Comparative Disc-electrophoretic Protein Analyses of Selected *Meloidogyne, Ditylenchus, Heterodera,* and *Aphelenchus* spp.[1]

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Abstract: Disc-electrophoretic separation of soluble proteins from whole nematode homogenates yielded band profiles useful for distinguishing selected species of *Meloidogyne* and *Ditylenchus,* and the genera *Heterodera,* and *Aphelenchus.* Certain protein bands were common to all the species of *Meloidogyne,* whereas other bands were specific. *Meloidogyne* spp. and *Heterodera glycines* shared some protein similarities, but other genera differed distinctly. Protein profiles of *Meloidogyne* spp. were not significantly altered by the host on which the nematode was cultured. *Key Words:* Disc-electrophoresis, Protein composition, chemotaxonomy, *Meloidogyne javanica, M. incognita, M. arenaria, M. hapla, Ditylenchus dipsaci, D. triformis, Heterodera glycines, Aphelenchus avenae.*

Differential hosts and perineal patterns have been valuable tools for distinguishing species of *Meloidogyne* Goeldi within given geographical regions but are less reliable in distinguishing populations from different regions. Also, individual species from different locations have caused variable host reaction (16). Mulvey (15) suggested cytogenetic studies of species of *Meloidogyne* might be used in taxonomic identification. Recent investigations have shown chromosome number often differs between populations of *M. javanica* (Trueb) Chitwood, *M. arenaria* (Neal) Chitwood and *M. hapla* Chitwood (18, 19, 20).

Electrophoretic separation of proteins has provided a valuable taxonomic tool for a variety of microorganisms, including fungi (2, 4, 7, 21), bacteria (9, 10, 11, 17) and protozoa (12). Benton and Myers (1) and Chow and Pasternak (3) reported disc-electrophoresis to be an excellent method for separating proteins of nematodes.

The objectives of this investigation were to use disc-electrophoresis to: (i) compare protein profiles of *Meloidogyne* spp. with those of other nematode genera; (ii) determine whether general protein profiles of *Meloidogyne* spp. were stable and characteristic for each species; (iii) determine whether the host on which the nematode was cultured altered these profiles; and (iv) determine whether protein profiles can supplement other criteria in the identification of *Meloidogyne* spp.

MATERIALS AND METHODS

NEMATODE SPECIES AND POPULATIONS: Four species of *Meloidogyne,* two species of *Ditylenchus* and one species each of *Heterodera* and *Aphelenchus* were investigated. The designations and sources of these species were: *M. javanica*; 7—North Carolina, 12—Rhodesia, 112—Australia, 217—Ivory Coast: *M. incognita* (Kofoid & White) Chitwood; 84—North Carolina, 116—Taiwan, 159—Ivory Coast; *M. hapla*; 23—England, 106—North Carolina, 268—Oklahoma; *M. arenaria*; 64—North Carolina: *Heterodera glycines* Ichinohe—North Carolina; *Aphelenchus avenae* Bastian—Wisconsin; *Ditylenchus dipsaci* (Kühn) Filipjev—North Carolina; *D. triformis* Hirschmann & Sasser—North Carolina.

The populations of *Meloidogyne* were greenhouse-cultured (24–30 C) on tomato, *Lycopersicon esculentum* Mill. (var. 'Rutgers') grown in a 3:1 mixture of sandy

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loam and coarse sand in 15.2-cm clay pots. These were watered weekly with one gm VHPF® (Miller Chemical Company, Baltimore 15, Maryland 21203) in 100 ml water.

H. glycines was cultured (24–30 C) in the same kind and size pot on soybean, *Glycine max* (L.) Merr. (var. 'Lee') grown in white silica sand and watered as needed with Hoagland's solution modified with 20 ppm iron supplied in iron citrate. *A. avenae* was cultured on *Rhizoctonia solani* Kühn grown on potato-dextrose agar in petri plates. *D. triformis* was similarly reared on *Pyrenochaeta terrestris* (Hans.) Gorenz, J. C. Walker and Larson, grown on potato-destrose agar and *D. dipsaci* was maintained on alfalfa callus using a modification of the method given by Krusberg (13). Alfalfa seeds were surface-sterilized 3 min in 1:1 (v/v) Clorox—95% ethyl alcohol, rinsed 8 times with sterile distilled water and aseptically germinated on 2% agar containing 0.1% sucrose and 0.1% proteose peptone (Difco). Twenty seedlings were aseptically transferred to each of 60 petri plates containing Krusberg's nutrient medium B (13), and inoculated by the addition of infected callus tissue.

