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# High-resolution DNA fingerprinting of parthenogenetic root-knot nematodes using AFLP analysis

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**Abstract**

**Amplified fragment length polymorphism (AFLP) analysis has been used to characterize 15 root-knot nematode populations belonging to the three parthenogenetic species *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. Sixteen primer combinations were used to generate AFLP patterns, with a total number of amplified fragments ranging from 872 to 1087, depending on the population tested. Two kinds of polymorphic DNA fragments could be distinguished: bands amplified in a single genotype, and bands polymorphic between genotypes (i.e. amplified in not all but at least two genotypes). Based on presence/absence of amplified bands and pairwise similarity values, all the populations tested were clustered according to their specific status. Significant intraspecific variation was revealed by AFLP, with DNA fragments polymorphic among populations within each of the three species tested. *M. arenaria* appeared as the most variable species, while *M. javanica* was the least polymorphic. Within each specific cluster, no general correlation could be found between genomic similarity and geographical origin of the populations. The results reported here showed the ability of the AFLP procedure to generate markers useful for genetic analysis in root-knot nematodes.**

*Keywords*: AFLP, fingerprinting, *Meloidogyne* spp., nematodes, parthenogenesis

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## Introduction

Root-knot nematodes (RKN) constitute the most widely distributed group of plant-parasitic nematodes. These biotrophic endoparasites cause extensive damage to a wide variety of economically important plants, and are responsible for world-wide annual loss, estimated at about 5% (Sasser & Carter 1985). Among the 55 described species, *Meloidogyne arenaria*, *M. incognita* and *M. javanica* are considered the three major species, accounting for 90% of this destruction (Lamberti 1979). These three species are homogenously encountered from temperate to tropical regions. In contrast to most *Meloidogyne* species, which are generally parasitic against a specific botanical family only, they are extremely polyphagous pests, with a wide host range of up to 3000 plant species including most of the commercial crops. Of particular concern is also the mode of reproduction of these three species, which is mitotic parthenogenesis. Although still hypothetical, the evolutionary pathway of RKN has been based on the following assumptions: (i) parthenogenetic nematodes evolved from ancestral forms that were amphimictic; (ii) all the mitotic parthenogenetic RKN probably evolved from meiotic parthenogenetic ancestors, or less likely, from amphimictic ones, following suppression of the meiotic process during maturation of the oocytes (Dalmasso & Bergé 1983; Triantaphyllou 1985).

The reasons for the widespread distribution of these three species remain unclear. Considering the fact that RKN are probably extremely ancient species, it seems likely that they are indigenous to most of the areas where they have been found. However, recent molecular data demonstrated a low level of infraspecific polymorphism within these three parthenogenetic species (Block *et al*. 1997b), which is not in agreement with the hypothesis of their indigenous origin. Comparisons of corresponding mtDNA coding sequences from *M. javanica* and *M. incognita* have indicated a very low nucleotide divergence, suggesting that their establishment as distinct species was a relatively recent event (Okimoto *et al*. 1991). Therefore, an alternative scenario could be both recent evolution and spread from a few centres of origins around most of the world through agricultural practices (e.g. infested plant-

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ing material), as has been demonstrated for potato cyst nematodes (Bakker *et al*. 1993). Considering the enormous host range of RKN, such an explanation for their very large geographical distribution has already been speculated (Trudgill 1995), but current knowledge of evolutionary relationships both between and within amphimictic and parthenogenetic species is too fragmentary to help infer the origin of (putative) ancestral *Meloidogyne*.

The recently described AFLP procedure, based on the selective amplification of restriction fragments from DNA of a given genotype, has provided a novel and very powerful DNA fingerprinting technique, with typically 50–100 fragments analysed simultaneously (Zabeau & Vos 1993; Vos *et al*. 1995). In this study, we evaluated the AFLP technique to investigate the genotypic diversity in populations of the three parthenogenetic RKN species, *M. arenaria*, *M. incognita* and *M. javanica*. The objective of this study is to provide information on infraspecific variation in RKN, as a preliminary step towards the understanding of their evolutionary relationships in connection with both their wide host range and their particular reproductive and parasitic strategies.

