Reticulate Evolution and the Origins of Ribosomal Internal Transcribed Spacer Diversity in Apomictic *Meloidogyne*

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Among root knot nematodes of the genus *Meloidogyne,* the polyploid obligate mitotic parthenogens *M. arenaria, M. javanica,* and *M. incognita* are widespread and common agricultural pests. Although these named forms are distinguishable by closely related mitochondrial DNA (mtDNA) haplotypes, detailed sequence analyses of internal transcribed spacers (ITSs) of nuclear ribosomal genes reveal extremely high diversity, even within individual nematodes. This ITS diversity is broadly structured into two very different groups that are 12%–18% divergent: one with low diversity (<1.0%) and one with high diversity (6%–7%). In both of these groups, identical sequences can be found within individual nematodes of different mtDNA haplotypes (i.e., among species). Analysis of genetic variance indicates that more than 90% of ITS diversity can be found within an individual nematode, with small but statistically significant (5%–10%; $P < 0.05$) variance distributed among mtDNA lineages. The evolutionarily distinct parthenogen *M. hapla* shows a similar pattern of ITS diversity, with two divergent groups of ITSs within each individual. In contrast, two diploid amphimictic species have only one lineage of ITSs with low diversity (<0.2%). The presence of divergent lineages of rDNA in the apomictic taxa is unlikely to be due to differences among pseudogenes. Instead, we suggest that the diversity of ITSs in *M. arenaria, M. javanica,* and *M. incognita* is due to hybrid origins from closely related females (as inferred from mtDNA) and combinations of more diverse paternal lineages.

Introduction

Species of the root knot nematode *Meloidogyne* exhibit extensive cytogenetic diversity, with many parthenogenetic forms, a large proportion of which are polyploid, obligate, mitotic parthenogens demonstrating a broad geographical distribution and host range diversity. Three apomictic species in particular, *M. arenaria, M. javanica,* and *M. incognita,* together with *M. hapla,* a species which has both mitotic and meiotic parthenogenetic races, are ubiquitous pests to agriculture (Taylor, Sasser, and Nelson 1982). Traditionally, species have been defined by host range tests, perineal patterns, and allozyme analysis (Taylor and Sasser 1978; Eisenback et al. 1981; Esbenshade and Triantaphyllou 1990), and more recently, mitochondrial DNA (mtDNA) diagnostics have been described (Powers and Harris 1993; Hugall et al. 1994; Stanton, Hugall, and Moritz 1997). Among populations of *M. arenaria, M. javanica,* and *M. incognita,* mtDNA diversity is very low (<0.6%) but strongly correlated with esterase allozyme phenotypes considered diagnostic for the species, with each esterase type having a single mtDNA haplotype (see fig. 1). *Meloidogyne hapla* is evolutionarily distinct and represents a separate origin of parthenogenesis from an unrelated maternal lineage (Hugall, Stanton, and Moritz 1997).

In contrast to the low levels of mtDNA diversity, these species have substantial nuclear genetic diversity, as reflected by allozymes, anonymous repeated sequences, and RAPDs (e.g., Esbenshade and Triantaphyllou 1987; Castagnone-Sereno et al. 1993; Castagnone-Sereno, Vanlerberghe-Masutti, and Leroy 1994). This contrast of low but strongly structured mtDNA diversity against higher nuclear diversity could be explained by multiple, possibly hybrid, origins from distinct but closely related sexual females, as has been established for a number of other polyploid parthenogenetic taxa (Densmore, Wright, and Brown 1989; Moritz et al. 1989; Moritz 1993; Dufresne and Hebert 1994) and has been suggested for some lineages of *Meloidogyne* by Triantaphyllou (1985).

To further investigate the distribution of genetic diversity within and among parthenogenetic lineages of *Meloidogyne,* we undertook an analysis of variation for nuclear ribosomal internal transcribed spacers (ITSs). The aims of our study were to test whether there was significant nuclear sequence variation among previously identified mtDNA types (=species) that may shed light on their origins and provide further tools for species identification.

Although nothing is known of the organization of rDNA clusters in *Meloidogyne,* incidental evidence (unpublished Southern blot analysis; Vahidi et al. 1988 on cloned deletion variants) suggests that it is complex. However, several sequence analyses of rDNA (including ITSs and intergenic spacers [IGSs]) have reported very similar or even identical sequences among *M. arenaria, M. javanica,* and *M. incognita* but also evidence of heterogeneity within individual nematodes (Adams and Powers 1996; Blok, Phillips, and Fargette 1997; Powers et al. 1997; Zijlstra, Uenk, and van Silfhout 1997).

