

Enzymatic Relationships and Evolution in the Genus *Meloidogyne* (Nematoda: Tylenchida)¹

P. R. ESBENSHADE AND A. C. TRIANTAPHYLLOU²

Abstract: Thirty populations of *Meloidogyne* of diverse geographic origin representing 10 nominal species and various reproductive, cytological, and physiological forms known to exist in the genus were examined to determine their enzymatic relationships. The 184 bands resolved in the study of 27 enzymes were considered as independent characters. Pair-wise comparisons of populations were performed in all possible combinations to estimate the enzymatic distances (ED) and coefficients of similarity (S). A phylogenetic tree was constructed. The apomictic species *M. arenaria*, *M. microcephala*, *M. javanica*, and *M. incognita* shared a common lineage. *M. arenaria* was highly polytypic, whereas conspecific populations of *M. javanica* and *M. incognita* were largely monomorphic. The mitotic and meiotic forms of *M. hapla* were very similar ($S = 0.93$), suggesting that the apomictic race B evolved only recently from the meiotic race A. The five remaining meiotic species (*M. chitwoodi*, *M. graminicola*, *M. graminis*, *M. microtyla*, and *M. naasi*—each represented by a single population) were not closely related to each other or to the mitotic species.

Key words: biochemistry, biosystematics, electrophoresis, enzymes, evolution, *Meloidogyne* spp., root-knot nematode, phylogeny, taxonomy.

The genus *Meloidogyne* comprises the most widely distributed and economically important plant-parasitic nematodes, commonly known as root-knot nematodes (20). Fifty-five species have been described from the roots of a wide variety of woody and herbaceous plants. Their taxonomy is based primarily on morphology of various life stages and to a lesser extent on host preferences (7).

Attempts to elucidate phyletic relationships of root-knot nematodes have been made in the last 15 years through extensive cytogenetic and some biochemical investigations. The cytogenetic studies have revealed that evolution in the genus *Meloidogyne* has been influenced by various modifications of the mode of reproduction and by the establishment of various degrees of polyploidy and aneuploidy (16, 21, 23). Of the 15 species that have been investigated cytogenetically, three are dip-

loid and reproduce exclusively by cross-fertilization (amphimixis). These species are limited geographically to a few localities, have restricted host ranges, and cause little economic damage. Six other species are also diploid and reproduce either by amphimixis or, facultatively, by meiotic parthenogenesis. They are more widely distributed, have less restrictive host ranges, and cause greater economic losses than the amphimictic species. The remainder of the species reproduce obligatorily by mitotic parthenogenesis (apomixis) and are polyploid or polyploid derivatives. These species are widely dispersed in all temperate and tropical regions of the world and have extensive host ranges. Three of the obligatorily parthenogenetic species (*M. arenaria*, *M. incognita*, and *M. javanica*) together with the facultatively parthenogenetic *M. hapla* are responsible for more than 90% of the estimated damage caused by root-knot nematodes to agricultural crops on a world-wide basis (7). Apparently the apomictic and facultatively parthenogenetic forms of *Meloidogyne*, which are assumed to have evolved from diploid, amphimictic ancestors, are the most successful as plant parasites (24). This conclusion agrees with observations indicating that the polyploid, parthenogenetic forms of many plants and animals can exploit a greater number of diverse environments than can

Received for publication 5 June 1986.

¹ Paper No. 10314 of the Journal Series of the North Carolina Agricultural Service, Raleigh, NC 27695. Use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Service of the products named nor criticism of similar ones not mentioned. This study was supported in part by the International *Meloidogyne* Project Contract No. AID/ta-C-1234 and by the National Science Foundation Grant BSR-8314908 AO1.

² Research Assistant and Professor, Department of Genetics, North Carolina State University, Raleigh, NC 27695.

We thank Ted H. Emigh for assistance in computer analysis.

TABLE 1. *Meloidogyne* populations examined electrophoretically.

Population number	Species	Race*	Chromosome number†	Mode of reproduction‡	Origin
1	<i>M. arenaria</i>	1	53-54	MtP	Nigeria
2		1	53	MtP	U.S.A., TX
3		2	48	MtP	Chile
4		2	54	MtP	U.S.A., GA
5		2	53	MtP	Guadeloupe
6		2	37	MtP	Colombia
7		2	53	MtP	Argentina
8	<i>M. microcephala</i>		36-40	MtP	Thailand
9	<i>M. javanica</i>		48	MtP	U.S.A., NC
10			42	MtP	Brazil
11	<i>M. incognita</i>	1	41	MtP	U.S.A., NC
12		1	42	MtP	El Salvador
13		1	42	MtP	China
14		2	45	MtP	Nigeria
15		2	33-34	MtP	Puerto Rico
16		3	43-44	MtP	U.S.A., NC
17		3	36	MtP	Argentina
18		4	36-39	MtP	U.S.A., TN
19		4	42	MtP	U.S.A., TX
20	<i>M. chitwoodi</i>		(14)	AMP + MeP	Holland
21	<i>M. graminicola</i>		(18)	AMP + MeP	U.S.A., LA
22	<i>M. graminis</i>		(18)	AMP + MeP	U.S.A., FL
23	<i>M. microtyla</i>		(19)	AMP	Canada
24	<i>M. naasi</i>		(18)	AMP + MeP	England
25	<i>M. hapla</i>	A	(15)	AMP + MeP	Canada
26		A	(17)	AMP + MeP	U.S.A., VA
27		A	(17)	AMP + MeP	France
28		A	(14)	AMP + MeP	Holland
29		B	48	MtP	Chile
30		B	30	MtP	Korea

* Numbers refer to host-specific races (20) and letters refer to cytological races (21).

