COMPARISON OF THREE DIFFERENT ESTERASE PHENOTYPE ISOLATES OF THE KONA COFFEE ROOT-KNOT NEMATODE, *MELOIDOGYNE KONAENSIS*

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ABSTRACT

The esterase phenotype of female *Meloidogyne konaensis* (MK) was originally described as a single fast band (F1). Culturing MK, originally isolated from coffee, on tomato resulted in the detection of two additional phenotypes (I1-F1 and I1). These three *M. konaensis* esterase phenotypes (MKF1, MKI1-F1 and MKI1) were evaluated for molecular and morphological behavior. The isolate MKI1-F1 exhibited two esterase bands (F1 and I1). The MKI1 exhibited a slow migrating band identical to that of *M. incognita*. All three isolates had an MDH phenotype N1, identical to that of *M. javanica* and *M. incognita*. On a 2-dimensional electrophoresis gel (2-DGE), the protein maps of all three isolates differed from that of *M. javanica*. There were similar protein spots between isolates of *M. konaensis*. Morphological comparison showed differences in the male heads and perineal patterns among three isolates. All MKF1 males had the typical high and rounded head shape and stylet shaft with protuberance. MKI1-F1 males also had the rounded head shape but the majority of head caps were lower than those of MKF1. The majority of MKI1 male heads caps was narrow, square and indented anteriorly.

Host range and pathogenicity differed among the three isolates. MKF1 had low reproduction on coffee, whereas MKI1-F1 and MKI1 failed to develop on coffee. The reproduction ratio on coffee by MKF1 was 1.1, and zero for MKI1-F1 and MKI1. In contrast, reproduction was high on tomato and cucumber. MKI1-F1 had the highest reproduction on tomato and cucumber which was two times more than MKF1 and MKI1, whereas MKF1 and MKI1 were similar. The reproduction ratio on tomato and cucumber was 110.9 and 40.8 by MKI1-F1, 45.9 and 9.3 by MKF1, and 47.6 and 17.6 by MKI1

respectively. Overall, MKF1 was the only isolate having the ability to infest coffee and MKI1-F1 was the most aggressive isolate of *M. konaensis* on tomato and cucumber.

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CHAPTER 1. INTRODUCTION

Meloidogyne konaensis Eisenback, Bernard, Schmitt is a root-knot nematode discovered in 1991 on coffee and described in 1994 (6). *M. konaensis* is morphologically distinct from other described *Meloidogyne* species. It has 2n = 44 chromosomes and reproduces by mitotic parthenogenesis. Besides morphologically diagnostic characters, the non-specific female esterase phenotype of F1 is also a useful biochemical approach for identification (6). Other root-knot species infecting coffee such as *M. paranaensis* and *M. querciana* have an identical F1 esterase phenotype (2).

In the past decades, the phenotypes of non-specific esterases, malate dehydrogenases, superoxide dismutases, and glutamate-oxaloacetate transaminases have proven to be very powerful characters for identification of the *Meloidogyne* species and characterization of new taxonomic forms (3, 4, 5, 7, 8, 10). In routine enzyme studies of *Meloidogyne* species, the thin-slab polyacrylamide gel electrophoresis has proven reliable and able to detect the phenotype of a single *Meloidogyne* female (4, 7, 10). Among these enzymes, esterase is the most useful and reliable for identification of major *Meloidogyne* species (3, 4, 5, 7, 8, 10).

Many of the major species of *Meloidogyne* exhibit a stable and characteristic esterase phenotype (7). *M. javanica* has a 3-band phenotype—J3. Esbenshade and Triantaphyllou (1985) investigated 291 populations representing 16 species of *Meloidogyne* originating from 65 countries. They observed the J3 esterase phenotype in 46 *M. javanica* populations but none of other species. Some species of *Meloidogyne* had

more than one esterase phenotype. Eighty-four percent of *M. arenaria* populations had one of three phenotypes. Most populations, 99% of *M. incognita* and 94% of *M. hapla* populations had species characteristic esterase phenotypes I1 and H1 (7), respectively. None of the other *Meloidogyne* species had consistent phenotypic patterns (7). *M. incognita* exhibited an I1 and S1 phenotype. *M. hapla* exhibited phenotypes H1 and A1 (7).

The esterase phenotype of *M. konaensis* also shows variation. *M. konaensis* populations with esterase phenotypes of two migrating bands and a single slow migrating band have been found. The double migrating bands phenotype has one band at the F1 level, and another band at the I1 level identical to *M. incognita* (7). This phenotype will be represented by the symbol I1-F1. The single slow migrating band phenotype has one band at the I1 level.

In our laboratory, *M. konaensis* has routinely been cultured on susceptible tomato cultivars. During a routine assay for culture purity, *M. konaensis* previously tested as F1 exhibited the I1 phenotype. The laboratory concluded the culture was contaminated with *M. incognita* and a new field isolation was made (Schmitt and Zhang, pers. comm). Several years later the culture appeared to be "contaminated" again and a third field isolation was made (Serracin and Schmitt, pers. comm). A single egg mass of this third culture of *M. konaensis* was maintained in a greenhouse isolated from other *Meloidogyne* species (Serracin et al., pers. comm).

An experiment was conducted to determine if temperature affected the esterase phenotype of *M. konaensis*. Eggs of *M. konaensis* were collected from a tomato culture,

inoculated onto *Lycopersicon esculentum* cv. Orange Pixie, and the plants placed in incubators set at constant 22°C, 25°C, and 33°C. Females were collected 30 days later and assayed for esterase phenotype. The experiment was repeated except that inoculum was collected from coffee plants. Regardless of temperature, females from the first run of the experiment exhibited the I1 phenotype and females from the second run of the experiment exhibited the F1 phenotype (Mordan-McCombs and Sipes, unpublished)

To test the effect of host on *M. konaensis* esterase phenotype, F1 *M. konaensis* eggs from coffee cultures were inoculated onto tomato and placed in the greenhouse. Populations were assayed for esterase phenotype and reinoculated onto tomato plants monthly. Only females with the F1 phenotype were recovered during the first 8 months (Sipes, unpublished). Nine months after being placed on tomato, females exhibiting the I1 were detected (Sipes, pers. comm).

In order to exclude the possibility of contamination, a follow up experiment was conducted in the laboratory (Sipes, pers. comm). 'Orange Pixie' tomatoes were transplanted into individual 35-ml test tubes filled with 30 ml of sterile sand. Four individual single egg mass lines of *M. konaensis* with the F1 phenotype were inoculated onto the plants. These plants were separated and gently watered to prevent splash from tube to tube. No other *Meloidogyne* cultures were maintained near these plants. Two single egg mass lines were from coffee (C1 and C2) and two from tomato (T1 and T2). Every 6 weeks, several females were teased from roots and assayed for esterase phenotype. Eggs were collected by shaking roots in a NaOCl solution (1) and inoculated on new tomato plants. All females assayed had the F1 phenotype until 9 months after

establishment. The esterase patterns of two females from C2 line had the I1 band. Nine months later, 18 months after establishment, one female from the C1 line had an I1-F1 pattern. With subsequent assays, more females with the I1-F1 phenotype were detected from C1. A single egg mass line of the single slow and two band phenotypes were established and maintained on tomato. The remaining lines continued to exhibit F1 phenotype.

Hussey hypothesized that host plants may influence a phytoparasitic nematode's metabolism and affect biochemical analyses (9). We hypothesize that nematode metabolism, as evident in biochemical assays, influences it's parasitic ability on particular host plants. For *M. konaensis*, coffee parasitism plays a strong selective pressure for the F1 esterase phenotype. The F1 phenotype *M. konaensis* is the result of selection on coffee. The other two esterase phenotypes fail to develop or develop very poorly on coffee. These same populations grow well on tomato.

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CHAPTER 2. ENZYMES AND SOLUBLE PROTEINS

Introduction

Esterases are enzymes that degrade esters by catalyzing the hydrolysis of short chain fatty acids. Esterases are important in the catabolism of fats and in nerve impulse transport through the hydrolysis of acetylcholine (11). Esterases are classified according to their susceptibility to specific inhibitors. Non-specific esterases are not completely inhibited by $10^{-6}M$ eserine, whereas cholinesterases are (11).

Non-specific esterase and malate dehydrogenase phenotypes are both very useful isozymes for identification of *Meloidogyne* species (13). Thin-slab electrophoresis techniques make the enzyme analysis of *Meloidogyne* convenient and time efficient. This thin-slab technique can detect these enzymes in one female of *Meloidogyne*. Since mixed root-knot nematode populations are found in nature, using one female assures sample purity and greater likelihood of detecting the variation among individuals within a population. Egg masses can be saved and used later as inoculum to obtain progeny of the individual females (13).

Two-dimensional electrophoresis (2-DGE) is a very powerful and widely used technique for analyzing complex protein mixtures with high resolution and sensitivity. This technique sorts proteins according two independent properties in two discrete steps. The first dimension, isoelectric focusing (IEF), separates proteins according to their isoelectric points. The second dimension, SDS-polyacrylamide gel electrophoresis (SDS-

PAGE), separates proteins according to their molecular weights. Protein studies have been conducted by this technique on bacteria, plants, animals and in human medicine (25). It was also successfully used on plant-parasitic nematodes in the Heteroderidae such as *Heterodera* (5, 6, 15, 17), *Globodera* (3, 4, 10) and *Meloidogyne* (9, 24, 25, 26, 27). 2-DGE can be used to detect specific proteins and study the phenetic relationships, genetic distance, and genetic variability among species (24, 25), races, pathotypes, and isolates (5, 16).

