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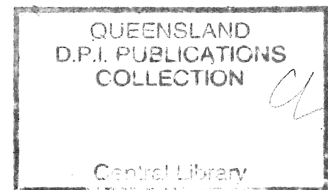

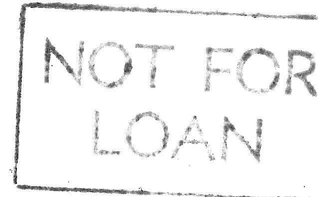

RURAL INDUSTRIES RESEARCH & DEVELOPMENT CORPORATION

FINAL REPORT

Project Title: Development of diagnostic probes for identification of species and races of root-knot nematode (*Meloidogyne* spp.)

Project Number: DAQ 103A

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A final report prepared for the Rural Industries R&D Corporation

(ii) NON-TECHNICAL SUMMARY

To control root-knot nematodes with the use of resistant cultivars or by rotation with non-host crops, it is necessary to identify the nematodes and their host ranges. However, traditional methods of identification are time-consuming, inaccurate and unreliable and most do not predict host range. More than 40 species have been described, some with more than one host race. The most common are *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* causing 95% of the damage worldwide.

Workers in Australia and overseas have detected a wide variety of DNA markers in an effort to differentiate species and races. However, with few exceptions, these studies compared only a small number of isolates. It is questionable whether those populations were characteristic of variation found in the field. The study requires a population genetics approach, with analysis of the distribution of molecular polymorphism within and among field isolates.

We have paid particular attention to polymorphisms in mitochondrial DNA (mtDNA) because of the large copy number of the mitochondrial genome, its small size, rapid evolutionary rate, ease of extraction and because the entire mitochondrial genome of *Meloidogyne javanica* has been sequenced.

We have examined a large number of Australian populations of *Meloidogyne* and characterised them by perineal pattern, standard host range, esterase phenotype and RFLP's of mtDNA. Using RFLP's of mtDNA, we have found 10 different mtDNA types, some of which correspond to those found by workers in the USA. We have also found additional mtDNA types. Esterase phenotypes corresponded perfectly with mtDNA type. Perineal pattern was not correlated with mtDNA type. Host range was reasonably well correlated with mtDNA type but there was a significant number of discrepancies. Also, we have found several novel host reactions which do not correspond to any known species or race.

Previous work by researchers in USA suggested greater than 3% sequence divergence between *M. javanica*, *M. incognita* and *M. arenaria*. However, our studies show less than 0.6% sequence divergence between these species. Nevertheless, there is more than 2% sequence divergence within *M. hapla* and more than 24% between *M. hapla* and the other species.

The standard host range test uses five hosts and only two reaction categories (resistant and susceptible) to represent the range of reactions of these species and races on more than 2,000 plant species. Reactions are often intermediate so the classification system forced populations into artificial groupings which may not have agricultural relevance. We propose that a host range test should use only extreme resistant and susceptible reactions.

Initial data show that some soybean cultivars, unlike the standard host range test, could distinguish between *M. arenaria* race 2 and *M. javanica* and between two host range types of haplotype D (*M. javanica*). These will be tested across a large number of populations of each haplotype.

We suggest that further studies of these nematodes should focus on well-defined molecular groups, whether or not these coincide with existing

taxonomic units. The aim of a host range test is to corroborate molecular differences. Where genetic differences occur, we need to show that these correlate with significant host range differences. Similarly, where host range differences occur, we must identify genetic differences to be able to distinguish different host races.

We have devised a PCR-based diagnostic for the mtDNA haplotypes identified so far. This is a simple test involving DNA amplification and RFLP analysis of the product. This test should be extended to differentiate further host races and for use with soil and root samples.

(iii) BACKGROUND

Root-knot nematodes are major pests of many agricultural and horticultural crops. Collectively, the various species of root-knot nematode affect nearly every agricultural, horticultural and ornamental crop grown (Table 1). Not only are yields affected but the quality and marketability of crops is also reduced. This nematode is currently estimated to cause crop losses of 5-20% under present control strategies, which usually depend on chemical nematicides (Bird 1978; O'Brien and Stirling 1991). Many of these chemicals are under threat because of health and environmental risks and efficacy problems and their high cost restricts their use to high value crops. We cannot assume that nematicides currently used will be available in the future.

Table 1. Crops affected by root-knot nematode in Australia.

Crop group	Crops
Tree and vine fruits	almond, grape, kiwifruit, nectarine, passionfruit, pawpaw, peach, plum
Vegetables	bean (mung, French, navy), beetroot, capsicum, carrot, celery, cucurbits (cucumber, melon, pumpkin), eggplant, lettuce, okra, onion, potato, sweet potato, tomato
Ornamentals	carnation, *Chrysanthemum, Dahlia*, gerbera, gladiolus, *Protea*, rose
Field crops	aloe vera, clover, cowpea, kenaf, lucerne, lupin, pigeon pea, peanut, soybean, sugarcane, tea, tobacco
Other horticultural crops	banana, ginger, pineapple, strawberry

Nematicides control all species and races of root-knot nematode but many non-chemical control methods are species-specific. To use sustainable management systems for root-knot nematodes based on crop rotation, resistant cultivars and biological control agents, it is necessary to detect and identify nematodes in root and soil samples. Current methods using

morphological features (Jepson 1987) and a standard host test (Eisenback *et al.* 1981) are insensitive, inaccurate and time-consuming. A molecular diagnostic would enable rapid and accurate detection and identification of root-knot nematodes in soil and root samples which would facilitate management recommendations.

(iv) OBJECTIVES

(a) To identify root-knot nematode species in a collection of single eggmass cultures from throughout Australia by traditional methods.

(b) To assess the genetic variation within and between the species of root-knot nematode in the culture collection.

(c) To compare the host ranges of different species and genetic races within a species of root-knot nematode.

(d) To develop a rapid and reliable genetic method for diagnosing species and races of root-knot nematode from soil samples and infected roots.

(v) INTRODUCTORY TECHNICAL INFORMATION

Identification of species of root-knot nematode has for many decades relied on morphological characters (Jepson 1987). The main character used has been the perineal pattern (i.e. the pattern of striae on the cuticle around the vulva and anus of the adult female). Although there are typical patterns for each of the four most common species, variability makes it unreliable. Other morphological characters include measurements of various structures. The most diagnostic appear to be the length of the female stylet, the distance from the head to the excretory pore, width of vulva, distance from anus to vulva and length of the tail of the second-stage juvenile. However, there is great variability and overlap of these measurements between species.

Esterase phenotype has been a useful character for differentiating some species (Esbenshade and Triantaphyllou 1990). Although morphological characters and esterase phenotypes may distinguish some species, they cannot differentiate host races within species. Furthermore, the standard host range test cannot distinguish between *M. javanica* and *M. arenaria* race 2.

Powers and Sandall (1988) found considerable variation in mtDNA restriction fragment patterns within and among 12 isolates representing different species and host races of *Meloidogyne* and this provided the basis for a subsequent assay using the polymerase chain reaction (PCR) and diagnostic *Hin*fI polymorphisms (Harris *et al.* 1990). However, the data obtained had some significant limitations. A small number of isolates were examined and some of these were misidentified (Hyman and Powers 1991) resulting in an overestimate of within-species variation. Moreover, the analyses of whole-genome restriction fragment variation (Powers and Sandall 1988; Powers *et al.* 1986) were potentially confounded by insertion/deletion events.

The analysis and use of RFLP's is greatly enhanced when the variable sites can be located, either by restriction site mapping or by reference to a sequence. The complete nucleotide sequence of a *M. javanica* mtDNA has been determined (Okimoto *et al.* 1991; D. Wolstenholme pers. comm.). This genome (Figure 1) contains the same number of proteins, rDNA and tRNA genes as other nematode mtDNA's but is distinguished by an apparently non-coding region of about 7 kb that includes ~36 copies of a 102 bp sequence and ~11 copies of a 63 bp sequence. Okimoto *et al.* (1991) also reported variation in the sizes of the repeat-containing restriction fragments among mtDNA from different races and species of *Meloidogyne*. They suggested that these differences, most likely caused by variation in repeat copy number, may provide a useful diagnostic tool.

Harris *et al.* (1990) were able to differentiate three haplotypes of *Meloidogyne* using *Hin*fI digestion of a 1.8 kb amplified product of the *cyt*b gene and an intergenic region. However, this test did not distinguish between *M. hapla* and *M. javanica* and no amplification was obtained for some populations of *M. arenaria*. This lack of amplification was resolved by a further primer located in the 16srDNA (T.O. Powers pers. comm.). Powers and Harris (1993) reported a test to differentiate between five haplotypes corresponding to *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla* and *M. chitwoodi*. This used an amplification product of the COII and 16srDNA genes and digestion with *DraI* and *Hin*fI.

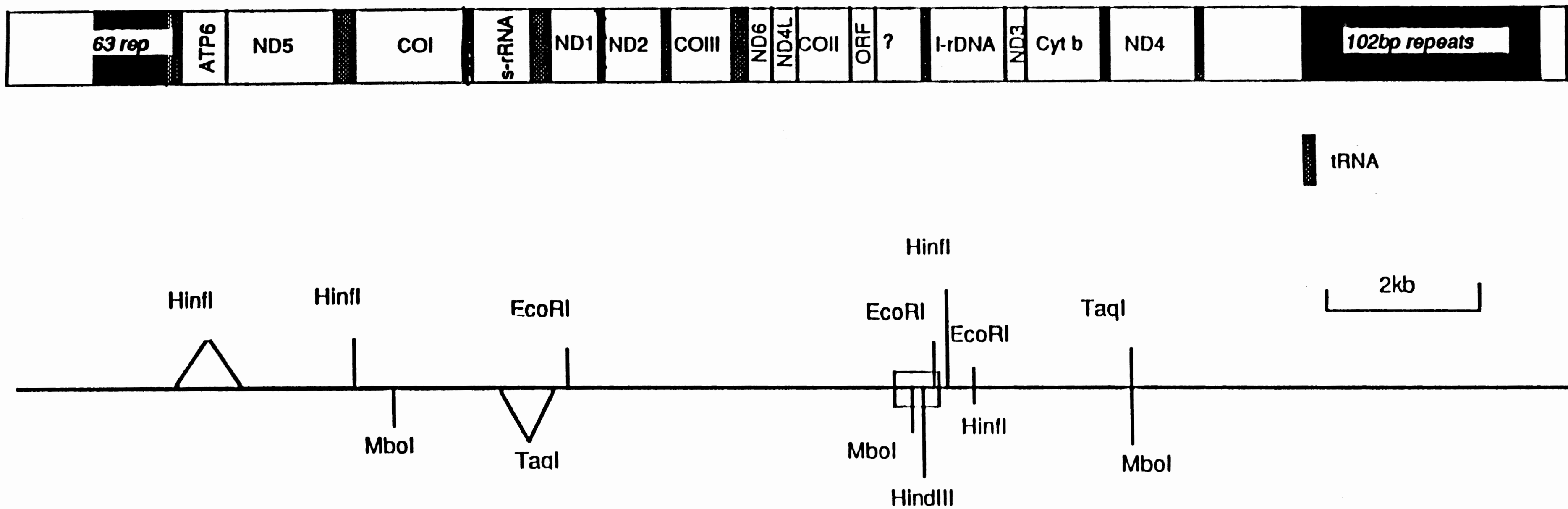
(vi) RESEARCH METHODOLOGY

Nematode populations were selected from the QDPI root-knot nematode collection which now contains over 100 single eggmass populations from throughout Australia. Eggs were removed from roots in 0.5% sodium hypochlorite (Southey 1986) for DNA and host range studies. Females were extracted from roots by shaking in 5% Clariphase(R) (containing pectinolytic enzymes) overnight. Twenty females of each population were examined (Jepson 1987) for perineal pattern, stylet length and position of excretory pore. Females for esterase studies were dissected by hand from roots. Esterase phenotypes were determined by the method of Esbenshade and Triantaphyllou (1985). Total and mitochondrial DNA were prepared from eggs as described in Dowling *et al.* (1990) except that eggs were homogenised by hand with mortar and pestle for 5 min before centrifugation.