† Plain numbers indicate somatic (2n) chromosome numbers; numbers in parentheses refer to the haploid (n) chromosome numbers.

‡ Mode of reproduction; AMP = amphimixis, MeP = meiotic parthenogenesis, and MtP = mitotic parthenogenesis.

their diploid, amphimictic counterparts, even to the point where they totally replace the sexual forms in some environments (13,18,27).

Biochemical research involving a small number of enzyme systems in the four most important *Meloidogyne* species has also contributed useful information about the enzymatic relationships of these nematodes. Both Dickson et al. (5) and Dalmaso and Bergé (3) found extensive enzymatic variation among the obligatorily parthenogenetic species *M. arenaria*, *M. javanica*, and *M. incognita*. Although these species shared some isozymes, they could clearly be differentiated from each other by their unique enzyme phenotypes. The mitotic and the meiotic forms of *M. hapla* were similar to each other but only distantly related to the

obligatorily parthenogenetic species. This observation suggested a distinct line of enzymatic evolution for each of these two groups.

We examined the enzymatic relationships of 30 selected populations of root-knot nematodes from around the world, representing 10 *Meloidogyne* species of known cytogenetic status, and evaluated statistically the variation encountered in 27 enzyme systems in an effort to draw some conclusions about the evolutionary relationships of these nematodes.

MATERIALS AND METHODS

The 30 *Meloidogyne* populations of this study were selected from among 800 populations of the International *Meloidogyne* collection of North Carolina State Univer-

TABLE 2. Electrophoretic gel systems used in the study of *Meloidogyne* spp.

Designation	Description
A1	7% polyacrylamide gel (4) gel buffer—0.378 M tris/HCl, pH 8.9 electrode buffer—0.005 M tris 0.038 M glycine, pH 8.3
A2	7% polyacrylamide gel (14) gel buffer—0.188 M tris/HCl, pH 8.6 electrode buffer—0.005 M tris 0.038 M glycine, pH 8.3
SC	13% starch gel (26) gel buffer—0.016 M L-histidine (free base) 0.002 M citric acid, pH 6.5 electrode buffer—0.065 M L-histidine (free base) 0.007 M citric acid, pH 6.5
SG	13% starch gel (26) gel buffer—0.010 M tris 0.003 M citric acid, pH 7.0 electrode buffer—0.135 M tris 0.043 M citric acid, pH 7.0
SL	13% starch gel (26) gel buffer—0.076 M tris 0.005 M citric acid, pH 8.65 electrode buffer—0.3 M boric acid 0.05 M sodium hydroxide, pH 8.45
SM	13% starch gel (26) gel buffer—0.052 M tris 0.008 M citric acid, pH 8.3 1 part electrode buffer mixed with 9 parts tris/citric acid buffer electrode buffer—0.19 M boric acid 0.04 M lithium hydroxide, pH 8.3

sity. They represent 10 nominal species and most of the cytological, reproductive, and physiological forms known to exist in the genus *Meloidogyne* (Table 1). All nematodes were propagated on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) with the exception of *M. graminicola* which was propagated on barnyard grass (*Echinochloa colonum* (L.) Link.), *M. graminis* on St. Augustine grass (*Stenotaphrum secundatum* (Walt.) Kuntze.), and *M. naasi* on barley (*Hordeum vulgare* L.). Female nematodes, extracted from infected roots of these

plants, were assayed for 27 enzymes by polyacrylamide or starch gel electrophoresis.

Crude extracts of whole nematodes were prepared as previously described (8), stored at -15°C , and separated anodally on polyacrylamide or starch gels using standard procedures (Table 2). Extracts of single nematodes were run on polyacrylamide gels, whereas extracts of five nematodes were used for starch gels. Up to 20 different samples were compared on the same gel.

Polyacrylamide gel electrophoresis was conducted at 5°C in 7% gel slabs ($0.7 \times 125 \times 175$ mm) at a constant voltage of 250 volts until the tracking dye had migrated 100 mm (4,17).

Starch gels were run horizontally and were prepared using 13% (w/v) Electro-starch (Otto Hiller) and 1.5% (w/v) sucrose. The crude nematode homogenate was absorbed onto filter paper wicks ($2 \times 11 \times 0.2$ mm, Whatman #3MM chromatography paper). Electrophoresis was conducted at 5°C at a constant power of 7 watts until the bromophenol blue tracking dye had migrated 100 mm.

Enzymatic activity was detected in gels using standard techniques (12,15) (Table 3). Gels were photographed and bands of activity were scored for each population for each enzyme in at least three replicate gels. Bands co-migrating in three or more gels were assumed to represent identical proteins.

For the statistical evaluation, pair-wise comparisons of populations were performed to determine the enzymatic distance (ED). These distance estimates were used to construct a phylogenetic tree according to the method of Fitch and Margoliash (10) contained in the Phylogeny Inference Package, PHYLIP (9). The program FITCH fits an unrooted tree with branch lengths proportional to the observed differences in taxa, whereas the program KITSCH assumes that all living species are contemporaneous and fits a rooted tree based upon evolutionary time, where branch lengths are constrained so

TABLE 3. Enzymes examined and gel systems used in the investigation of *Meloidogyne* spp.