RECOVERY OF NEMATODES: Young adult females of *Meloidogyne* spp. were recovered from host plants by a modification of the technique reported by Dropkin *et al.* (6) Fifteen to 25 host plants were inoculated with 6 to 10 egg masses and grown in a greenhouse at 24–35 C for 40 to 45 days. Approximately 80 g of chopped roots were placed in 200 ml of "macerating enzyme," Pectinol 59-L® (Rohm & Haas Co., Bristol, Penn. 19007), diluted 1:1 (v/v) with distilled water in a 500-ml Erlenmeyer flask and constantly agitated on a wrist-action shaker at 90 oscillations/min for 5–6 hr at 22–25 C. The softened roots were decanted onto a 30 and 60-mesh sieves and subjected to a high-pressure water spray to dislodge the females from the softened roots and collect them on the 60-mesh sieve. The cellular plant debris and females were poured into a beaker and decanted several times to remove as much of the plant debris as possible. The females, which were withdrawn with a pipette and thoroughly washed with distilled water, were separated from the remaining plant cells by adding 20–30 ml of a 1 M sucrose solution. Any plant cells or egg masses remaining with the females were removed with a pipette.

A. avenae was cultured for 28 days, *D. triformis* for 60 days and *D. dipsaci* for 55 days then Baermann extracted.

Cysts of *H. glycines* were washed from soybean plants which had been inoculated five weeks earlier. Cysts in the yellow stage were picked out with a pipette.

PREPARATION OF NEMATODE EXTRACTS FOR ELECTROPHORESIS OF SOLUBLE PROTEINS: The females of *Meloidogyne* spp. and cysts of *H. glycines* were concentrated in most cases by removing the water with a pipette. However, in some cases, the females were concentrated on an 8-μ Millipore filter, subjected to 760 mm Hg suction until the water was removed. Females and cysts were weighed, then homogenized in an ice-cooled glass homogenizer.

A. avenae, D. triformis, and *D. dipsaci* were concentrated on a Millipore filter as described above, frozen with liquid nitrogen and homogenized in a porcelain mortar.

All nematodes were homogenized in a cold solution of 0.4 M sucrose, 0.57 mM ascorbic acid and 0.64 mM cysteine-HCl buffered at *p*H 7.8 with 0.1 M Tris (hydroxymethyl) aminomethane, sterilized by 0.22-μ Millipore filtration and stored at 2 C until used. The extraction buffer was added at the rate of one ml per 0.6 g wet weight of nematodes. The homogenates were then centrifuged in glass tubes for 20 min (49,000 $\times$ *g*; 0 C) and the clear supernatant was retained for electrophoretic analysis. Protein concentration was determined according to the method of Lowry

