* *Values are means in μm and ± standard error*
** *Similar letter indicates no significant difference at P = 0.05 with Multiple Range Test*

Table 3. Stylet length of populations of *M. ethiopica* by locality and host plant.

Population no.	Locality	Host plant	Stylet length (μm) (mean) *
Sd-t-423	Sheled	Tomato	13.91 ± 0.13 a**
Mk-b-433	Meki	Snap bean	14.28 ± 0.28 ab
Mk-b-435	Meki	Snap bean	14.62 ± 0.12 abc
Mu-t-468	Mutulu	Tomato	14.90 ± 0.31 bc
Ji-bt-603	Jimma	Beetroot	14.04 ± 0.11 ab
Ji-bt-604	Jimma	Beetroot	14.56 ± 0.28 ab
Ji-c-605	Jimma	Cabbage	14.15 ± 0.15 ab
Ji-c-606	Jimma	Cabbage	14.55 ± 0.21 ab
Ji-c-607	Jimma	Cabbage	13.91 ± 0.18 ab
Gu-e-803	Gunchere	'Enset'	15.40 ± 0.37 cd
Gu-e-805	Gunchere	'Enset'	15.96 ± 0.35 d
Gu-e-807	Gunchere	'Enset'	15.96 ± 0.14 d

* *Values are means in μm and ± standard error*
** *Similar letter indicates no significant difference at P = 0.05 with MRT*

Table 4. Number and percentage of populations of *Meloidogyne* spp. in location and crops isolated.

Species	No. of locations	No. of crops isolated from	No. of Populations	Percentage
M. incognita	11	3	29	58
M. javanica	6	4	9	18
M. ethiopica	5	5	12	24
Total			50	100

Discussion

The three *Meloidogyne* species identified in this study were previously recorded (O'Bannon 1975) and no new species were encountered. 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. The identity of these populations is, however, confirmed through cytogenetic and isozyme methods in a similar study (Wondirad, Unpublished). Due to such variability, *M. incognita* and *M. javanica* used to have ten and seven synonyms, respectively (Eisenback & Triantaphyllou 1991).

Some of the variability is genetically controlled and some are the result of external environmental factors (Triantaphyllou & 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 & 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, perineal pattern is the basic taxonomic character. 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 was higher in *M. incognita* than in *M. javanica* and *M. ethiopica*. The minimum and maximum stylet lengths found in this study, however, were within the reported range (Jepson, 1987).

Eisenback (1985) 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 lengths observed in two populations of *M. incognita* could not be explained with respect to morphological and other parameters examined. 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 14μm parasitize graminae or cyperaceae, and 71% of species with stylet length greater than 16μm primarily parasitize woody hosts. In 14-16μm 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. Stylets are used in two ways, to penetrate tissues and cells and as a passage for nematode secretions and food (Eisenback 1985). 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. In addition, the nature of 'Enset', being perennial and vegetatively propagated, would give a conducive coexistence for a unique adaptation to emerge. 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.

M. incognita was the most encountered species followed by *M. ethiopica* and *M. javanica*. The high incidence of *M. incognita* was in agreement with its worldwide distribution (Sasser 1987). In tropical soils, the economically important species after *M. incognita* are *M. javanica*, *M. arenaria* and *M. hapla* (Sasser 1979). According to Netscher & Sikora (1990) and Shepherd & 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 (1979) ranked *M. ethiopica* ninth in Africa. The high incidence of *M. ethiopica* in this study, however, indicated its potential as an important parasitic species.

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