Enzyme	Gel system*	Total number of bands
Oxidoreductases		
Glycerol-3-phosphate dehydrogenase		
GPD E.C. 1.1.1.8	A1	5
Lactate dehydrogenase		
LDH E.C. 1.1.1.27	SC	6
Malate dehydrogenase		
MDH E.C. 1.1.1.37	A1	5
Malic enzyme		
ME E.C. 1.1.1.40	A1	8
Isocitrate dehydrogenase		
ICD E.C. 1.1.1.42	SC	6
Phosphogluconate dehydrogenase		
PGD E.C. 1.1.1.44	SC	7
Glucose-6-phosphate dehydrogenase		
G6PDH E.C. 1.1.1.49	A1	4
D-Aspartate oxidase		
DASOX E.C. 1.4.3.1	SL	3
Lipoamide dehydrogenase		
LADH E.C. 1.6.4.3	SC	5
Diaphorase (NADH)		
DIA E.C. 1.6.2.2	A1	5
Catalase		
CAT E.C. 1.11.1.6	A1	4
Superoxide dismutase		
SOD E.C. 1.15.1.1	A1	6
Transferases		
Glutamate-oxaloacetate transaminase		
GOT E.C. 2.6.1.1	A1	10
Glutamate pyruvate transaminase		
GPT E.C. 2.6.1.2	A1	8
Hexokinase		
HK E.C. 2.7.1.1	SG	11
Phosphoglucomutase		
PGM E.C. 2.7.5.1	SC	14
Hydrolases		
Esterase		
EST E.C. 3.1.1.1	A2	11
Acid phosphatase		
ACP E.C. 3.1.3.2	A1	9
α -Glucosidase		
GLU E.C. 3.2.1.20	A1	2
α -Galactosidase		
GAL E.C. 3.2.1.22	A1	5
α -Mannosidase		
MAN E.C. 3.2.1.24	A1	6
α -Fucosidase		
FUC E.C. 3.2.1.51	A1	5
Peptidase 1 (Trp-Gly)		
PEP1 E.C. 3.4.11	SL	7
Peptidase 2 (Gly-Trp)		
PEP2 E.C. 3.4.11	SL	4
Lyase		
Fumarate hydratase		
FH E.C. 4.2.1.2	A1	6

TABLE 3. Continued.

Enzyme	Gel system*	Total number of bands
Isomerases		
Triose phosphate isomerase		
TPI E.C. 5.3.1.1	SM	8
Glucose phosphate isomerase		
GPI E.C. 5.3.1.9	SG	14

* For explanation of gel composition see Table 2.

that the total length from the root of the tree to any population sampled is the same.

The same data were used to calculate Jaccard's coefficient of similarity (S) (6,19).

RESULTS

Because of various technical difficulties and the parthenogenetic reproduction of many of these nemtodes, genetic studies have not been conducted; thus, it is difficult to translate the observed electrophoretic phenotypes into genotypes (Fig. 1, Table 4). Consequently, no attempt was made to assign specific genes or alleles to each enzyme locus studied. Instead, the 184 bands of electrophoretic activity detected for the 27 enzymes (Table 3) were evaluated as independent characters. In pair-wise comparisons of all populations, in all possible combinations, the presence or absence of a particular band of a given enzyme was scored without regard to the size of the band or the intensity of staining. The ED between two populations is expressed by the number of bands by which these populations differ from each other (Table 5, upper triangle). Jaccard's coefficient of similarity (S) is expressed as a fraction, equal to the number of bands for which two populations are identical (matches), divided by the same number plus the number of mismatches; the negative matches (where two populations that are compared lack a band which is present in some other population) are disregarded (Table 5, lower triangle).

Meloidogyne arenaria (populations #1-7) was found to be highly variable in that no two populations of this species were enzy-

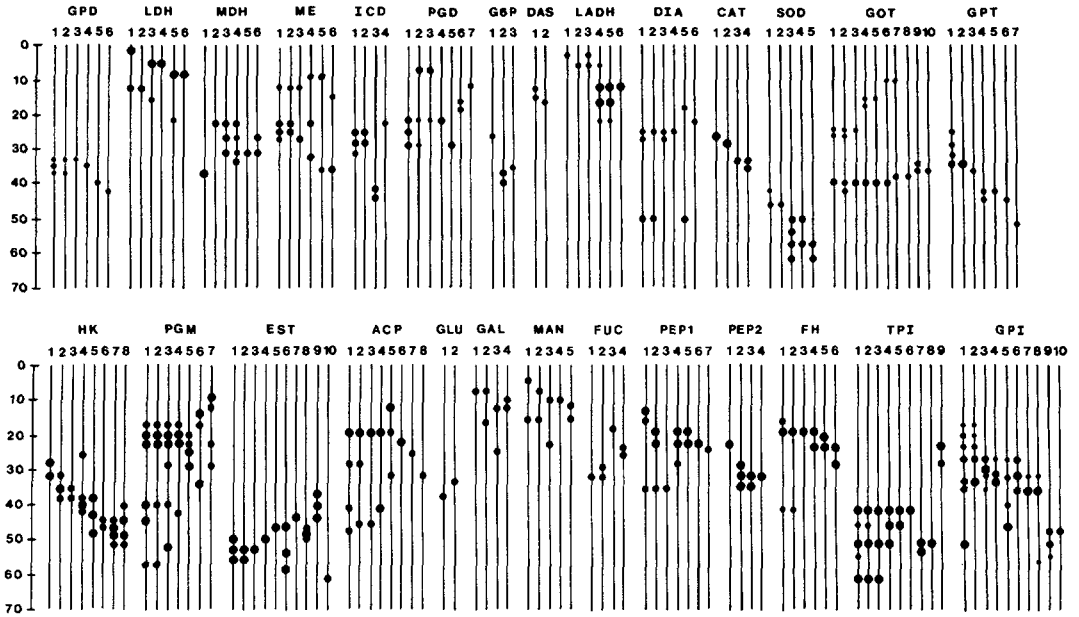


FIG. 1. Phenotypes of enzymatic activity (designated numerically) of 27 enzymes observed in the study of 30 populations of *Meloidogyne*. For enzyme designations see Table 3.