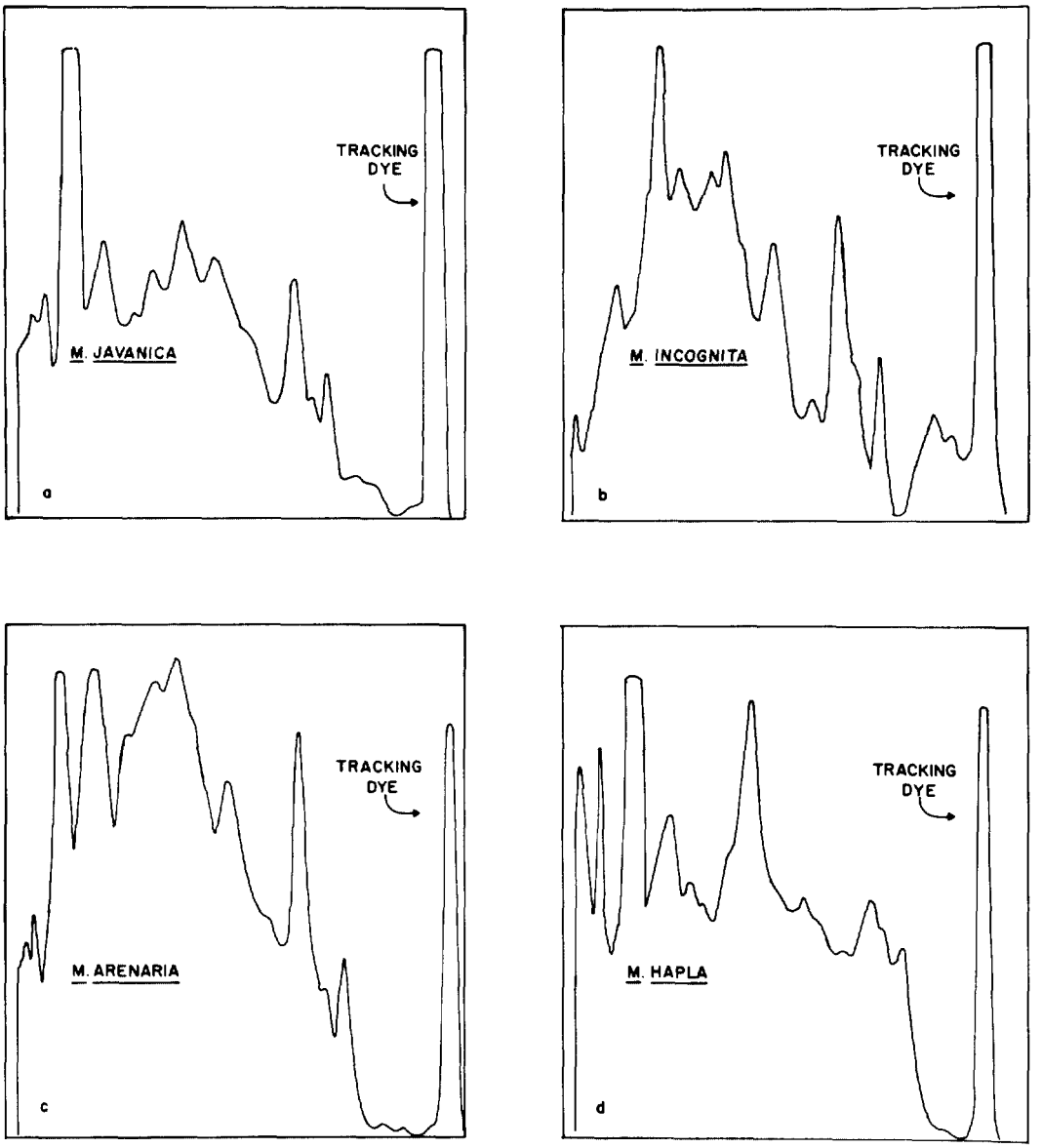

FIG. 1. Densitometric tracings of the protein profiles of four species of *Meloidogyne*.

et al. (14). All preparations were stored on ice until needed and could be held up to 4 days without alteration of protein profiles.

ELECTROPHORESIS: Negatively charged proteins were separated by disc-electrophoresis as described by Davis (5). Up to 12 gel tubes were run simultaneously. The nematode protein (0.5 mg) was added directly onto the 'spacer' gel as described by Gilbert & Goldberg (8). A current of three ma per tube was applied until the tracking dye (bromophenol blue) had migrated to the origin