The objective of these experiments was to elucidate differences among the non-specific esterases, malate dehydrogenase, and soluble proteins in three different *M. konaenesis* isolates.

Materials and methods

Three isolates of *M. konaensis* differing in esterase phenotype were used. MKF1 (F1) was an isolate from *Coffea arabica* var Typica Selection Guatemala in Kona Hawaii which exhibits a single fast band esterase (F1) typical of *M. konaensis*. MKI1-F1 (I1-F1) was an isolate derived from an MKF1 single egg mass line which exhibits a two band esterase phenotype composed of the F1 band and the I1 band. The MKI1 was an isolate from weedy amaranth in a Kealakekua, Hawaii coffee field. A single egg mass was taken from each isolate and propagated on 'Orange Pixie' tomato. The isolates were cultured on tomato for 4 months or less. *M. javanica*, maintained on 'Orange Pixie' tomato, was used as a reference phenotype on the all gels. *M. incognita* maintained on 'Orange Pixie' tomato was used for comparison.

An automated PhastSystem (Amersham Pharmacia Biotech) was used to conduct

the electrophoresis. Proteins of single females were separated on native polyacrylamide gradient 10-15 gels and then stained for esterase or malate dehydrogenase isozymes. The electrophoresis included three stages. The first stage was run at 5°C at 400V, 10.0 mA, 2.5W for 10 Vh. The second stage was run at 5°C at 400V, 1.0mA, 2.5W for 2Vh. The final stage was run at 5°C at 400V, 10.0mA, 2.5W for 200Vh. After electrophoresis, the gel was stained for either esterase (14) or malate dehydrogenase (14). The gel was covered with fresh stain solution, and incubated at 37°C for 1 hour (14). After staining, gel was placed in fixative solution (10% acetic acid, 10% glycerol) for 1 hour. Then it was dried and kept in cellophane.

Field survey. A field survey was conducted for esterase phenotypes of *Meloidogyne* species on weeds found in a coffee field in Kealakekua Hawaii. Forty three *M. konaensis* females were teased from two amaranth plants collected from a Kealakekua coffee field and frozen. Soil was collected from the coffee field and placed into three 15-cm-diameter pots. One 'Orange Pixie' tomato plant was transplanted into each pot. Six months after planting, 68 females were teased from tomato galls and used for esterase phenotype analysis along with those from amaranth.

Esterase and malate dehydrogenase assay. Pear shaped females were teased from tomato roots and placed in 0.16M NaCl. Eight females were transferred into a 1.5ml epp tube and frozen (-20 °C) until used. Before electrophoresis, the females were thawed. Eight well PhastGel sample stamps were each filled with one female and 0.6µl extract buffer (20% sucrose, 2% Triton X-100, 0.01% Bromophenol Blue) (13). Each female was crushed with a small cooper rod. The nematode homogenate was loaded onto a 0.5µl

sample applicator that was inserted into the cathode slot of a PhastSystem applicator arm. Each gel contained a *M. javanica* female as a reference. Fifty females of MKF1 from 6 plants, 30 females of MKI1-F1 from 10 plants and 45 females of MKI1 from 6 plants were assayed for esterase phenotypes. Three females of each MK isolate from three plants were assayed for malate dehydrogenase phenotypes.

Two-dimensional electrophoresis comparison. At least fifty females of each isolate were extracted from tomato roots and frozen in 0.16M NaCl. Before electrophoresis, the females were thawed and washed three times with a 10mM Tris HCl, pH 7.4. Females were crushed with a plastic pestle in 10µl 10mM Tris HCl and 30µl homogenizing solution of 9 M urea, 5% (v/v) β-mercaptoethanol and 2% ampholytes (4), pH range 2.5-5 and 5-8 (Pharmalyte, Amersham Pharmacia Biotech). The homogenates were centrifuged at 10,000 g for 10 minutes and supernatants frozen at -20°C. The protein concentration was estimated by measuring the shift of extinction of coomassie Blue G-250 at 595nm (7). Concentration was adjusted to 2µg/µl with the homogenate solution (24).

Low molecular weight marker kit (LMW) protein (Amersham Pharmacia Biotech) was dissolved in 200µl of 10mM Tris-HCl, 2% SDS, 0.1M DTT, 0.01% bromophenol blue, and 1mM EDTA, pH 8.0. The solution was diluted by at least 50-fold in 1× sample buffer and heated for 5 minutes at 95-100 °C for use.

The PhastSystem (Amersham Pharmacia Biotech) was used to conduct this electrophoresis. Both dimensions were run horizontally with running conditions microprocessor controlled (1). The First dimension IEF consisted of a 3-9 gradient gel

washed twice in distilled water (5 minutes each time) followed by a 10 minute wash in 10% glycerol. The gel was then dried. The dried gel was rehydrated for 30 minutes in 8 M urea, 0.5% Nonidet P-40, and 7.5% Pharmalyte mixture (pH2.5-5+pH5-8 in the ratio 1:1) (1). The surface water of the IEF gel was removed and the gel placed on the separation bed. A 3µl nematode homogenate sample was applied on the cathode as a continuous line across the width of the gel with a sample applicator.

The IEF was run for 800 volthours (Vh). The first step was run at 200V, 2.5mA, 3.5W, 15°C for 30Vh. The second step was run at 2000V, 2.5mA, 3.5W, 15°C for 770Vh. After focusing, the area where electrodes rested was removed. The gel was cut into two 4-mm-wide strips and stored at -20 °C. Upon use, the strip was equilibrated for 2 minutes in 0.112 M Tris/HAc, 1% DTT, and 2.5% SDS followed by 2 minutes in 0.112 M Tris/HAc, 2.5% SDS, 0.260 M iodoacetamide, and 0.001% BPB and adjusted to pH 6.4 (18).

The second dimension SDS-PAGE consisted of placing the equilibrated strip gel side down on the stacking gel zone of a gradient 10-15 polyacrylamide gel. SDS buffer strips were inserted into the holder and the LMW marker applied. The electrophoresis was run for 70 Vh. The fist step consisted of a run at 250 V, 2.5 mA, 3.0 W, 15 °C for 5 Vh. The second step was run at 250 V, 5.0 mA, 3.0 W, 15 °C for 5 Vh. The final step was run at 250 V, 10.0 mA, 3.0 W, 15 °C for 60 Vh.

A silver staining method was used to show proteins (23). After electrophoresis, the SDS-polyacrylamide gel was fixed by incubating the gel for 4 hours with gentle shaking in the solution of ethanol:glacial acetic acid:water (30:10:60). The fixing solution was

discarded, 30% ethanol was added to cover the gel, and the gel incubated for 30 minutes. The 30% ethanol incubation was repeated once. The gel was washed twice with deionized water for 10 minutes. The gel was next placed in a 0.1% solution of AgNO3, freshly diluted from a 10% stock, for 30 minutes with gentle shaking. After staining, both sides of the gel were gently washed for 20 seconds. each. An incubation in an aqueous solution of 2.5% NaCO4 and 0.02% formaldehyde with gentle agitation followed. The incubation continued until the desired contrasting protein bands were obtained and stopped by washing the gel several times with deionized water, 10 minutes per wash.

Results

During the fist 6 months of MKF1 culture from coffee on tomato, no esterase change was detected. The esterase pattern of MKI1-F1, maintained on tomato for 3 years and MKI1 maintained on tomato for 14 months did not change.

Field survey. The females collected from amaranth exhibited two esterase phenotypes: 79% F1 (Fig. 2.1A) and 21% I1 (Fig. 2.1B). Females removed from tomatoes grown in the infested field soil exhibited all three phenotypes. F1 phenotype accounted for 69%. The I1 phenotype represented 27% of the females. The I1-F1 phenotype accounted for the remaining 4% of the females (Fig. 2.2). This was the first time the I1-F1 was recovered from field soil.

Esterase. Each isolate esterase phenotype was consistent among all females tested (Table 2.1). MKF1 exhibited F1 in all females tested. MKI1-F1 had one band at the F1 level and another band at I1 level identical to *M. incognita*. MKI1 had a single slow migrating band at the I1 level (Fig. 2.3).