matically identical (Table 4). Populations #3 from Chile and 7 from Argentina were the closest to each other, differing only by two enzymatic bands, for a high coefficient of similarity of 0.97. Most populations differed by more than 11 bands, for a coefficient of similarity of less than 0.85.

Many population pairs of *M. incognita* (populations #11–19) were identical, and others differed only by a small number (1–4) of bands, for a coefficient of similarity of more than 0.93.

Meloidogyne hapla (populations #25–30) showed an intermediate degree of similarity between *M. arenaria* and *M. incognita*. Two population pairs were identical, and the coefficient of similarity was more than 0.92 for all population pairs investigated. The two populations (#29 and 30, Fig. 2) of cytological race B (mitotic) were identical for all 184 enzyme bands and differed only slightly from populations of race A (meiotic). Specifically, race B populations had an additional band of activity for LADH and GPI that was not detected in race A populations, whereas race A populations had an additional band of GOT

activity not detected in race B populations (Table 4).

The two populations of *M. javanica* (#9 and 10) were enzymatically identical.

The remaining species (*M. chitwoodi*, *M. graminicola*, *M. graminis*, *M. microtyla*, and *M. naasi*) were represented by a single population each; therefore, no intraspecific variation could be determined. All these species were quite distinct from each other and from the major species previously discussed.

The ED determined from the pair-wise comparisons of 30 populations (Table 5) were used to construct a statistically optimal phylogenetic tree (Fig. 2) according to the method of Fitch and Margoliash (10). The programs FITCH and KITSCH contained in the Phylogeny Inference Package, PHYLIP, were run 28 and 20 times, respectively. A total of 48 separate trees thus produced were compared to determine what effect the order in which the populations were entered into the programs and also changes of certain other program parameters would have on the structure of the phylogenetic tree. It was

TABLE 4. Enzyme phenotypes* (designated numerically) observed in 30 populations of *Meloidogyne* spp.

Pop. #	GPD	LDH	MDH	ME	ICD	PGD	G6P DH	DAS OX	LAD H	DIA	CAT	SOD	GOT	GPT	HK	PGM	EST	ACP	GLU	GAL	MAN	FUC	PEP 1	PEP 2	FH	TPI	GPI
1	1	6	3	1	1	1	1	1	5	1	1	3	1	2	4	1	2	1	1	4	2	1	4	2	4	1	9
2	1	6	2	2	1	1	1	1	6	4	1	3	1	2	4	1	1	2	1	4	2	1	4	2	4	3	9
3	1	2	2	2	1	1	1	1	6	2	1	3	1	2	4	1	1	1	1	4	2	1	4	2	4	1	9
4	1	6	3	1	1	1	1	1	4	1	1	3	1	2	4	2	2	1	1	4	2	1	5	2	4	3	9
5	1	5	2	2	1	1	1	1	5	1	1	4	2	2	4	1	2	2	1	4	2	1	4	2	4	2	9
6	2	2	2	2	1	5	1	1	4	1	1	4	1	2	4	1	2	1	1	4	2	1	4	2	4	3	10
7	1	2	2	2	1	1	1	1	6	2	1	3	1	2	4	1	1	1	1	4	2	1	4	2	4	3	9
8	2	2	2	2	1	1	1	1	4	3	1	4	1	2	4	4	3	2	1	4	2	1	4	2	4	3	10
9	1	6	2	2	1	5	1	1	5	1	1	4	3	2	5	4	6	2	1	4	2	1	4	2	4	3	9
10	1	6	2	2	1	5	1	1	5	1	1	4	3	2	5	4	6	2	1	4	2	1	4	2	4	3	9
11	3	6	2	2	1	2	1	1	6	2	1	5	4	2	8	3	5	4	1	4	2	1	4	2	4	3	5
12	3	6	2	2	1	3	1	1	6	2	1	5	4	2	8	3	5	4	1	4	2	1	4	2	4	3	5
13	3	6	2	2	1	2	1	1	6	2	1	5	4	2	8	3	5	4	1	4	2	1	4	2	4	3	5
14	3	6	2	2	1	2	1	1	6	2	1	5	5	2	8	3	5	4	1	4	2	1	4	2	4	3	5
15	3	6	2	2	1	3	1	1	6	2	1	5	4	2	8	3	5	4	1	4	2	1	4	2	4	3	5
16	3	6	2	2	1	3	1	1	6	2	1	5	5	2	8	3	5	4	1	4	2	1	6	2	4	3	5
17	3	6	2	2	1	2	1	1	6	2	1	5	4	2	8	3	5	4	1	4	2	1	4	2	4	3	5
18	3	6	2	2	1	3	1	1	6	2	1	5	4	2	8	3	5	4	1	4	2	1	4	2	4	3	5
19	3	6	2	2	1	3	1	1	6	2	1	5	4	2	8	3	5	4	1	4	2	1	4	2	4	3	5
20	N	4	6	3	2	7	3	N	6	2	3	2	9	7	1	7	7	3	1	3	4	3	2	1	6	5	2
21	6	1	5	4	4	4	3	N	2	6	3	2	6	6	3	5	9	6	2	1	4	1	7	N	1	6	10
22	6	1	4	4	4	4	3	N	2	6	3	1	6	4	2	5	9	7	2	N	4	N	7	4	1	4	1
23	5	3	1	6	3	5	1	N	3	5	4	2	7	1	2	5	8	3	1	2	5	2	1	4	2	8	3
24	N	4	6	3	3	7	3	N	2	6	3	2	8	5	6	5	10	8	1	1	3	N	3	4	3	7	4
25	4	3	1	5	3	6	2	2	1	5	2	2	9	3	7	6	4	5	1	3	1	4	1	3	5	9	7
26	4	3	1	5	3	6	2	2	1	5	2	2	9	3	7	6	4	5	1	3	1	4	1	3	5	9	8
27	4	3	1	5	3	6	2	2	1	5	2	2	9	3	7	6	N	5	1	3	1	4	1	3	5	9	7
28	4	3	1	5	3	6	2	2	1	5	2	2	9	3	7	6	4	5	1	3	1	4	1	3	5	9	7
29	4	3	1	5	3	6	2	2	3	5	2	2	10	3	7	6	4	5	1	3	1	4	1	3	5	9	6
30	4	3	1	5	3	6	2	2	3	5	2	2	10	3	7	6	4	5	1	3	1	4	1	3	5	9	6