Among known or suspected meiotic hybrid taxa, diversity of ribosomal ITSs has been attributed to the retention of, and perhaps reticulate evolution between, the heterologous genomes (Wendel, Schnabel, and Seelanan 1995; Buckler and Holtsford 1996; Odorico and Miller 1997; Roelofs et al. 1997). While in meiotic interspecific hybrids gene conversion appears to able to replace one parental rDNA with the other (Hillis et al.

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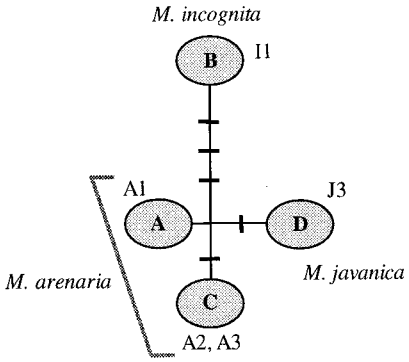

FIG. 1.—Summary of mtDNA and esterase phenotype diversity found for three species of *Meloidogyne*. Esterase codes are next to mtDNA haplotypes in ovals, with single-base differences marked on branches (from Hugall et al. 1994; Hugall, Stanton, and Moritz 1997; Stanton, Hugall, and Moritz 1997). MtDNA is based on sequence data from 1,188 bp covering 16S rDNA and ND3.

1991; Wendel, Schnabel, and Seelanan 1995), the nature of concerted evolution among rDNA copies in mitotic clonal lineages has not been investigated, although theoretically, it could still be effective (Schlötterer and Tautz 1994; Birky 1996).

Given all the complications of polyploidy, mitotic parthenogenesis, and concerted evolution, we took a population genetic approach to assessing the distribution of ITS genetic diversity within an individual, and within and among clonal taxa, which a priori we suggest be mtDNA groups.

Materials and Methods

Nematode Isolates

Isolates from 17 *Meloidogyne* populations were used in this study. Fourteen of these were previously described (Hugall et al. 1994). The other three were *M. javanica* isolate 126, an *M. chitwoodi* isolate from the U.S.A. (T. Powers, personal communication), and *M. triformophila* isolate 160 from New Zealand (C. Mercer, personal communication). The karyotype and mode of reproduction of this last isolate are not known, but it appears to be closely related to *M. graminicola* based on morphological, mtDNA, and allozyme analyses (Bernard and Eisenback 1997; Mercer, Starr, and Miller 1997; unpublished data) and is probably a diploid and facultatively sexual species, as is *M. chitwoodi*. The remainder are polyploid obligate mitotic parthenogens (Triantaphyllou 1966, 1985; Esbenshade and Triantaphyllou 1987). Based on chromosome counts, one of the *M. hapla* isolates (isolate 113) appears to be of polyploid apomictic race B.

All isolates were maintained as single-egg mass cultures. The DNA extraction of an isolate is therefore from the offspring of a single adult nematode. Sequence analysis of multiple clones from each isolate therefore samples the within-individual genome diversity. For isolate 126, this is literally the case, with the DNA extracted from a single animal. All isolates have been typed allozymically for esterase; all but 126, J, and 160 have been typed by the North Carolina differential host test (Hartman and Sasser 1985); and all but NQ5 have been typed by perineal pattern (see Hugall et al. 1994). The identity of the DNA extracts used in the ITS analysis were confirmed using the PCR-RFLP test described in Stanton, Hugall, and Moritz (1997).

DNA Extractions

DNA was purified by standard methods based on phenol-chloroform extraction of proteinase-K-digested pooled egg masses from a single pot culture, as described in Hugall et al. (1994) and Stanton, Hugall, and Moritz (1997). Isolate 126 was extracted using 5% Chelex (Bio-Rad) from a single adult female excised from a root gall.