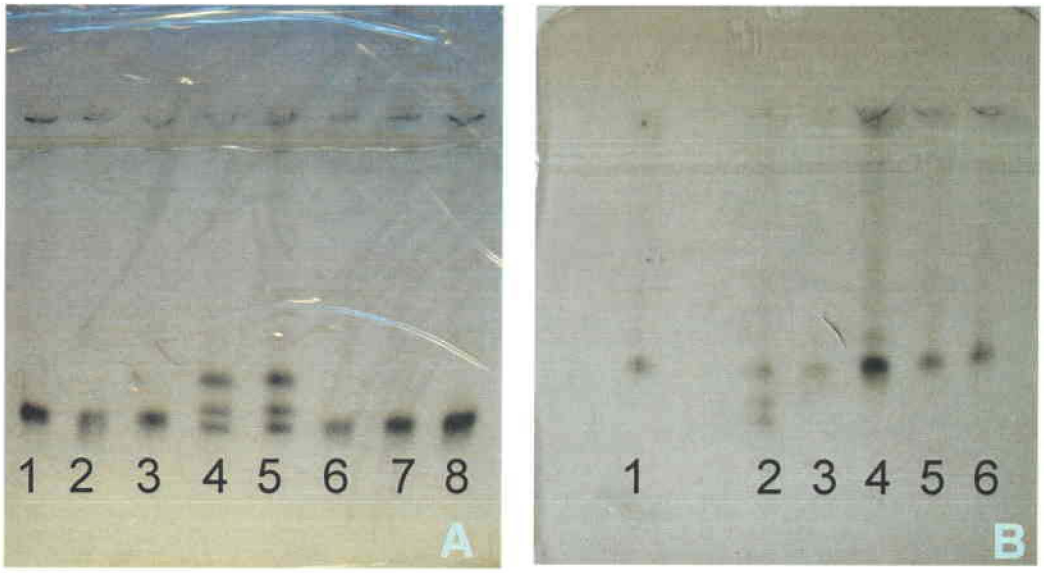

Figure 2.1 Esterase phenotypes of *Meloidogyne* species isolated from amaranth growing in a coffee field in Kealakekua, Hawaii.

A) *M. javanica*: lanes 4 and 5; MKF1: lanes 1, 2, 3, 6, 7 and 8.

B) *M. javanica*: lane 2; MKI1: lanes 1, 3, 4, 5, and 6.

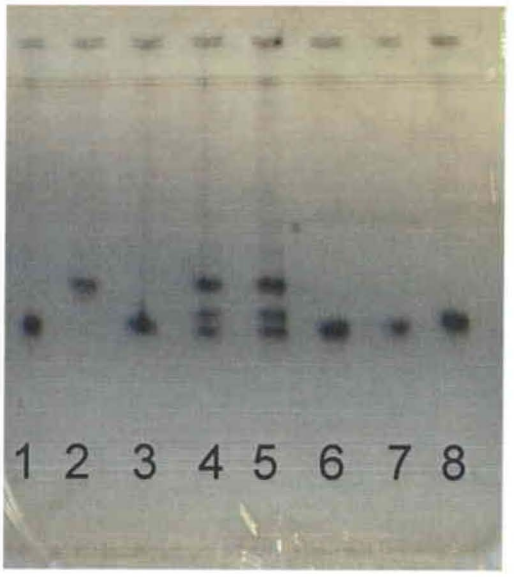

Figure 2.2 Esterase phenotypes survey of *Meloidogyne* species on tomatoes grown in infested soil from a coffee field in Kealakekua Hawaii. *M. javanica*: lanes 4 and 5; MKF1: lane 1, 3, 6, 7, 8; MKI1: lane 2.

Malate dehydrogenase. Malate dehydrogenase did not differentiate the isolates of *M. konaensis* from *M. javanica* and *M. incognita.* All females tested were phenotype N1 (Fig. 2.4).

Two-dimensional electrophoresis. On a 2-dimensional electrophoresis gel (2-DGE), the protein maps of all three isolates differed from the one of *M. javanica.* There were similar protein spots between isolates of *M. konaensis* (Fig. 2.5).

Table 2.1 Esterase phenotypes of three *Meloidogyne konaensis* isolates on tomato.

Isolates	Number of plants	Number of females observed	% phenotype
MKF1	6	50	100
MKI1-F1	10	30	100
MKI1	6	45	100

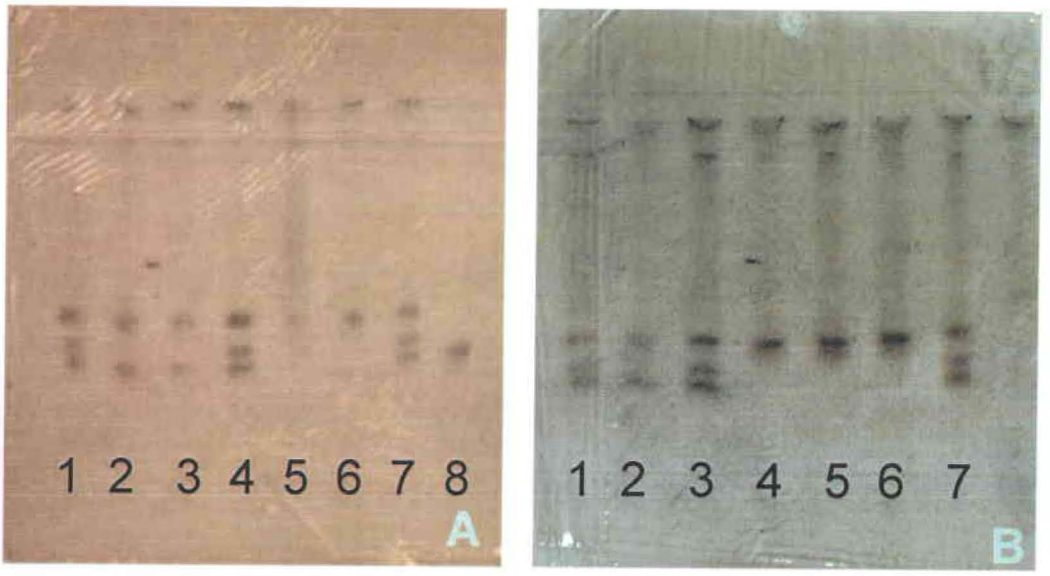

Figure 2.3 Esterase phenotypes of three isolates of *Meloidogyne konaensis* on tomato. **A)** MKF1: lane 8; MKI1-F1: lanes 2 and 3; MKI1: lane 6; *M. javanica*: lanes 1, 4, and 7; *M. incognita*: lane 5. **B)** MKI1-F1: lane 2; MKI1: lanes 4 and 6; *M. javanica*: lanes 1, 3 and 7; *M. incognita*: lane 5.

Figure 2.4 Malate dehydrogenase phenotypes of three isolates of *Meloidogyne konaensis* on tomato. MKF1: lane 8, MKI1-F1: lanes 2 and 3, MKI1: lane 6, *M. javanica*: lane 1, 4 and 7, and *M. incognita*: lane 5.

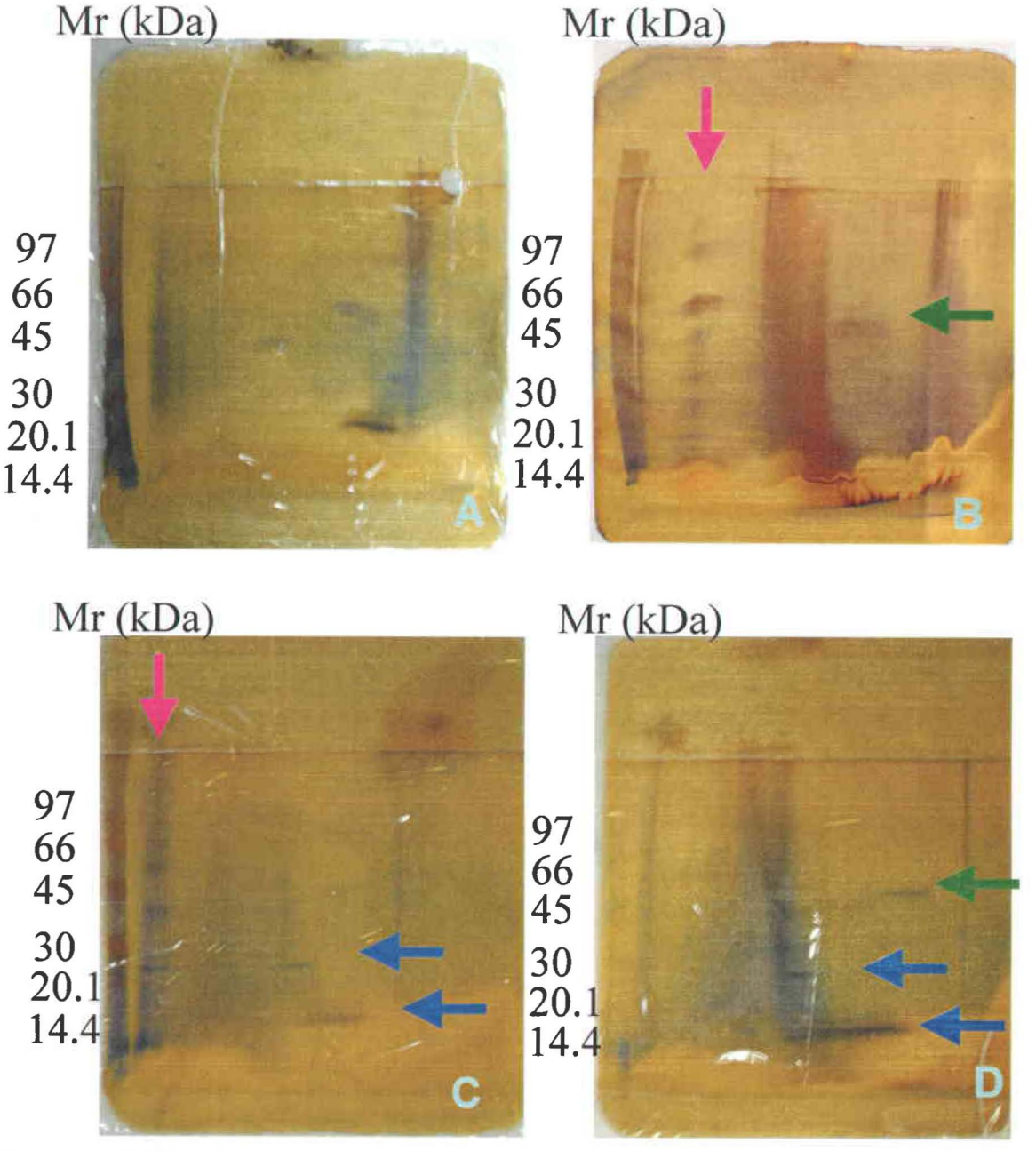

Figure 2.5 Two-dimensional electrophoresis gels of three isolates of *Meloidogyne konaensis* and *Meloidogyne javanica* on tomato. **A)** *M. javanica*; **B)** *M. konaensis* MKF1; **C)** *M. konaensis* MKI1-F1; **D)** *M. konaensis* MKI1. Arrows in the same color indicate similar protein spots between isolates.