## Materials and methods

### Meloidogyne *spp. populations and DNA extraction*

The name and geographical origin of nematode populations are reported in Table 1. Each nematode population used in this study consisted of a field isolate and originated from a single female. Except for the six populations kindly provided by Dr Vivian Block, SCRI Dundee (nos 10, 17, 18, 23, 24 and 34), all the nematodes have been maintened on tomato plants in the INRA Antibes greenhouse collection for several years, and were identified, at the species level, according to their isoesterase electrophoretic pattern (Dalmasso & Bergé 1978).

**Table 1** Parthenogenetic *Meloidogyne* species and populations used in this study

| Species | Code | Geographic origin |
|---|---|---|
| *Meloidogyne arenaria* | AN7 | Monteux, France |
| | AN22 | Espiguette, France |
| | no. 10 | Ivory Coast |
| | no. 34 | French West Indies |
| *Meloidogyne incognita* | AN11 | Calissane, France |
| | AN5 | Adiopodoumé, Ivory Coast |
| | INCR2 | California, USA |
| | AN31 | Taiwan |
| | AN1 | Valbonne, France |
| | no. 17 | Burkina Faso |
| | no. 18 | Chad |
| *Meloidogyne javanica* | AN40 | Oualidia, Morocco |
| | AN41 | La Réunion Island |
| | no. 23 | Burkina Faso |
| | no. 24 | Spain |

For each nematode population, total genomic DNA was isolated from 100 to 200 µL of second-stage juveniles pooled together. Nematodes were frozen in liquid nitrogen, ground by mortar and pestle and total genomic DNA was extracted as follows. The powder was incubated in extraction buffer (5 M NaCl, 1 M Tris pH 8, 0.5 M EDTA, 2.3% SDS, 0.5% triton) at room temperature, washed in homogenization buffer (5 M NaCl, 0.2 M sucrose, 0.5 M EDTA) at room temperature and incubated in lysis buffer (2 M tris pH 9.2, 0.5 M EDTA, 2.3% SDS) at 55 °C for 30 min and at room temperature for a further 10 min. The sample was then purified according to a phenol/chloroform procedure (Sambrook *et al*. 1989). Following ethanol precipitation, DNA was resuspended in 1× TE buffer to a final concentration of 500 ng/µl and stored at – 20 °C.

### *AFLP procedure*

The AFLP procedure was performed as originally described (Zabeau & Vos 1993; Vos *et al*. 1995) with some minor modifications (choice of the rare cutter enzyme, amount of DNA restricted and final volume of enzymatic reactions, cycling programs and number of amplification cycles in both the preselective and selective PCR reactions).

Adapters and oligonuceotides used in this study were supplied from Eurogentec. The sequences of the adapters were as follows.

*Hin*dIII adapter, 5´-CTCGTAGACTGCGTACC-3´,
3´-CATCTGACGCATGGTCGA-5´

*Mse*I adapter, 5´-GACGATGAGTCCTGAG-3´,
3´-TACTCAGGACTCAT-5´

Preselective and selective primers were derived from primers H + 0 (5´-GACTGCGTACCAGCTT-3´) and M + 0 (5´-GATGAGTCCTGAGTAA-3´), which are complementary to the core of the adapter sequences. Preselective primers had one additional A nucleotide at their 3´ end (H + A, M + A). Selective primers had three additional nucleotides at their 3´ end (H + AAA, M + AAA, M + AAC, M + AAG, M + AAT, M + ACA, M + ACC, M + ACG, M + ACT, M + AGA, M + AGC, M + AGG, M + AGT, M + ATA, M + ATC, M + ATG, M + ATT).

### *DNA restriction and ligation of adapters*

One microgram of DNA from each sample was double digested with 10 units each of *Hin*dIII and *Mse*I for 3 h at 37 °C in RL buffer (10 mM Tris pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 50 ng/µl BSA) in a final volume of 80 µL. Then, 10 µL of a mixture containing 5 pmoles of *Hin*dIII adapter, 50 pmoles of *Mse*I adapter, 1 unit of T4

DNA ligase, 1 mM ATP in RL buffer was added to 50 µL of the digestion and incubated for 3 h more at 37 °C. After ligation, the reaction mixture was diluted 10-fold in 1× TE buffer and stored at – 20 °C.