PCR Amplification

Two sets of primers were used, and the sequence positions are described with respect to *Caenorhabditis elegans* rDNA of Ellis, Sulston, and Coulson (1986). The first (ITS2.1, ITS2.2) was designed for highly conserved regions of the 18S and 28S ribosomal RNA genes immediately flanking the ITS region (ITS2.1, 18S position 1636, CGTAGGTGAACCTGCGGAAGGATC; ITS2.2, 28S position 2693, CCTGGTTAGTTTCTTTT CCTCCGC). These primers should, in principle, recognize all known ribosomal targets, and in some cases, they amplified several spurious ITS-like sequences (as determined by phylogenetic analysis of 5.8S rDNA gene), presumably due to fungal contamination in some of the DNA extracts. For all ITS clones analyzed here, the 5.8S genes formed a robust monophyletic group with respect to *Heterodera avenae* and *Naccobus aberrans*, representatives of tylenchid sister genera (GenBank accession numbers U12389 and U71376), and are considered to represent *Meloidogyne*. The second set of primers (MP581, MITS2) was designed to amplify ITS sequences among *M. arenaria, M. javanica,* and *M. incognita,* so as to preferentially exclude one of the two groups of ITS sequences obtained in the first round of PCR (see below) (MP581, 5.8S position 2187, ATTTGTGCGAACTGCAGAAACC; MITS2, 28S position 2645, GCTCTCGACTGAGTTCAGGTC). All amplifications were done using 4 μM primer, 1.7 mM MgCl, 0.2 U Promega *Taq* polymerase I with approximately 40 ng of genomic DNA template in a 25-μl volume with annealing at 50°C for 40 s and extensions at 72°C for 1 min.

Cloning and Sequencing

PCR products were end-filled with Klenow DNA Polymerase I, size-selected on 1.5% agarose gels, and blunt-end-cloned into Bluescript pKS+ cut with *Eco*RV. For the full-length ITS clones made with ITS2.1 and ITS2.2 primers, colonies were screened with RFLP testing of mini-prep plasmid DNA which were then cycle-sequenced with primers end-labeled with [γ-^{33}P]ATP using combinations of vector primers KS17 and SK20 and the original PCR primers ITS2.1 and ITS2.2. For the second series of clones made using M581 and MITS2, colonies were screened directly by PCR with vector primers T3 and T7, and the product was gel-purified and

Table 1
Sizes and Base Contents of the ITS Regions and the Intervening 5.8S rDNA

Species	No. of Clones	ITS1	5.8S	ITS2
Meloidogyne arenaria, M. javanica, and *M. incognita,* mtDNA haplotypes A, B, C, and D				
Group 1	11	219–223 (62.0)	158 (54.5)	107–108 (76.8)
Group 2	11	215–224 (63.0)	157–158 (52.1)	109–113 (74.8)
Meloidogyne trifoliophila	6	227 (66.8)	157 (53.7)	120 (83.3)
Meloidogyne chitwoodi	7	219 (64.7)	157 (54)	107 (81.3)
Meloidogyne hapla				
Group 3	3	223 (64.4)	156–158 (51.8)	113–114 (78.6)
Group 4	6	217–219 (62.2)	156–158 (53.8)	108 (73.6)

NOTE.—Numbers in parentheses are percentages of A+T content. For the obligate parthenogenetic species, results are presented for each of the groups outlined in figure 2.

sequenced using ABI dye terminator automated sequencing. For most of these clones, both strands were sequenced.

Statistical Analyses

Nucleotide sequences were aligned using CLUSTAL V (Higgins, Bleasby, and Fuchs 1992). Sequence statistics, pairwise differences, and estimates of divergence were determined with MEGA (Kumar, Tamura, and Nei 1993). Nucleotide diversities were estimated with REAP (McElroy et al. 1992). Nucleotide diversities and divergences are based on observed numbers of differences only. Gaps were treated as missing data with pairwise exclusion. Unrooted networks were developed by neighbor-joining and maximum-parsimony algorithms (using tree bisection-reconnection [TBR] with all characters unordered and unweighted) in MEGA and PAUP (Swofford 1993). Analyses of the distribution of genetic variance with and without molecular distance measures were done using analysis of molecular variance (AMOVA) (Excoffier, Smouse, and Quattro 1992) and significance tested using 1,000 permutations. Evidence of recombination among sequences was explored using RETICULATE (Jakobsen and Easteal 1996) and the runs method of Stephens (1985). A subset of sequences from this study are available in the GenBank database (accession numbers AF077082–AF077091). Multiple alignments have not been included but are available from the University of Queensland Department of Zoology internet site http://www.zoology.uq.edu.au/research/.