The standard host range test is described by Taylor and Sasser (1978). Plants were inoculated with 6,000 eggs each with treatments replicated four times. The host plants used were *Capsicum frutescens* L. (capsicum) cv. California Wonder, *Gossypium hirsutum* L. (cotton) cv. Deltapine 16, *Arachis hypogaea* L. (peanut) cv. Florunner, *Lycopersicon esculentum* Mill. (tomato) cv. Tiny Tim, *Nicotiana tabacum* L. (tobacco) cv. NC95 and *Citrullus vulgaris* Schrad. (watermelon) cv. Charleston Gray. After 60 days, roots were washed and stained with 0.15% phloxine B to highlight eggmasses.

The standard host test uses eggmass ratings according to the following scale: 0 = 0 eggmasses per plant, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = >100. If more than 100 eggmasses were found, no more were counted. In the standard differential host test, plants with average ratings of 2 or less are classified as resistant and those with ratings greater than 2 are classified as

Figure 1. Positions of ten polymorphic restriction enzyme sites and a 529 bp deletion on the *Meloidogyne* mitochondrial DNA moleculae (bottom) aligned with the gene map that also shows the positions of the 63 bp and 102 bp tandem repeats. The deletion in haplotype A is shown as a box and encompasses *Eco*RI, *Hin*dIII and *Mbo*I sites.



susceptible. The reaction of each population to the six hosts is used to assign a species and race (Table 2).

Further information on host range was obtained by screening large numbers of plant genotypes with selected nematode populations to identify possible sources of resistance in plants easily grown in the glasshouse. Firstly, four nematode populations representing haplotypes A, B and D were tested against 50 plant genotypes (Table 3). This revealed possible differences within soybean and cowpea. These, along with other plant genotypes, were tested for resistance to five nematode populations representing haplotypes A, B, C, and D and another population with haplotype D but with the type 2 novel host range (Table 4). Populations of *M. hapla* were tested against cultivars of crops with potential for variation in resistance (Table 5).

MtDNA haplotypes were initially identified from RFLP's produced by digestion with *Eco*RI, *Dra*I, *Mbo*I, *Hin*dIII and separation of end-labelled fragments on 1.5% agarose and 3.5% polyacrylamide gels (Hugall *et al.* 1994). Later, the complete restriction map of mtDNA of *M. javanica* (D. Wolstenholme pers. comm.) and high resolution gels were used to identify all restriction sites. Primers designed from the *M. javanica* sequence (D. Wolstenholme pers. comm.) (Table 6; Figure 2) and other primers (Harris *et al.* 1990; Powers, T.O. pers. comm.) were used to sequence the $tRNA^{His}$, 16srDNA, ND3 and 5' end of *cyt*b genes. This provided 2161 bp of sequence from representatives of haplotypes A, B, C, D, G and H.

A 1.8 kb fragment, identified as the *M. hapla* homologue to that sequenced in haplotypes A, B, C, D, G and H (16srDNA, ND3, cytb), was cloned from population 113 and sequenced. From this, primers were designed to amplify and sequence this region in all *M. hapla* populations (populations 48, 102, 113, 114 and 150). A 102 bp tandem repeat of population 113 of *M. hapla* 113 was characterised by sequencing. Nuclear rDNA was assessed for representatives of haplotypes by sequencing the ITS/5.8srDNA and intergenic spacer (IGS) regions using conserved primers.

(vii) DETAILED RESULTS

Perineal pattern was poorly correlated with other characters measured (Table 7). Host range was reasonably well correlated with esterase phenotype and mtDNA haplotype but there were significant discrepancies and the appearance of novel host range types (Table 8). Other morphological measures (Table 9) did not correlate well with perineal pattern, host range or biochemical characters.

Initial screening identified soybean and cowpea as possible sources of variation in resistance and were easily grown in the glasshouse. Further screening showed that reproduction on four cultivars of soybean, cvs. Dragon, Manark, Forrest and Triton, could differentiate the haplotypes of *M. arenaria* race 2 and *M. javanica*. It confirmed the difference between novel host range type 2 and other standard host ranges (Table 4).

Sequence divergence between haplotypes, other than those corresponding to *M. hapla*, was less than 0.6% (Figure 3). Sequence divergence between populations of *M. hapla* were greater than 2% while divergence between the

Table 2. Usual responses of the four common *Meloidogyne* species and their reactions to the standard differential Host Test and novel reactions of some Australian populations.

Meloidogyne species and race		Differential host plants*					
		CP	CT	PN	TB	TM	WM
M. incognita	race 1	+	-	-	-	+	+
	race 2	+	-	-	+	+	+
	race 3	+	+	-	-	+	+
	race 4	+	+	-	+	+	+
M. arenaria	race 1	+	-	+	+	+	+
	race 2	-	-	-	+	+	+
M. javanica		-	-	-	+	+	+
M. hapla		+	-	+	+	+	-
novel (1)		+	-	-	+	+	-
novel (2)		-	-	-	-	+	?
novel (3)		-	-	-	+	+	-
novel (4)		-	-	+	+	+	-
novel (5)		+	-	+	-	+	-
novel (6)		+	-	+	-	+	+

* CP, *Capsicum frutescens* (capsicum) cv. California Wonder; CT, *Gossypium hirsutum* (cotton) cv. Deltapine 16; PN, *Arachis hypogaea* (peanut) cv. Florunner; TB, *Nicotiana tabacum* (tobacco) cv. NC95; TM, *Lycopersicon esculentum* (tomato) cv. Tiny Tim; WM, *Citrullus vulgaris* (watermelon) cv. Charleston Gray.

Table 3 Screening for wide differences in host range showing eggmass ratings[a]

Population	15	35	44	51
Haplotype	A	B	D	D
Standard host range	i1	i1	i2	j/a2
Aeschynomene elegans	2	2	2	2
Aeschynomene brasiliana	-	-	0	0
Aeschynomene histrix	-	0	0	-
Aeschynomene villosa	0	-	2	2
Aeschynomene sp.	2	-	2	2
Aeschynomene falcata	2	-	2	1
Aeschynomene paniculata	0	0	1	2
Aeschynomene filosa	0	0	2	2
Avena sativa cv. Minhafer	2	1	1	1
Avena sativa cv. Cooba	0	1	0	0
Avena sativa cv. Saia	0	1	0	0
Avena sativa cv. Garry	1	1	1	1
Axillaris	3	4	3	2
Axonopus sp.	0	2	0	0
Bromus inermis	3	3	3	3
Canavalia ensiformis	3	2	3	4
Cenchrus cilliaris cv. Biloela	1	2	3	2
Cyamopsis senegalensis	2	2	2	2
Cyamopsis tetragonoloba	2	2	2	2
Desmodium intortum	1	1	1	0
Desmodium uncinatum	2	1	2	1
Glycine max cv. Coker 136	0	0	0	0
Glycine max cv. Bossier	0	1	0	0
Glycine max cv. Coker 338	0	0	0	0
Glycine max cv. Cobb	0	0	0	0
Glycine max cv. Bragg 5	0	0	0	0
Glycine max cv. Dragon	2	3	0	0
Glycine max cv. Blackhawk	0	0	0	0
Glycine max cv. Davis	3	0	3	2
Indigofera suffriticosa	2	-	3	1
Indigofera schimperi	0	-	-	2
lucerne cv. L 220	0	0	0	0

lucerne cv. WL 516	1	0	0	0
lucerne cv. Nova	3	1	0	0
lucerne cv. Sequel	0	0	0	0
Okra cv. Clemson Spineless	4	4	5	5
Pennisetum glaucum	3	3	3	3
Sorghum cv. Martin	4	1	2	2
tomato BTN 280	3	3	1	3
tomato BTN 828	4	3	4	4
tomato BTN 826	4	4	4	4
tomato BTN 824	2	2	1	1
tomato BTN 825	2	3	2	3
tomato BTN 827	1	1	2	2
tomato BTN 627	2	1	1	1
Vigna unguiculata cv. Eureka	4	5	4	5
Vigna unguiculata cv. Red Caloona	2	4	4	4
Zea mays cv. PS Hycorn 40	4	4	4	3
Zea mays cv. Rush DK 529	3	3	3	2
Zea mays cv. XL80 Super Grit	2	2	1	2

[a] Eggmass rating: 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = >100 eggmasses per plant.

Table 4 Extended host range testing of populations other than *M. hapla* showing numbers of eggmasses per plant after inoculation with 5,000 eggs

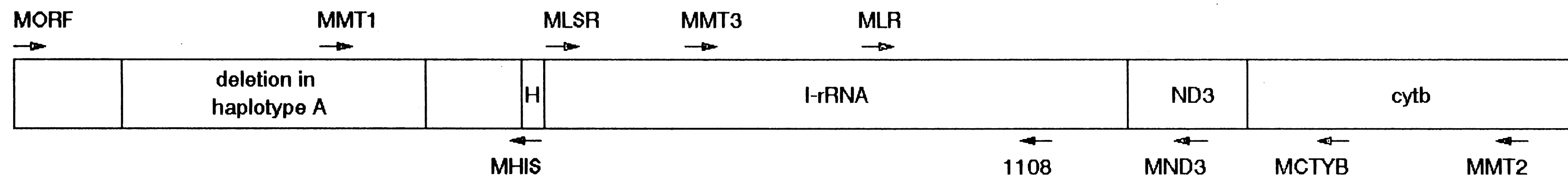
Population		39	44	78	G	NQ5
Haplotype		A	D	D*	B	C
Species	Cultivar					
Tomato	Tiny Tim	>100	>100	>100	>100	>100
	Zola	0	13	71	15	11
Soybean	Forrest	0	92	5	0	58
	Centaur	1	29	-	2	0
	Dragon	0	0	33	65	79
	Manark	0	93	2	9	2
	Actolac	-	40	>100	>100	>100
	Davis	1	90	>100	>100	>100
	Triton	1	>100	4	41	2
Cowpea	Havana	75	63	>100	73	>100
	Caloona	0	53	68	19	>100
	Chinese Red	88	82	>100	>100	>100
	Red Caloona	1	35	87	21	98
	Blackeye 5	88	>100	>100	>100	>100
	Cristando	>100	41	94	94	80
	Soutter	31	>100	>100	>100	>100
	Santiago	4	45	79	>100	>100
Okra	Clemson Spineless	>100	>100	>100	>100	>100
Sweet potato	LO323	39	0	9	9	0
	Resisto	0	0	1	0	0
	Abundance	9	0	0	1	0
	Roso Blanco	47	49	11	>100	98
	Beerwah Gold	38	88	57	88	47

D*, Haplotype D with novel host range type 2.