### *Preselective amplification*

Five µl of primary template (resulting from double digestion and adapter ligation) was mixed with 75 ng of H + A and 75 ng of M + A primers, 0.2 mM of all four dNTPs, 0.5 units *Taq* in 1× *Taq* buffer (Appligene) in a final volume of 50 µL. Amplifications were performed in a Biometra TRIO-Thermoblock thermal cycler for 28 cycles with the following cycle program: 1 min at 94 °C, 1 min 20 s at 60 °C, 1 min at 70 °C.

### *Selective amplification*

The H + AAA primer (5 ng) was labelled using $^{33}$P-dATP and T4 polynucleotide kinase for 45 min at 37 °C and 15 min at 70 °C. Amplification was performed as described above using 5 ng of labelled and 30 ng of unlabelled selective primer (M + three additional nucleotides) and 1 µL of pre-amplified DNA previously fivefold diluted in 1× TE buffer. The cycle profile was as follows: 1 min 94 °C, 1 min 20 s at 65 °C, 1 min at 72 °C. The annealing temperature was subsequently reduced each cycle by 0.7 °C for the next 12 cycles, and was continued at 56 °C for the remaining 24 cycles.

### *Gel analysis*

The PCR products were mixed with one volume formamide dye (98% formamide, 10 mM EDTA, 0.025% each of xylene cyanol and bromophenol blue) and denatured for 3 min at 90 °C. Five µl of the mix was loaded onto a 4.75% denaturing polyacrylamide gel and electrophoresed in 1× TBE buffer at 50 W for 2 h. The gels were dried on Whatmann 3 mm paper and X-ray films were exposed for 2–3 days at room temperature.

### *Data analysis*

Autoradiographs were visually evaluated on a bench viewer. Every experiment was repeated at least once, and only DNA fragments consistently present or absent between repeats were taken into account for the analysis. The presence or absence of a DNA fragment in a genotype was considered as a binary character, and DNA fingerprints were converted to a matrix of similarity values ($F$) using the formula $F = 2\,Nxy/(Nx + \mathrm{Ny})$, where $Nx$ = number of fragments in genotype x, $Ny$ = number of fragments in genotype y and $Nxy$ = number of fragments shared by genotypes x and y (Nei & Li 1979). To simplify data treatment, quantitative polymorphisms (i.e. variable band intensities and/or reproducible faint bands) were not taken into account. Cluster analysis was based on similarity matrices using the UPGMA (Sneath & Sokal 1973) and neighbour-joining (Saitou & Nei 1987) methods performed with PHYLIP 3.5c software (Felsenstein 1993). Relationships between nematode populations were visualized as dendrograms.

## Results

Sixteen primer combinations were used to generate AFLP fingerprinting of seven *Meloidogyne incognita* populations, four *M. arenaria* populations and four *M. javanica* populations. Depending on the primer combination used and the nematode genotype analysed, 26–96 amplified DNA fragments were resolved on a single lane. The total number of amplified fragments obtained ranged from 872 to 944, from 1008 to 1046 and from 1041 to 1087 for *M. incognita*, *M. arenaria* and *M. javanica* populations, respectively. Under the reaction conditions described, the size of the amplified DNA fragments ranged approximately from 50 to 600 bp. Two kinds of polymorphic DNA fragments could be distinguished: bands amplified in a single genotype, and bands polymorphic between genotypes (i.e. amplified in not all but at least two genotypes). Figure 1 represents typical fingerprints obtained with different primer combinations and shows both types of polymorphisms.

The UPGMA analysis grouped the 15 *Meloidogyne* populations into three clusters corresponding to their respective species (Fig. 2). *Meloidogyne arenaria* and *M. javanica* appeared closer to one another than to *M. incognita*, as indicated by the interspecific similarity values ($0.74 < F < 0.76$ between *M. incognita* and *M. javanica* populations; $0.71 < F < 0.80$ between *M. incognita* and *M. arenaria* populations; $0.87 < F < 0.90$ between *M. arenaria* and *M. javanica* populations). The same clustering and relationships between the three species were displayed in the neighbour-joining dendrogram (data not shown).