Results

Analysis of Full-Length ITS Sequence Diversity

Using the universal primers ITS1.1 and ITS2.2, 44 clones were sequenced from 10 isolates, including 2 facultative sexual parthenogenetic species. Multiple clones were sequenced from all individual isolates. Sizes ranged from 483 to 510 bp, of which 156–158 bp was 5.8S rDNA. Three of the clones were found to be incomplete, missing large sections of ITS1. As the PCR product was size-selected, this subsequent size difference was probably an artifact of cloning. The ITS2 is smaller, more A+T-rich than ITS1 (table 1), and more variable (table 2). Secondary-structure analysis revealed ITS1 and ITS2 to be essentially unique sequences, with one clone (C77-8) having an ~25-bp inversion in the ITS1. Across the whole data set, both ITS1 and ITS2 exhibit extensive sequence diversity, making alignments somewhat subjective; in comparison, 5.8S had lower sequence variation and unambiguous alignment. Plotting variable sites per 25-bp moving window (data not shown) shows the 5.8S and the small 5′ fragment of 28S to have the least variability, with ITS2 being the most variable. This is also reflected in the diversities of each segment (table 2).

Six and seven clones, respectively, were sequenced from a single isolate from each of the two facultative sexual parthenogenetic species *M. trifoliophila* and *M. chitwoodi.* Sizes were 504 and 483 bp, respectively. Each isolate had two variants with low overall sequence diversity (0.1%–0.2%). This would suggest that *Taq* polymerase error has made a minimal contribution to apparent diversity. Divergence between the species averaged 12% across the entire ITS and was 2.3% for 5.8S (table 2).

Thirty-one clones were sequenced from eight polyploid parthenogenetic isolates: one each of mtDNA haplotypes A (*M. arenaria* isolate NQ1), C (*M. arenaria* isolate 77), and D (*M. javanica* isolate 42); two of hap-

Table 2
Nucleotide Diversities of ITS Sections

Group	ITS1	5.8S	ITS2
Meloidogyne arenaria, M. javanica, and *M. incognita* mtDNA haplotypes A, B, C, and D			
Group 1	0.010	0.008	0.007
Group 2	0.042	0.009	0.119
Between groups 1 and 2[a]	0.086	0.087	0.228
Meloidogyne trifoliophila–Meloidogyne chitwoodi[a]	0.195	0.023	0.325
Meloidogyne hapla			
Group 3	0.014	0.021	0.012
Group 4	0.012	0.018	0.019
Between groups 3 and 4[a]	0.111	0.096	0.648
Between groups 2 and 3[a]	0.091	0.043	0.500

NOTE.—All values are based on uncorrected proportional distance. Note that the estimates of divergence among groups for ITS1 and ITS2 are approximate because of uncertainties in alignment.

[a] Average of pairwise divergences between groups.

