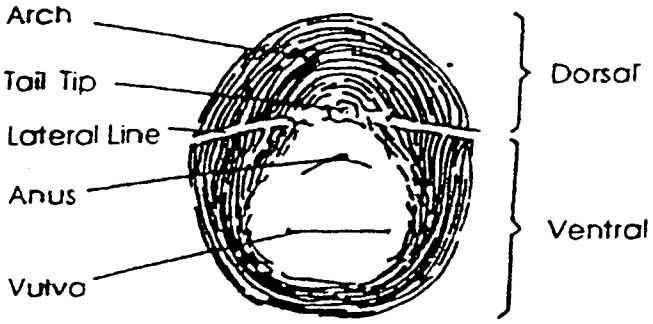
Identification by Perineal Pattern



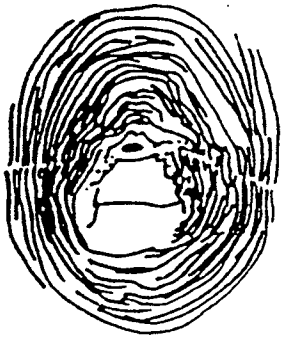
Meloidogyne Perineal Pattern

Previous methods of identifying Meloidogyne species were limited to the methods setting up a host range test, which can take up to 3 months, and creating a slide of the perineal section of the adult female nematode to use as a fingerprint (see right). After this section has been mounted, visual observation of the patterns of lines created by the cuticle determine the species of Rootknot.

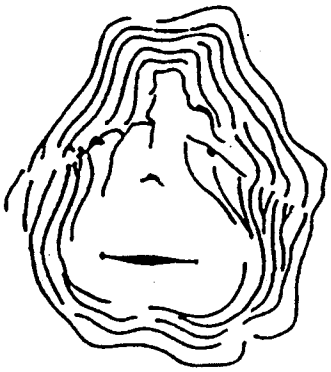
Most species have unique characters that distinguish them from the other species in the genus, however the variation among these characters creates some ambiguity in species determination. The examples to the left of *M. javanica*, *M. incognita*, *M. chitwoodi*, and *M. hapla* give illustrations of the variation in perineal patterns within a species, as well as the difficulty in differentiating between species. Using the PhastSystem decreases the amount of variation within a species and increases the ability to distinguish one species from another.

M. javanica

M. incognita

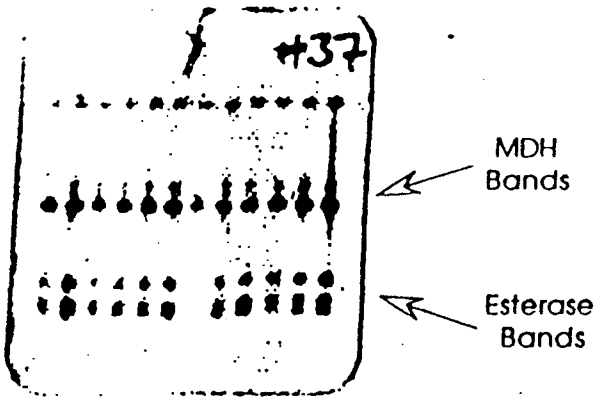


M. chitwoodi



M. hapla

Isozyme Electrophoresis to Identify Root-Knot Species

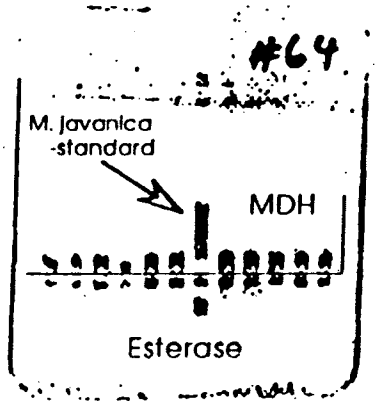


M. javanica

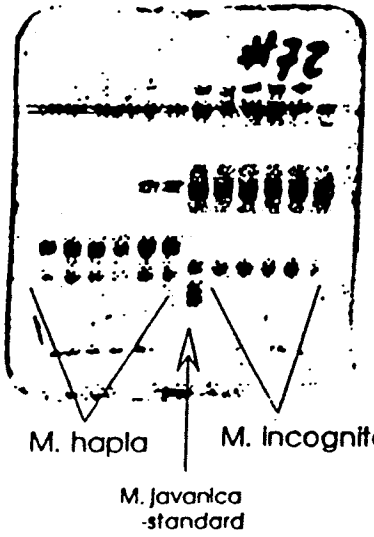
At Extension Nematology we have been supplementing traditional identification procedures (see info on perineal patterns) with a new method of identification for Root-Knot nematode. Isozyme Electrophoresis utilizes the fact that different species of Root-knot nematode have unique forms of isozymes. If we do gel electrophoresis on whole nematodes we can look at the differences in the nematodes and determine which species are in which fields.

