Molecular Polymorphism and Phylogenetic Relationship in some Meloidogyne spp.: Application to the Taxonomy of Meloidogyne

A. DALMASSO and J. B. BERGE[1]

Abstract: Proteins and various isozymes were investigated by direct analysis of single specimens in order to check molecular genetic variability, which is not rare in *Meloidogyne* species in spite of parthenogenetic reproduction. Variability was found in esterases, α-glycerophosphate, malate dehydrogenases, and some other proteins. Other loci appear monomorphic in the genus (for example, catalase). Distinct pools of genes are in a relative accordance with the common described species. Characteristic electrophoretograms are given for *M. arenaria, M. javanica, M. incognita, M. hapla,* and *M. naasi,* and it appears that nonspecific esterases are a useful tool supplementing morphology for specific characterization. Because the biochemical evidence is less subjective than the morphological, we believe it is more reliable. *Key Words:* genetic, electrophoresis, proteins, enzymes, Nematoda.

Polymorphism within a species is a universal phenomenon. Its relation with phylogeny and speciation can be investigated on several levels; we think the biochemical level is most promising. At that level several classes of chemicals have been used to study polymorphism. In attempting to understand why individuals of the same species differ it would be ideal to analyze the genes themselves. Unfortunately, quantitating nucleic acids and establishing their base sequences are time-consuming processes which require larger amounts of material than are generally available from a single nematode specimen. Analysis of some other biochemical components is less arduous. Secondary metabolites and proteins may be useful in elucidating polymorphism. Since secondary metabolites are the result of numerous interactions generally involving several enzymes, their utility in elucidating polymorphism is limited. Proteins, however, are the products of the nucleic acids and are almost a direct reflection of the structure and work of the genes. Their utility in research on polymorphism, therefore, is nearly ideal. Electrophoretic analysis of proteins from individual nematodes has already been demonstrated (2). DNA differences between individuals can be detected from the electrophoretic migrations of their proteins; but that represents only a part of molecular variability since amino acid substitution can change the protein without substantially changing the physical properties affecting electromobility (7, 15). Nevertheless, electrophoretic characterization of the proteins of a significant portion of a gene pool could confirm or establish speciation on a genetic basis.

This procedure for establishing species might be very useful in the genus *Meloidogyne,* where many populations exhibit atypical taxonomic characters in perineal patterns, host ranges, and karyotypes. It is well known that intermediate perineal patterns are very confusing (17).

Received for publication 13 March 1978.

[1] Institut National de la Recherche Agronomique, Station de Recherches sur les Nématodes, 123, Boulevard du Cap, B.P. 78, 06602 Antibes, France.

Host ranges may be misleading, especially with mixed populations, or even confounded when certain pathotypes are involved (1, 18, 20). Species determination on the basis of chromosome numbers has made much progress (23, 24, 25) but requires cytological examination of several specimens, and two species may have the same chromosome number. Studies have shown that some *Meloidogyne* reproduce by mitotic parthenogenesis (23, 24). With that mode of reproduction, mutations would be genetically isolated. Thus, a multitude of clones could result, further complicating the task of taxonomists.

Molecular genetics addresses the problem of speciation in parthenogenetic organisms (2, 4, 5), and electrophoresis has begun to be used as a taxonomic tool (8, 9, 10, 11, 13, 26), although the results were obtained with protein extracts of several specimens. Thus genetic analysis was impossible at intra- and interspecific levels. This work is an attempt to assess biochemical polymorphism for its application to the taxonomy of *Meloidogyne*.

MATERIALS AND METHODS

Nematode populations: Five known and one unknown species of *Meloidogyne*, comprising a total of 83 populations from various locations, were used:

M. arenaria: France 17, Switzerland 2, Spain 1, Italy 1.

M. incognita: French Antilles 8, France 2, Romania 1, Ivory Coast 1, Switzerland 1, uncertain origin 1, Morocco 1.

Meloidogyne sp.: (Seville 36 chromosomes): Spain 1.