Table 5 Extended host range testing of *M. hapla* showing numbers of eggmasses per plant after inoculation with 5,000 eggs

Population		113	114	115	150	156
Haplotype		F	I			
Species	Cultivar					
Cucumber	Green Gem	>100	>100	>100	>100	>100
Strawberry	Redlands Crimson	91	>100	2	39	77
Lettuce	Green Mignonette	96	87	>100	>100	94
Lucerne	Sequel	56	95	34	67	75
Peanut	Florunner	71	>100	56	>100	56
Carrot	All Seasons	96	>100	>100	16	>100
Tomato	Tiny Tim	>100	>100	>100	>100	>100

Figure 2. Mitochondrial DNA of *Meloidogyne* showing polymerase chain reaction primer sites.



H, $tRNA^{His}$

Table 6. Primers used in this study

Primer name	Sequence	Gene	3' position	Reference
C2F3	5'-GGTCAATGTTCAGAAATT-3'	COII	?	Powers and Harris (1993)
MORF	5'-ATCGGGGTTTAATAATGGG-3'	ORF	9298	this study
MMT1	5'-TAAATCAATCTGTTAGTGAA-3'	intergenic	9827	Harris *et al.* (1990)
MHIS	5'-AAATTCAATTGAAATTAATAGC-3'	$tRNA^{His}$	10018	this study
TRNAH	5'-TGAATTTTTTATTGTGATTAA-3'	$tRNA^{His}$	10054	this study
MMT3	5'-GAAAAATAAAAAAATTTTGTT-3'	l-rRNA	10212	T.O. Powers pers. comm.
MLR	5'-ATGATTTTTGTGTCTGCTCA-3'	l-rRNA	10414	this study
1108	5'-TACCTTTGACCAATGACGCT-3'	l-rRNA	10436	Powers and Harris (1993)
MND3	5'-TTTCCCAACCTATTAAAACCTCT-3'	ND3	10972	this study
MCYTB	5'-AATCTGCTCCATTTAACT-3'	cyt b	11214	this study
MMT2	5'-ATAAACCAGTATTTCAAACT-3'	cyt b	11492	Harris *et al.* (1990)

Table 7 Characterisation of Australian populations of root-knot nematode

Pop	Host	Region[1]	Perineal pattern[2]	Standard host range[2]	Esterase phenotype[3]	mtDNA haplotype[4]
1	banana	BR	j	ja2		D
3	pigeon pea	BR	j	ja2?		D
5	kiwi fruit	MO	j	ja2?	J3	D
11	grape	DD	j	ja2	J3	D
12	grape	DD	a	#1	S2M	G
15	pineapple	MO	a	i1		A
16	kiwifruit	MO	j	ja2	J3	D
18	banana	MO	a			D
19	ginger	MO	a	i1		
20	sugarcane	MO				
21	sugarcane	MO	j	ja2?		D
33	pasture	FN		i2		C
35	banana	FN	a	i1	I1	B
37	sugarcane	FN	j	ja2	J3	D
39	grape	FN	a	i2	A2	A
40	grape	FN	j		J3	D
42	sugarcane	FN	j	i2	J3	D
44	peanut/maize	BU	a	i2	J3	D
46	banana	MO				D
47	banana	MO	a	ja2		D
48	kiwi fruit	MO	h	h	H1	E
50	tobacco	FN		ja2		
51	tomato	BU	a	ja2	J3	D
53	peanut	MO	h		J3	D
54	kiwifruit	MO				D
58	pineapple					D
60	african violet		j	ja2		D
63	tobacco	MO	j	ja2		D
65	melon	NT	a	ja2	a2	A
66	banana	FN	j			

68	sweet potato	BU	a		a1	C
69	leucaena	BR	j	ja2		D
70	tomato	BU	j			D
71	babaco	MO	a			D
72	lucerne	MO	a	ja2	J3	D
73	heliconia	FN	a		J3	
74	Leucospernum	DD		ja2?		
75	grape	VIC	j	ja2?	A3	A
76	stonefruit	BR	j	ja2?		
77	ginger	MO	a	ja2?	A1	H
78	cucurbits	NO	a	#2	J3	D
79	pawpaw	NO	a	i2?	I1	B
82	lucerne	SA	j	ja2	J3	D
83	lucerne	SA	j	ja2	J3	D
84	kiwi fruit	SA	j			
85	passion fruit	SA	j	?		D
86	pecan	SA	j	i2	J3	D
87	fig	SA	j	ja2		
88	pomegranate	SA	a	#2		D
89	avocado	SA	j			D
90	pistachio	SA	j		J3	D
91	grape	SA	j	ja2?		D
92	grape	SA	-	ja2		
93	ginger	MO	a			D
94	ginger	MO	j	ja2	J3	D
95	almond	SA	j			
96	okra	SA	j	ja2?		
97	grape	SA	j			
98	grape	SA	j	#2	J3	D
99	carrots	WA	h			
100	silver beet	NSW	i			
101	grape	SA	j	ja2	J3	
102	carrot	WA	h	#4	H1	J
103	tomato	SA	j			

104	carrot	WA	h/j			
105	maize	FN	a			
106	tomato	WA	j	ja2		
107	cucumber	SA	a			
110	kiwi fruit	MO	j			
111	nightshade	WA	j			
113	peanut	MO	h	#5	H1	F
114	peanut	FN	j	a1	H1	I
115	peanut	FN	h	?		
116	banana	MO	a	?		
118	tobacco	VIC	j		J3	
121	tobacco	VIC	a	ja2	J3	
122	tobacco	VIC		?		
123	tobacco	VIC		ja2	J3	
125	tobacco	VIC	j	i2	J3	
126	tobacco	VIC	a	?	J3	
127	tobacco	VIC	a	i2?	J3	
128	tobacco	VIC	j/a	?		
129	banana	MO		?		
130	banana	MO		ja2		
132	cucumber	SA		i2		
133	banana	MO		ja2		
134	carrot	BR		ja2		
135	african violet	BR		i2?		
147	white clover	TAS			J#	
150	parsnip White Gold	TAS				
151	lucerne					
156	riceflower	NSW				
157	mullinbimbi couch	FN				
A	tomato	BU	j	#3		D
B	tomato	MO	j	ja2	J3	D
C	banana	FN	j	ja2?	J3	D
D	olive	SW	j	i2		D

G	Thunbergia	FN	i	i1	I1	B
H	home garden	BU	i	i2	I1	B
I	vegetable	NO	j	i1	I1	B
J	dolichos	NO	i		I1	B
K	sugarcane	NO	j			B
NQ1	tobacco	FN	a	ja2	A2	A
NQ2	tobacco	FN	j	ja2	J3	D
NQ5	tobacco	FN	?	ja2	A1	C
NQ7	tobacco	FN	a	ja2	A1	C
V	duboisia	BU	j		J3	D
X	carnation	FN	j			D
Y	taro	FN	j	ja2	A2	A
Z	banana	FN	i	i2	I1	B

[1] NSW, NT, SA, TAS, VIC, WA - New South Wales, Northern Territory, South Australia, Tasmania, Victoria, Western Australia; BR, BU, DD, FN, MO, NO, SW - Brisbane, Bundaberg, Darling Downs, Far North, Moreton, Northern, South West shires of Queensland

[2] a, j, i, h - *M. arenaria*, *M. javanica*, *M. incognita*, *M. hapla*. Numbers following indicate host race. # - novel host reaction

[3] A, J, I, H - *M. arenaria*, *M. javanica*, *M. incognita*, *M. hapla*. Numbers following are esterase type designations by Esbenshade and Triantaphyllou (1985).

[4] Letters refer to haplotypes as designated in Hugall *et al.* (1993).

Table 8 Identification of *Meloidogyne* populations by standard host range test and mitochondrial DNA haplotype.

Pop	Differential host test[a]			Correlation with standard host races[b]	mtDNA haplotype[c]
	Numerical	Rating	Identity		
Y	000453	RRRSSS	j/a2	j,a2	C
11	000555	RRRSSS	j/a2	j,a2	D
47	000555	RRRSSS	j/a2	j,a2	D
51	000534	RRRSSS	j/a2	j,a2	D
60	000344	RRRSSS	j/a2	j,a2	-[e]
63	000555	RRRSSS	j/a2	j,a2	-
65	000555	RRRSSS	j/a2	j,a2	A
72	000555	RRRSSS	j/a2	j,a2	D
82	000555	RRRSSS	j/a2	j,a2	D
92	000455	RRRSSS	j/a2	j,a2	-
101	000555	RRRSSS	j/a2	j,a2	-
106	000555	RRRSSS	j/a2	j,a2	-
NQ1	000555	RRRSSS	j/a2	j,a2	A
NQ2	000555	RRRSSS	j/a2	j,a2	D
NQ5	000555	RRRSSS	j/a2	j,a2	C
NQ7	000555	RRRSSS	j/a2	j,a2	C
B	100454	RRRSSS	j/a2	i2,j,a2	D
1	200555	RRRSSS	j/a2	i2,j,a2	D
16	100554	RRRSSS	j/a2	i2,j,a2	D
69	200555	RRRSSS	j/a2	i2,j,a2	D
87	100454	RRRSSS	j/a2	i2,j,a2	-
94	100555	RRRSSS	j/a2	i2,j,a2	D
19	500055	SRRRSS	i1	i1	-
35	400044	SRRRSS	i1	i1	B
G	500155	SRRRSS	i1	i1,i2	B
15	300155	SRRRSS	i1	i1,i2	A
H	500555	SRRSSS	i2	i2	B
Z	400452	SRRSSS	i2	i2	B
39	400455	SRRSSS	i2	i1,i2	A

D	300554	SRRSSS	i2	i2,j,a2	D
33	300553	SRRSSS	i2	i2,j,a2	C
42	300555	SRRSSS	i2	i2,j,a2	D
44	400555	SRRSSS	i2	i2,j,a2	D
86	300555	SRRSSS	i2	i2,j,a2	D
48	405451	SRSSSR	h	h	F
114	505455	SRSSSS	a1	a1	E
102	203551	RRSSSR	novel (4)	h	E
113	405250	SRSRSR	novel (5)	h	E
115	503055	SRSRSS	novel (6)	i1	-
A	100451	RRRSSR	novel (3)	i2,j,a2	D
12	300250	SRRSSR	novel (1)	?	G
78	00014-	RRRRS-	novel (2)	?	D
88	00014-	RRRRS-	novel (2)	?	D
98	00004-	RRRRS-	novel (2)	?	D

[a] Reactions of host plants are listed in the following order: *Capsicum frutescens* (capsicum) cv. California Wonder; *Gossypium hirsutum* (cotton) cv. Deltapine 16; *Arachis hypogaea* (peanut) cv. Florunner; *Nicotiana tabacum* (tobacco) cv. NC95; *Lycopersicon esculentum* (tomato) cv. Tiny Tim; *Citrullus vulgaris* (watermelon) cv. Charleston Gray. An average eggmass rating of <2 indicates resistance (R); an average rating of >2 indicates susceptibility (S); plants with a rounded-off, numerical rating of 2 were assigned an R or S rating depending on whether the original value was ≤ 2 or >2. For example, ratings of 1.5-2.0 are considered indicative of resistance (R), ratings of 2.1-2.4 indicative of susceptibility (S).
[b] Significant ($r>0.811$, $P<0.05$) when tested by correlation against standard host reactions i.e. '+' reactions in Table 1 were given a rating of 5 and '-' reactions a rating of 0.
[c] Hugall *et al.* (1993, in prep)
[d] j, *M. javanica*; a, *M. arenaria*; i, *M. incognita*; h, *M. hapla*. Numbers denote standard host races.
[e] not tested.

