Nem 100 (lab 15)

Nematode Hydroponic culture and identification of Meloidogyne spp.

1. Morphological characters
   Juveniles (J2)
   Males
   Females
2. Host differentials for species and races

3. Protein based approaches
   Monoclonal and polyclonal antibodies

Isozyme analysis
Esterase (EST)
Malate Dehydrogenase (MDH)

4. DNA analysis
Mitochondrial DNA polymorphism
Ribosomal DNA polymorphism
Others

Isozyme analysis.

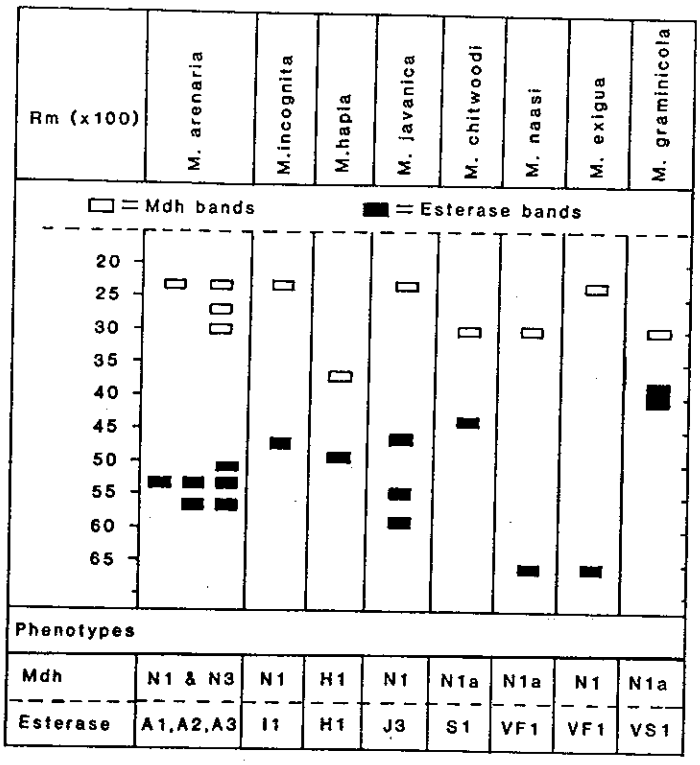


FIGURE 12 Species-specific phenotypes of malate dehydrogenase (Mdh) and esterases helpful in differentiating eight species of *Meloidogyne*. On the same polyacrylamide gel, malate dehydrogenase bands are bluish, those of esterases black. Phenotype designations correspond to those of Esbenshade and Triantaphyllou (1989). (Illustration provided by A. C. Triantaphyllou.)

In vitro synthesis of DNA via amplification

Requirements

- oligonucleotide primers
- DNA polymerase (thermostable polymerase)
- dNTP's
- Enzyme cofactors (i.e., Mg ++)
- Buffered environment for enzyme activity
- Sometimes other facilitators (proteins, DMSO)
- Mineral oil if a heated lid isn't part of the PCR machine

Basic procedure

- Heat denaturation of taxon DNA (94 C)
- Annealing temperature appropriate for primers (G/C
- Extension temperature for DNA polymerase (~72 C)
- Repeat cycle 25-30 times
- Post-incubation at 72 C (5 minutes or >) to extend ss DNA