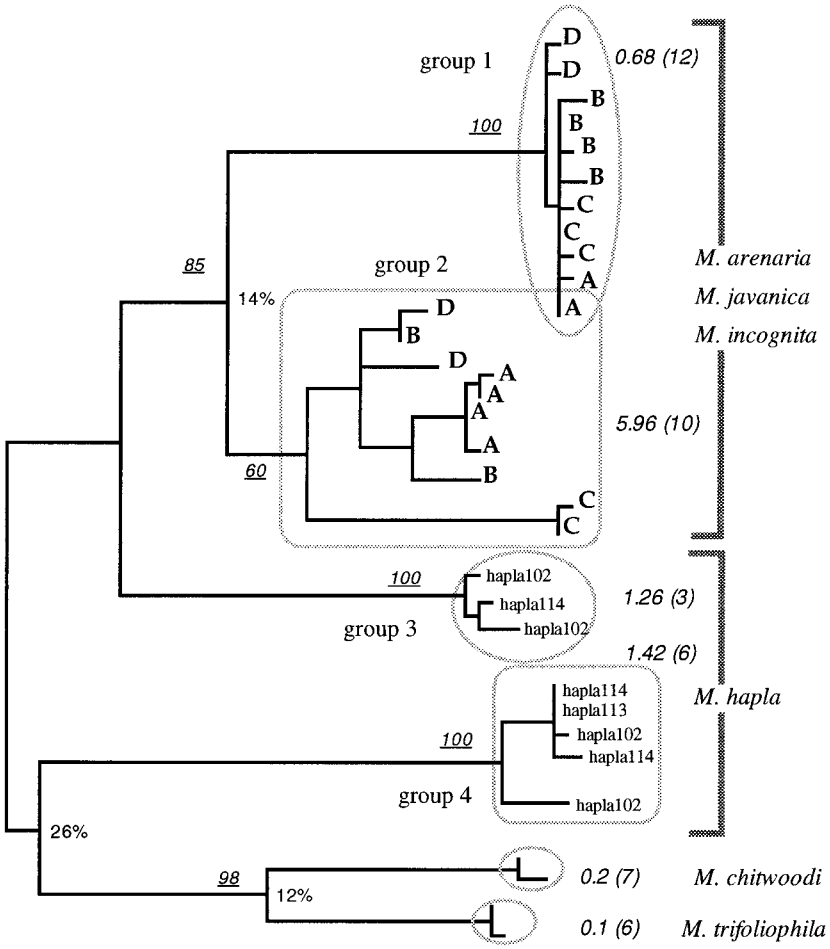

FIG. 2.—Maximum-parsimony network of ITS sequences within and among species; unweighted midpoint 50% consensus network of 5.8S rDNA and ITS sequences amplified with ITS1 and ITS2 primers. Species designations are on the right. The letters A, B, C, and D refer to mtDNA haplotypes described in Hugall et al. (1994) and Stanton, Hugall, and Moritz (1997) corresponding to *M. arenaria*, *M. javanica*, and *M. incognita*. Names of *M. hapla* clones refer to isolates described in Hugall et al. (1994) and Hugall, Stanton, and Moritz (1997). Numbers adjacent to each group outlined are percentages of nucleotide diversities and are followed by numbers of clones (in brackets). Percentage values at nodes are average divergences between groups. Underlined numbers at nodes are bootstrap values for 100 replicates.

lotype B (*M. incognita* isolates J and H), and three isolates of *M. hapla* (102, 113, and 114). The 31 clones from the eight isolates yielded 26 distinct sequences with between one and six variants per isolate; within-individual (isolates) sequence divergence ranged up to 18% in *M. arenaria*, *M. incognita*, and *M. javanica* and 26% in *M. hapla*.

The sequence diversity for the full-length ITS clones can be represented as a maximum-parsimony unrooted network (fig. 2). This analysis uses 300 bp covering some of ITS1, 5.8S, and ITS2 to maximize the number of sequences available; the tree is essentially the same including all of ITS1 where available. Two features worth addressing are the degree of clustering and the diversities of the clusters.

The sequences cluster as six groups: one each for *M. trifoliophila* and *M. chitwoodi*, two for the species associated with mtDNA haplotypes A, B, C, and D (*M. arenaria*, *M. incognita*, and *M. javanica*), labeled groups 1 and 2 in figure 2, and two for *M. hapla* (groups 3 and 4). This overall pattern is present independently in all three domains ITS1, 5.8S, and ITS2 (table 2). Bootstrap analyses of the full length and also of the 5.8S-ITS2 subset support the clustering of all but the group 2 sequences found among *M. arenaria*, *M. incognita*, and *M. javanica*.

Each isolate of the parthenogenetic species, except *M. hapla* 113 (two clones only) included sequences from each of two divergent groups. Within the isolates of *M. arenaria*, *M. javanica*, and *M. incognita*, one rDNA group (group 1 in fig. 2) had low diversity (0.7%), with several examples of identical sequences in different isolates, whereas the other had high diversity (6%). As both of these groups had similarly low diversity in 5.8S (0.8% vs. 0.9%), the extra diversity of group 2 is due to the ITS regions. Divergences between representatives of the two groups ranged from 14% to 18% overall, with an average of 8.6% for 5.8S. Each of the two groups in *M. hapla* had moderate diversity (1.3%–1.4%), with diversity spread evenly across the three regions (table 2). Average divergence between the two groups was 25% (9.6% for 5.8S). Among the three regions of the six groups of ITSs, only divergences be-

Table 3
Sequence Variants and Nucleotide Diversities Among Group 2–Specific ITS Clones

Isolate (haplotype)	No. of Clones	No. of Variants	% Nucleotide Diversity
NQ1 (A)	12	12	7.5
75 (A)	13	12	6.9
39 (A)	12	11	6.7
H (B)	13	13	7.9
J (B)	12	9	5.0
35 (B)	13	9	4.4
77 (C)	12	9	8.2
NQ5 (C)	13	12	6.1
NQ7 (C)	14	13	10.7
42 (D)	12	9	3.5
72 (D)	12	12	9.5
126 (D)	13	9	5.1
Subtotals for mtDNA haplotype groups[a]			
A	37	29	7.1
B	38	22	5.8
C	39	23	8.3
D	37	23	6.2

NOTE.—All values are based on uncorrected proportional distance.
[a] Nucleotide diversity estimated from all clones in each mtDNA group.

tween 5.8S and ITS2 were correlated (Kendall's tau = 0.428, $P < 0.05$).