Table 9. Morphometric data relating to single eggmass populations of *Meloidogyne*

Pop	ST	DOGO	EP	EP:ST	V	A	V/A
1	13.9-16.7 15.53 0.91	3.3-5.6 4.15 0.70	31.1-72.2 48.16 11.15	2.2-4.3 3.10 0.65	-	-	-
3	15.2-24.8 19.6 3.28	2.4-4.8 3.4 1.03	33.6-96.0 62.6 22.07	1.4-6.3 3.4 1.62	16.8-28.8 23.4 3.24	16.0-19.2 18.0 1.14	0.9-1.6 1.3 0.18
5	13.2-15.6 14.6 0.76	1.1-5.0 3.2 1.05	22.2-93.3 45.8 20.4	1.5-6.3 3.1 1.35	22.4-28.0 25.6 2.57	16.0-20.8 18.8 1.86	1.2-1.6 1.4 0.11
11	9.3-17.8 15.9 2.40	3.9-6.7 4.8 0.76	43.3-88.9 71.1 14.67	2.6-9.5 4.7 1.86	22.4-28.8 25.8 1.89	17.6-22.4 19.5 1.62	1.0-1.5 1.3 0.12
12	13.3-19.4 15.2 1.64	3.6-4.4 4.1 0.33	37.8-72.2 51.4 13.60	2.4-5.4 3.4 0.98	20.0-24.8 22.1 1.39	15.2-20.0 18.2 1.41	1.0-1.4 1.2 0.11
15	13.3-17.8 16.1 1.17	3.9-6.1 4.7 0.60	35.0-126.7 52.8 27.5	2.1-8.1 3.3 1.80	17.6-26.4 22.5 2.43	14.4-25.6 17.7 2.88	0.8-1.6 1.3 0.21
16	14.4-16.6 15.7 0.82	2.8-5.0 3.6 0.70	25.0-76.7 43.6 14.93	1.7-4.6 2.8 0.83	-	-	-
18	11.1-18.6 15.9 1.91	3.9-5.0 4.5 0.42	31.7-70.0 54.2 12.24	1.9-4.5 3.4 0.76	-	-	-
19	15.0-16.7 15.9 0.53	3.3-7.8 5.1 1.29	24.4-50.6 35.5 7.19	1.6-3.3 2.2 0.46	20.8-30.4 24.2 3.02	16.0-21.6 19.1 1.53	1.1-1.7 1.3 0.19
20	15.0-17.8 17.0 1.03	2.8-4.7 4.1 0.64	31.7-72.2 49.3 13.47	1.8-4.6 2.9 0.91	-	-	-
21	14.4-17.8 16.5 0.97	3.3-5.5 4.5 0.72	33.3-60.6 47.7 9.66	2.1-3.6 2.9 0.51	27.2-36.8 31.6 2.63	21.6-30.4 27.0 2.29	1.0-1.4 1.2 0.12
35	13.3-1.76 15.3 1.26	2.8-5.0 3.9 0.72	19.6-53.3 29.7 9.85	1.1-4.0 2.0 0.80	19.2-26.4 23.3 2.23	12.0-25.6 18.5 3.36	0.8-2.0 1.3 0.27
37	10.6-16.1 14.3 1.55	3.3-5.0 3.8 0.61	12.8-93.3 36.0 21.52	1.0-8.8 2.7 2.22	19.2-30.4 25.3 3.29	14.4-24.8 19.8 2.74	0.8-2.0 1.3 0.30
39	12.2-16.7 15.0 1.50	4.2-6.1 4.9 0.63	32.2-78.9 57.3 19.55	2.3-6.5 3.9 1.55	20.0-27.2 23.5 2.28	15.2-24.8 17.5 2.76	0.9-1.7 1.4 0.20

40	13.3-18.2 15.9 1.33	2.8-6.4 4.7 1.08	35.1-78.9 52.2 15.30	2.1-5.3 3.3 1.19	22.4-29.6 25.5 2.06	13.6-21.6 17.5 2.22	1.1-1.9 1.5 0.23
42	13.9-18.9 15.7 1.88	3.9-5.6 4.4 0.65	23.3-45.6 34.1 8.39	1.5-2.7 2.2 0.42	-	-	-
44	12.2-15.0 13.7 0.75	3.9-7.2 5.7 1.11	21.1-92.8 44.0 19.17	1.5-6.8 3.2 1.47	17.6-30.4 22.3 4.23	12.8-27.2 19.0 4.43	0.7-1.9 1.2 0.35
47	-	-	-	-	24.0-33.3 29.0 2.53	15.2-28.0 20.3 3.22	0.9-1.9 1.5 0.26
48	14.4-23.2 18.5 2.85	2.4-8.0 4.4 1.85	36.0-75.2 58.1 16.88	2.2-4.2 3.1 0.86	16.0-27.2 19.9 2.43	12.8-22.4 16.5 2.67	0.8-1.7 1.2 0.20
50	-	-	-	-	24.8-32.0 27.5 2.02	18.4-24.0 21.4 1.65	1.1-1.5 1.3 0.12
51	-	-	-	-	21.6-31.2 26.4 2.51	18.4-32.0 23.8 3.21	0.9-1.3 1.1 0.14
53	6.4-14.4 12.9 2.40	2.4-5.6 4.5 0.86	23.3-47.2 33.6 7.09	1.6-7.4 2.9 1.65	21.6-24.8 23.5 1.6	15.2-20.8 18.4 2.32	1.1-1.5 1.3 0.14
54	-	-	-	-	17.6-20.0 19.4 1.04	15.2-16.8 16.5 0.72	1.1-1.2 1.2 0.02
58	-	-	-	-	17.6-29.6 23.2 3.11	16.8-27.2 19.8 2.89	0.8-1.5 1.2 0.17
60	11.2-21.6 14.6 3.32	3.2-5.6 4.0 0.60	20.0-66.4 34.0 12.01	1.6-3.2 2.3 0.57	20.0-27.2 22.7 1.97	15.2-26.4 20.2 2.78	0.8-1.6 1.1 0.21
63	13.6-22.4 17.1 2.53	2.4-4.8 2.9 0.80	34.4-88.8 48.0 17.06	1.9-4.0 2.8 0.81	24.0-27.2 23.8 1.68	16.8-25.6 20.7 3.18	1.0-1.5 1.3 0.17
64	14.4-19.2 16.9 1.79	2.4-3.2 2.6 0.37	28.0-88.8 61.2 20.46	1.5-5.8 3.7 1.44	-	-	-
66	8.0-17.6 15.9 2.59	3.2-6.0 3.9 1.04	24.8-64.0 38.5 13.01	1.4-3.9 2.5 0.89	20.0-27.2 23.8 1.68	15.2-28.0 19.6 3.08	0.9-1.6 1.2 0.19
68	16.8-19.2 17.9 1.13	3.2-5.6 4.8 0.96	32.8-55.2 43.0 7.10	1.9-2.9 2.4 0.36	14.4-24.0 18.6 3.02	12.8-26.4 17.9 3.65	0.8-1.3 1.1 0.15

69	14.4-23.2 18.6 3.29	3.2-4.8 3.9 0.70	36.8-62.4 50.8 8.06	2.1-3.6 2.8 0.49	21.6-26.4 23.7 1.58	16.0-20.0 17.7 1.28	1.2-1.7 1.4 0.16
70	12.8-20.0 17.4 2.37	2.4-6.4 3.7 1.15	24.0-64.0 44.2 14.53	1.2-3.3 2.6 0.81	21.6-29.6 24.8 4.2	16.8-19.2 17.9 1.22	1.1-1.8 1.4 0.33
71	-	-	-	-	22.4-30.4 26.1 2.36	14.4-28.8 21.1 3.29	0.9-1.9 1.3 0.21
72	11.2-24.0 18.2 3.41	2.4-4.8 3.8 0.85	28.0-92.0 49.2 21.47	1.2-4.4 2.8 0.94	20.8-25.6 23.6 1.70	14.4-24.8 19.4 3.47	0.9-1.5 1.2 0.20
73	13.6-21.6 16.1 2.25	2.4-5.6 3.8 1.07	25.6-110.4 51.4 24.61	1.8-6.3 3.1 1.27	20.0-28.0 23.7 2.17	16.8-24.8 20.2 2.43	0.9-1.5 1.2 0.19
75	16.8-17.6 17.2 0.57	5.6-5.6 5.6 0.00	36.0-36.0 36.0 0.00	2.0-2.1 2.1 0.07	16.8-26.4 22.3 2.30	16.0-21.6 18.3 1.63	1.0-1.5 1.2 0.17
77	9.6-24.8 16.4 4.32	2.4-5.6 3.6 1.06	32.8-64.0 43.6 9.05	1.6-4.9 2.9 1.12	15.2-26.4 21.7 2.52	11.2-21.6 18.3 2.48	0.9-1.5 1.2 0.15
78	14.4-24.8 18.8 3.97	2.4-5.6 3.6 1.05	24.0-82.4 53.8 18.52	1.7-4.9 2.9 1.14	20.0-26.4 23.1 1.73	16.0-22.4 19.1 2.22	0.9-1.5 1.2 0.15
79	11.2-20.0 16.2 2.85	2.4-4.8 3.6 0.83	24.0-91.2 46.8 15.88	1.3-5.6 3.0 1.28	17.6-23.2 19.6 2.49	14.4-28.0 18.6 6.35	0.8-1.3 1.1 0.21
83	13.6-20.8 17.0 2.76	2.4-6.4 4.7 1.68	25.6-60.0 37.0 13.89	1.4-3.0 2.2 0.67	21.6-28.0 25.7 2.28	15.2-20.8 17.8 1.48	1.2-1.7 1.5 0.13
84	17.6-47.6 27.9 13.40	3.2-4.8 4.0 0.92	48.0-64.0 56.0 9.24	2.1-3.6 2.9 0.91	16.0-19.2 17.1 1.20	8.8-16.8 13.6 2.72	1.0-2.2 1.3 0.44
85	17.6-22.4 20.5 1.47	2.4-5.6 3.6 1.21	20.0-81.6 52.5 17.20	1.0-4.1 2.6 0.82	19.2-25.6 23.4 1.63	16.0-28.8 19.1 3.53	0.8-1.6 1.3 0.24
86	19.2-22.4 20.2 1.51	2.4-4.8 3.4 1.01	28.0-68.0 47.4 16.43	1.5-3.5 2.4 0.87	21.6-28.8 25.4 2.35	13.6-22.4 18.7 2.18	1.0-1.9 1.4 0.20
87	-	-	-	-	17.6-28.0 22.4 2.91	14.4-24.0 17.3 2.40	0.9-1.7 1.3 0.24
88	15.2-20.8 18.0 2.07	3.2-6.4 4.3 1.20	36.0-60.8 45.7 10.39	1.9-3.6 2.6 0.76	15.2-34.4 21.9 4.69	13.6-22.4 18.0 2.32	1.0-1.7 1.2 0.23