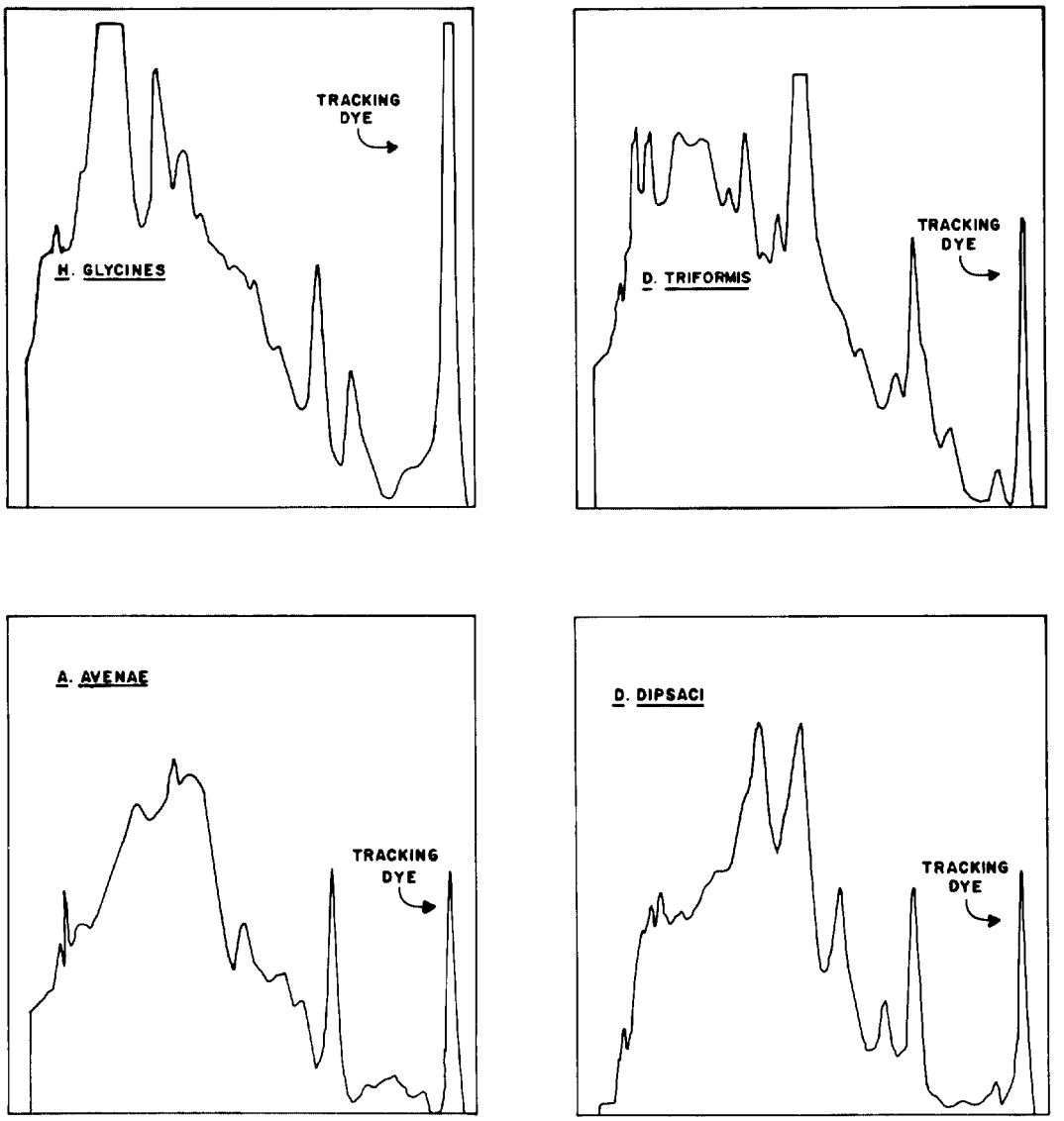

FIG. 2. Densitometric tracings of protein profiles of four nematode genera.

of the small-pore gel at which time the current was increased to five ma per tube. When the tracking dye had moved to within 1 cm of the anodic end of the gel tube, the power was turned off and the gels were removed. The gels were treated for the demonstration of protein bands by simultaneous staining and fixation with 0.1% amido Schwartz in 7% acetic acid, destained electrophoretically and scanned with a recording electrophoretic microdensitometer. The relative electrophoretic mobility (E_f) of each protein was calculated as the ratio of the movement of the band to that of the tracking dye.

TABLE 1. Disc-electrophoretic analyses of raw extracts of four *Meloidogyne* spp. (E_f is ratio of movement of protein band and bromophenol blue tracking dye) (Average of two replicates).

Protein bands	E_f ratios			
	M. javanica 7—NC	*M. incognita* 84—NC	*M. hapla* 106—NC	*M. arenaria* 64—NC
a	.03	.02	.02 (57%)†	.03
b	.06	.07 (75%)	.06	.06
c	.12	.11	—‡	.12
d	.14 (10%)	.15 (50%)	.14	—
e	.20	.22	.22	.20
f	.27	.28	.29	.28
g	.32	—	—	.32
h	—	.35	.34	—
i	.39	.39	.39	.39
j	—	.43	.44	.42
k	.48	.50	.49	.50
l	.57	—	.56	—
m	—	.60	.60	.60
n	.64 (23%)	—	—	—
o	.68	.66	.66	.67
p	.72	.70	.73	.72
q	.76	.76	.76	.76
r	—	—	.80	—
s	.83 (90%)	.84	—	.85
t	.88 (90%)	.89	.90 (14%)	.90
u	.95 (90%)	.93	—	.96

† Percentage of tests in which a band was observed in gels of all experiments on the species. The remainder was observed 100% of the time.
‡ Minus sign (–) indicates no band was observed.