M. javanica: France 3, Morocco 1, Abou Dhabi 1, Iraq 1.

M. hapla: France 31, Italy 2, Bulgaria 2, Hungary 1, Morocco 1.

M. naasi: France 3.

The populations were reared on tomato, *Lycopersicon esculentum* var. Marmande, in greenhouses at 20–25 C.

Electrophoresis: Individual specimens were crushed in 0.005 ml 0.1 M Tris HCl buffer, pH 8, containing 17% sucrose, 0.1% ascorbic acid, and 0.1% cystine hydrochloride in a bottom-sealed 1.1-mm-ID microhematocrit tube. The macerates were centrifuged at 9,000 *g* for 15 min. The supernatant fluids were used as the protein sources and placed on a spacer gel. Electrophoresis was performed on polyacrylamide gels cast as slabs (System 2) or as cylinders in microhematocrit tubes (Systems 1, 3, 4) (2, 16).

System 1: Acrylamide 7%, pH 8.9, 60 volts, bromophenol migration 5 cm, carried in cylindrical electrophoresis tank.

System 2: Acrylamide 7%, pH 8.9, 120 volts, bromophenol migration 7 cm, carried with Pharmacia apparatus.

System 3: Acrylamide 4%, pH 8.9, 60 volts, bromophenol migration 5 cm, carried in cylindrical tank.

System 4: Acrylamide 4.5%, pH 7.5, 60 volts, bromophenol migration 5 cm, carried in cylindrical tank.

Electrophoretic migration distances of proteins and enzymes relative to the migration distances of the bromophenol-blue fronts (R_f) were corrected for differences between tubes. Independent close values were confirmed by competing the two proteins on same gels (or using slab gels).

Upon completion of electrophoresis the gels were removed from the microhematocrit tubes with water pressure and incubated in developing reagent until bands appeared.

Developing reagents: For proteins we used 1% aqueous Coomassie Brillant Blue R250 and 7% acetic acid (1.19 v:v), and destained the blackground with several changes of 7% acetic acid. We also identified malate dehydrogenase (MDH), glycerolphosphate dehydrogenase (GPDH), glucose-6-phosphate dehydrogenase (G6PDH), and 6-phosphogluconate dehydrogenase (6 PGDH), catalase, and esterase using published procedures (2, 3, 12, 21). Tetrazolium oxidases were developed either on slab gels (System 2) or simultaneously with the dehydrogenases. They appeared as uncolored bands on the dark background of gel.

To establish variability in each locus, the electrophoretic analysis of individuals derived from wild populations was replicated 80 times; analysis of individuals from

TABLE 1. Electrophoretogram patterns[a] produced by proteins from different *Meloidogyne* species in Coomassie-stained gels.

Protein	R_f	*Meloidogyne* species					
		M. hapla	*M. naasi*	*M. incognita*	*M.* sp. (Seville)	*M. javanica*	*M. arenaria*
b	0.08-0.10	+++	+++	+++	+++	+++	+++
d	0.14	+	+	+	+	+	+
	0.17	+	−	+	−	+	−
faint-bands	0.21-0.24	+	−	+	−	+	+
	0.26	+	−	+	−	−	−
e	0.28-0.33	++	−	+	+	+	+
f	0.50-0.52	−	++	++	++	++	++
g	0.52-0.60	++	++	++	++	++	++
h	0.57-0.60	++	++	++	++	++	++

[a]Band intensity is indicated by the number of + signs.

egg mass populations was replicated 20 times. A total of 22,000 individual specimens were analyzed for this study.