* Graphic representations of the phenotypes of all the enzymes can be seen in Figure 1. N = no bands were detected. For explanation of enzyme designations see Table 3.

TABLE 5. Enzymatic distances* (upper triangle) and coefficients of similarity† (lower triangle) among 30 populations of *Meloidogyne*.

Pop. #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1		13	9	4	12	15	11	22	25	25	39	40	39	38	40	41	39	40	40	77	88	94	98	89	104	105	103	104	105	105
2	.83		8	13	9	18	6	17	22	22	34	35	34	33	35	36	34	35	35	81	81	90	86	86	95	96	96	95	96	96
3	.88	.89		13	15	16	2	21	28	28	36	37	36	35	37	38	36	37	37	83	83	90	89	90	97	98	98	97	98	98
4	.94	.82	.83		13	13	11	18	23	23	38	38	38	36	38	39	38	38	38	82	84	91	94	85	102	103	101	102	100	100
5	.84	.87	.80	.83		13	13	14	17	17	38	38	38	36	38	39	38	38	38	82	84	93	90	89	98	99	97	98	99	99
6	.80	.75	.78	.82	.82		12	13	22	22	34	35	34	33	35	36	34	35	35	79	81	90	85	82	97	97	98	97	98	98
7	.86	.91	.97	.85	.82	.83		19	26	26	34	35	34	33	35	36	34	35	35	83	81	90	85	88	95	96	96	95	96	96
8	.71	.76	.72	.76	.80	.81	.74		17	17	35	36	35	34	36	37	35	36	36	76	72	81	82	79	94	95	93	94	93	93
9	.68	.70	.64	.70	.76	.69	.66	.75		0	32	33	32	31	33	34	32	33	33	76	82	88	81	82	89	90	88	89	90	90
10	.68	.70	.64	.70	.76	.69	.66	.75	1		32	33	32	31	33	34	32	33	33	76	82	88	81	82	89	90	88	89	90	90
11	.54	.56	.56	.54	.54	.56	.57	.54	.57	.57		1	0	1	1	4	0	1	1	71	77	84	77	72	83	84	82	83	82	82
12	.53	.56	.54	.54	.53	.55	.56	.53	.56	.56	.98		1	2	0	3	1	0	0	70	76	83	78	71	82	83	81	82	81	81
13	.54	.56	.56	.54	.54	.56	.57	.54	.57	.57	1	.98		1	1	4	0	1	1	71	77	84	77	72	83	84	82	83	82	82
14	.54	.57	.56	.56	.55	.56	.58	.55	.58	.58	.98	.97	.98		2	3	1	2	2	70	76	83	76	69	82	83	81	82	81	81
15	.53	.56	.54	.54	.53	.55	.56	.53	.56	.56	.98	1	.98	.97		3	1	0	0	70	76	83	78	71	82	83	81	82	81	81
16	.53	.55	.54	.53	.52	.54	.55	.51	.57	.57	.98	.95	.98	.95	.95		4	3	3	69	73	80	75	68	79	80	78	79	78	78
17	.54	.57	.56	.54	.54	.56	.57	.54	.57	.57	1	.98	1	.98	.98	.93		1	1	71	77	84	77	72	83	84	82	83	82	82
18	.53	.56	.54	.54	.53	.55	.56	.53	.56	.56	.98	1	.98	.97	1	.95	.98		0	70	76	83	78	71	82	83	81	82	81	81
19	.53	.56	.54	.54	.53	.55	.56	.53	.56	.56	.98	1	.98	.97	1	.95	.98	1		70	76	83	78	71	82	83	81	82	81	81
20	.18	.15	.15	.16	.16	.15	.14	.16	.16	.16	.18	.22	.18	.19	.22	.22	.18	.22	.22		63	60	73	48	72	73	72	72	73	73
21	.11	.10	.11	.13	.10	.11	.12	.14	.10	.10	.10	.11	.10	.11	.11	.11	.10	.11	.11	.12		20	62	45	84	85	83	84	83	83
22	.13	.11	.13	.14	.11	.10	.12	.14	.10	.10	.12	.13	.12	.13	.13	.13	.12	.13	.13	.22	.62		70	48	93	94	92	93	90	90
23	.12	.15	.14	.14	.13	.14	.15	.15	.16	.16	.17	.17	.17	.18	.17	.18	.17	.17	.17	.14	.18	.20		49	64	66	65	64	61	61
24	.10	.08	.07	.12	.07	.09	.07	.09	.08	.08	.13	.13	.13	.14	.13	.14	.13	.13	.13	.25	.22	.27	.32		65	66	64	65	61	61
25	.09	.10	.09	.09	.09	.08	.10	.08	.11	.11	.14	.14	.14	.14	.14	.14	.14	.14	.14	.13	.01	.04	.24	.14		1	1	0	3	3
26	.09	.09	.09	.09	.09	.08	.10	.08	.11	.11	.13	.14	.13	.14	.14	.14	.13	.14	.14	.12	.01	.04	.21	.14	.98		2	1	4	4
27	.09	.09	.08	.09	.09	.08	.09	.08	.11	.11	.14	.14	.14	.14	.14	.14	.14	.14	.14	.13	.01	.04	.23	.15	.98	.96		1	4	4
28	.09	.10	.09	.09	.09	.08	.10	.08	.11	.11	.14	.14	.14	.14	.14	.14	.14	.14	.14	.13	.01	.04	.24	.13	1	.98	.98		3	3
29	.09	.09	.09	.09	.09	.08	.10	.09	.11	.11	.15	.15	.15	.15	.15	.15	.15	.15	.15	.14	.01	.06	.27	.18	.94	.93	.92	.94		0
30	.09	.09	.09	.09	.09	.08	.10	.09	.11	.11	.15	.15	.15	.15	.15	.15	.15	.15	.15	.14	.01	.06	.27	.18	.94	.93	.92	.94	1	