Analysis of Group 2 ITS Diversity Among *M. arenaria, M. incognita,* and *M. javanica*

To further examine the partitioning of variation within and among the three species *M. arenaria, M. incognita,* and *M. javanica,* and the mtDNA variants that diagnose them (A+C, B, and D, respectively, fig. 1), we focused on the relatively diverse assemblage of sequence variants (group 2 in fig. 2) by designing primers that should exclude the other, less diverse, group 1 sequences. To limit a priori bias, one primer, MITS2, was designed to amplify all sequences, whereas M581 was located in the 5.8S gene but with mismatches at the 3′ end to variants that unambiguously defined group 1 sequences. A total of 12–14 clones were sequenced for each of three isolates representing each of the four mtDNA types (resulting in 151 clones in total; table 3).

The 151 clones included 89 different sequence variants (ignoring gaps), for a total nucleotide diversity of 7.2%. This diversity is similar to the estimate from the original 10 sequences obtained in the first analysis (~6%). Each nematode isolate was found to have a large number of sequence variants (9–13 per isolate) (table 3). Sequence diversity within isolates ranged from 4.4% to 10.7%. Twenty of the 89 sequence variants were represented by multiple clones ($N = 2$–14), with 19 of these being from different isolates. The most common variant was found in six isolates spanning mtDNA haplotypes A, B, and C.

Owing to the large number of variants, neighbor joining rather than parsimony was used to visualize the diversity as a network of similarity. Presenting the network as a midpoint rectangular phylogram is not meant to imply anything about the nature of the evolution of these many ITS variants; it is merely a succinct way of

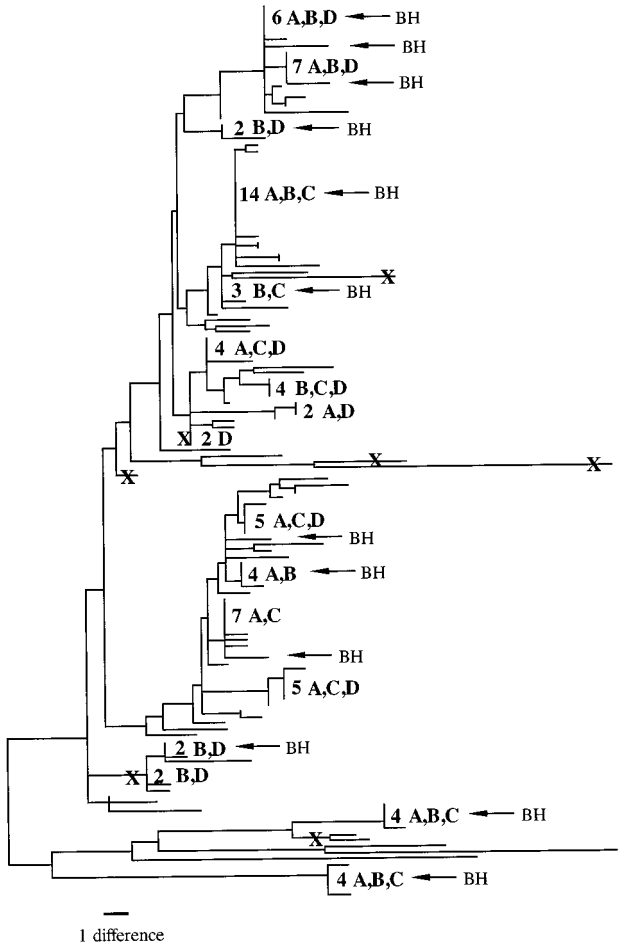

FIG. 3.—Neighbor-joining network of group 2 ITS sequences from *M. arenaria, M. incognita,* and *M. javanica* (complete ITS2 and ~80 bp of 5.8S amplified with MITS2 and MP581 primers). The analysis is based on unweighted absolute number of differences and is midpoint-rooted. Multiple identical clones from different individual isolates are marked by the number of clones, adjacent to the mtDNA haplotypes of these isolates. Also shown are the 12 sequences cloned from isolate BH (mtDNA haplotype B, *M. incognita*). Potential recombinant clones mentioned in the text are marked with an "X". Scale shows one difference.