Discussion

These experiments showed that the esterase phenotypes F1, I1-F1 and I1 of three *M. konaensis* isolates on tomato were very different and all the tested females of each isolate exhibited consistent phenotypes. During this study, the cultural conditions and electrophoresis process were consistent for all the three isolates. The same *M. konaensis* adult stage females were used because some enzyme profiles have been found to be age dependent, including esterase (8, 12, 21). Hussey and Dickson (12, 20) found no difference in esterase patterns of *M. incognita* on three different host plants. Overall esterase is a relatively stable enzyme but Ishibashi (22) reported that some variation occurred in esterase profiles when nematodes were propagated on different host plants. Host influence on nematode enzyme production and activity varies with enzymes and host plants (20). During this study, tomato clearly affected *M. konaensis* esterase profiles.

This study showed that the change in esterase phenotype was not rapid. After MKF1 was transferred from coffee to tomato for 9 months, the changes in esterase phenotypes were detected. Greet and Firth (19) stated that host proteins which occur in the gut of nematodes may interfere with the protein analysis. If esterase profiles were influenced by host plant proteins, the change should have been detected since the fist generation. This was not the case. The factors that initiate the esterase change are speculative. I hypothesize that *M. konaensis* adapts to different hosts by changing its esterase.

MKF1 has demonstrated that its esterase phenotype is not stable on tomato, but the esterase phenotypes of MKI1-F1 and MKI1 were found very stable on tomato. No

any other esterase phenotype was ever detected in MKI1-F1 and MKI1 isolate cultures. During this study, no MKI1-F1 esterase phenotype was detected directly from field. Although only observed in the laboratory, it is very probable that changes in the frequency of esterase phenotypes occur in nature. The survey showed that field populations of *Meloidogyne* species collected from amaranth had F1 and I1 phenotypes just like those in the laboratory. *Meloidogyne* species populations on tomato grown in infested coffee field soil also exhibited F1, I1-F1 and I1 phenotypes. But because the tomatoes have been grown for 6 months, it is not clear that I1-F1 and I1 were originally from field or developed after propagation on tomato. Thus, a similar process probably occurs in the field as we observed in the lab. It's very possible that the F1 esterase phenotype is not stable on other plants just like on tomato. Schmitt and Zhang (unpublished) surveyed vegetables in the Kona district but found no F1 phenotypes, whereas the nematode exhibited a wide host range in a greenhouse study. It appears insufficient to use only the F1 esterase phenotype to diagnose and identify *M. konaensis*. I1-F1 is currently a unique esterase phenotype to *M. konaensis*. More field surveys are suggested especially for I1-F1 phenotype.

These experiments showed that all the three *M. konaensis* isolates had the same malate dehydrogenase phenotype N1 which was similar to *M. javanica* and *M. incognita*. Malate dehydrogenase was not affected by culturing on tomato.

The ultimate goal of the 2-DGE was to uncover genotypic differences among three *M. konaensis* isolates important to parasitism. Proteins are a manifestation of the sequence of nucleotides in a gene and analyses of these macromolecules can provide a

reliable approach for comparing genotypes of organisms (21). Compared to protein analysis, amino acid analysis is very laborious (21). At interspecific and intraspecific levels, the high degree of protein resolution provided by 2-DGE makes it possible to identify a large number of gene products (2, 4, 25).

Other physiological changes surely occurred but were not assayed. In the future, genotypic differences among three *M. konaensis* isolates need to be analyzed and other enzymes important for taxonomy must be evaluated for multiple phenotypes. Superoxide dismutase and glutamate-oxaloacetate transaminase are possible markers because these enzymes have good taxonomic utility. Other molecular phenotypes which may change in relation to parasitism are deserving of study.

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CHAPTER 3. MORPHOLOGICAL DIFFERENCES OF THREE *MELOIDOGYNE KONAENSIS* ISOLATES

Introduction

Several morphological characters are important for the identification of *Meloidogyne konaensis*. The most useful preliminary diagnostic character of *M. konaensis* is morphology of the male stylet (3). Other diagnostic characters include the morphology of the perineal pattern and female stylet, the shape of the male head, the tail shape and location of the phasmids on the second-stage juveniles (3). Actually, all of these aspects should be assessed to have confidence in the identification of the nematode.

The perineal pattern of *M. konaensis* is variable with coarse striae. The dorsal arch is rounded to squarish and the perivulval region is free of striae (3).

The male head cap of *M. konaensis* is high, rounded and tapers posteriorly. The most characteristic aspect of *M. konaensis* is the 6-12 large projections protruding from the shaft of the stylet (3).

The second-stage juvenile tail of *M. konaensis* is often distinctly curved ventrally with annulations being larger posterior to than anterior. The tail terminus has a distinct hyaline region. Phasmids are small and located in the ventral incisure of the lateral field, posterior to anus (3).

Since variation in population is likely to occur, especially since there is known variation in esterase phenotypes, the objective of these experiments was to compare the

important diagnostic characters of male head and stylet morphology, the female perineal pattern and second juvenile tails among three *M. konaensis* isolates differing in esterase phenotypes.

Materials and methods

Three isolates of *M. konaensis* differing esterase phenotype were used to determine morphological variation within the species. The first population, isolated from *Coffea arabica* var Typica seletion Guatamala in Kona, HI, had a single fast band esterase phenotype (MKF1), typical of the described species. The second population (MKI1-F1) was subcultured from the MKF1 population by selection over 9 generations on tomato. This MKI1-F1 isolate has a two band esterase phenotype (F1 and I1). The third population (MKI1) was isolated from weeds in a Kealukekua, Hawaii commercial coffee field. MKI1 has a single slow band esterase phenotype similar to the band in *M. incognita*. Each isolate was from a single egg mass and propagated on 'Orange Pixie' tomato for at least 1 generation to increase their number. MKF1 on tomato was 4 months or less to prevent an esterase change. The male head shape, stylet, and female perineal patterns of the *M. konaensis* as described by Eisenback et al. were used as reference for comparison (3).

Male head shape and stylets. Tomato roots were carefully removed from pots and gently washed with tap water to dislodge soil. Males were extracted from the soil by centrifugation. Twenty males specimens were examined using light microscopy immediately after mounting.

Perineal patterns. Females of each isolate were teased from roots of 30-day-old tomato

plants. Twenty perineal patterns of each isolate were cut and examined (4).

Second-stage juvenile tails. Eggs of each *M. konaensis* isolate were collected (1) and placed onto a 2-cm-diameter screen with 20 µm opening. These screens were placed in a glass petridish containing 5 ml tap water. Twenty second-stage juveniles (J2) of each isolate were picked after 48 hours of incubation and mounted on slides for examination.

Results

Male head shape and stylet. Slight differences were observed among the head shape of the three isolates (Table 3.1). All MKF1 males had the typical described high and rounded head caps (3) (Figure 3.1 A-B). The head caps of the majority of MKI1-F1 were lower than those of MKF1 (Figure 3.1 C-D). All the MKI1-F1 male head caps were round and similar to those of the *M. konaensis* description (3) (Figure 3.1 C-D). For MKI1, 16 of the male heads caps were narrow, square and indented anteriorly (Figure 3.1 E-F). Four of MKI1 males had head caps that were narrow but rounded.

The stylet shafts of MKF1 males were not smooth. Two to 4 obvious projections were observed on all 20 MKF1 male stylets (Figure 3.1 A-B). The majority, 95%, of the MKI1-F1 male stylets had projections on the shaft and were identical to MKF1 males (Figure 3.1 C-D). Nine of the MKI1 male stylets had smooth shafts and 11 had projections (Fig. 3.1 E-F).

Perineal patterns. There were some variations among the three isolates of MK perineal patterns (Table 3.2). The majority of MKF1 and MKI1 perineal patterns resembled the described *M. konaensis* type (Figure 3.2). Their dorsal arches were rounded to squarish. The perivulval region was free of striae. The phasmids were small, directly to the side

Table 3.1 Comparison of male head morphology of three isolates of *Meloidogyne konaensis* differing in esterase phenotypes under light microscopy.