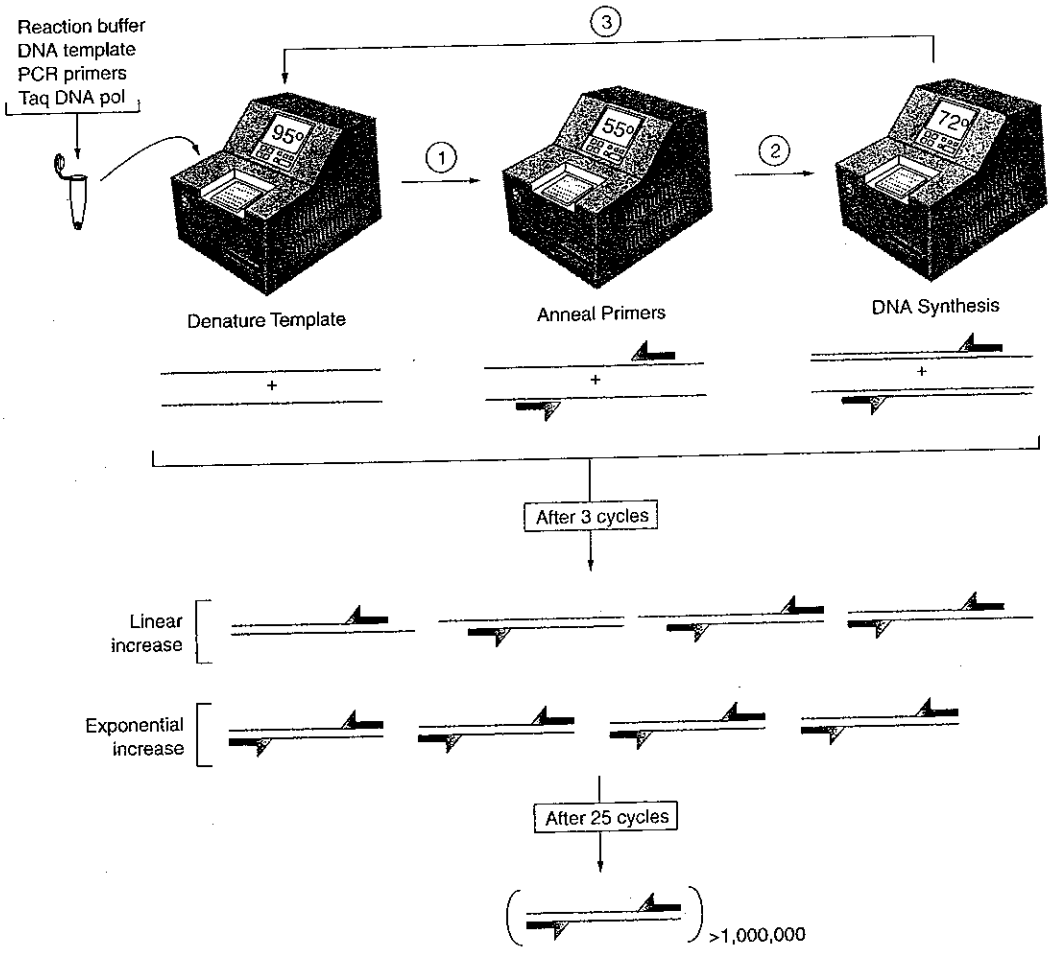


Figure 2 Each PCR cycle requires three temperature steps to complete one round of DNA synthesis. Before cycle 1 can be initiated, the double-strand DNA target must be heat-denatured to provide single-strand regions for primer annealing. Because the primer molecules are in a vast molar excess over the number of target DNA molecules, primer annealing is a rapid bimolecular reaction that is promoted by a temperature shift to ~55°C. The extension reaction is maximal at the thermal optimum for the DNA polymerase, which is 72°C. After three rounds of cycling, each template molecule in the reaction generates four complete PCR products that are flanked by primer sequences, as well as four partial PCR products. Complete PCR products accumulate exponentially until the reaction components become limiting (~30 cycles).