RESULTS

Proteins (Systems 1 and 4): Anionic electrophoresis using System 1 revealed 10-15 main protein bands, with nine most obvious. All nine were not present in all the *Meloidogyne* species studied (Table 1). The band at R_f 0.28-0.33 stained more intensely for *M. hapla* than for any other species. *M. hapla* did not appear to have a protein at R_f 0.50–0.52 even when extracts from several females were combined whereas other species had a moderately intense band at this position. The major protein band in all species electrophoresed with System 1 was at R_f 0.09; electrophoresis with System 4 resolved this band at R_f 0.67 for *M. arenaria, M. javanica, M. incognita,* and *Meloidogyne* sp. Seville, and at nearly R_f 0.70 for *M. naasi* (Table 2).

Of the 37 populations of *M. hapla* studied, 10 had a double band, at R_f 0.60 and 0.65, and 27 had a single wide band, at R_f 0.60–0.62. Progeny had the same features as their mothers. To elucidate these bands further, antiserum was elaborated with protein from the single wide band. The serological test indicated that the proteins were closely related, if not the same. That finding has been confirmed by SDS bidimensional electrophoresis: four main identical bands are obtained from the two kinds of females and bands (Bergé & Dalmasso, in press). This indicates also that several structural genes are involved for the major protein complex.

Esterase (System 1): M. hapla has been reported to have at least two loci, β and b, coding for esterase synthesis. β-esterases are not active on β-naphthyl acetate, in contrast to b-esterases (2). The β locus has at least two alleles, electrophoretically detectable at R_f 0.19 and 0.24. Homozygous individuals have one or the other of these alleles, heterozygotes have both of the alleles (Table 3). The b locus also has two alleles, one a nonfunctional defective gene, the other demonstrable at R_f 0.33. The esterase zymogram pattern for *M. arenaria, M. incognita,* and *M. javanica* is essentially like that for *M. hapla.* One of the two alleles at the β locus from the Seville *Meloidogyne* population has an R_f in common with the other *Meloidogyne* populations (R_f 0.24), but the second allele (R_f 0.34) is unique. The b locus in *M. arenaria* and *M. javanica* had three bands (alleles), at R_f 0.30, 0.36, 0.38, but strong differences in respective intensities of these three bands permitted distinction between the two species. In *M. naasi* the b locus had a single esterase band, at R_f 0.46. The β locus had two bands, at R_f 0.28 and 0.34. No cathodic esterases were found for any *Meloidogyne* population.

Malate dehydrogenase (System 3): The general zymogram features for MDH (Table 4) were similar to those reported for the esterases. The more anionically moving bands were more intense than the slower-moving band. *M. javanica* and *M.*

TABLE 2. Electrophoretograms of major protein from *Meloidogyne* species.

	Meloidogyne species and chromosome number (n)						
R_f	*M. hapla* n = 16-17 2n = 45	n = 14 to 17	*M. naasi* n = 18	*M. incognita* 2n = 40-43	*M.* sp. Seville 2n = 36	*M. javanica* 2n = 46-47	*M. arenaria* 2n = 50-52 2n = 56
0.60-0.62	+	+					
0.65		+					
0.67				+	+	+	+
0.70			+				