Observed male	MKF1		MKI1-F1		MKI1	
	Head cap	Stylet	Head cap	Stylet	Head cap	Stylet
1	High rounded	Projections*	Round	Projections	Narrow, high, flat	Smooth
2	High rounded	Projections	Round	Projections	Narrow, high, flat	Smooth
3	High rounded	Projections	Round	Projections	Narrow, high, flat	Smooth
4	High rounded	Projections	Round	Projections	Narrow, high, flat	Smooth
5	High rounded	Projections	Round	Smooth	Narrow, high, flat	Projections
6	High rounded	Projections	Round	Projections	Narrow, high, flat	Projections
7	High rounded	Projections	Round	Projections	Narrow, high, flat	Projections
8	High rounded	Projections	Flat	Projections	Narrow, high, flat	Projections
9	High rounded	Projections	Round	Projections	Narrow, high, round	Smooth
10	High rounded	Projections	Round	Projections	Narrow, high, flat	Projections
11	High rounded	Projections	Round	Projections	Narrow, high, round	Smooth
12	High rounded	Projections	Round	Projections	Narrow, high, round	Projections
13	High rounded	Projections	Round	Projections	Narrow, high, round	Projections
14	High rounded	Projections	Round	Projections	Narrow, high, flat	Projections
15	High rounded	Projections	Round	Projections	Narrow, high, flat	Smooth
16	High rounded	Projections	Round	Projections	Narrow, high, flat	Projections
17	High rounded	Projections	Round	Projections	Narrow, high, flat	Projections
18	High rounded	Projections	Round	Projections	Narrow, high, flat	Smooth
19	High rounded	Projections	Round	Projections	Narrow, high, flat	Projections
20	High rounded	Projections	Round	Projections	Narrow, high, flat	Smooth

*Projections: Two to 4 small spots protruding from male stylet shaft.

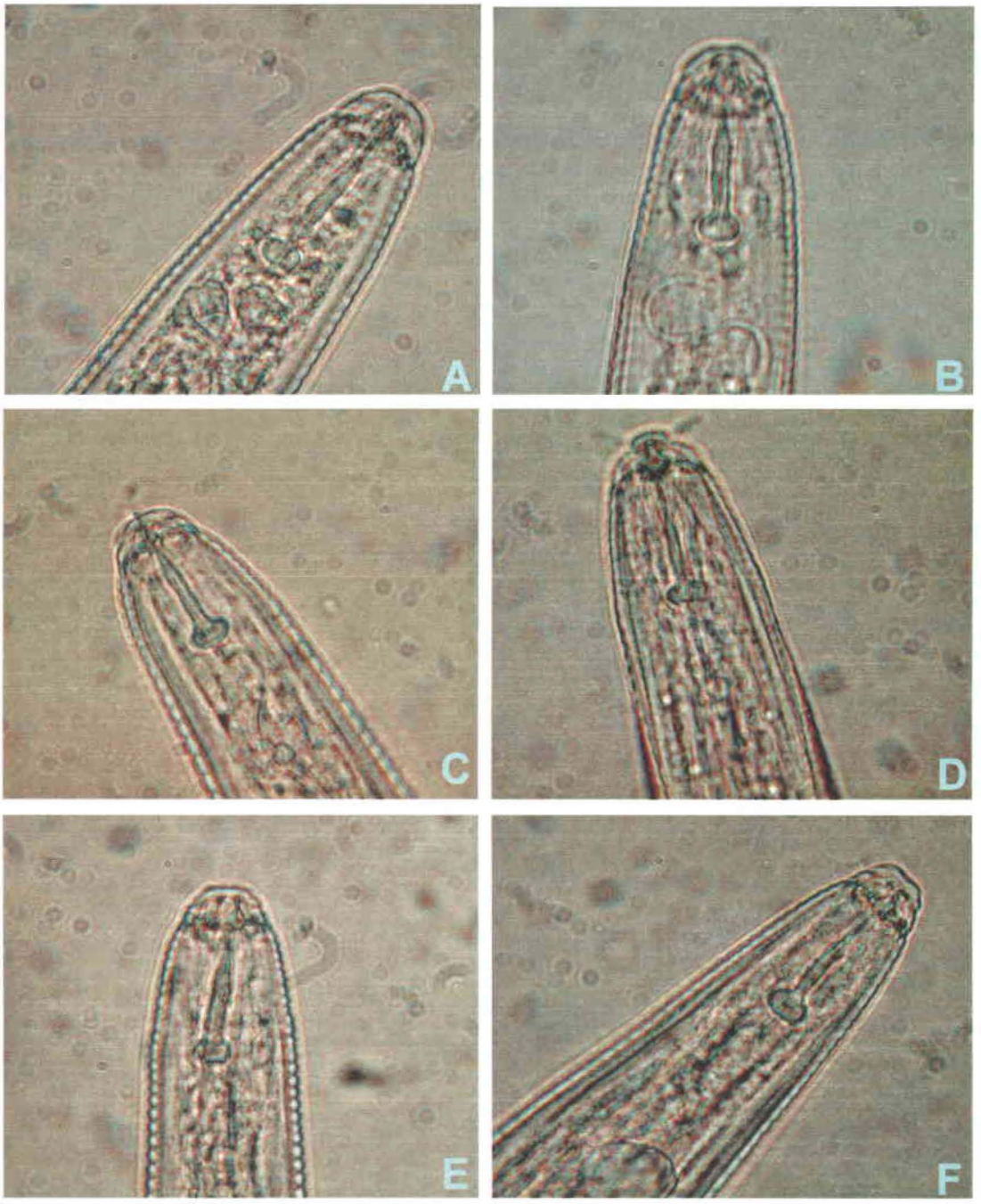

Figure 3.1 Male heads of three isolates of *Meloidogyne konaensis* observed at 40 × magnification. **A-B)** *M. konaensis* isolate MKF1. **C-D)** *M. konaensis* isolate MKI1-F1. **E-F)** *M. konaensis* isolate MKI1.

Table 3.2 Comparison of perineal pattern morphology of three isolates of *Meloidogyne konaensis* differing in esterase phenotypes under light microscopy.

Observed	MKF1		MKI1-F1		MKI1	
patterns	Dorsal arch	Lateral lines	Dorsal arch	Lateral lines	Dorsal arch	Lateral lines
1	Square	No	Square	Long, strong	Round	Long
2	Square	No	Square	Long, strong	Round	Weak, short
3	Square	Strong	Square	Long, strong	Round	Long
4	Round	Weak	Square	Long, strong	Round	No
5	Square	No	Square	Long, strong	Round	No
6	Square	No	Square	Long, strong	Wrinkled	No
7	Round	Short	Square	Long, strong	Wrinkled	Long
8	Square	Short	Square	Long, strong	Round	Long
9	Round	Short, weak	Square	Long, strong	Round	No
10	Square	No	Square	Long, strong	Round	Weak, long
11	Round	No	Square	Long, strong	Round	Weak, long
12	Square	No	Square	Long, strong	Round	Weak, long
13	Square	No	Square	Long, strong	Round	Weak, long
14	Round	No	Square	Long, strong	Round	Weak, long
15	Square	Long	Square	Long, strong	Round	Weak, short
16	Round	Short, weak	Square	Long, strong	Round	No
17	Square	No	Square	Long, strong	Round	No
18	Round	No	Square	Long, strong	Round	Weak, short
19	Square	Long	Square	Long, strong	Wrinkled	Weak, short
20	Square	No	Square	Long, strong	Wrinkled	No