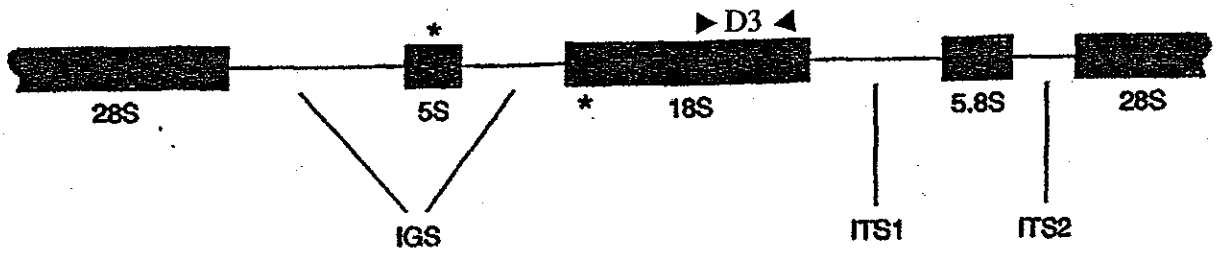
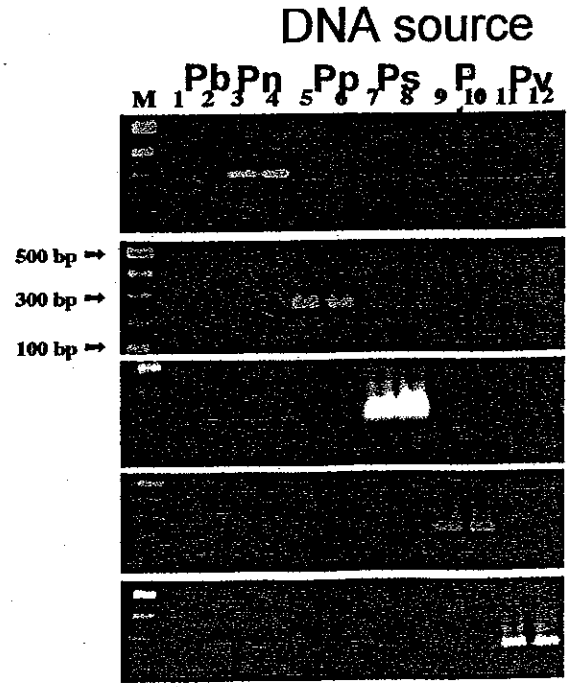
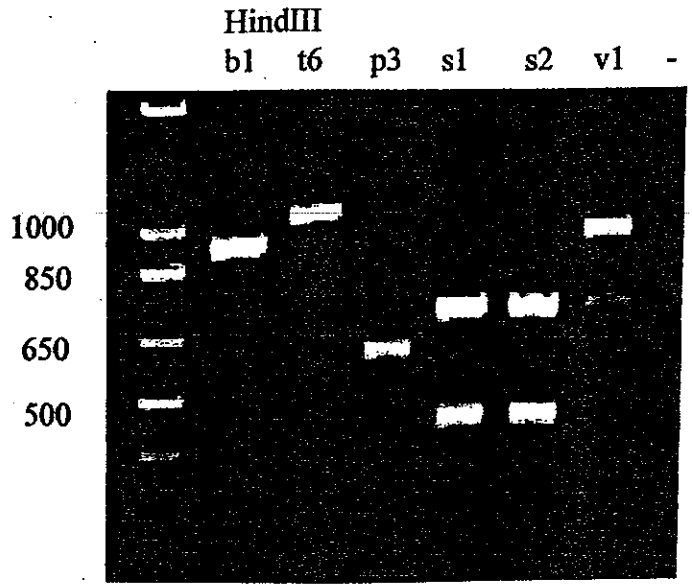


FIG. 1. Diagram of the ribosomal cistron as observed in *M. arenaria*. "*" indicates the position of the PCR primers used to amplify the intervening IGS between the 18S and 5S genes.



Pratylenchus Species –specific primers developed from D loop sequence.
JON 36:144



Pratylenchus species can also be distinguished by amplification of ITS followed by HdIII digestion followed by gel electrophoresis (Jack Qiu, unpublished)

Fig 4. Species specific primer sets amplify corresponding species

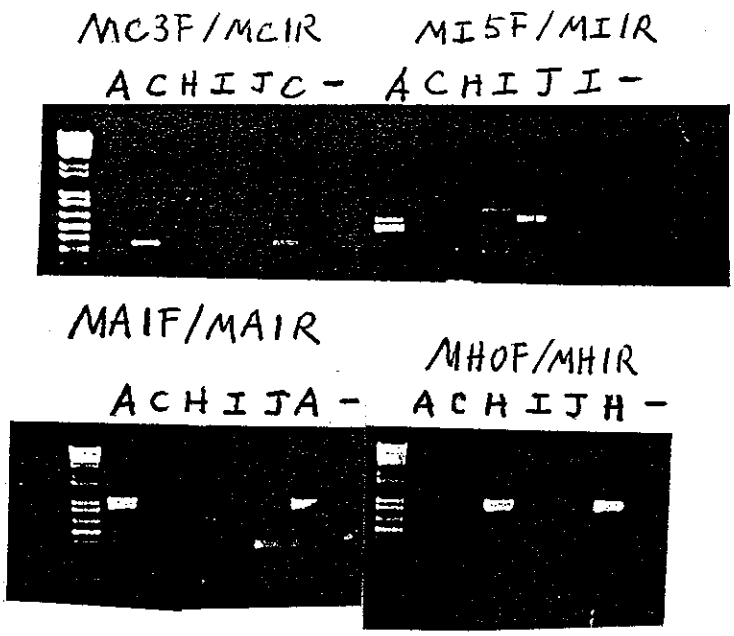
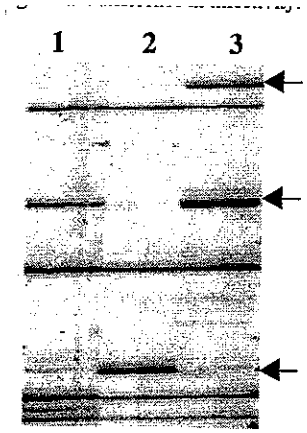