presenting the data. The original 10 sequences from cloning the full ITS are distributed across the tree (not indicated), suggesting that they were a reasonable subsample. Individual isolates (and, thus, the mtDNA groups) have many different variants spread across the network (e.g., isolate BH, fig. 3). Conversely, specific variants are found in different individuals of different mtDNA types (A–D, fig. 3). While bootstrap analyses reinforce the existence of a number of discrete clusters in addition to the groups of identical sequences, given the complexity of these, a statistical approach to partitioning the diversity is preferable to a tree-based approach (see below). Of the sequences cloned (marked "X" in fig. 3), several are consistent with being "recombinants" (based on runs and compatibility tests of Stephens [1985] and Jakobsen and Easteal [1996]; $P < 0.05$, see fig. 3). It is not possible to tell whether these derive from the *Meloidogyne* genome or are artifacts of

Table 4
Hierarchical Analysis of Genetic Variance Using AMOVA

Comparison	Within Individual		Among Individuals in Groups		Among Groups	
	% Variance	*P*	% Variance	*P*	% Variance	*P*
mtDNA groups	92.6	<0.001	1.9	NS[a]	5.5	0.001
Variants with $N > 1$	90.5	<0.001	1.0	NS	8.5	0.001
Other groupings						
Among host range	94.1	0.002	5.8	0.001	0.1	NS
Among perineal pattern	93.7	<0.001	7.0	0.001	−0.7	NS

[a] Not significant at $P = 0.05$.

PCR (e.g., Bradley and Hillis 1997), but some recombinant sequences derived from several different isolates are probably present in the original genomic DNA.

Analysis of Molecular Variance

An AMOVA (table 4) was used to partition the genetic diversity and test whether there was any hierarchy of ITS sequence variation among individuals, in particular among the proposed clonal lineages defined by mtDNA type (fig. 1). AMOVA revealed that most (93%) of the variation occurred within individual isolates and that there was a small (5.5%) but significant ($P < 0.05$) partitioning of variation between the four mtDNA types. Considering only the alleles that occurred more than once (and thus were less likely to be PCR artifacts), the amount of variation among mtDNA types increases slightly (8.5%) and remains significant. In contrast, AMOVA analyses among host range or perineal pattern classes (both more ambiguous approaches to species identification, see Stanton, Hugall, and Moritz 1997) reveal lower and nonsignificant differentiation (table 4).

Discussion

The object of this ITS study was to test for further correlation of nuclear gene variation with mtDNA markers that appear to be diagnostic for the species (Hugall et al. 1994). Two key observations are that there is (1) extraordinarily high heterogeneity of rDNA sequences within apomictic individuals and (2) low but significant correlation of rDNA diversity with mtDNA types.

Interpretation of rDNA Heterogeneity

In looking for possible causes of the ITS diversity, two observations need to be considered. First, each of the apomictic taxa have two divergent groups of sequences (as typified by the 5.8S rDNA gene), whereas the sexual species have one. Second, within *M. arenaria, M. incognita,* and *M. javanica,* there is one relatively uniform group of sequences and a second far more heterogeneous assemblage. Below, we consider three hypotheses that attempt to explain these observations.

First, the two groups of ITS sequences could represent nonhomologous loci, for example, different clusters of rDNA on nonhomologous chromosomes with no concerted evolution between the paralogous sets. The relative absence of heterogeneity within either of the sexually reproducing species versus the presence of two groups in *M. arenaria, M. incognita,* and *M. javanica* and in the independently evolved *M. hapla* argues against duplicate rDNA loci in ancestral *Meloidogyne.* However, given that *M. chitwoodi* and *M. trifoliophila* may form a clade with respect to the other species, they could share a loss of one locus. This hypothesis, however, does not readily explain the high diversity of group 2 ITS sequences relative to those in group 1.