Intraspecific variation was also revealed by AFLP, with DNA fragments polymorphic among populations within each of the three species tested (Fig. 1). The proportions of polymorphic amplified fragments were 9.7%, 4.4% and 3.3% in *M. arenaria*, *M. incognita* and *M. javanica*, respectively. Thus, *M. arenaria* appeared as the most variable species, and *M. javanica* was the least. The estimated similarity among *M. arenaria* populations averaged 0.90 and ranged from 0.89 to 0.91, while it averaged 0.96 and 0.97 and ranged from 0.91 to 0.99 and from 0.95 to 0.99 among *M. incognita* and *M. javanica* populations, respectively. Within each specific cluster, no general correlation could be found between genomes and geographical origin (Fig. 2). Some populations originally collected from very

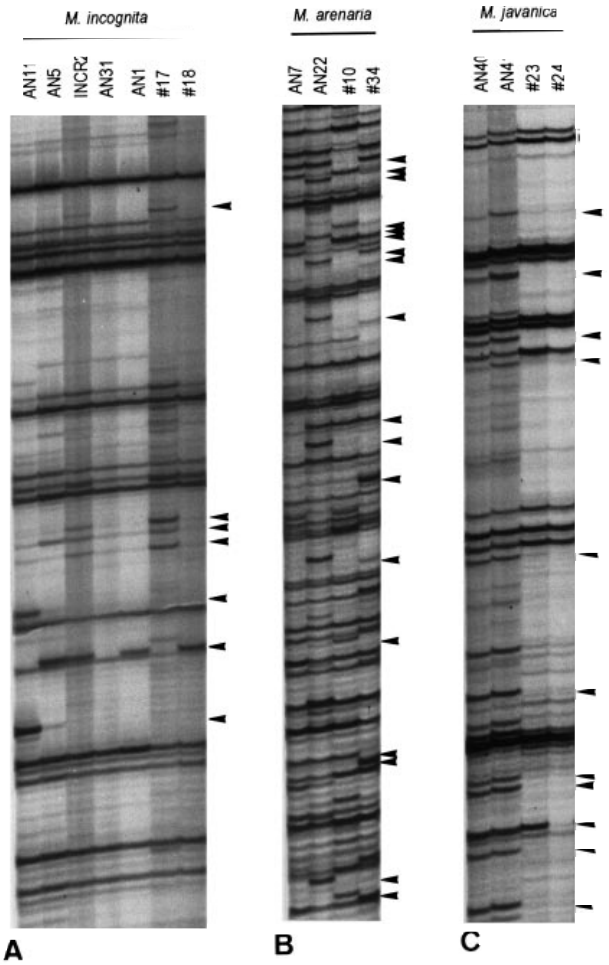


**Fig. 1** Examples of infraspecific AFLPs generated from genomic DNA of *Meloidogyne* populations. (A) *Meloidogyne incognita* populations and primer combination H + AAA/M + ATC. (B) *Meloidogyne arenaria* populations and primer combination H + AAA/M + AGC. (C) *Meloidogyne javanica* populations and primer combination H + AAA/M + ACC. Arrows indicate representative polymorphic DNA fragments. Population codes are given in Table 1.

distant areas displayed very high similarity values (for example, $F$ = 0.99 for *M. incognita* populations AN1 (France) and AN31 (Taiwan), or *M. javanica* populations no. 23 (Burkina Faso) and no. 24 (Spain)). On the contrary, some populations from close geographical origins were not clustered together. For example, *M. incognita* populations AN1 (Valbonne) and AN11 (Calissane) were both collected in the south-east of France, but they were not closely related on the basis of their AFLP patterns, as shown by the UPGMA dendrogram (Fig. 2).

## Discussion

The present study was conducted to evaluate the application of the recently described AFLP technology as a new method for DNA fingerprinting in parthenogenetic nematodes of the genus *Meloidogyne*. The results demonstrated that AFLP is a powerful method for the characterization of infraspecific polymorphism among populations of *M. arenaria*, *M. incognita* and *M. javanica*. Reproducibility of AFLP banding patterns was very high, thus lending support to the conclusions derived from the analysis. To our knowledge, this is the first time that AFLP has been used to characterize genomic variability of RKN species.

