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Diagnostic Probes Targeting the Major Sperm Protein Gene That May Be Useful in the Molecular Identification of Nematodes

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Abstract: Discrimination of closely related nematode species is typically problematic when traditional identification characteristics are prone to intraspecific variation. In this study, a molecular approach that can distinguish *Pratylenchus penetrans* and *P. scribneri* is described. The approach uses universal primers in conjunction with polymerase chain reaction (PCR) to amplify equivalent fragments of the major sperm protein (*msp*) gene from any nematode. This gene fragment typically includes an intron of variable sequence. The presence of this highly variable segment in an otherwise conserved gene sequence allows *P. penetrans* and *P. scribneri* to be distinguished by either a species-specific amplification or by dot-blot hybridization. The approach is potentially of general utility in species-specific identification of nematodes.

Key words: identification, intron, lesion nematode, molecular biology, nematode, *Pratylenchus penetrans*, *Pratylenchus scribneri*, sperm protein.

Accurate, specific identification of nematode infestations is required for successful crop management practices (2). Morphological techniques can be used in many, but not all cases, and therefore alternative approaches have been developed to augment or supplant identification based on morphological characteristics. Biochemical approaches have included isozyme analysis (10,21), two-dimensional polyacrylamide gel electrophoresis (11), and serological techniques that use polyclonal or monoclonal antibodies (14,27). Molecular approaches to identification originally relied on restriction fragment length polymorphism (RFLP) of genomic (5,9,12,16) and mitochondrial (13,23) DNA. The emergence of polymerase chain reaction (PCR) technology (19), which can amplify small amounts of DNA, has revolutionized the practical utility of molecular approaches in medical diagnostics. Not surprisingly, PCR approaches have been found to show promise in nematode identification and systematics (33).

Two types of PCR-based approaches have been used in nematode detection. The first of these, random amplified polymorphic DNA (RAPD) analysis, utilizes random primers to generate distinct amplification markers that can be used for nematode identification (6,7). This technique has been used to identify geographically distinct groupings of *Pratylenchus vulnus* (24). RAPD-PCR is advantageous in that it requires no isotopic labelling nor does it require extensive sequence knowledge of the target genome. However, multiple primers or primer combinations must be tried to develop a suitable marker array. A promising alternative approach is PCR amplification of distinct mitochondrial or genomic DNA sequences (3,15,22). In the work presented here the particular advantages of an identification scheme based on the genes encoding the major sperm protein (MSP) are explored. MSP is a 127 amino acid protein that is known to be a major component of the cytoskeleton of the amoeboid sperm of *Ascaris suum* (4,30). It is encoded by a multicopy gene family (the *msp* genes) that varies in size from 1 to 4 copies in filarial nematodes (29) to at least 35 copies in *Caenorhabditis elegans* (32). The organization of the *msp* family has been studied in *C. elegans* (32), where the genes are loosely clustered in several dis-

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tinct chromosome regions but nevertheless encode essentially the same peptide. Each *C. elegans msp* gene is preceded by a presumptive promoter, and each apparently represents a separate transcription unit (1,17). *Msp* genes have been found in every nematode in which they have been sought, including free-living soil (1,17) and plant-parasitic (20,28), animal-parasitic (4), and entomopathogenic (29) nematodes. MSP has not yet been detected in any non-nematode species; hence, the presence of one or more *msp* genes appears to be a characteristic nematode feature. In most species, the *msp* genes appear to be interrupted by a single intron. In the case of *Ascaris,* the existence of an intron has been experimentally proven by comparison of genomic DNA sequence to previously published cDNA sequence (4). All but one of the *C. elegans msp* genes lacks the putative intron (1); however, this appears to be atypical among nematode species. To date, genomic DNA has been examined from 21 nematode species (1,4,17,20,26,29,31; Setterquist et al., unpubl.). This sequence evidence suggests that intron-containing *msp* genes are present in all 21 species.

The potential utility of the *msp* gene in nematode detection stems from the fact that it encompasses highly species-specific sequences, i.e., those of the putative intron within predictably conserved flanking regions, the *msp* coding region. Thus it is easy to obtain sequence information for any species of interest using gene-specific amplification primers to conserved coding sequences that flank the intron. The resulting sequence includes a variable region that can be used as a hybridization target in subsequent identification and a conserved region that can serve as a positive control. This study demonstrates an *msp*-based detection system that can distinguish *P. scribneri* from *P. penetrans.*

Materials and Methods

Nematode isolates: Two isolates of *Pratylenchus penetrans* were obtained. The first isolate (*Pp*OR), obtained from L. Al-Banna and V. Williamson at the University of California, Davis, consisted of nematodes originally associated with mint plants in Oregon and subsequently maintained in the greenhouse at the University of California, Davis. The second isolate of *P. penetrans* (*Pp*MD), originally collected in Talbot County, Maryland, and an isolate of *Pratylenchus scribneri* were obtained from D. Chitwood of the USDA in Beltsville, Maryland.