Fig 5. Genetic polymorphism of different isolates of M.hapla can be resolved by PCR based approach (Amplified Fragment Length Polymorphism, AFLP) . Patterns produced with M.hapla isolates HSL (1), HRO (2) and HSMB (3) are compared. Arrows indicate polymorphic bands that distinguish these isolates.



Reference:
Miesfeld R.L.1999. Applied Molecular Genetics Wiley-Liss, Inc. pp 143-145

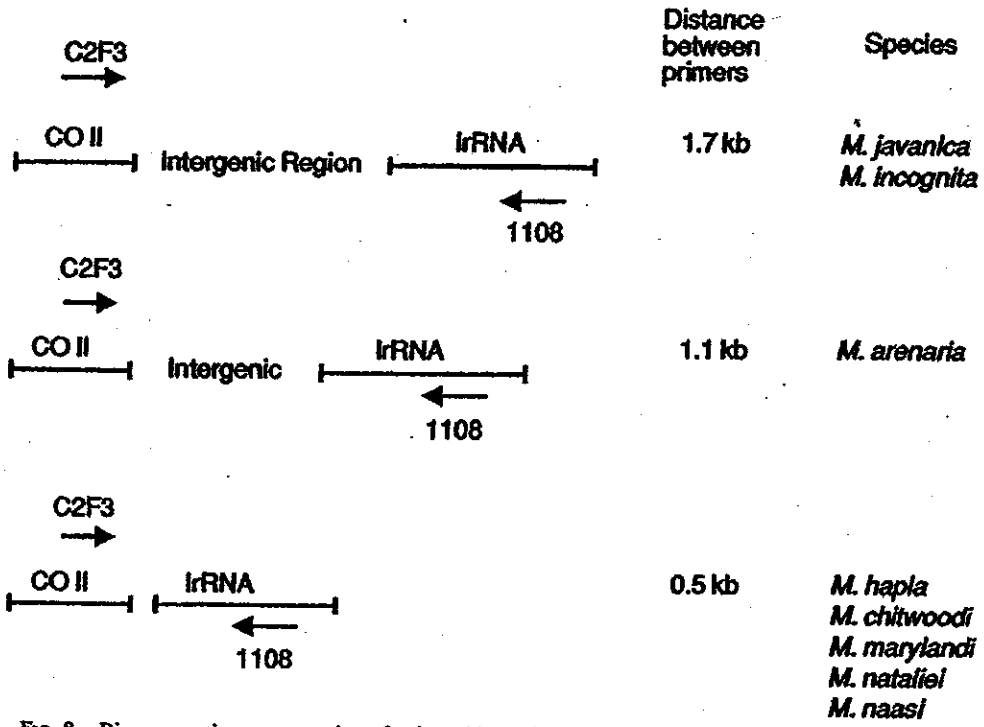


FIG. 2. Diagrammatic representation of primer binding sites on the *Meloidogyne* mitochondrial genome. Primer #C2F3 anneals to the coding strand of the cytochrome oxidase subunit II (COII) gene and primer #1108 anneals approximately 450 bp downstream from the start of the lrRNA gene. The intergenic region varies in size among the different *Meloidogyne* species.

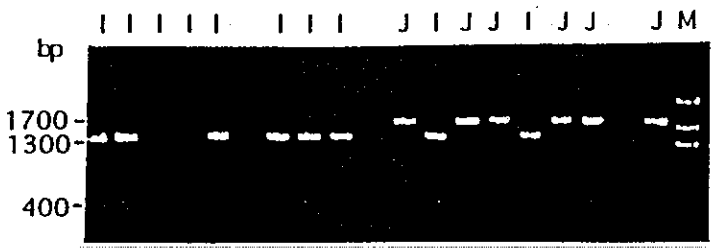


Figure 3 PCR amplification of mitochondrial DNA from single juveniles in a mixed population. Single juveniles from a mixed population of *Meloidogyne incognita* and *M. javanica were* amplified using PCR primers and conditions described in Powers and Harris (1993) except that individuals were picked with an insect pick (minuetin) and disrupted with the pick in the PCR tube before buffer was added. DNA was digested with *Hin*fI and electrophoresed on a 1.5% agarose gel. Lanes marked "J" were identified as *M. javanica* and those marked "I" were identified as *M. incognita*