The development of the polymerase chain reaction (PCR) technology has offered new perspectives in the field of RKN diagnosis. Primers specific to mtDNA sequences (Powers & Harris 1993; Stanton *et al*. 1997) or satellite DNA sequences (Castagnone-Sereno *et al*. 1995) allowed unambiguous specific characterization of RKN, even from a single individual. DNA fingerprinting by random amplified polymorphic DNA (RAPD) analysis was also successfully used, but the infraspecific polymorphisms detected remained rather low, especially within both *M. incognita* and *M. javanica* species (Baum *et al*. 1994; Castagnone-Sereno *et al*. 1994; Cenis *et al*. 1993). A noteworthy result of the present study is the finding that AFLP displayed a higher rate of polymorphism among RKN species and populations compared to that obtained with RAPD, as previously demonstrated for the potato cyst nematode species *Globodera pallida* and *G. rostochiensis* (Folkertsma *et al*. 1996).

At the specific level, the genetic relationships inferred from this study are in close agreement with RAPD data: *M. arenaria* and *M. javanica* were found to be closer to each other than to *M. incognita* (Baum *et al*. 1994; Castagnone-Sereno *et al*. 1994). Also, the rather low variability in *M. incognita* and *M. javanica* compared to that observed among *M. arenaria* populations is consistent with RAPD analysis, and could suggest that the 'species' *M. arenaria* may be a composite group including distinct lineages. Cytological studies indicated the occurrence of a major triploid form in *M. arenaria* (with a somatic chromosome number larger than 50), along with less frequent diploid (2n = 30–38) and hypotriploid (2n = 40–48) isolates (Triantaphyllou 1985). Based on this, it has been assumed that the taxonomic status of the diploid and hypotriploid forms was rather uncertain. Previous studies of isoenzyme variation revealed low diversity within both *M. javanica* and *M. incognita*, but high diversity within *M. arenaria* (Esbenshade & Triantaphyllou 1987). More recently, a RFLP analysis showed a significant dichotomy of mtDNA within *M. arenaria* compared to other parthenogenetic RKN species (Hugall *et al*. 1994). Our data are in good agreement with all of these studies, which when considered together strongly suggest that *M. arenaria* as presently defined may not be a discrete genetic unit. Compared with potato cyst nematodes (Folkertsma *et al*. 1996), which are

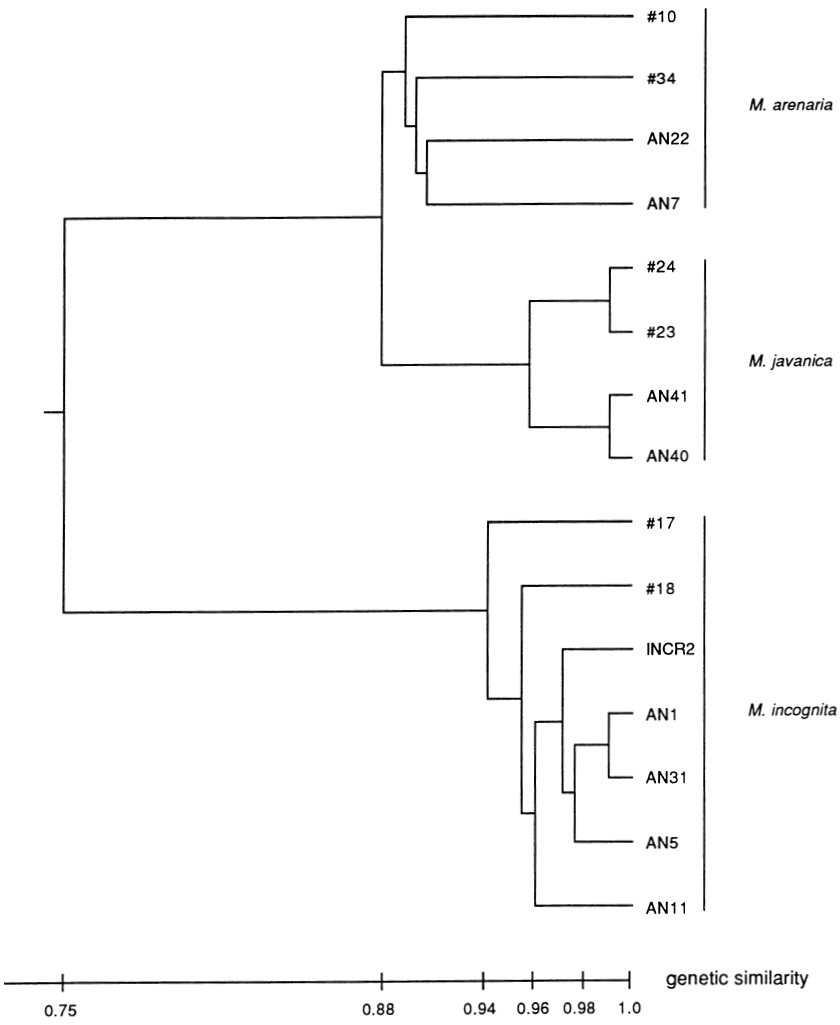


**Fig. 2** UPGMA dendrogram of 15 *Meloidogyne* populations based on presence/absence of AFLP bands. Population codes are given in Table 1.