RESULTS

Each nematode genus investigated had a characteristic electrophoretic protein profile (Figure 1 and 2).

A total of 21 protein bands were resolved for the four *Meloidogyne* spp. Ten (a, b, e, f, i, k, o, p, q, t) bands were present in all four spp., six others (c, d, j, m, s, u) were identified in three (not always the same three) of the four spp., three (g, h, l) were found in only two of the species, and two (n, r) appeared in just one (see Table 1).

Quantitative relations between the proteins extracted from the *Meloidogyne* spp. are shown by the densitometric tracings in Figure 1 and 3. These were qualitatively and quantitatively stable within populations over a period of 2 years. Bands *o*, *p* and *q* were easily and consistently obtained in *Meloidogyne* and may be of taxonomic or evolutionary value. Bands *e* through *n* showed differences between species that may be found to be diagnostically important (see Figure 1).

Similarly analyzed extracts from *A. avenae* and *D. triformis* yielded 14 and 15 bands, respectively, and *D. dipsaci* and *H. glycines* both had only 12. Several E_f's of these bands were similar, but their densitometric tracings differed widely (see Figure 2).

A comparison of the average E_f values of bands for four populations of *M. javanica* revealed a striking similarity (Table 2). In one out of ten cases band *c* occurred as two distinct bands for *M. javanica* (7-NC and 217—Iv. Coast) (Figure 3, a and c). Nine-tenths of the time, however, a single heavily stained band appeared in this zone for *M. javanica* (7—NC) (Figure 1, a). Apparently this band was an aggregate of proteins, separable into two bands on some occasions. Otherwise, densitometric tracings of the four populations exhibited a very close resemblance (Figure 3).

The E_f values for protein bands resolved from four extracts of *M. javanica* (7—NC) isolated from tomato, tobacco, cucumber, and wheat at different times were essentially identical.

DISCUSSION

The results show disc-electrophoretic analyses of soluble proteins can be used to help characterize and identify nematodes. Markedly different protein profiles were obtained for different genera, indicating that appreciable intergeneric differences probably exist among the Nematoda. The morphologically-based familial (Heteroderidae, Filipjev) relationship of *Heterodera* and *Meloidogyne* is also reflected in the similarity of their electrophoretic protein composition. Probably many of their proteins are homologous which could be confirmed by immunoelectrophoresis or other definitive qualitative analyses.