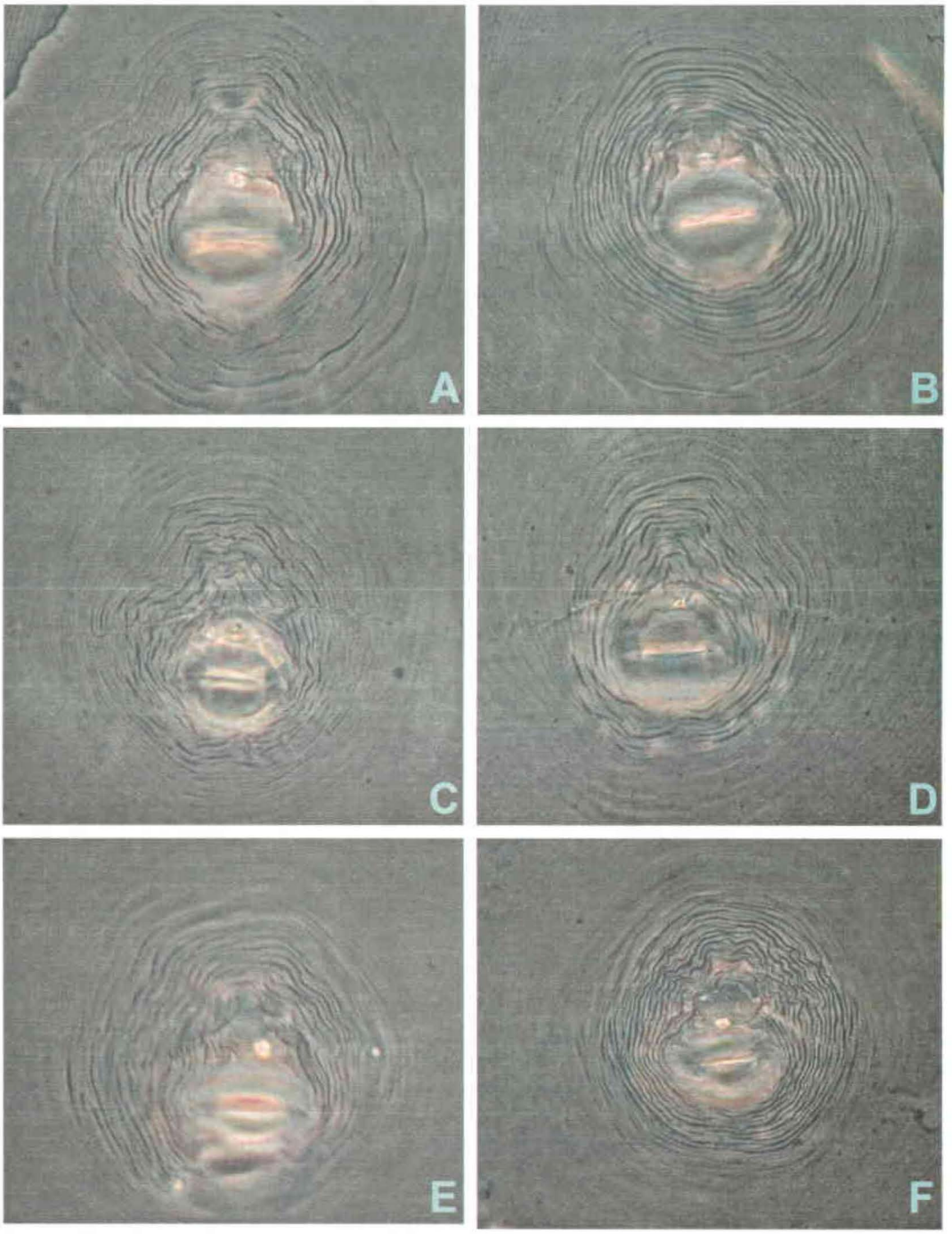

Figure 3.2 Perineal patterns of three isolates of *Meloidogyne konaensis* observed at 40 × magnification. **A-B)** *M. konaensis* isolate MKF1. **C-D)** *M. konaensis* isolate MKI1-F1. **E-F)** *M. konaensis* isolate MKI1.

and posterior to the anus (Figure 3.2 A-B, E-F). MKI1 perineal patterns were highly varying from a relatively smooth rounded arch to a very wavy one and the high dorsal arches of MKI1 were round (Figure 3.2 E-F). The majority patterns of MKI1 had lateral lines, varying from long to short (Table 3.2). Most, 65%, of MKF1 observed had a high dorsal arch which was squarer than MKI1. The other MKF1 perineal patterns were round like MKI1 (Figure 3.2 A-B). Lateral lines on MKF1 were strong to absent. Three patterns had long lateral lines. Six of the lines within the pattern were short, and the remainders lacked lateral lines (Table 3.2). Like MKF1 and MKI1, the dorsal arches of MKI1-F1 were also rounded to squarish (Figure 3.2 A-B). The perivulval region was free of striae and the phasmids were small, directly to the side and posterior to the anus similar to the described. All the MKI1-F1 patterns had long and strong lateral lines (Figure 3.2 C-D). Most of the low dorsal arches in MKI1-F1 were square but narrower than those of the MKF1 and MKI1 isolates (Fig. 3.2 C-D).

Second stage juvenile tails. No differences were found in J2 morphology among the three isolates. As in the original description of *M. konaensis*, the tails of all J2 were usually distinctly curved ventrally (Fig. 3.3). The posterior annulations were large. The hyaline tail terminus was distinct and the tail tips were pointed (Fig. 3.3).

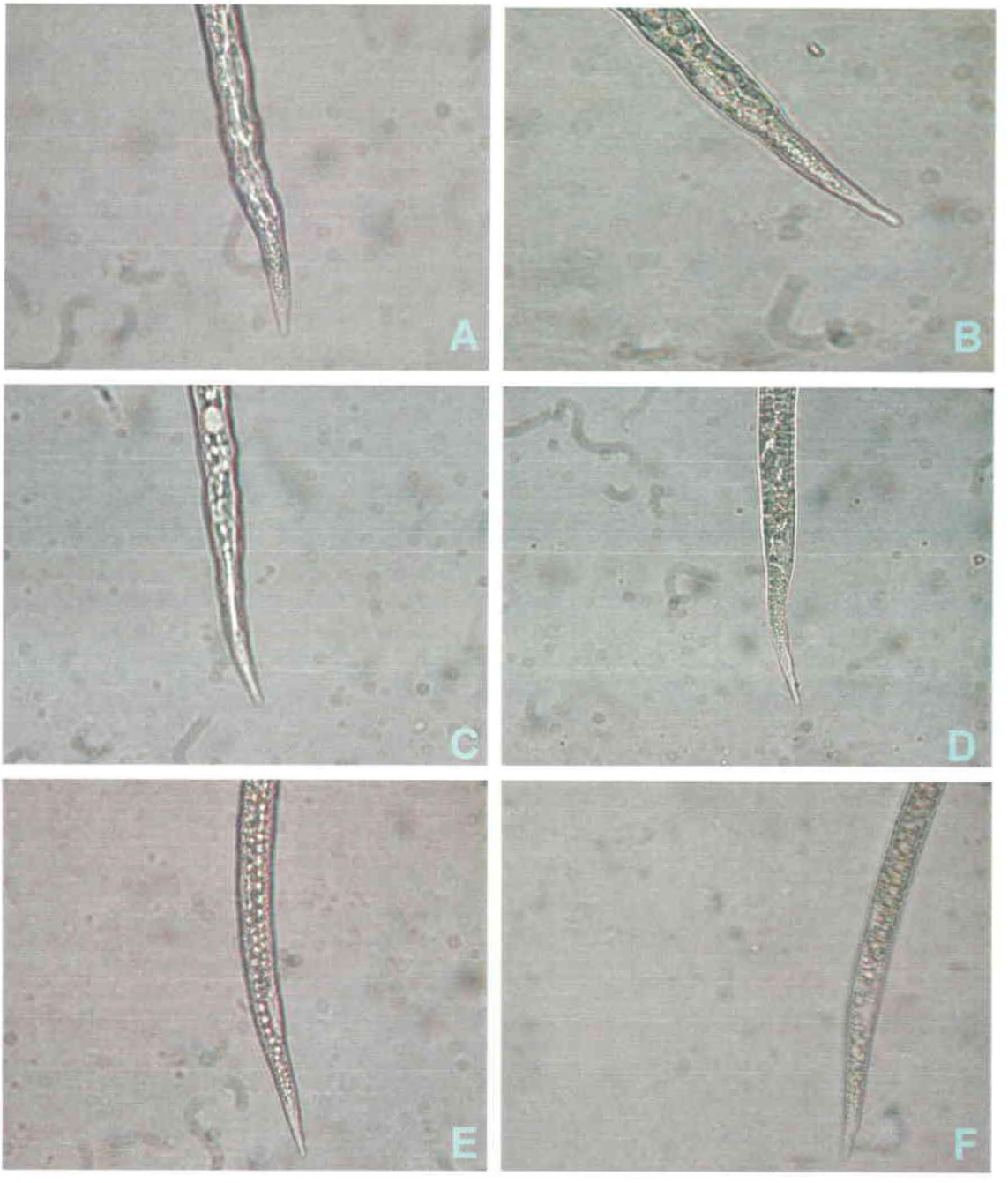

Figure 3.3 Tails of second-stage juveniles of three isolates of *Meloidogyne konaensis* observed at 40 × magnification. **A-B)** *M. konaensis* isolate MKF1. **C-D)** *M. konaensis* isolate MKI1-F1. **E-F)** *M. konaensis* isolate MKI1.

Discussion

The discovery of multiple esterase phenotypes in *M. konaensis* made this character impractical for identification of the species. The identification aspect is even more confounded since there are also morphological differences in perineal patterns, male head shape and male stylets.

Although esterase phenotype and even the male head shape of MKI1 were similar to *M. incognita*, the perineal patterns of MKI1 females suggested it was not *M. incognita* (2). In this study, MKI1 was isolated from amaranth in a coffee field. Future studies are suggested to use the one isolated from a MKF1 single egg mass culture which exhibit the I1 esterase phenotype.

These morphological and physiological changes probably are associated with parasitism on coffee and tomato. *M. konaensis* male head, stylet and female perineal pattern are both diagnostic characters. Feeding and reproduction are both related to nematode parasitism so these morphological changes may be the specific adaptations to the different hosts. It is also probable that the F1 phenotype makes MKF1 adapted to coffee, whereas those of MKI1-F1 and MKI1 make these isolates less adapted to coffee.

Thus, several experiments must be conducted to investigate the parasitism of three MK isolates on coffee and tomato. Other vegetable cultivars should also be inoculated with a MKF1 single egg mass to monitor the esterases on these plants. If any change is detected, that will be very helpful for us to understand *M. konaensis* and incidence in field surveys.