amphimictic organisms, AFLP nevertheless revealed here an overall high homogeneity within each parthenogenetic RKN species (even within *M. arenaria*). In the case of soil organisms with a low potential of population dispersal, this (relative) lack of genetic variation could be considered as the result of a mechanism allowing conservation of the best adapted genotype for its specific environment. Assuming that parthenogenesis is likely to provide such a selective advantage, it may therefore represent, in RKN, an alternative to the classical hypothesis of the advantages of amphimixis (i.e. increased progeny fitness because of reciprocal gene exchange; Kondrashov 1993).

Within each of the three RKN species, the cluster analysis did not reveal any correlation between genomic similarity and geographical origin of the populations. Such a result is in good agreement with a recent RAPD work on tropical *Meloidogyne* spp. which showed that grouping of lines within a species did not reflect their geographical provenance (Block *et al*. 1997b). Even though the number of populations tested here is rather low and should be increased to strengthen the informative value of our data, this study nevertheless provides arguments for the hypothesis that RKN may not be indigenous throughout their current geographical distribution (Block *et al*. 1997a; Trudgill 1995). The fact that populations which are very similar at the genome level were found in widely separated areas (Table 1) suggests that they could share a common centre of origin. The presence of populations with very low genomic similarity in the same region could be the result of random dissemination from a number of centres of origin and juxtaposition through agronomical practices rather than extreme genetic drift from a common and local ancestor.

From a practical point of view, the ability to accurately detect polymorphisms between RKN populations is of outstanding importance for the design of effective integrated control of these parasites. Plant resistance is currently the most efficient and environmentally sound method of controlling RKN, but the implementation of crop rotations including resistant cultivars requires

specific and subspecific identification of these pests. The fact that most *Meloidogyne* species (including *M. arenaria*, *M. incognita* and *M. javanica*) reproduce by parthenogenesis precludes any Mendelian genetical approach with these nematodes (i.e. crossing experiments), making it sometimes difficult to infer relationships between (unidentified) groups within this genus. Moreover, the occurrence of virulent populations able to reproduce on plants carrying resistance genes further complicates the situation (Castagnone-Sereno 1994). Therefore, a better knowledge of both frequency and distribution of virulent isolates is urgently needed. Thus, research focusing on the identification of molecular markers able to accurately discriminate at a number of levels (species, populations, pathotypes) should be encouraged. Within the scope of subsequent ecological and epidemiological studies, AFLP fingerprinting could be further extended to a broad survey of populations present in both cultivated areas and natural native ecosystems, in order to evaluate the impact of coevolution between host plants and parthenogenetic RKN on their biodiversity.

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## References

Bakker J, Folkertsma RT, Rouppe van der Voort JNAM, de Boer J, Gommers FJ (1993) Changing concepts and molecular approaches in the management of virulence genes in potato cyst nematodes. *Annual Review of Phytopathology*, **31**, 169–190.

Baum TJ, Gresshoff PM, Lewis SA, Dean RA (1994) Characterization and phylogenetic analysis of four root-knot nematode species using DNA amplification fingerprint and automated polyacrylamide gel electrophoresis. *Molecular Plant–Microbe Interactions*, **7**, 39–47.

Block VC, Ehwaeti M, Fargette M, Kumar A, Phillips MS, Robertson WM, Trudgill DL (1997a) Evolution of resistance and virulence in relation to the management of nematodes with different biology, origins and reproductive strategies. *Nematologica*, **43**, 1–13.

Block VC, Phillips MS, McNicol JW, Fargette M (1997b) Genetic variation in tropical *Meloidogyne* spp. as shown by RAPDs. *Fundamental and Applied Nematology*, **20**, 127–133.