Second, there could have been independent formation of pseudogenes in *M. hapla* and the ancestor of *M. arenaria, M. incognita,* and *M. javanica.* The presence of pseudogenes is supported by the cloning of deletion mutants from *M. arenaria* by Vahidi et al. (1988) and by our unpublished data. However, the level of difference between the functional locus and the putative pseudogenes is not in keeping with the patterns of variation across the ITS regions, particularly the conservation of the 5.8S segment (table 2). Buckler, Ippolito, and Holtsford (1997) identified pseudogenes by shifts in base content and thermodynamic stability, but in *Meloidogyne,* the two ITS groups do not differ significantly in percentage of A+T. While recognizing that some of the observed variation could represent pseudogenes, the above observations seem inconsistent with this as an explanation of the presence of major dichotomies in both of the mitotic parthenogenetic complexes examined.

Third, the structuring and high diversity of ITS genetic variation could be due to hybrid origins of the polyploid obligate mitotic parthenogens, *M. arenaria, M. incognita,* and *M. javanica,* and also of the evolutionarily independent *M. hapla.* According to this hypothesis, the distinct groups of rDNA would be derived from different sexual species with divergent sequences, with subsequent attenuation of interchromosomal concerted evolution. This interpretation is at odds with the earlier suggestions that apomictic *M. hapla* (race B) arose by direct transition from facultative meiotic *M. hapla* (race A) (Triantaphyllou 1985, 1991), but the transition to parthenogenesis and polyploidy is often complex (e.g., Tinti and Scali 1996).

Following this interpretation, the low-diversity group 1 sequences in *M. arenaria, M. javanica,* and *M. incognita* reflect the low diversity of mtDNA and may reflect recent common inheritance from a single shared maternal ancestral species. The heterogeneous group 2 sequences would derive from paternal contributions

from diverse sexual species to these polyploid lineages, perhaps enhanced by effects of reticulate evolution among the different genomes. This hypothesis mirrors interpretations of complexes of hybrid-parthenogenetic lineages of lizards, which also have low diversity for mtDNA but high diversity for nuclear genes (Densmore, Wright, and Brown 1989; Moritz et al. 1989; Moritz 1993), and of some invertebrate polyploid apomictic taxa, such as Ostracodes (Little and Hebert 1997).

Meloidogyne hapla also has two groups, and this parallel pattern in an evolutionarily independent parthenogen reinforces the hybrid origin hypothesis. It is not possible to ascribe maternal or paternal origin to the two types of ITS in *M. hapla*, but the overall within-group diversity matches previous studies of genomic diversity (allozyme and nuclear DNA), being less than that seen among *M. arenaria, M. incognita,* and *M. javanica* (Esbenshade and Triantaphyllou 1987; Castagnone-Sereno et al. 1993).

Although we favor the third hypothesis on the basis of the available evidence, rigorous proof would require demonstration of a single rDNA locus and identification of multiple parental species by comparison of genetic profiles of the parthenogenetic lineages with those of sexually reproducing species of *Meloidogyne* using single-copy nuclear genes. Regrettably, the sexual relations of parthenogenetic *Meloidogyne* are poorly known (Triantaphyllou 1985; Trudgill 1995).

Correlation of rDNA Diversity and mtDNA Groups

Detailed analysis of sequence diversity among group 2 sequences for *M. arenaria, M. incognita,* and *M. javanica* suggests a low but significant correlation with mtDNA markers (table 4). In contrast, there was no correlation with phenotypic measures previously used to identify species—host range and perineal pattern. Combined with the absolute correlation of mtDNA and isozyme patterns, this supports the suggestion that the mtDNA markers provide appropriate diagnostics. However, the high level of within-individual variation for rDNA sequences precludes their use for diagnostic purposes.

If our hypothesis of hybrid origins is true, summarizing net multilocus data as genetic distances (as has been done in all analyses of *Meloidogyne* nuclear diversity to date) may lead to spurious phylogenetic interpretations (Sanderson and Doyle 1992; Funk et al. 1995). Typically, these earlier analyses were interpreted under the assumptions of divergent phylogenesis producing a pattern of relationships wherein *M. arenaria* and *M. javanica* are closely related, forming a sister group to *M. incognita,* with *M. hapla* as a divergent lineage. However, as this approach will obscure hybrid ancestry or other forms of reticulation among species, we suggest that the mode of evolution of these species and relationships among parthenogenetic and sexually reproducing species of *Meloidogyne* should be revisited through sequence analysis of single-copy genes (for example, the extensive analyses of Gaut and Dobely 1997), including a wide variety of sexual as well as parthenogenetic species.

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