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CHAPTER 4. HOST PARASITISM

Introduction

Although *M. konaensis* is commonly known as the Kona coffee root-knot nematode, tomato and cucumber cultivars are better hosts than coffee (8). The life cycle of *M. konaensis* on coffee is nearly twice as long as on tomato. *M. konaensis* requires 26 days at 25°C to develop on tomato and 48 days at 25°C to reach adulthood on coffee (9). Currently, *M. konaensis* has only been found on coffee and weeds in coffee fields (4, Schmitt, unpublished).

Meloidogyne konaensis has a broad host range based on greenhouse test (8). Zhang et al. (1994) evaluated 32 plant species which included 54 vegetable cultivars, 12 field crop cultivars, one *Gardenia* sp., and two weed species. Among these plants, only peanut and 'Decicco' broccoli were nonhosts. The reproductive ratio (Pf/Pi) was greater than 1 on 81% of the plant cultivars. The majority of cultivars (57%) were categorized as good host (Pf/Pi>5.0). A sizeable portion (24%) of the cultivars were categorized as fair hosts (5.0=Pf/Pi>1) and 16% cultivars were classified as poor hosts (8).

Variations in parasitism exist among populations of other *Meloidogyne* species (5). Four physiological or "host" races of *M. incognita* can be identified by their ability to reproduce on 'Deltapine 61' cotton and 'NC 95' tobacco. Two host races of *M. arenaria* are defined by their ability to reproduce on peanut. The basic difference between race 1 and race 2 of *M. chitwoodi* is their differential reproduction on "Thor" alfalfa and "Red Cored Chantenay" carrot (7). These races of *M. incognita*, *M. arenaria* and *M. chitwoodi*

are not distinguishable from each other on the basis of morphological characters (5).

Given the range of reproductive behavior already observed in *M. konaensis*, such inability to parasitize a host could also occur. A *M. konaensis* isolate exhibiting an I1 esterase phenotype did not reproduce well on coffee (Sipes, unpublished). The objective of this experiment was to compare the parasitism of three isolates of *M. konaensis*, differing in esterase phenotypes, on coffee, cucumber, and tomato.

Materials and methods

Three *M. konaensis* isolates, MKF1, MKI1-F1 and MKI1, differing in esterase phenotypes were used. MKF1 was cultured from *Coffea arabica* cv. Typica selection Guatamala from Kona, Hawaii and has a F1 esterase phenotype. MKI1-F1 was derived from an MKF1 single egg mass line and has an I1-F1 esterase phenotype. MKI1 was cultured from weeds in a commercial coffee field in Kealakekua, Hawaii which has an I1 esterase phenotype. Each isolate was cultured from a single egg mass on 'Orange Pixie' tomato 4 months or less.

The reproduction of each isolate was evaluated on *Coffee arabica* cv. Typica Selection 502, *Lycopersicon esculentum* cv. Orange Pixie, and *Cucumis sativus* cv. Sweet Success. A randomized complete block design was used. The experiment was conducted in the greenhouse (17°C~40°C). The experiment was repeated once. Plants were watered daily and fertilized weekly with water-soluble Grow More fertilizer (N:P:K (20:20:20)).

Seeds were germinated in vermiculite and then transplanted into 10-cm-diameter clay pots filled with 360 cm^3 sterilized media (50% field soil high in clay content and 50% sand). Coffee and tomato seedlings were transplanted upon reaching in the first true

leaf stage. Cucumbers were transplanted 4~5 days after germination. In the fist run of the experiment, coffee seedlings were inoculated 60 days after transplanting. In the second run of the experiment, coffee seedlings were inoculated 108 days after transplanting. Other plants were inoculated 14 days after transplanting.

Inoculum was extracted by agitating infected roots in NaOCl solution (2). The egg suspension was adjusted to yield 600 eggs/ml and 5ml aliquots were applied to each plant.

Plants were removed from soil 60 days after inoculation. The shoots were cut at the soil line and weighed. The roots were gently washed to remove soil and then they were rated for galling using a scale of 0-100% (1). The root fresh weight was recorded. Eggs were extracted from egg matrix with 1% NaOCl solution (2), collected on a 20 μm mesh screen, and centrifuged. The shoots and roots were oven-dried at 70°C for 2 days and weighed. The total number of second-stage juveniles and eggs extracted from roots were combined to give a final nematode population density (Pf). Reproduction ratios (Pf/Pi) were then calculated. The number of second-stage juveniles and eggs per gram of fresh root was also calculated.

Data analysis. Analysis of variance was conducted (SAS Institute, Cary, NC). The data from the two runs of the experiment were first tested for homogeneity of variance. If the preliminary analysis as combined repeats was not significantly different between two repeats ($P > 0.05$), the two sets of data were analyzed for variance separately. The F test was conducted to test the difference between the mean square errors of two sets of data. If there was not significant difference between the two mean square errors the two sets of

data were then combined.

The number of eggs and second-stage juveniles, eggs and second-stage juveniles per gram of fresh root weight, and reproduction ratios were log 10 transformed ($\log_{10}$ X+1). The reproduction ratios on coffee were also square root transformed and analyzed. The Waller-Duncan k-ratio t-test was used to separate treatment means when treatments were significantly different.

Results

The homogeneity analysis showed no significant difference in all the factors between runs of the experiment except for dry shoot weight. The data of dry shoot weight of each run were analyzed separately. The F-test showed that the mean squares of errors of other dependent values between two runs were not significantly different ($P>0.05$). Consequently two sets of data of these dependent values were combined for analysis.

Coffee was a host for MKF1 but not for MKI1-F1 nor MKI1. The mean number of eggs and second-stage juveniles on coffee was 3,402 for MKF1 and zero for MKI1-F1 and MKI1 (Table 4.1). More than twenty eggs and second-stage juveniles were found in 2 replicates of MKI1, but these were not *Meloidogyne* species. The reproduction ratio, number of eggs and second-stage juveniles per gram of fresh root and gall index on coffee was 1.1, 1,723 and 15% for MKF1 respectively, and zero for the other two isolates ($P<0.05$) (Table 4.2, 4.3 and 4.4).

Tomato was a good host for all the three isolates with MKI1-F1 having the greatest reproduction (Table 4.1). The number of eggs and second-stage juveniles produced by MKI1-F1 was 2.4 times greater than that produced by MKF1 and 2.3 times greater than

Table 4.1 The number of eggs and second-stage juveniles (J2) of three isolates of *Meloidogyne konaensis* on coffee, tomato and cucumber 60 days after inoculation with 3,000 eggs.

Isolate	Coffee	Tomato	Cucumber	Mean
MKF1	3,402 a	137,800 b	28,090 b	56,431 b
MKI1-F1	0 b	332,536 a	122,280 a	151,605 a
MKI1	0 b	142,933 b	52,818 b	65,250 b
Mean	1,134	204,423	67,729	

Numbers in a column followed by the same letter are not different according to Waller-Duncan's k-ratio t test (k=100).

Table 4.2 Reproduction ratio (Pf/Pi) of three isolates of *Meloidogyne konaensis* on coffee, tomato and cucumber 60 days after inoculating with 3,000 eggs.

Isolate	Coffee	Tomato	Cucumber	Mean
MKF1	1.1 a	45.9 b	9.3 b	18.8 b
MKI1-F1	0 b	110.9 a	40.8 a	50.6 a
MKI1	0 b	47.6 b	17.6 b	21.7 b
Mean	0.4	68.1	22.6	

Numbers in a column followed by the same letter are not different according to Waller-Duncan's k-ratio t test (k=100).

Table 4.3 The number of eggs and second-stage juveniles per gram of fresh root of three isolates of *Meloidogyne konaensis* on coffee, tomato and cucumber 60 days after inoculating with 3,000 eggs.

Isolate	Coffee	Tomato	Cucumber	Mean
MKF1	1,723 a	12,123 b	5,237 b	6,361 b
MKI1-F1	0 b	42,338 a	10,736 a	17,691 a
MKI1	0 b	11,785 b	7,418 b	6,402 b
Mean	574	22,082	7,797	

Numbers in a column followed by the same letter are not different according to Waller-Duncan's k-ratio t test (k=100).

Table 4.4 The mean gall index of three isolates of *Meloidogyne konaensis* on coffee, tomato and cucumber 60 days after inoculating with 3,000 eggs.

Isolate	Coffee	Tomato	Cucumber	Mean
MKF1	15% a	80% b	90% a	62% a
MKI1-F1	0 b	90% a	80% a	57% a
MKI1	0 b	80% b	80% a	53% b
Mean	5%	83%	83%	

Numbers in a column followed by the same letter are not different according to Waller-Duncan's k-ratio t test (k=100).

reproduction by MKI1 ($P<0.05$). Reproduction by MKF1 and MKI1 was similar (Table 4.1) ($P>0.05$). The reproduction ratio of MKI1-F1 was two times more than the other two isolates (Table 4.2). The mean of the number of eggs and second-stage juveniles per gram of fresh root of MKI1-F1 was 42,338 which was greater than MKF1 or MKI1 on tomato (Table 4.3). The mean of gall index of MKI1-F1 was also greater than the other two isolates. The gall index was 90%, 80% and 80% for MKI1-F1, MKF1 and MKI1 respectively ($P<0.05$) (Table 4.4).