* Number of isozyme band differences between each population pair.

† Jaccard's coefficient of similarity (6,19).

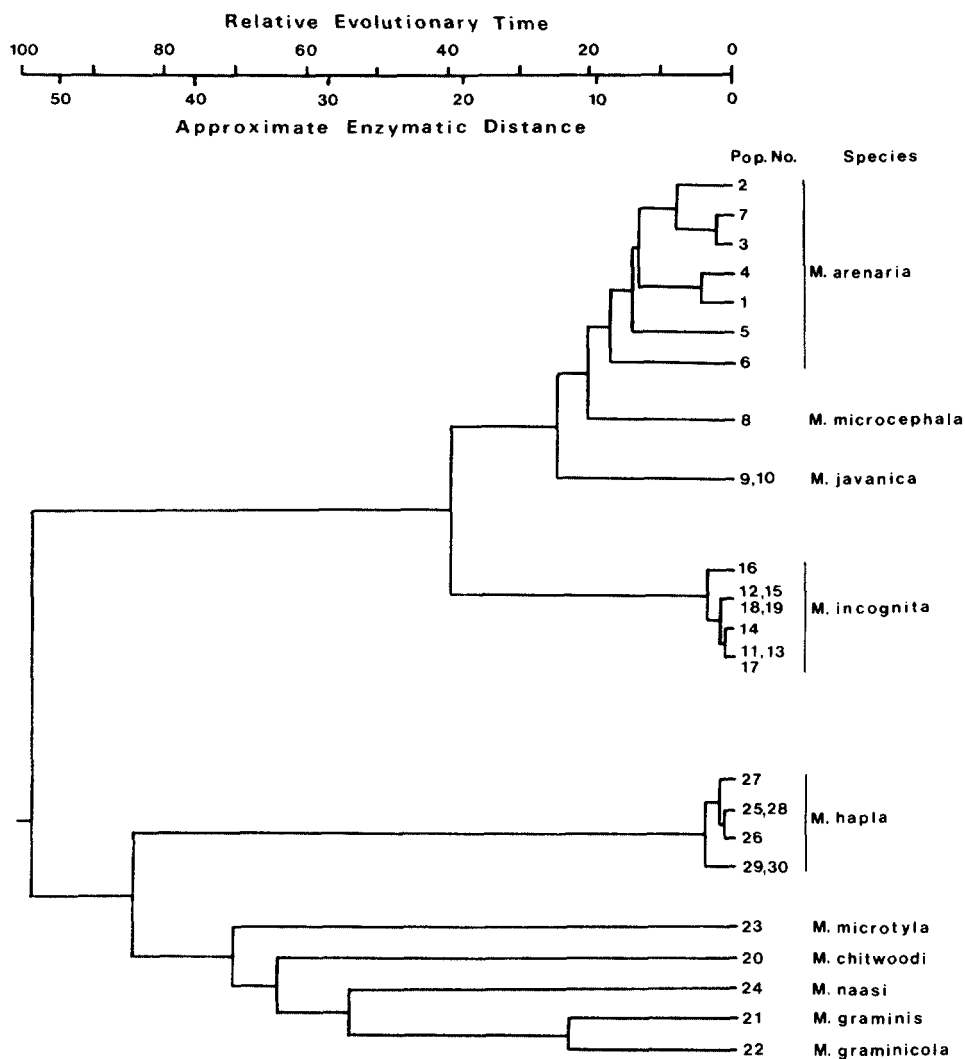


FIG. 2. Phylogenetic tree of 30 populations, representing 10 *Meloidogyne* species, derived from evaluation of 27 enzymes, using the Fitch-Margoliash algorithm and the KITSCH program of Felsenstein (9).

found that the tree-branch points and lengths were stable under a variety of conditions.