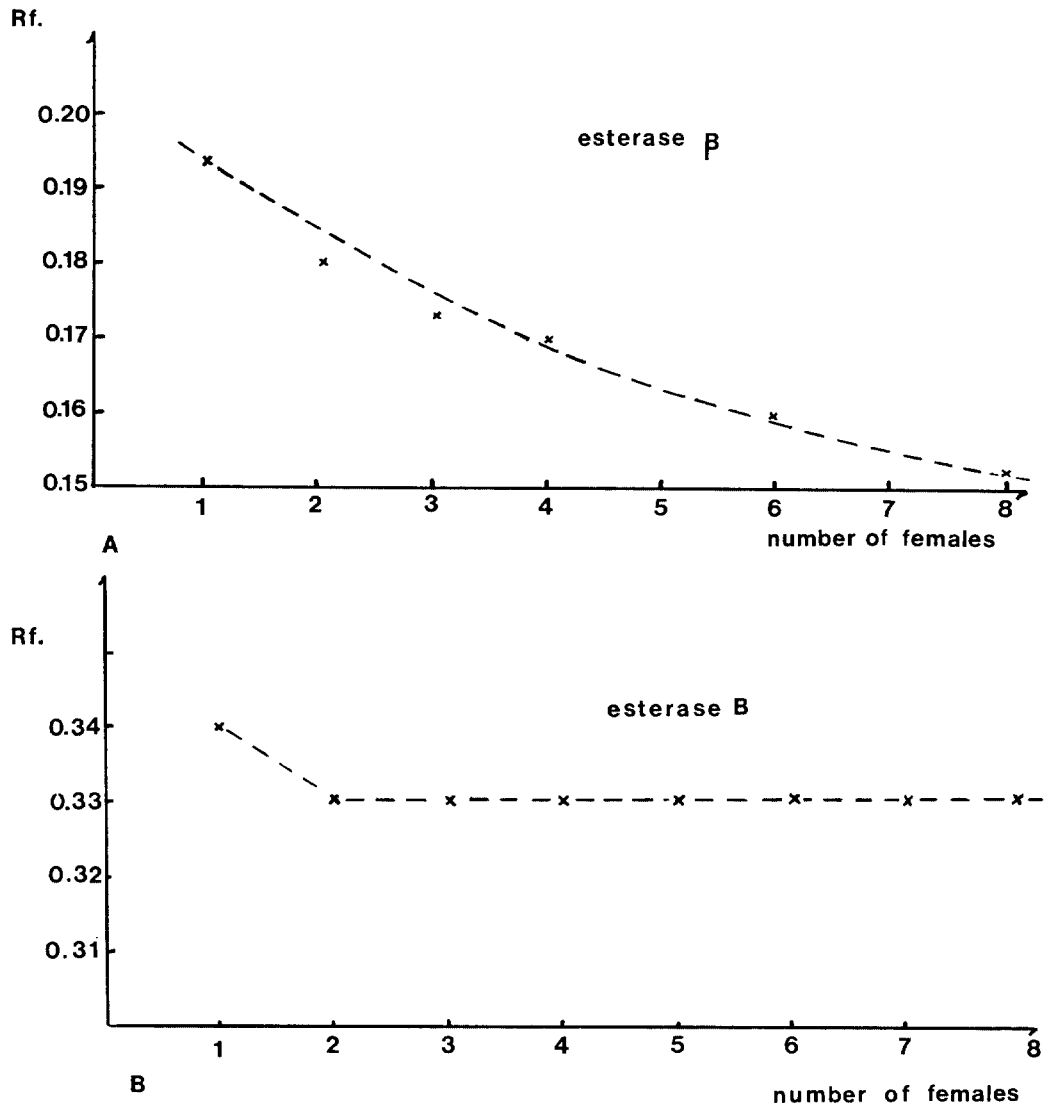

FIG. 1 (A–B). Influence of amounts of esterase β (A) and esterase b (B) on migration of these proteins in microhematocrit tubes.

arenaria had weak bands at R_f 0.19 and 0.24 and strong bands at R_f 0.30 and 0.35. An additional band was observed at R_f 0.40 in the Grau du Roi population of *M. arenaria*. The less anionic bands (R_f 0.19 and 0.24) were absent in *M. incognita* but all seven populations had strong bands at R_f 0.30 and 0.35. *M. naasi* had only one definite band, at R_f 0.35. *M. hapla* did not have any bands at an R_f in common with the other species. Its sole MDH band was at R_f 0.50. Nicotinamide adenosine diphosphate (NADP) did not function as a cofactor, so it was not malic enzyme.