Cucumber was a good host for all the three isolates. The number of eggs and second-stage juveniles of MKI1-F1 on cucumber was 4.4 times greater than MKF1 and 2.3 times greater than MKI1 (Table 4.1). The reproduction ratio of MKI1-F1 was also 4.4 times greater than MKF1 and 2.3 times greater than MKI1 on cucumber (Table 4.2). The same trend held true for the number of eggs and second-stage juveniles per gram of fresh root mean of 10,736, 5,237 and 7,418 for MKI1-F1, MKF1 and MKI1 respectively ($P<0.05$) (Table 4.3). The gall index were not different ($P>0.05$). The gall indice was 80%, 90% and 80% for MKI1-F1, MKF1 and MKI1 respectively (Table 4.4). In both repeats of the experiment, some cucumber plants died 20 days after inoculation with *M. konaensis,* whereas the noninoculated control plants did not. The 3000 egg inoculum level seriously retarded cucumber growth and even was lethal.

Based on the average number of three isolates of *M. konaensis,* reproduction on each host 60 days after inoculation, tomato was the best host, cucumber was an intermediate host, and coffee was the poorest host to the three nematode isolates (Table 4.1, 4.2 and 4.3). The average number of eggs and second-stage juveniles of three isolates

developing on tomato was 204,423 which was 180 times greater than on coffee and 3 times greater than on cucumber (Table 4.1). The average number of mean of three isolates reproduction ratio on tomato was 68.1 greater than 22.6 on cucumber or 0.4 on coffee ($P<0.05$) (Table 4.2). The same trend held for the eggs and second-stage juveniles per gramfresh root (Table 4.3). The average number of gall index was 83% on tomato and cucumber, and 5% on coffee ($P<0.05$) (Table 4.4).

Based on the mean of the number of reproduction of each isolate averaged over three hosts 60 days after inoculation, the MKI1-F1 isolate had the highest reproduction among the three isolates ($P<0.05$) (Table 4.1, 4.2, 4.3). The mean number of eggs and second-stage juveniles produced by MKI1-F1 averaged over three hosts was 151,605 which was 2.7 times greater than the 56,431 produced by MKF1 and 2.3 times greater than 65,250 produced by MKI1 (Table 4.1). Reproduction by MKF1 and MKI1 was similar ($P>0.05$) (Table 4.1). The averaged number of reproduction ratio of MKI1-F1 was 50.6 which was two times greater than MKF1 and MKI1 (Table 4.2). The averaged number of eggs and second-stage juveniles per gram of fresh root by MKI1-F1 was 17,691 which was two times greater than the other two isolates ($P<0.05$) (Table 4.3). The average gall index for MKF1 and MKI1-F1, 62% and 57% was higher ($P>0.05$) than the gall index 53% for isolate MKI1 ($P<0.05$) (Table 4.4).

No differences in wet and dry root and shoot weight of tomato, cucumber or coffee were found among three nematode isolates (Table 4.5 and 4.6). Nor was a significant isolate and host interaction detected for wet and dry root and shoot weight ($P>0.05$).

Table 4.5 Wet and dry root weight of tomato, cucumber and coffee infested by three isolates of *Meloidogyne konaensis* 60 days after inoculation with 3,000 eggs.

Isolate	Fresh roots (g)			Dry roots (g)		
	Coffee	Tomato	Cucumber	Coffee	Tomato	Cucumber
MKF1	2.1	11.6	10.2	0.2	1.0	0.6
MKI1-F1	2.2	9.8	13.3	0.2	1.0	0.9
MKI1	2.3	13.1	8.9	0.2	0.8	0.6

Numbers in a column are not different according to Waller-Duncan's k-ratio t test (k=100).

Table 4.6 Wet and dry shoot weight of tomato, cucumber and coffee infested by three isolates of *Meloidogyne konaensis* 60 days after inoculation with 3,000 eggs.

Isolate	Fresh shoots (g)			Dry shoots (g)					
	Coffee	Tomato	Cucumber	Coffee		Tomato		Cucumber	
				1	2	1	2	1	2
MKF1	3.2	13.0	12.5	0.6	1.0	1.5	2.2	1.5	.
MKI1-F1	3.6	11.3	13.5	0.7	1.0	1.3	1.9	1.5	.
MKI1	3.6	15.5	16.3	0.6	1.1	1.5	2.7	0.6	.

Numbers in a column are not different according to Waller-Duncan's k-ratio t test (k=100).

(1) Data from the fist repeat experiment; (2) Data from the second repeat experiment; (.) Missing data.

Discussion

All *M. konaensis* isolates were originally derived from populations that developed on coffee, but differences in host ranges and parasitism exist among them. All the three *M. konaensis* isolates reproduced on tomato and cucumber. MKI1-F1 and MKI1 failed to develop on coffee but MKF1 showed low reproduction on coffee. A similar phenomenon was observed in other experiments caused out in our lab: a MKI1-F1 culture and a MKF1 culture have been grown in greenhouse nearly 2 years. No eggs nor any stage of nematodes were found in MKI1-F1 culture, but some eggs and second-stage juveniles of *Meloidogyne* were found in the MKF1 culture (Sipes, pers. comm). Since MKF1 has the

ability to infect coffee while the other two isolates can not, MKF1 can be differentiated from the other two MK isolates by host range on coffee and may have use as a parasitism marker for *M. konaensis* on coffee. Pathogenic variation also existed on tomato and cucumber among the three isolates. For both tomato and cucumber, MKI1-F1 was the most aggressive isolate. MKI1-F1 had the highest reproduction which was two times more than MKF1 and MKI1, whereas MKF1 and MKI1 were similar. So MKI1-F1 also can be differentiated from the other two isolates by reproduction on tomato or cucumber. Thus all three MK isolates can be differentiated by host range and reproduction on coffee, tomato and cucumber.

Coffee is a poor and intolerant host of *M. konaensis.* Zhang et al. reported a gall index of 6 and 8 on 3- and 5-month old seedlings respectively, 4 months after a similar inoculation density (3,750 eggs) of the same coffee cultivar (8). The higher gall index observed by Zhang et al. was probably caused by the longer growth period. More generations were produced in 4 months compared to the 2 months of the current experiment.

This experiment also confirmed that tomato and cucumber are very good hosts for *M. konaensis* (8). All the MK isolates had high reproduction ratio on tomato and cucumber. This experiment also showed that tomato is a better host compared to coffee and cucumber. Each MK isolate had higher reproduction ratio on tomato than on cucumber.

Esterase change is apparently related to nematode parasitism. Esterase may have a role in the establishment of the host-parasite relationship in coffee or may be linked to

those genes. Additional studies are needed to elucidate the mechanism. Those genes or related molecular organisms were suggested to be investigated to understand the molecular mechanism.

Parasitism can be a driving force in speciation (6). The host-parasite relationship is a powerful force in adaptation and speciation, such as The unstable environment of a host could increase the selection (6). Coffee may offer a more unstable or hard environment (root tissue is hard) for *M. konaensis* than tomato and favor a particular esterase phenotype such as MKF1. After MKF1 feeding on tomato, the selection pressure changed, the new host-parasite relationship established and may drive the physiological and morphological adaptations. MKI1-F1 and MKI1 were possibly the result of esterase adaptations. Although MKI1-F1 and MKI1 established on tomato, they can not parasitize coffee. We hypothesized that in nature coffee plays a strong selection pressure for all the *M. konaeasis* isolates but only MKF1 can adapt this environment.

Some adaptive long-term strategies employed by nematodes to overcome patchy distribution of hosts include egg mass production, dispersal of inseminated females, dispersal by phoresy, utilization within the life cycle of resting stages, and development of a parthenogenesis mode of reproduction (3, 6). Parthenogenesis can lead to polyploidy and polyploidy may lead to differing phenotypes (3, 6). *M. konaensis* has a mitotic parthenogenesis and is polyploid (4). Parthenogenic polypoidy may explain why there are morphological and biochemically different phenotypes in *M. konaensis*. Many *Meloidogyne* species have biotypes. The same maybe true for *M. konaensis*.

It is a widely accepted precept that natural selection generally fashions new features

from pre-existent ones and phylogenetic pathways are constrained by prior genetic history. The development of characters and ecological adaptations already present in ancestral form (6). The genetic basis of parasitism adaptation of *M. konaensis* is not clear. A hypothesis is that multiple genes can give different expression patterns and subsequent phenotypes under different environments (e.g. hosts). Genetic variations lead to the physiological and phenotypical differences which were observed.

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APPENDIX. ESTERASE AND MALATE DEHYDROGENASE (MDH) STAINING SOLUTIONS

Esterase stain solution for one gel:	
0.1M Potassium phosphate buffer, pH 7.4	25 ml
EDTA	0.0075g
Fast Blue RR salt	0.015g
α - Napthylacetate acetate (dissolved in 0.5 ml acetone	
add dropwise to the buffer while stirring)	0.01g
Malate dehydrogense stain solution for one gel:	
Mdh stock solutions (can be stored at 5 °C)	
Stock solution A	
Sodium carbonate	10.6 g
L-Malic acid	1.34 g
Water up to 100 ml	
Stock solution B	
TRIS	6.06 g
Adjust to pH 7.1 with 2N HCl	
Water up to 100 ml	
MDH stain solution	
Stock solution A	10 ml
Stock solution B	15 ml
NAD	50 mg
NBT or MTT	30 mg
Water, 50 ml	
PMS in 26 ml water	2 mg
MDH bands will appear as blue bands in the gel	