89	16.0-22.4 18.6 2.41	2.4-4.8 3.4 0.82	40.0-82.4 55.1 15.12	1.8-5.2 3.0 1.01	16.0-28.8 23.8 3.32	12.8-22.4 17.5 2.68	1.0-2.1 1.4 0.21
90	16.8-23.2 20.6 2.11	2.4-5.6 4.1 1.03	32.0-72.8 49.4 12.75	1.6-3.8 2.4 0.64	20.0-29.6 24.5 3.09	15.2-20.0 18.3 1.55	1.0-1.7 1.3 0.20
91	-	-	-	-	20.0-33.6 26.2 2.99	14.4-28.0 19.2 3.06	1.0-1.8 1.4 0.21
92	15.2-23.2 19.1 2.61	2.4-4.8 3.9 0.93	30.4-64.8 45.9 9.88	1.4-4.1 2.4 0.67	19.2-29.6 23.3 3.26	14.4-25.6 18.1 2.81	1.0-1.5 1.3 0.15
93	11.2-23.2 18.0 4.49	2.4-4.8 3.9 1.06	27.2-72.8 45.7 19.80	1.5-4.1 2.6 0.95	16.8-30.4 24.6 2.92	15.2-23.2 18.3 2.06	0.9-1.7 1.4 0.16
94	12.8-25.6 16.7 3.65	2.4-5.6 4.1 1.16	25.6-60.8 42.0 11.93	1.7-3.4 2.5 0.64	20.0-28.0 23.2 2.38	14.4-22.4 17.9 2.20	1.1-1.5 1.3 0.12
95	19.2-23.2 21.0 1.48	3.2-5.6 4.3 0.80	25.6-92.0 51.5 19.46	1.1-4.4 2.5 1.00	16.0-30.4 23.2 2.64	14.4-28.0 18.8 2.77	0.8-1.8 1.3 0.19
97	-	-	-	-	16.0-20.8 18.2 2.20	13.6-28.0 18.6 6.48	0.7-1.2 1.0 0.19
101	-	-	-	-	17.6-31.2 23.1 3.19	12.0-28.8 18.9 3.80	0.8-1.6 1.3 0.21
103	-	-	-	-	15.2-29.6 25.4 3.47	4.0-201.6 30.5 45.84	0.1-6.2 1.5 1.30
104	-	-	-	-	16.8-23.2 20.1 2.65	16.0-24.8 17.9 3.46	0.9-1.4 1.1 0.16
106	-	-	-	-	19.2-28.0 24.3 2.67	15.2-20.8 18.1 1.78	1.0-1.7 1.4 0.16
A	11.1-16.7 14.8 1.83	3.3-5.6 4.6 0.84	27.8-53.3 40.5 10.83	1.7-3.8 2.8 0.74	19.2-28.8 24.4 2.74	16.0-23.2 18.5 2.35	0.9-1.6 1.3 0.17
B	14.4-16.7 15.7 0.74	2.8-4.8 4.0 0.62	25.6-72.2 47.2 14.70	1.7-5.0 3.0 0.99	21.6-29.6 25.4 2.61	16.8-23.2 20.6 1.97	1.1-1.5 1.2 0.11
C	12.2-17.8 15.9 1.57	3.3-5.6 4.6 0.74	27.8-67.8 44.3 14.10	1.7-4.7 2.8 0.91	22.4-35.2 27.7 3.70	17.6-36.0 23.1 3.80	0.8-1.6 1.2 0.21

D	12.2-16.9 14.9 1.68	1.1-6.7 4.1 1.48	31.1-94.4 51.3 24.30	0.7-7.0 3.0 1.83	26.4-40.0 32.7 3.81	22.4-32.8 25.8 3.07	0.8-1.5 1.3 0.22
G	13.3-16.1 15.2 0.84	2.2-4.4 3.3 0.69	21.1-71.1 41.3 15.00	1.3-4.5 2.7 0.94	16.8-26.4 22.2 2.50	16.0-24.8 20.7 2.54	0.8-1.5 1.1 0.2
H	13.8-18.0 15.7 1.41	3.1-5.0 4.1 0.58	17.8-36.7 26.4 6.37	1.1-2.6 1.7 0.47	20.0-26.4 23.6 0.92	20.0-26.4 22.1 1.95	0.9-1.2 1.1 0.12
I	13.3-16.7 15.7 0.93	3.3-6.7 4.6 1.10	43.3-77.8 58.8 11.35	2.8-5.8 3.8 0.92	20.0-28.0 25.4 2.47	12.8-24.0 18.6 3.41	1.1-2.0 1.4 0.31
J	15.0-16.1 15.6 0.41	2.2-3.9 3.2 0.52	21.1-38.9 26.7 4.8	1.4-2.5 1.7 0.32	18.4-26.4 23.7 2.46	16.8-24.0 20.0 2.12	1.0-1.4 1.2 0.12
K	15.6-17.2 16.3 0.59	2.8-5.6 4.3 0.94	23.3-67.8 46.3 14.19	1.5-4.4 2.8 0.86	17.6-30.4 25.5 3.74	17.6-23.2 19.5 2.17	1.0-1.6 1.3 0.18
V	12.2-17.2 15.2 1.71	2.8-4.4 3.7 0.54	20.0-74.4 42.9 18.00	1.6-4.7 2.8 1.09	22.4-35.2 26.5 3.40	16.0-30.4 21.4 4.13	0.9-1.6 1.3 0.17
X	13.9-17.8 16.5 1.01	2.2-4.7 3.6 0.85	36.7-88.9 53.4 17.6	2.2-6.4 3.3 1.28	20.0-37.6 27.6 5.37	22.4-35.2 26.7 3.45	0.7-1.5 1.1 0.26
Y	15.6-19.4 17.0 1.14	3.3-5.1 4.1 0.65	25.6-53.3 35.1 8.89	1.4-5.0 2.4 1.09	23.2-36.8 30.0 4.34	23.2-34.4 29.9 3.69	0.8-1.1 1.0 0.12
Z	14.4-16.7 15.7 1.13	3.3-3.9 3.7 0.32	27.8-31.1 28.9 1.93	1.7-2.2 1.9 0.26	25.6-32.8 28.6 2.35	19.2-27.2 22.5 2.59	1.1-1.4 1.3 0.09

Values are range, mean and standard deviation of 20 individuals, respectively.

ST, length of stylet; DOGO, distance from dorsal oesophageal gland opening to base of stylet; EP, distance fom head to excretory pore; V, width of vulva; A, distance from anus to vulva.

Table 10 Sequence divergence between haplotypes of *Meloidogyne* based on restriction fragment length polymorphisms (above diagonals) and sequence (below diagonals).

Haplotype	A	B	C	D	G	H
A (*M. arenaria*)	-	0.4	0.4	0.6	0.4	0.6
B (*M. incognita*)	0.1	-	0.3	0.4	0.3	0.4
C (*M. arenaria*)	0.1	0.4	-	0.4	0.3	0.1
D (*M. javanica*)	0.3	0.5	0.4	-	0.4	0.6
G (*M. hispanica*)	0.1	0.4	0.2	0.3	-	0.4
H (*M. arenaria*)	0.1	0.3	0.0	0.4	0.3	-

Haplotype	E	F	I
E (*M. hapla*)	-	6.0	12.0
F (*M. hapla*)	2.0	-	4.0
I (*M. hapla*)	2.4	0.6	-
D (*M. javanica*)	24	23	26

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63Dlsrna    AGAGATATAAGGAGATTTAATTTTTAAGTTAAATCCAATTCGTAATGTTTGGAATTTACCAAGGTAGAATTATACGTTAATTTAGAAGAATTGTTGAAAAGAATGAATTCTTAATGG
77CHlsrna   AGAGATATAAGGAGATTTAATTTTTAAGTTAAATCCAATTCGTAATGTTTGGAATTTACCAAGGTAGAATTATACGTTAATTTAGAAGAATTGTTGAAAAGAATGAATTCTTAATGG
GBlsrna     AGAGATATAAGGAGATTTAATTTTTAAGTTAAATCCAATTCGTAATGTTTGGAATTTACCAAGGTAGAATTACACGTTAATTTAGAAGAATTGTTGAAAAGAATGAATTCTTAATGG
12Glsrna    AGAGATATAAGGAGATTTAATTTTTAAGTTAAATCCAATTCGTAATGTTTGGAATTTACCAAGGTAGAATTATACGTTAATTTAGAAGAATTGTTGAAAAGAATGAATTCTTAATGG
113lsrna    TTTTTTTTAAAAATAGATGTTATGACAGGTGTTATAAGGGTATTAAATTATAATTTTGAAATATTGGGTATTTTACGGGCAATGTTCCGAAATTTGTGGAGTTAATCATTCTTTTAT
                 * ***  *  *  *  *  *   ** *      *     * *  **  * ***   *   * *  *          *     *  ***   **       *  *  *