α-Glycerophosphate dehydrogenase (System 2): α-GPDH was in accordance with the previous observations on esterase b. *M. incognita* and *Meloidogyne* sp. (Seville; 36 chromosomes) were characterized by a single strong α-GPDH band, R_f 0.35. *M. javanica* had two slight bands, at 0.38 and 0.40, the latter one being a little stronger. *M. arenaria* populations presented more variability and many of them gave zymograms similar but weaker than those of *M. javanica*. Some, such as the "Grau du Roi" population, had three α-GPDH bands in the same interval. Two others, from "Villefranche-sur-Saône" and "St-Sylvestre," show only one obvious band, at 0.38. This

TABLE 3. Electrophoretogram patterns[a] for esterase isozymes in different populations of *Meloidogyne* sp.

Meloidogyne species	Chromosome number	R_f for esterases from different loci							
		β alleles			b alleles				
		0.19	0.24	0.34	0.30	0.33	0.36	0.38	0.46
M. hapla	n = 15-16-17 2n = 45	++				+++			
	n = 14-15-17		++			+++			
	n = 16-17	++	++			+++			
	n = 16-17		++			b⁻(null)			
M. naasi	n = 18		(+)[b], +						++
M. incognita	2n = 40-43	+	+		+++				
Meloidogyne sp. (Seville)	2n = 36		+	+	+++				
M. javanica	2n = 46-47	+	+		+++		++	++	
M. arenaria	2n = 50-52	+			+		++	++	
	2n = 50-52		+		+		++	++	
	2n = 50-52	+	+		+		++	++	

[a]Band intensity is indicated by the number of + signs.
[b]See text.

TABLE 4. Electrophoretogram patterns[a] for malate dehydrogenase isozymes in different populations of *Meloidogyne* spp.

Meloidogyne species	Chromosome number	R_f for malate dehydrogenases					
		Locus 1		Locus 2			
		0.19	0.24	0.30	0.35	0.40	0.50
M. hapla	n = 14-15 16-17 2n = 45	0	0				+++
M. naasi	2n = 18	0	0	?	+++		
M. incognita	2n = 40-43	0	0	+++	++		
Meloidogyne sp. (Seville)	2n = 36	0	0	+++	++		
M. javanica	2n = 46-47	+	+	+++	++		
M. arenaria	2n = 50-52 2n = 56	+	+	+++	++		
	Grau du Roi 2n = 50-51	+	+	+++	++	+	

[a]Band intensity is indicated by the number of + signs.

migration difference between the *M. incognita* group and *M. arenaria* and *M. javanica* was confirmed on slab gels. *M. incognita* and *Meloidogyne* sp. (36 chromosomes) have the less mobile band; the αGPDH mobility is intermediate in *M. javanica* and *M. arenaria*.

All populations of *M. hapla* and *M. naasi* code for a single band, respectively at 0.40 and 0.35, but five female *M. naasi* had to be used to get this band. One population of *M. naasi* from Villons-les-Buissons gave no band even with ten females (Table 5).

The α-GPDH 0.40 allele in *M. hapla*, on one hand, and in *M. javanica* and *M. arenaria*, on the other, is nevertheless nearly distinguishable in 7% acrylamide gel slab at pH 8.9. The fastest band is given by *M. hapla* allele.

Glucose-6-phosphate dehydrogenase (System 1): This enzyme, which is one of the accesses of the phosphate pathway, is NADP-dependent. Few investigations have been carried on. G6PDH appears as a large diffuse band that is faster in *M. hapla* than in *M. arenaria, M. javanica* and *M. incognita*.

It is interesting that the four dehydrogenases are always faster in *M. hapla* than in the other species.

Catalase (System 3): Only a single catalase-active site appears on gel in all populations and species studied. It is located at 0.33. This very constant protein is revealed by pyrogallol oxidation or other phenolic substrates: gallic acid, hydroquinon, caffeic acid. The last substrate gives a very slight band. Activity is strong in all specimens, although very evanescent because of the thinness of microgels; so the migration distances must be recorded immediately.

It will be of interest to investigate this locus in closely related genera.