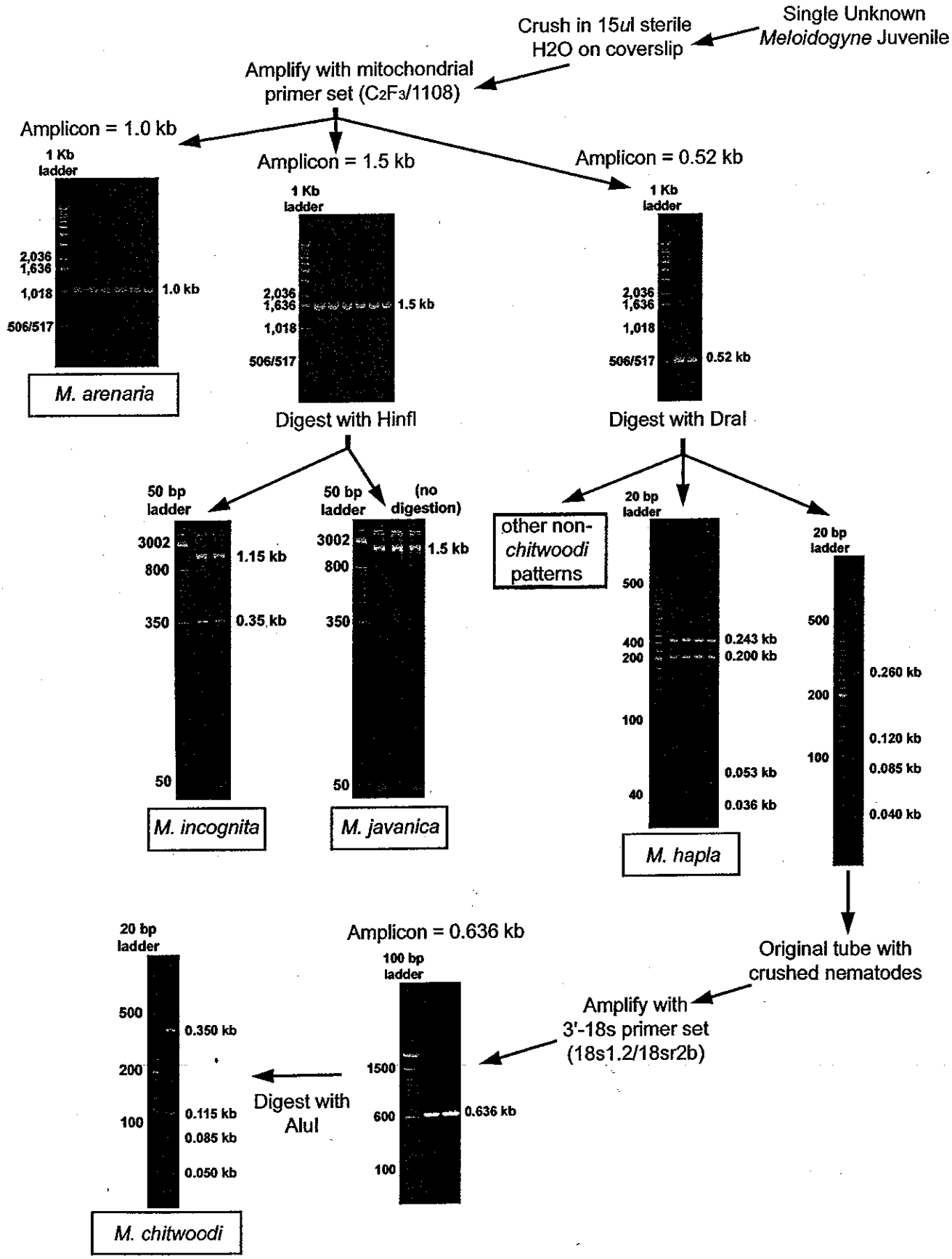


FIG. 2. Flow chart depicting the PCR/RFLP steps used to identify *Meloidogyne* species in this study.