                                                                                     tRNAhis                  |-> lRNA
63Dlsrna    AAACAGTAAGGATATTTTTTATATAATTATTTTTAATAATATTAAAAATAAAGCTATTAATTTCAATTGAATTTTTTATTGTGATTAAAAAAGTTTTTGGCTAAATTATTTTTTTAG
77CHlsrna   AAACAGTAAGGATATTTTTTATATAATTATTTTTAATAATATTAAAAATAAAGCTATTAATTTCAATTGAATTTTTTATTGTGATTAAAAAAGTTTTTGGCTAAATTATTTTTTTAG
GBlsrna     AAACAGTAAGGATATTTTTTATATAATTATTTTTAATAATATTAAAAATAAAGCTATTAATTTCAATTGAATTTTTTATTGTGATTAAAAAAGTTTTTGGCTAAATTATTTTTTTAG
12Glsrna    AAACAGTAAGGATATTTTTTATATAATTATTTTTAATAATATTAAAAATAAAGCTATTAATTTCAATTGAATTTTTTATTGTGATTAAAAAAGTTTTTGGCTAAATTATTTTTTTAG
NQ1Alsrna   -----------------------------------------------------------------------------------------------AAAGTTTTTGGCTAAATTATTTTTTTAG
113lsrna    ACCTATTATAATTGAAGTAGTTTTATTTGATTTTTTTAAGAATTATGTTTAAGCTAATAATTTCTAGTGAATTTTTTATTGTGATTAAAAAGTTAATCAGCTATTTTATTTT--GAA
150lsrna    ------------------------------------------------------------------------------------------------------TAGCTATTTTATTTT--GAA
114lsrna    -----------------------------------------------------------------------------------------------AAAAGTTAATCAGCTATTTTATTTT--GAA
48lsrna     ------------------------------------------------------------------------------------------------------TAGCTATTTTATTTT--GAA
            *   * **    *    *   * *  **  ****  **  * * *   * ****** ******* * ************************  *  *  ****  *******   *

63Dlsrna    ATTATTTTTTATTGTTGAAAAAATTAAAAACAAATTGTTTTTTACAATAATTAAAATTTATAATATTTCAATTTTTAATTTTT-AGTTTAAATAAAAATTACAAATATGAAAAATAA
77CHlsrna   ATTATTTTTTATTGTTGAAAAAATTAAAAACAAATTGTTTTTTACAATAATTAAAATTTATAATATTTCAATTTTTAATTTTT-AGTTTAAATAAAAATTACAAATATGAAAAATAA
GBlsrna     ATTCTTTTTTATTGTTGAAAAAATTAAAAACAAATTGTTTTTTACAATAATTAAAATTTATAATATTTCAATTTTTAATTTTT-AGTTTAAATAAAAATTACAAATATGAAAAATAA
12Glsrna    ATTATTTTTTATTGTTGAAAAAATTAAAAACAAATTGTTTTTTACAATAATTAAAATTTATAATATTTCAATTTTTAATTTTT-AGTTTAAATAAAAATTACAAATATGAAAAATAA
NQ1Alsrna   ATTATTTTTTATTGTTGAAAAAATTAAAAACAAATTGTTTTTTACAATAATTAAAATTTATAATATTTCAATTTTTAATTTTT-AGTTTAAATAAAAATTACAAATATGAAAAATAA
113lsrna    ATTATTTTTTATTGTTGAAAAAATTAATAATAATTTATTGTTAATAAAAATTAAAATTTATAATATTTTA-TTTTTATTTTTT-AGTTTAAATAAAAAAAATAAAATTTATATATAT
150lsrna    ATTATTTTTTATTGTTGAAAAAATTAATAATAATTTATTGTTAATAAAAATTAAAATTTATAATATTTTA-TTTTTATTTTTT-AGTTTAAATAAAAAAAATAAAATTTATATATAT
114lsrna    ATTATTTTTTATTGTTGAAAAAATTAATAATAATTTATTGTTAATAAAAATTAAAATTTATAATATTTTA-TTTTTATTTTTTTAGTTTAAATAAAAAAAATAAAATTAATATATAT
48lsrna     ATTATTTTTTATTGTTGAAAAAATTAATAATAATTTATTGTTAATAAAAATTAAAATTTATAATATTTTA-TTTTTATTTTTT-AGTTTAAATAAAAATAATAAAATTTATATATAT
            *** ********************** ** ** ** ** ** * ** ******************** * ****** ***** ***************  * ***  * * * ***
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Figure 3. Sequences of 16srDNA, tRNA^{His}, *cyt*b and ND3 genes of populations 63 (haplotype D), 12 (haplotype G), 77 (haplotype C), G (haplotype B), NQ1 (haplotype A), 48, 113, 114, 150 (*M. hapla*), 63 and 12.

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63Dlsrna      AAAAATTTTGTTATAAATTAAATTAATTTATTTATATTAAAAATATTTTTT-ATAA-------TTTTAATTTTTTTTATTTTTTTAAAAAAAAAA-TAATTTAATTATAATTATTTAT
77CHlsrna     AAAAATTTTGTTATAAATTAAATTAATTTATTTATATTAAAAATATTTTTT-ATAA-------TTTTAATTTTTTTTATTTTTTTAAAAAAAAAA-TAATTTAATTATAATTATTTAT
GBlsrna       AAAAATTTTGTTATAAATTAAATTAATTTATTTATATTAAAAATATTTTTT-ATAA-------TTTTAATTTTTTTTATTTTTTTAAAAAAAAAA-TAATTTAATTATAATTATTTAT
12Glsrna      AAAAATTTTGTTATAAATTAAATTAATTTATTTATATTAAAAATATTTTTT-ATAA-------TTTTAATTTTTTTTATTTTTTTAAAAAAAAAA-TAATTTAATTATAATTATTTAT
NQ1Alsrna     AAAAATTTTGTTATAAATTAAATTAATTTATTTATATTAAAAATATTTTTT-ATAA-------TTTTAATTTTTTTTATTTTTTTAAAAAAAAAA-TAATTTAATTATAATTATTTAT
113lsrna      AAAAATTTTGTTATAAATTATAATAGTTTATTTATTT-------GTTTTTT-ATAATAAATAATAGGATTTTTTTTTATTATTTTATAAAAAAAAATAATTTAATTATAATTATTAAA
150lsrna      AAAAATTTTGTTATAAATTATAATAGTTTATTTATTT-------GTTTTTT-ATAATAAATAATAGTTTTTTTTTTTATTATTTTATAAAAAAAAATAATTTAATTATAATTATTAAA
114lsrna      AAAAATTTTGTTATAAATTATAATAGTTTATTTATTT-------ATTTTTTTATAATAAATAATAGGATTTTTTTT-ATTATTTTATAAAAAAAAATAATTTAATTATAATTATTAAA
48lsrna       AAAAATTTTGTTATAAATTATAATAGTTTATTTGTTT-------ATTTTTT-ATAATAAAAAATAGTATTTTTTTT-ATTTTTTTATAAAAAAAAATAATTTAATTATAATTATTAGA
              ******************** * ** ******* * *       ****** ****       *      ******* *** ***** ******** *******************

63Dlsrna      TTAAATTTAATAATTAAATATAAAATTTTTTATTAAATAAATTTAATAATAAATGTTTTTTAAATTCTTTGAGGTTTTGATTTTTGATTTTTTGTTTCTGCTCATTGTTAAAGAAAA
77CHlsrna     TTAAATTTAATAATTAAATATAAAATTTTTTATTAAATAAATTTAATAATAAATGTTTTTTAAATTCTTTGAGATTTTGATTTTTGATTTTTTGTTTCTGCTCATTGTTAAAGAAAA
GBlsrna       TTAAATTTAATAATTAAATATAAAATTTTTTATTAAATAAATTTAATAATAAATGTTTTTTAAATTCTTTGAGGTTTTGATTTTTGATTTTTTGTTTCTGCTCATTGTTAAAGAAAA
12Glsrna      TTAAATTTAATAATTAAATATAAAATTTTTTATTAAATAAATTTAATAATAAATGTTTTTTAAATTCTTTGAGGTTTTGATTTTTGATTTTTTGTTTCTGCTCATTGTTAAAGAAAA
NQ1Alsrna     TTAAATTTAATAATTAAATATAAAATTTTTTATTAAATAAATTTAATAATAAATGTTTTTTAAATTCTTTGAGGTTTTGATTTTTGATTTTTTGTTTCTGCTCATTGTTAAAGAAAA
113lsrna      AGTAATTTTGTTATTTAGAATAATATATTAATATAAATAACAAAAATATTAAATATTTTTTAAATTTTTTGGGGATTTAAGTTATAATTTTTTGTGTCTGCTCAGTGAATTTAAATA
150lsrna      AGTAATTTTGTTATTTAGAATAATATATTAATATAAATAACAAAAATATTAAATATTTTTTAAATTTTTTGGGGATTTAAGTTATAATTTTTTGTGTCTGCTCAGTGAATTTAAATA
114lsrna      AGTAATTTTGTTATTTAGAATAATATATTAATATAAATAACAAAAATATTAAATATTTTTTAAATTTTTTGGGGATTTAAGTTATAATTTTTTGTGTCTGCTCAGTGAATT------
48lsrna       AGTAATTTTGTTATTTAGAATAATATATTAATATAAATAAAAAAAATATTAAATATTTTTTAAATTTTTTGGGAATTTAAATTATAATTTTTTGTGTCTGCTCAGTGAATTTAAATA
                 *****  * *** *  **** ** **     *******    **** ***** *********** **** *  *** * ** * ********* ******** **      ** *

63Dlsrna      GCACTTTTAGCGTGATTGGCCAAAGGTAGCGAGGTAATTTGTTTTTTTATTGAATTCTAGTATGAATGGATTTTTTGTTATT
77CHlsrna     GCACTTTTAGCGTGATTGGTCAAAGGTAGCGAGGTAATTTGTTTTTTTATTGGATTCTAGTATGAATGGATTTTTTGTTATT
GBlsrna       GCACTTTTAGCGTGATTGGTCAAAGGTAGCGAGATAATTTGTTTTTTTATTGGATTCTAGTATGAATGGATTTTTTGTTATT
12Glsrna      GCACTTTTAGCGTGATTGGTCAAAGGTAGCGAGGTAATTTGTTTTTTTATTGGATTCTAGTATGAATGGATTTTTTGTTATT
NQ1Alsrna     GCACTTTTAGCGTGATTGGTCAAAGGTAGCGAGGTAATTTGTTTTTTTATTGGATTCTAGTATGAATGGATTTTTTGTTATT
113lsrna      GCACTTTTAGCGTGATTAGTCAAACCA
150lsrna      GCACTTTTAGCGTGATTAGTCAAACCA
48lsrna       GCACTTTTAGCGTGATTAGTCAAACCA
              ***************** ******
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63Dcytb    -------------------------------------------------------AAACGAAATCTAAAATAAAATAAAGCCTTTAATATATGTAATATAACTAATATAAAAAATAAAG
12Gcytb    -------------------------------------------------------AAACGAAATCTAAAATAAAATAAAGCCTTTAATATATGTAATATAACTAATATAAAAAATAAAG
113cytb    GATCAAATTAAGGCATATCCTAAAAAATTACTAAACCTACAAATCAAACTATCTTCAAACGATAGCTAAAAAAAAATAAAGCTTTTAAAATATGTAAAAATAACATCATAAAAAAAAAAG
150cytb    ---------------------------------------------------------------------------------------------------------------------
48cytb     ---------------------------------------------------------------------------------------------------------------------
                                                                  ****** * ****** ********** ***** ******** *  *  *  ******** ****