Tetrazolium oxidase (System 1): Several proteins have tetrazolium oxidative activity in *Meloidogyne* spp. On slab gels strong or weak oxidative bands are formed (Fig. 2): *M. incognita* and *Meloidogyne* sp. (Seville, 36 chromosomes), have two bands set close together. The slow band in *M. arenaria* and *M. javanica* probably corresponds to the fastest one in *M. incognita*. The other strong band is the same in the two groups. In *M. hapla* there is only one large band, which is slightly slower than that for the other species tested.

TABLE 5. Electrophoretogram patterns[a] for α-glycerophosphate dehydrogenase isozymes (α-GPDH) from different populations of *Meloidogyne* spp.

Meloidogyne species	Chromosome number	R_f for α-GPDH				
		0.35	0.35	0.39	0.40 (slow)	0.40 (fast)
M. hapla	n = 14-15 16-17 2n = 45					+++
M. naasi	n = 18	+++				
M. incognita	2n = 40-43	+++				
Meloidogyne sp. (Seville)	2n = 36	+++				
M. javanica	2n = 46-47		+		+	
M. arenaria	2n = 50-52 2n = 56		trace		trace	
	2n = 50-52	weak	trace	weak	trace	
	2n = 50-52		+++			

[a]Band intensity is indicated by the number of + signs.

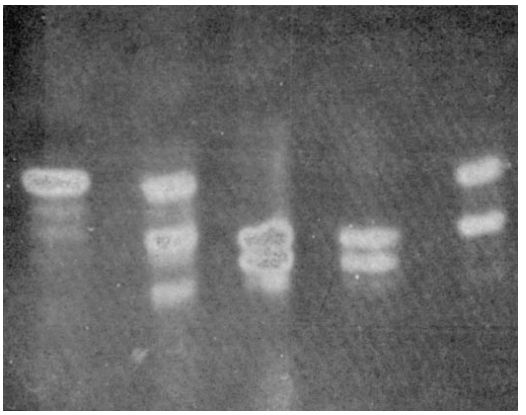

FIG. 2. Tetrazolium oxidases from (left to right) *Meloidogyne hapla, M. arenaria, M. incognita, Meloidogyne* sp. Seville, and *M. javanica*. Dark bands correspond to α-GPDH.

DISCUSSION

By extrapolating data from the literature about *Meloidogyne* karyotypes (25), it appears that the number of protein and enzyme alleles is correlated with chromosome numbers. That is true for total allele number of a single female as well as for total allele number of the species. In *M. hapla* only one allele is generally present at each locus when one specimen is examined, except in heterozygotes resulting from amphimixis. It had been assumed that the basic chromosome number may be n = 9 or n = 18 (25). Present observations, especially on *M. hapla,* indicate that the species is diploid. An exception, however, is the apparent triploid population (Saulcy: 45 chromosomes), which was found to be a homozygote for all loci investigated. That could be explained either by a recent triploid origin or by chromosome splits due to a holocentric phenomenon. In the latter case the population would still be considered a special diploid form. This hypothesis has cytological support. At early metaphase, 14 to 17 chromatic spots appear that could be assimilated to diakinetic bivalents. The same feature occurs in *Meloidogyne* sp. (Seville), in which the 36 chromosomes are well individualized in late metaphase, whereas early in meiosis the number appears lower (14). This species would therefore also be diploid. That is confirmed by the fact that more than two different alleles for a presumed locus have never been found. The same situation occurs in *M. incognita* in spite of some supernumerary chromosomes (polysomy), which may be either chromosomes resulting from incomplete polyploidy or pieces of chromosomes.

Another step in the evolutionary process of *M. javanica* and *M. arenaria* occurs where there are frequently more than two alleles for the same locus and in the same individual; for example, esterase b at R_f 0.30, 0.36, and 0.38. Two other loci show the same character in *M. arenaria.* That seems to agree with the triploid condition of *M. arenaria* (25). The less frequent occurrence of triple alleles in *M. javanica* probably indicates that this species is less polysomic, i.e., trisomic for some chromosomes only.