Castagnone-Sereno P (1994) Genetics of *Meloidogyne* virulence against resistance genes from solanaceous crops. In: *Advances in Molecular Plant Nematology* (eds Lamberti F, De Giorgi C, Mck Bird D), pp. 261–276. Plenum Press, New York.

Castagnone-Sereno P, Vanlerberghe-Masutti F, Leroy F (1994) Genetic polymorphism between and within *Meloidogyne* species detected with RAPD markers. *Genome*, **37**, 904–909.

Castagnone-Sereno P, Esparrago G, Abad P, Leroy F, Bongiovanni M (1995) Satellite DNA as a target for PCR-specific detection of the plant-parasitic nematode *Meloidogyne hapla*. *Current Genetics*, **28**, 566–570.

Cenis JL (1993) Identification of four major *Meloidogyne* spp. by random amplified polymorphic DNA (RAPD–PCR). *Phytopathology*, **83**, 76–80.

Dalmasso A, Bergé JB (1978) Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp. application to the taxonomy of *Meloidogyne*. *Journal of Nematology*, **10**, 323–332.

Dalmasso A, Bergé JB (1983) Enzyme polymorphism and the concept of parthenogenetic species, exemplified by *Meloidogyne*. In: *Concepts in Nematode Systematics* (eds Stone AR, Platt HM, Khalil LF), pp. 187–196. Academic Press, London and New York.

Esbenshade PR, Triantaphyllou AC (1987) Enzymatic relationships and evolution in the genus *Meloidogyne* (Nematoda: Tylenchida). *Journal of Nematology*, **19**, 8–18.

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle, USA.

Folkertsma RT, Rouppe van der Voort JNAM, de Groot KE *et al.* (1996) Gene pool similarities of potato cyst nematode populations assessed by AFLP analysis. *Molecular Plant– Microbe Interactions*, **9**, 47–54.

Hugall A, Moritz C, Stanton J, Wolstenholme DR (1994) Low, but strongly structured mitochondrial DNA diversity in root-knot nematodes (*Meloidogyne*). *Genetics*, **136**, 903–912.

Kondrashov AS (1993) Classification of hypotheses on the advantage of amphimixis. *Journal of Heredity*, **84**, 372–387.

Lamberti F (1979) Economic importance of *Meloidogyne* spp. in subtropical and mediterranean climates. In: *Root-knot Nematodes (*Meloidogyne *spp.). Systematics, Biology and Control* (eds Lamberti F, Taylor CE), pp. 341–357. Academic Press, New York.

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA*, **76**, 5269–5273.

Okimoto R, Chamberlin HM, MacfarlaneJL, Wolstenholme DR (1991) Repeated sequence sets in mitochondrial DNA molecules of root knot nematodes (*Meloidogyne*): nucleotide sequences, genome location and potential for host-race identification. *Nucleic Acids Research*, **19**, 1619–1626.

Powers TO, Harris TS (1993) A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology*, **25**, 1–6.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2th edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sasser JN, Carter CC (1985) Overview of the International *Meloidogyne* Project 1975–84. In: *An Advanced Treatise on Meloidogyne*, Vol. 1 (eds Sasser JN, Carter CC), pp. 19–24. North Carolina State University Graphics, Raleigh.

Sneath PHA, Sokal RR (1973) *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. WH Freeman and Co., San Fransisco, CA.

Stanton J, Hugall A, Moritz C (1997) Nucleotide polymorphisms and an improved PCR-based mtDNA diagnostic for

parthenogenetic root-knot nematodes (*Meloidogyne* spp.). *Fundamental and Applied Nematology*, **20**, 261–268.

Triantaphyllou AC (1985) Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. In: *An Advanced Treatise on Meloidogyne*, Vol. 1 (eds Sasser JN, Carter CC), pp. 113–126. North Carolina State University Graphics, Raleigh.

Trudgill DL (1995) Origins of root-knot nematodes (*Meloidogyne* spp., Nematoda) in relation to their cultural control. *Phytoparasitica*, **23**, 191–194.

Vos P, Hogers R, Bleeker M *et al*. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Zabeau M, Vos P (1993) Selective restriction fragment amplification: A general method for DNA fingerprinting. European Patent Application no. 92402629.7. Publication number EP, 0534858, A:1.

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