63Dcytb    AAACTATATTAAAATGAATTAAACGAATTAACCAACCATTATTAACCTCAATTATTAAATATTGAATTGAATCAAAAGAAAAATTTCTTCTAGATTCATAAAATAAACTTAATAATAAAC
12Gcytb    AAACTATATTAAAATGAATTAAACGAATTAACCAACCATTATTAACCTCAATTATTAAATATTGAATTGAATCAAAAGAAAAATTTCTTCTAGATTCATAAAATAAACTTAATAATAAAC
113cytb    AAACTAAATTAAAATGAATCAATCGAATTAATCAACCTTTATTTACTTCAATTATTAAATATTGAATTGAATTAAAAGAAAAATTAGACTCAGAACTATAATATAACCTTAATATTAAAC
150cytb    ---------------------------------------------------------------------------------------------------------------------
48cytb     ---------------------------------------------------------------------------------------------------------------------
           ****** ************ ** ******** ***** ***** ** ************************* ************      ***   **** **** ******* *****

                                                                                             MCYTB                            stop codon
63Dcytb    CAGAAATTACTTGCATAAAAAAAAATAATTCCTAAAAAACTACCAAAATTAAAAAAATAACAAAATAAAATCTGCTCCATTTAACTTTAATATTTTTTTAAAAAATTTTCTACAAAAATC
12Gcytb    CAGAAATTACTTGCATAAAAAAAAATAATTCCTAAAAAACTACCATAATTAAAAAAATAACAAAATAAAATCTGCTCCATTTAACTTTAATATTTTTTTAAAAAATTTTCTACAAAAATC
113cytb    CTGAAATTACTTGGATAATAAAAATAAATCCTAATATTCTACCATAATTAAAATTGTAATTCAAATAAATCGACTCCATTTAACTTCAACATTTTTTTAAAAAAA----CTATAATACTC
150cytb    ------------------------------------------------------------------------------------------TTTTTTTAAAAAAA----CTATAATACTC
114cytb    ------------------------------------------------------------------------------------------TTTTTTTAAAAAAA----CTATAATACTC
48cytb     --------------------------------------------------------------------------------------------TTTTTAAAAAAAAAA--CTATAATACTC
           * *********** **** ******** ****** *   ****** ********    ***    **   *****   ************* ** **************       *** ** * **

             Cytb | ND3               <- transcription
63Dcytb    AAATTAATTTAATATATTTTCATTCCAAAACTAAAGTTATCATAATAAAAATAATTAATAAAAAAATTATTCAATAAATCAAATTTAAATTAAAATAAATAAATAAAAATAAAAATA-TT
12Gcytb    AAATTAATTTAATATATTTTCATTCCAAAACTAAAGTTATCATAATAAAAATAATTAATAAAAAAATTATTCAATAAATCAAATTTAAATTAAAATAAATAAATAAAAATAAAAATA-TT
113cytb    AAATTAATTTAATATATTTTCATTCTAAAAATAAAGTTATAATAATAAAAAAAATAATTATTATAACTATTCAATAAACTAAATAATAATTAAAAAAAACAAATAAAAAAAAAAAATAATT
150cytb    AAATTAATTTAATATATTTTCATTCTAAAAATAAAGTTATAATAATAAAAAAAATAATTATTATAACTATTCAATAAACTAAATAATAATTAAAAAAAACAAATAAAAAAAAAAAATAATT
114cytb    AAATTAATTTAATATATTTTCATTCTAAAAATAAAGTTATAATAATAAAAAAAATAATTATTATAACTATTCAATAAACTAAATAATAATTAAAAAAAACAAATAAAAAAAAAAAATAATT
48cytb     AAATTAATTTAATATATTTTCATTCTAAAAATAAAGTTATAATAATAAAAAAAATAATTATTATAACTATTCAATAAACTAAATAATAATTAAAAAAAACAAATAAAAAAAAAAAATAATT
           ************************* **** ********* ********** *** * **   * ** ***********  ****     ******** *** ********* ******* **

63Dcytb    CCAGATCAAATAAAATAAAAATTAAAATAATTATAAAAAAATGAATTCTATAACTAAATAGTAATTTTCCCAAATA-ATTAAAACCTCTTTCGAAATAAGAATTTTTCATTAACTTATTT
12Gcytb    CCAGATCAAATAAAATAAAAATTAAAATAATTATAAAAAAATGAATTCTATAACTAAATAGTAATTTTCCCAAATA-ATTAAAACCTCTTTCGAAATAAGAATTTTTCATTAACTTATTT
113cytb    CTAAATCAAATAAAACAAAAATTATAACCAAACAAAAAAAATGTATATTAATACTAAATA-TATTTTTACCTAACCTATTAAAACCTCTCTCAAAAAAAGAGTTTTTTATTTTTTTATAT
150cytb    CTAAATCAAATAAAACAAAAATTATAACCAAACAAAAAAAATGTATATTAATACTAAATA-TATTTTTACCTAACCTATTAAAACCTCTCTCAAAAAAAGAGTTTTTTATTTTTTTATAT
114cytb    CTAAATCAAATAAAACAAAAATTTTAACCAAACAAAAAAAATGTATATTAATACTAAATA-TATTTTTACCTAACCTATTAAAGCCTCTCTCAAAAAAAGAGTTTTTTATTTTTTTATAT
48cytb     CTAAATCAAATAAAACAAAAATTATAACTAAACAAAAAAAATGTATATTAATACTAAATA-TATTTTTACCTAACCTATTAAAACCTCTCTCAAAAAAAGAGTTTTTTATTTTTTTATAT
           * * *********** *******  **  *     ********* **  **  ******** ** **** ** **    ****** ***** ** *** **** ***** ***    **** *

                                                                      ND3 | lRNA
63Dcytb    TTTTTTTTATTAAAAAAAAAAGTTACAAAAAAAAATCCTAAAGAAAAAATACCATAAATATTAATTAAAAAATTTAATTTAAAATAATTTTCCTTTCGTACTATTTATTTTCTAA-----
12Gcytb    TTTTTTTTATTAAAAAAAAAAGTTACAAAAAAAAATCCTAAAGAAAAAATACCATAAATATTAATTAAAAAATTTAATTTAAAATAATTTTCCTTTCGTACTATTTATTTTCTAA-----
113cytb    TTTTTTTTAGTAAATATAAAAGTTAGAAAAAAAAAAATCATA--ATTAATAAAAAAATTACTAAT-AAAAAATTTAATTATAAATAATTATCCTTTCGTACTGTTTATTTTCTCAAAATA
150cytb    TTTTTTTTAGTAAATATAAAAGTTAGAAAAAAAAAAATCATA--ATTAATAAAAAAATTACTAAT-AAAAAATTTAATTATAAATAATTATCCTTTCGTACTGTTTATTTTCTCAAAATA
114cytb    TTTTTTTTAGTAAATATAAAAGTTAGAAAAAAAAAAATCATA--ATTAATA---------------------------------------------------------------------
48cytb     TTTTTATTAGTAAATATAAAAGTTAGAAAAAAAAAAATCATA--GTTAATAAAAAAATTACTAAT-AAAAAATTTAATTTAAATAATTATCCTTTCGTACTGTTTATTTTCTCAAAATA
           ***** *** **** * ******** *********     * *       ****  * ** ** **** *************  ******** ************ ********** *
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M. hapla group and the other haplotypes was more than 20% (Table 10; Figure 4). Several restriction sites are concentrated around tRNA^{His} to *cyt*b (Figure 1). In *M. arenaria* and *M. hapla*, there is a deletion immediately 5' to tRNA^{His}. This deletion is 529 bp in *M. arenaria*, much larger in *M. hapla* and contains several restriction sites. In two cases, a single nucleotide substitution causes more than one change in restriction sites.

The mtDNA of *Meloidogyne* contains a region of tandem repeats of a 102 bp sequence. *M. hapla* has a larger mtDNA genome (25-27 kb compared with ~20 kb in *M. javanica*) than the other species due to longer non-coding regions with greater duplication of tandem repeats. Some populations of *M. hapla* also contained a possible 1.3 kb duplication. There was no obvious relationship between the sequences of the 102 bp repeats in *M. hapla* and *M. javanica*. Within population 113 of *M. hapla*, there was about 5% length difference and about 5% sequence difference between repeats.

MtDNA haplotypes could be distinguished by restriction analysis of PCR amplification products (Table 11). Amplification using the primers, MLSR and MCYTB, and digestion with *Hin*fI distinguishes *M. hapla* and haplotypes B, D and (A, C and G). A size difference in the amplification product using MORF and TRNAH distinguishes haplotype A from C and G.

Amplification with MLSR and MCYTB and digestion with *Mnl*I distinguishes (*M. hapla* and haplotype B), C and (A, D and G). Size differences in the MORF-TRNAH product distinguishes haplotype A from D and G. Haplotypes D and G can be distinguished by digestion with *Afl*III. *M. hapla* can be distinguished from haplotype B because it does not amplify with MORF-TRNAH. Simultaneous amplification with these two primer sets may provide a simpler test.

To distinguish different haplotypes of *M. hapla*, the MLSR-CYTB product may be cut with *Ase*I or *Dra*I. This distinguishes haplotypes F and I from E and J. These two groups may correspond to the cytogenetic races A and B.

Studies of the ITS region of 5.8srDNA were complicated by multiple loci and hybrid polyploidy. However, the results were consistent with mtDNA haplotypes. Results of studies in a 600 bp region of the IGS between 5srDNA and 18srDNA were easier to interpret and were consistent with mtDNA groupings. Haplotypes A, C and D were identical, haplotype B was very slightly divergent (<0.5%) while *M. hapla* was very divergent with two main types corresponding to populations 113 and 114.

(viii) <u>DISCUSSION</u>

Of a total of 662 populations collected worldwide in a previous study, about 47% were *M. incognita*, 40% *M. javanica*, 7% *M. arenaria* and 6% *M. hapla* (Taylor *et al.* 1982). In the current study, we found a similar distribution of populations when using the standard host test. However, we also found that 14% of populations induced host reactions which were novel, i.e. an identity could not be based on the standard host test.