No good explanation has been given for the differences in activities (indicated by the color intensity of the bands) of the different alleles from the same locus. The catalytic action of the enzymes may be influenced by epigenic modulation, although other possibilities are not excluded (19).

Intrapopulation polymorphism is not very extensive in *Meloidogyne.* It is slight in *M. hapla,* perhaps in correlation with amphimixis. It does not exist in natural conditions in mitotic forms, but does occur in different populations of the same species (see α-GPDH in *M. arenaria*).

Paradoxically, heterozygosity is higher in obligatory parthenogenetic species (for example, *M. arenaria* and *M. javanica*) than in meiotic ones. Heterozygosity is protected by mitotic reproduction and favored by polysomy and polyploidy. That also explains their world-wide distribution. *M. hapla* seems to compensate for its low heterozygosity percentage by a higher rate of polymorphism. A high percentage of homozygosity would not be surprising, even in amphimictic forms, because spatial isolation and imbreeding must increase the rate of homozygosity, progressively pulling away lethal homozygotes. This high rate of homozygosity precedes and prepares the transition to parthenogenetic reproduction, as noted recently (6), and may be given as explanation for the high occurrence of this reproductive process in soil nematodes.

These data may be practical for taxonomic purposes. Molecular identifications

TABLE 6. Approximate allelic differences between species

$$\% = \frac{100 \times \text{number of different bands for paired species}}{\text{number of studied bands totaled from 15 presumed loci}}$$

	M. hapla	*M. naasi*[a]	*M.* sp. (Seville)	*M. incognita*	*M. javanica*	*M. arenaria*
M. hapla	0	65	75	70	87	87
M. naasi[a]		0	33	33	72	67
Meloidogyne sp. (Seville)			0	11	52	48
M. incognita				0	46	42
M. javanica					0	10
M. arenaria						0

[a]Twelve loci were inventoried.

are especially useful for classifying mixed populations. Nonspecific esterases are a good tool for this purpose.

Discriminant alleles established on zymograms split the mitotic species complex into two main groups: *M. arenaria* and *M. javanica;* and *M. incognita* and *Meloidogyne* sp. (Seville, 36 chromosomes). This is in good agreement with classification by chromosome numbers, the first group having more than 43 chromosomes, and the latter less than 45, and agrees also with Sasser's host range (20).

The data (Table 6) indicate that *M. arenaria* and *M. javanica* are distinct but closely related species; a Spanish strain (from Seville) that possesses 36 chromosomes appears more similar to *M. incognita* than the two previous ones. As expected, *M. hapla* and *M. naasi* remain independent branches on the *Meloidogyne* phylogenetic tree.

LITERATURE CITED

1. BERGE, J. B., A. DALMASSO, and M. RITTER. 1974. Influence de la nature de l'hôte sur le développement et le déterminisme du sexe du nématode phytoparasite Meloidogyne hapla. C.r. Acad. Agr., Séance du 20 Oct.: 946-952.
2. BERGE, J. B., and A. DALMASSO. 1976. Variations génétiques associées à un double mode de reproduction parthénogénétique et amphimictique chez le nématode Meloidogyne hapla. C.r. Acad. Sci., Paris, Série D, 282: 2087-2090.
3. BREWER, G. J., and C. F. SING. 1970. An introduction to isozyme techniques. Academic Press, Lond. 186 pp.
4. CHRISTENSEN, B., V. BERG, and J. JELNES. 1976. A comparative study on enzyme polymorphism in sympatric diploid and triploid forms of Lumbricillus lineatus (Enchytracidae, Oligochaeta). Hereditas 84:41-48.
5. CHRISTENSEN, B., and J. JELNES. 1976. Sibling species in the oligochaete worm Lumbricillus rivalis (Enchytraeidae) revealed by enzyme polymorphisms and breeding experiments. Hereditas 83:237-244.
6. CUELLAR, E. 1977. Animal parthenogenesis. A new evolutionary ecological model is needed. Science 197:837-843.
7. DICKINSON, W. J., and D. T. SULLIVAN. 1975. In: "Gene-Enzyme systems in Drosophila." Results and problems in cell differentiation. Edit. Springer-Verlag. Berlin Heidelberg New-York, volume 6, 163 pp.
8. DICKSON, D. W. 1969. The application of disc electrophoresis on studying the taxonomy of species of Meloidogyne and other nematode genera. Ph.D. Thesis, North Carolina State Univ., Raleigh: 100 pp.
9. DICKSON, D. W., D. HUISINGH, and J. N. SASSER. 1971. Dehydrogenases and alkaline phosphatases and esterases for chimotaxonomy of selected Meloidogyne, Ditylenchus, Heterodera and Aphelenchus. J. Nematol. 3:1-16.
10. EVANS, A. A. F. 1971. Taxonomic value of gel electrophoresis of proteins from mycophagous and plant parasitic nematodes. Int. J. Biochem. 2:72-79.