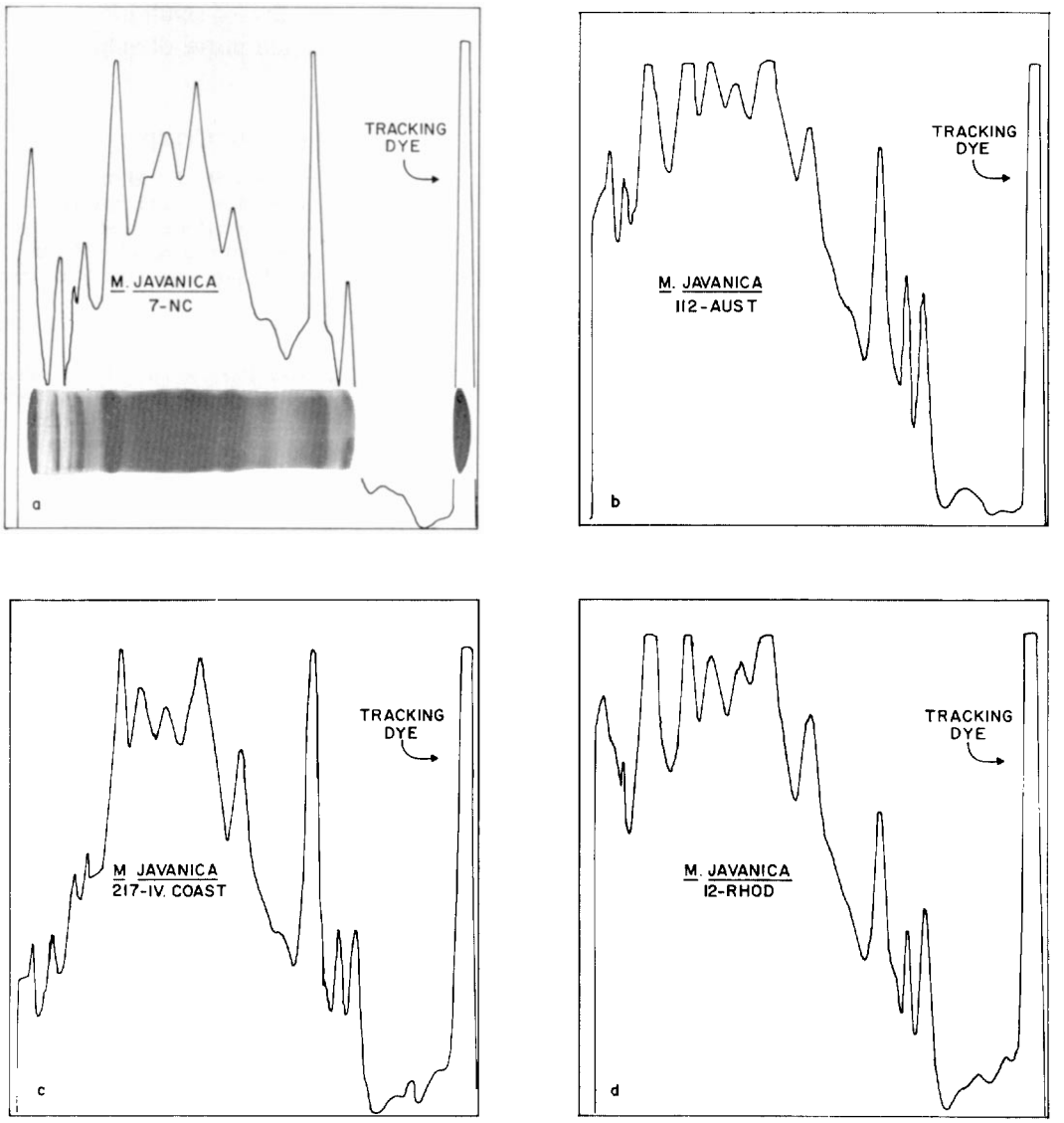

FIG. 3. Densitometric tracings of the protein profilesof four populations of *Meloidogyne javanica*. A photograph of gel is included with the tracing of *M. javanica* (7—NC).

Conversely, the *Ditylenchus* spp. exhibited little protein similarity and those of *A. avenae* differed from all the others.

Disc-electrophoresis can also be used to distinguish various species in the genus *Meloidogyne*. Although consistent and reproducible differences were detected between the protein profiles of these species, these were minor and represented only a small portion of the total proteins present. All four species shared 48% of the proteins and over 78% were shared by at least three of the four species. These results, as well as differences in staining intensities of the proteins as repre-

TABLE 2. Disc-electrophoretic analyses of raw extracts of *Meloidogyne javanica* from four geographical locations. (NC = North Carolina, Rhod = Rhodesia, Aust = Australia, Iv. Coast = Ivory Coast) [E_f is ratio of movements of protein band and bromophenol blue tracking dye (Avg. of two replicates)].

Protein bands	E_f ratios			
	7—NC	12—Rhod	112—Aust	217—Iv. Coast
a	.03	.02	.02	.03
b	.06	.06	.07	.07
c	.12	.12	.12	.12
d	.14 (10%)†	—‡	—	.14
e	.20	.22	.21	.22
f	.27	.27	.27	.27
g	.32	.33	.32	.33
h	.39	.40	.40	.40
i	.48	.49	.50	.49
j	.57	.58	.58	.58
k	.64 (23%)	—	—	—
l	.68	.66	.66	.66
m	.72	.72	.71	.72
n	.76	.76	.75	.75
o	.83 (90%)	.84	.83	.83
p	.88 (90%)	.88	.87	.87
q	.95 (90%)	.96	.94	.94

† Percentage of tests in which a band was observed in gels of all experiments on the species. The remainder was observed 100% of the time.
‡ Minus sign (–) indicates no band was observed.

sented by the heights of peaks on accompanying densitometric tracings, suggest a very close taxonomic relationship among the species.

Furthermore, disc-electrophoresis is a dependable technique for separating species of *Meloidogyne* collected from various geographical regions around the world. Of the various populations of each of the species of *Meloidogyne* tested, no differences could be detected in the protein profiles within the same species. In addition, no differences were detected when the nematodes were cultivated on various hosts.

This method appears to be of value as a complement to other methods which are presently being used to identify species of *Meloidogyne*. The results reported here show that while protein profiles of species of *Meloidogyne* are similar, differences exist which are probably indicative of small interspecific differences and should prove of value in taxonomic studies.

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