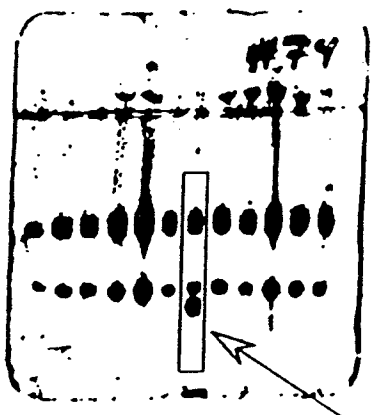
The advantage of using isozyme electrophoresis is that the quantitative differences between species shows itself more clearly than with perineal patterns. The gel above consists of twelve individual nematodes (from a greenhouse culture) that we electrophoresed. Eleven of the twelve nematodes clearly give the same results. If you look at the three *M. javanica* perineal patterns (next page), you can see that the variation within a species is much greater in the perineal shapes than in the isozymes.

If you look at the three gels below, you can easily see differences between the different species. The *M. hapla* nematodes have a low MDH band and an Esterase band that is just a little lower (far right picture, #64), while both the *M. incognita* (at right, #72) and *M. javanica* (at top, #37, and bottom left, #74) have much higher MDH bands. You can see the difference between these two nematodes in the lower Esterase bands; the *M. javanica* has three bands instead of just the one that the *M. incognita* has (these are a little more difficult to tell apart here because of the quality of the picture, not clarity of the gel).

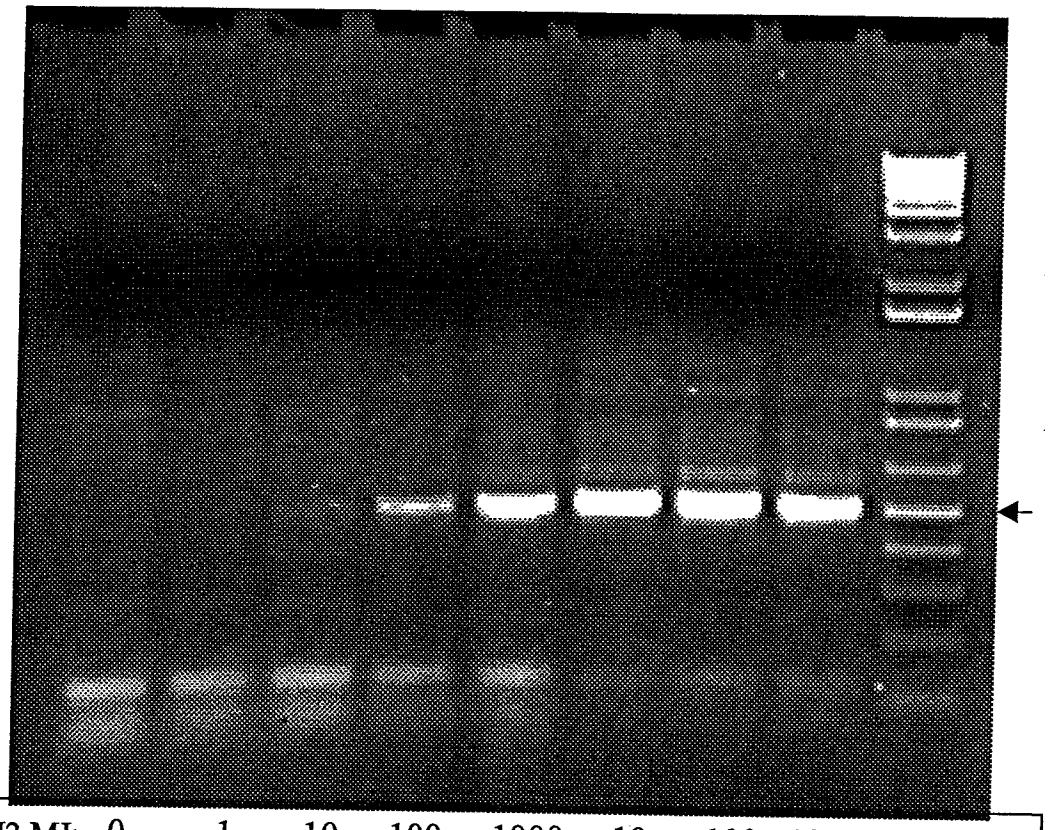


M. hapla





PCR Assay to Identify Root Knot Nematode Species from Soil Samples



#J2 MJ: 0 1 10 100 1000 10 100 1000 marker

SOIL EXTRACTS + MJ **PURE CULTURE MJ**

Major Steps:

- Baermann funnel extraction
- Sugar flotation
- Frozen-thaw and proteinase digestion
- PCR (polymerase chain reaction)
- Gel electrophoresis

Advantages:

- Efficiency
- Sensitivity
- Species specific identification
- Possible quantification

Provided by Jack J. Qiu from Drs. Becky B. Westerdahl and Valerie M. Williamson's labs, Department of Nematology, University of California, Davis, CA 95616, for 33rd California Nematology Workshop (3/27/2001).