11. FRIEDMAN, P. A., E. G. PLATZER, and J. E. EBRY. 1977. Species differentiation in Caenorhabditis elegans. J. Nematol. 9:197-203.
12. HUSSEY, R. S., and L. R. KRUSBERG. 1971. Disc electrophoretic patterns of enzymes and soluble proteins of Ditylenchus dipsaci and D. triformis. J. Nematol. 3:79-84.
13. HUSSEY, R. S., J. N. SASSER, and D. HUISINGH. 1972. Disc electrophoretic studies of soluble proteins and enzymes of Meloidogyne incognita and M. arenaria. J. Nematol. 4:183-189.
14. JANATI, A. 1977. Etude comparative de deux populations sympatriques de Meloidogyne appartenant au complexe M. incognita-M. arenaria. D.E.A. Biol. Anim. Acad. Montpellier, Univ. Sci. Tech. Languedoc, 51 pp.
15. LEWONTIN, R. C. 1974. The genetic basis of evolutionary change. Columbia Univ. Press: 346 pp.
16. MAURER, H. R. 1971. Disc electrophoresis and related techniques of polyacrylamide gel electrophoresis. W. de Gruyter Ed., 222.
17. NETSCHER, C. 1971. Réflexions sur la variabilité de quelques espèces de Meloidogyne. Conf. O.E.P.P., 27-29 Oct. 1971 (comm. orale).
18. RIGGS, R. D., and N. N. WINSTEAD. 1959. Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. Phytopathology 49:716-724.
19. ROEHRDANS, R. L., and J. C. LUCCHESI. 1977. Transcriptional control and dosage compensation in Drosophila melanogaster. Nature 269:243-245.
20. SASSER, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes Meloidogyne spp. Bull. A77 Univ. Maryland: 1-30.
21. SHAW, C. R., and R. PRASAD. 1969. Starch gel electrophoresis of enzymes. A compilation of recipes. Biochem. Genet. 4:297-320.
22. SUOMALAINEN, E., and A. SAURA. 1973. Genetic polymorphism and evolution in parthenogenetic animals. I. Polyploid Curculionidae. Genetics 74:489-508.
23. TRIANTAPHYLLOU, A. C. 1962. Oogenesis in the root-knot nematode, Meloidogyne javanica. Nematologica 7:105-113.
24. TRIANTAPHYLLOU, A. C. 1963. Polyploidy and parthenogenesis in the root-knot nematode, Meloidogyne arenaria. J. Morphol. 113: 489-499.
25. TRIANTAPHYLLOU, A. C. 1969. Gametogenesis and chromosomes of two root-knot nematodes, Meloidogyne graminicola and M. naasi. J. Nematol. 1:62-71.
26. TRUDGILL, D. L. 1972. Electrophoresis of proteins from different populations of the potato cyst nematode. Ann. Appl. Biol. 71: 278-280.