DISCUSSION

The present biochemical study generally supports previous assumptions about the evolutionary relationships of some members of the genus *Meloidogyne* (21,24) and provides new information regarding the interrelationships of additional species and various reproductive and cytogenetic forms.

Except for the mitotically parthenoge-

netic populations of *M. hapla*, all obligatorily (mitotic) parthenogenetic populations belonging to various species (*M. arenaria*, *M. microcephala*, *M. javanica*, and *M. incognita*) are grouped together on a major branch of the phylogenetic tree, opposite the branch that contains the amphimictic and facultatively parthenogenetic species. A similar phylogenetic arrangement was also suggested in an earlier enzymatic investigation of the four prevalent species of *Meloidogyne* (5). Such an arrangement indicates a close phyletic relationship and a monophyletic origin of the

mitotically parthenogenetic species (except *M. hapla* race B) without, however, indicating any amphimictic progenitors of these species. In reality the mitotically parthenogenetic forms may have evolved more recently from amphimictic progenitors which by chance have not been included in this investigation. Such progenitors may exist in low frequencies, occur in limited geographical areas of the world, or may have become extinct and been replaced entirely by the more successful mitotic forms. It is evident that it will be difficult to draw any definitive conclusions about the pathway of derivation of these obligatorily parthenogenetic forms until additional information regarding their direct ancestors becomes available.

Among the mitotic species, *M. microcephala* is closely related to *M. arenaria*. This is consistent with general morphology (2) and various cytogenetic features (24). *M. javanica* also is closely related to *M. arenaria*, and this relationship has been recognized earlier in biochemical (3,5) and cytological studies (24). *M. incognita* is distantly related to the other mitotic species ($S = 0.53-0.58$) distinct from that of the other species. This conclusion is supported by several unique cytological features such as the clumping of the chromosomes during a very prolonged prophase stage in maturing oocytes (22).

All amphimictic or facultatively parthenogenetic species are enzymatically distantly related to each other and to the mitotic species. This wide enzymatic divergence suggests that these species are older than the mitotic species. The only exception involves *M. graminis* and *M. graminicola* which are quite similar ($S = 0.62$). *M. hapla*, which is distinct from the other species of this group by its inability to infect graminaceous plants and by the reduced chromosome numbers ($n = 14-17$, compared with $n = 18$ of the other species), has branched off the phylogenetic tree quite early. In addition, *M. hapla* has evolved further cytogenetically with the establishment of a mitotically partheno-

genetic form (race B). This form is enzymatically similar to the predominant, facultatively parthenogenetic form (race A) and probably is of recent origin.

From the preceding discussion it can be concluded that the phylogenetic tree of Figure 2, derived from enzymatic data, is in good agreement and supports well previous knowledge about interrelationships of root-knot nematodes based on morphological, host range, and cytogenetic data. Still, one should consider that, for the construction of the phylogenetic tree, we recognized each of the 184 bands as an independent biochemical or genetic character. This assumption disregards the effects of protein structure (monomeric-polymeric), polyploidy (more than two alleles per locus) and heterozygosity (additional bands in the heterozygotes) on the banding pattern of each enzyme. All these aspects need to be investigated and clarified before a more authoritative phylogenetic tree can be constructed. In the present tree, the enzymatic and genetic distances undoubtedly are overestimates of the true phyletic distances among the species studied. If the rate of evolution in these nematodes as estimated by electrophoretic differences is assumed to be the same as that described for the cytochrome c molecule of various organisms, then the value of 100 for relative evolutionary time given in Figure 2 would correspond to approximately 43 million years (9,10). Again this number may be an overestimate of actual evolutionary time.

Intraspecific variation was minimal in all species studied with two or more populations, suggesting little enzymatic evolution within species. The same conclusion was reached in an earlier investigation in which a large number of conspecific populations were examined with regard to four enzyme systems (8). A similar conclusion was reached in a comparison of general proteins and specific enzymes from several pathotypes of the cyst nematodes *Globodera rostochiensis* and *G. pallida* (11). The coefficient of similarity was very high ($CS =$

0.95–1.00) among pathotypes of the same species and only slightly lower (CS = 0.88–0.92) between the two species. Such findings suggested a recent speciation of these organisms, which are also closely related in many other respects. Butler et al. (1) reported a close enzymatic relationship for two conspecific populations of the free-living nematode *Caenorhabditis elegans* obtained from different localities, whereas two “sister” species, *C. elegans* and *C. briggsae*, exhibited a large amount of enzymatic divergence.

The most extensive intraspecific variation was detected in *M. arenaria*, a species known to be morphologically, physiologically, and cytogenetically highly variable (2,24). Still, all populations of *M. arenaria* were grouped together in the phylogenetic tree (mean enzymatic distance = 11.2 ± 4.2) and were clearly separated from all the other species except *M. microcephala* and possibly *M. javanica*. Actually, all populations of *M. arenaria* were monomorphic, but extensive enzymatic variation was detected among populations, especially within the triploid form. This variation may indicate multiple origins for populations classified as *M. arenaria*. Alternatively, the variation may indicate that *M. arenaria* is an old species that has diverged considerably through multiple mutations and adaptations to different environments.