The main constraint on achieving all of the original objectives has been the lack of traditional methods to identify populations accurately. Furthermore,

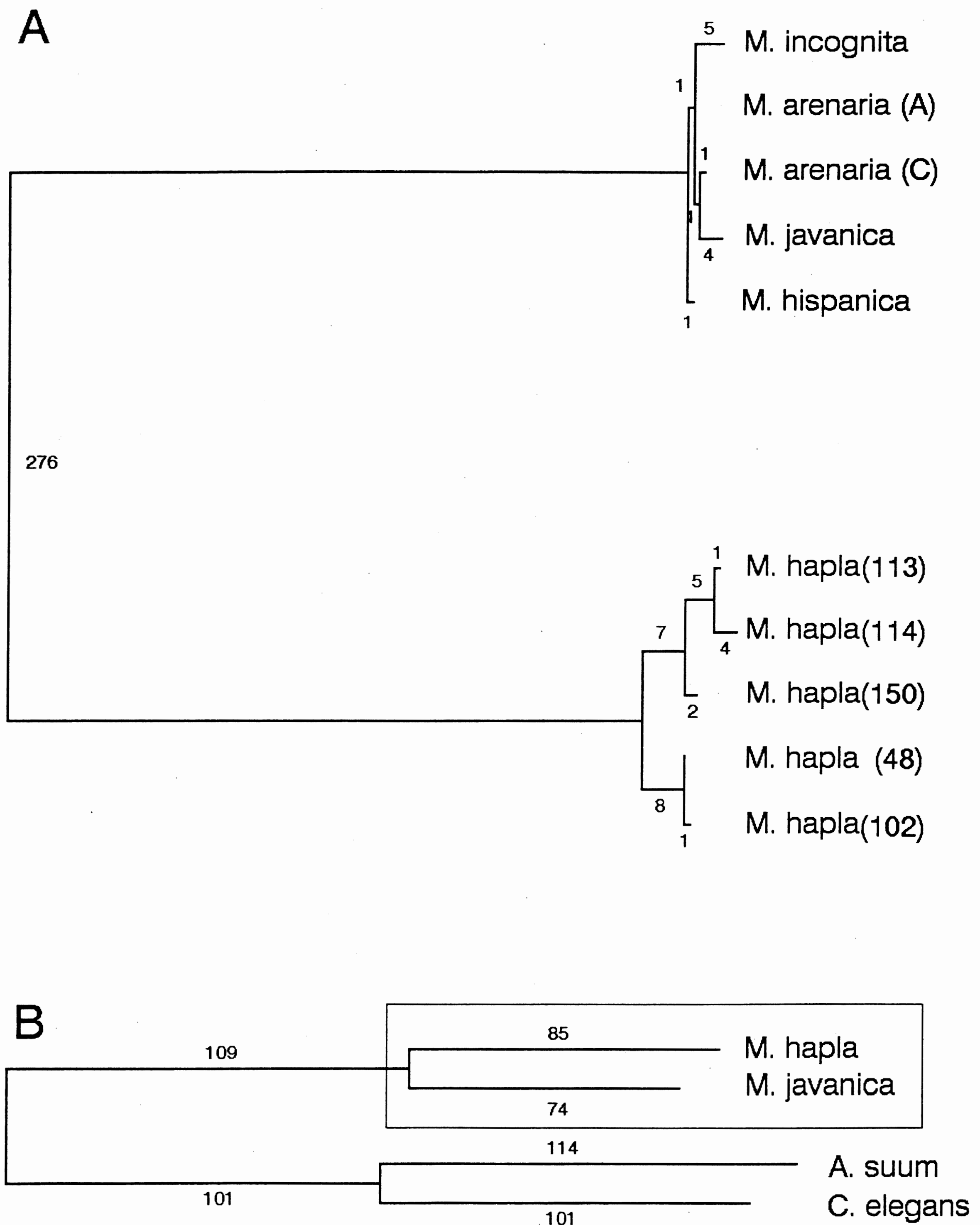

Figure 4. A, Relationships between *Meloidogyne* spp. based on ~2000 bp of mtDNA; B, Divergence of *M. javanica* from *M. hapla* relative to *Ascaris suum* and *Caenorhabditis elegans* based on 16s rDNA. Values indicate relative difference.

Table 11 MtDNA sequence polymorphisms among five haplotypes of mtDNA-PCR products and restriction enzymes diagnostic for them.

Primer set[a]	MORF-MHIS (9238-10040)						MLSR-MCYTB (10034-11232)					
Position[a]	9407	9410	9484	9534	9771	9921	10087	10384	10461	10480	10673	11257
Haplotype												
B	A	T	A	G	C	C	C	G	A	G	G	T
A	G	G	-[b]	-	-	-	A	G	G	G	A	T
C	G	G	A	A	C	C	A	A	G	G	A	T
D	G	G	A	A	T	T	A	G	G	A	A	T
G	G	G	T	G	C	C	A	G	G	G	A	A
Diagnostic restriction enzyme					*Mae*II	*Afl*III	*Hin*fI	*Mnl*I	*Mnl*I	*Eco*RI/ *Hin*fI		
Affected haplotype[b]					-D	-D	+B	-C	-B	+D/-D		

[a] numbers refer to position in the entire sequence of mtDNA of *M. javanica* (Okimoto *et al.* 1993).
[b] -, nucleotide deleted
[c] +, -; gain and loss of site, respectively.

during this project, we have revealed that overseas work was misleading because only a few populations representative of species and races were used and molecular differences between populations had been overestimated and because some of their populations had been misidentified. Our mtDNA groupings are corroborated by other genetic data, e.g. esterase phenotype. However, these molecular groupings conflict with identification of species and races by the standard host range test leading us to question the validity of the existing taxonomy.

The standard host range test, which was developed in the USA has been used for 40 years to provide a preliminary indication of nematode identity. The main disadvantage of the standard host range test is that it forces populations into artificial groups based on numbers of eggmasses on plants. There are further potential sources of error when assessing low numbers of eggmasses. The test also does not cope with species other than the four most common, such as *M. hispanica* and other variations as exhibited by the novel reactions, although novel reactions may represent rare species.

In this study, cotton was resistant and tomato susceptible to all root-knot nematode populations. Therefore, in effect, only four plant genotypes were being used to differentiate several species and races of *Meloidogyne*. It is probably an oversimplification to assume that the reactions of even the four most common *Meloidogyne* species and their races on four plant cultivars represent the total pathotype variability, especially as this genus is known to attack more than 2000 plant species. If the set of differentials were expanded, more differences among populations should be demonstrated. Our work suggests that some soybean cultivars may be useful for further differentiation of molecular groupings.

The standard host test was poorly correlated with perineal pattern, indicating that morphology was not a reliable indicator of pathogenicity. Hugall *et al.* (1994) suggest that further molecular groupings are required in haplotypes A and D to be consistent with the standard host test. Similarly, further host range data are required in these haplotypes so that pathogenicity is consistent with molecular groupings. We propose to assign plants with up to 5 eggmasses as resistant and more than 30 eggmasses as susceptible and not to assign intermediates to groups. A more useful host test could be devised by concurrently determining molecular groupings of populations and the host range of these groupings in the cropping systems of interest. Then it would be possible to predict control strategies based on crop rotation and resistant cultivars.

There is very low divergence between and diversity within haplotypes A, B, C, D, G and H. This, together with no discernible phylogenetic order between haplotypes and essentially fixation of esterase phenotype with mtDNA haplotypes (except in haplotype A), is explained by recent independent parthenogenetic origins of each haplotype from minor variants of the same female lineage. *M. hapla*, however, has a distinct female lineage. It appears that *M. hapla* has the ancestral gene order. *M. javanica*, *M. incognita* and *M. arenaria* have insertions and rearrangements of this gene order with subsequent deletion in *M. arenaria*.

Previous studies reported much greater sequence divergence between *M. javanica*, *M. incognita* and *M. arenaria* than did this study because they were

misled by length variations not due to nucleotide changes, the deletion in haplotype A and the multiple restriction site changes caused by single mutations. Our degree of error is much less also because we used higher resolution gels which distinguish more restriction sites. The wide divergence of *M. hapla* from the other species is consistent with other molecular data for nuclear genes e.g. allozyme and nuclear repeat studies. Our studies have also quantified divergence. Previous mtDNA studies confused the relationship between *M. hapla* and the other species because they lacked resolution and detail and because some populations had been misidentified.

(ix) IMPLICATIONS AND RECOMMENDATIONS

A polymerase chain reaction-based diagnostic test is now available to identify major molecular groups of *Meloidogyne*. These groups are generally related to host range. However, more work is needed to corroborate molecular grouping and to make these consistent with host range groups. Progress is now being made towards subdividing molecular groups using nuclear ribosomal DNA and RAPD studies. We also need to explore agriculturally relevant attributes of molecular groups by assessing an extended group of differential hosts.

When a diagnostic test is available to predict host range, this will catalyse the use of non-host rotation crops and resistant cultivars to control root-knot nematode. Industries threatened by severe yield losses due to lack of suitable chemical nematicides will then be able to rely on more sustainable management strategies.

(x) DESCRIPTION OF INTELLECTUAL PROPERTY

This project has not produced developments of immediate commercial significance. However, it is possible that a PCR-based diagnostic test may be commercialised.

(xi) TECHNICAL SUMMARY

As a result of this project, techniques for DNA preparation have been refined and used to isolate mtDNA and nuclear DNA from eggs of the root-knot nematode collection. Standard techniques for DNA analysis have been modified and refined for routine use with root-knot nematode DNA.

The root-knot nematode collection contains more than 100 populations from throughout Australia. Of these populations, 74 have been characterised by standard host range, 89 by perineal pattern, 69 by morphometric data, 51 by esterase phenotype and 66 by mtDNA restriction site variants. This has divided the collection into ten molecular groupings. Esterase phenotype is a character in addition to the original objectives set but has been found to correlate well with molecular groupings. Morphometric data and perineal pattern have been found to be too variable to be of use and will not be continued.

Previous studies revealed substantial variation in mtDNA apparently due to restriction site changes and variation in copy-number of 63 bp and 102 bp tandem repeats, but were hampered by small sample sizes and, in one case, misidentified specimens. We have extended the analysis of *Meloidogyne* mtDNA by (i) examining restriction site polymorphisms among a large number of Australian populations that have also been characterised for perineal pattern, standard host range and esterase phenotype, (ii) using higher resolution electrophoretic techniques and (iii) mapping variable restriction sites with reference to the complete nucleotide sequence. Our analysis reveals far less sequence divergence (<0.6%) between variants than previously reported.

There was perfect correlation between mtDNA haplotype and esterase phenotype. In contrast, there were several discrepancies between traditional methods of identification and between these and either of the biochemical measures. Combined with the conceptual difficulties of delineating species among closely related parthenogenetic forms, our data suggest that further development of diagnostics for *Meloidogyne* should focus on well-defined genetic groups, whether or not these coincide with existing taxonomic units.

Amplification of mtDNA regions between the open reading frame (ORF) and the *cyt*b gene and digestion with *Hin*fI or *Mnl*I will distinguish six molecular groups while digestion with *Ase*I or *Dra*I will differentiate two groups of *M. hapla*. In developing this, six new primers have been devised and more than 2000 bp of sequence for the ten haplotypes have been determined.

Progress has been made towards elucidating discrepancies between molecular groupings and host range by extending the host range test from the standard differential hosts. To date, 50 plant genotypes have been assessed for their ability to distinguish between different root-knot nematode types and several are of potential use. Four cultivars of soybean show promise in distinguishing haplotypes A, C and D and in confirming differences in host range within haplotype D.

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