We detected few isozymic differences within each of the species *M. hapla*, *M. incognita*, and *M. javanica*, although there was considerable cytogenetic variation present in all these species (Tables 1, 4). Similarly, Turner et al. (25) found only slight enzymatic divergence in three distinct cytotypes of the goodeid fish *Ilyodon furcidens* and concluded that changes in enzymes are not correlated with evolution of the karyotype.

The occurrence of mitotic parthenogenesis in two widely separated groups (i.e., the one including *M. arenaria*, *M. microcephala*, *M. javanica*, and *M. incognita* and the other containing *M. hapla* race B) demonstrates that evolution toward mitotic

parthenogenesis in *Meloidogyne* occurred independently at least twice.

LITERATURE CITED

- Butler, M. H., S. M. Wall, K. R. Luehrsen, G. E. Fox, and R. M. Hecht. 1981. Molecular relationships between closely related strains and species of nematodes. *Journal of Molecular Evolution* 18:18–23.
- Cliff, G. M., and H. Hirschmann. 1984. *Meloidogyne microcephala* n. sp. (Meloidogynidae), a root-knot nematode from Thailand. *Journal of Nematology* 16:183–193.
- Dalmasso, A., and J. B. Bergé. 1983. Enzyme polymorphism and the concept of parthenogenetic species exemplified by *Meloidogyne*. Pp. 187–196 in A. R. Stone, H. M. Platt, and L. F. Khalil, eds. *Concepts in nematode systematics*. New York: Academic Press.
- Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Annals of the New York Academy of Sciences* 121:404–427.
- Dickson, D. W., D. Huisinigh, and J. N. Sasser. 1971. Dehydrogenases, acid and alkaline phosphatases, and esterases for chemotaxonomy of selected *Meloidogyne*, *Ditylenchus*, *Heterodera*, and *Aphelenchus* spp. *Journal of Nematology* 3:1–16.
- Dunn, G., and B. S. Everitt. 1982. *An introduction to mathematical taxonomy*. London: Cambridge University Press.
- Eisenback, J. D., H. Hirschmann, J. N. Sasser, and A. C. Triantaphyllou. 1981. *A guide to the four most common species of root-knot nematodes (Meloidogyne species) with a pictorial key*. Raleigh: North Carolina State University Graphics.
- Esbenshade, P. R., and A. C. Triantaphyllou. 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* 17:6–20.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.
- Fitch, W. M., and E. Margoliash. 1967. Construction of phylogenetic trees. *Science* 155:279–284.
- Fox, P. C., and H. J. Atkinson. 1984. Isoelectric focusing of general protein and specific enzymes from pathotypes of *Globodera rostochiensis* and *G. pallida*. *Parasitology* 88:131–139.
- Harris, H., and D. A. Hopkinson. 1976. *Handbook of enzyme electrophoresis in human genetics*. Amsterdam: North-Holland Biomedical Press.
- Jackson, R. C. 1976. Evolution and systematic significance of polyploidy. *Annual Review of Ecology and Systematics* 7:209–234.
- Janati, A., J. B. Bergé, A. C. Triantaphyllou, and A. Dalmasso. 1982. Nouvelles données sur l'utilisation des isoesterases pour l'identification des *Meloidogyne*. *Révue de Nematologie* 5:147–154.
- Komuniecki, R., and H. J. Saz. 1979. Purification of lipoamide dehydrogenase from *Ascaris* muscle mitochondria and its relationship to NADH:NAD+

transhydrogenase activity. *Archives of Biochemistry and Biophysics* 196:239-247.

16. Lapp, N. A., and A. C. Triantaphyllou. 1972. Relative DNA content and chromosomal relationships of some *Meloidogyne*, *Heterodera*, and *Meloidodera* spp. (Nematoda: Heteroderidae). *Journal of Nematology* 4:287-291.

17. Ornstein, L. 1964. Disc electrophoresis. I. Background and theory. *Annals of the New York Academy of Sciences* 121:321-329.

18. Smith, S. G. 1971. Parthenogenesis and polyploidy in beetles. *American Zoologist* 11:341-349.

19. Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. San Francisco: W. H. Freeman and Co.

20. Taylor, A. L., and J. N. Sasser. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh: North Carolina State University Graphics.

21. Triantaphyllou, A. C. 1979. Cytogenetics of root-knot nematodes. Pp. 85-109 in F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (*Meloidogyne* species) systematics, biology, and control. New York: Academic Press.

22. Triantaphyllou, A. C. 1981. Oogenesis and the chromosomes of the parthenogenetic root-knot nematode *Meloidogyne incognita*. *Journal of Nematology* 13:95-104.

23. Triantaphyllou, A. C. 1983. Cytogenetic aspects of nematode evolution. Pp. 55-71 in A. R. Stone, H. M. Platt, and L. F. Khalil, eds. Concepts in nematode systematics. New York: Academic Press.

24. Triantaphyllou, A. C. 1985. Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. Pp. 113-126 in J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne*, vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.

25. Turner, B. J., T. A. Grudzien, K. P. Adkisson, and R. A. Worrell. 1985. Extensive chromosomal divergence within a single river basin in the goodeid fish, *Ilyodon furcidens*. *Evolution* 39:122-134.

26. Wendel, J. F., and C. W. Stuber. 1984. Plant isozymes: Enzymes studied and buffer systems for their electrophoretic resolution in starch gels. *Isozyme Bulletin* 17:4-11.

27. White, M. J. D. 1973. Animal cytology and evolution. Cambridge: Cambridge University Press.