Design of amplification primers for msp genes from different nematode species: DNA sequences for *msp* genes from *Globodera rostochiensis* (20), *C. elegans* (1,17) *A. suum* (4), *Onchocerca volvulus* (29), and *Dictyocaulus viviparus* (26,31) were aligned and the amino acid sequence deduced. Conserved sequence segments in the N-terminal region, KIVFNAP (amino acids 16–23 in *C. elegans*) and the C-terminal region, WFQGDGMV (amino acids 107–114) were used in the design of amplification primers mspF and mspR (Fig. 1). Using these primers, more than 80% of the coding region of the *msp* gene is typically amplified from a nematode genome template.

PCR amplification, DNA purification, cloning, and sequencing of nematode msp genes: MspF and mspR primers were used to amplify *msp* gene segments from crude lysates

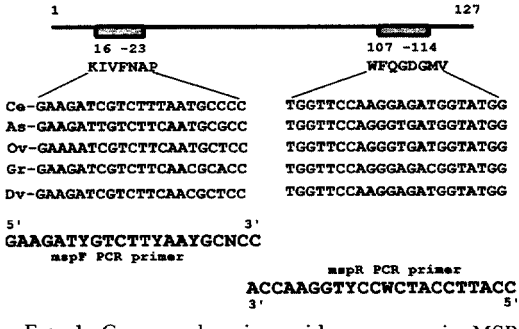

FIG. 1. Conserved amino acid sequences in MSP employed for the design of msp PCR primers. Top line indicates the Msp polypeptide, with boxes 16–23 and 107–114 indicating the highly conserved amino acid sequence shown below. Corresponding nucleotide sequence coding for these regions in *Caenorhabditis elegans* (Ce), *Ascaris lumbricoides* var. *suum* (As), *Onchocerca volvulus* (Ov), *Globodera rostochiensis* (Gr), and *Dictyocaulus viviparus* (Dv) are shown. The degenerate forward and reverse primers, mspR and mspF, used in this study are shown.

or purified genomic DNA. Ten to fifty nematodes were placed in 25 µl of lysis buffer (60 µg/ml Proteinase K in 10 mM Tris [pH 8.2], 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween, and 0.05% gelatin), heated to 60°C for 10 minutes, and then heated to 100°C for 5 minutes. Lysate (2.5 µl) was added to standard PCR reaction components: Primer mspF and mspR (1 µM) dNTPs (0.2 mM), $MgCl_2$ (2.0 mM), 5 µl ten-fold concentrated PCR reaction buffer (500 mM KCl, 100 mM Tris-HCl [pH = 9.0], 1% Triton X-100), 2 units *Taq* DNA polymerase and H_20 to 50 µl.

Each PCR reaction was placed in a thermocycler (MJ Research PTC 100 Thermocycler, MJ Research, Waterton, MA), where the following cycling parameters were applied: 94°C denaturing step for 2 minutes; 30 amplification cycles consisting of 94°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1 minute; and a final 72°C extension step for 5 minutes. The PCR reaction products were analyzed by electrophoresis on a 1.5% agarose gel containing ethidium bromide in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0).

PCR products from *P. penetrans* and *P. scribneri* were ligated into a T-tailed vector (Bluescript II KS [+/−] (18). Nucleotide sequences of cloned inserts were determined with Sequenase Version 2.0 (USB, Cleveland, OH) according to the manufacturer's instructions. KS and SK primers, which anneal to the 5′ and 3′ regions of pBluescript that flank the insert, were used to obtain the *msp* nucleotide sequences.

Msp nucleotide sequence analysis: DNA sequences obtained from the literature or determined in the laboratory were entered into computer files and deposited in GenBank. Sequences Ps 1–4 have accession numbers U57588–U57591, PpOR 1 and 2 have U57748 and U57749, and PpMD3–6 have U57750 and U57829–U57831. Sequence comparison was facilitated by using programs in the GCG sequence analysis package (Version 8.0; Genetics Computer Group Madison, WI) running on a SUN SPARCstation 20. Multiple-sequence alignments were established and maintained using the GDE (Genetic Data Environment) package.

Species-specific PCR amplification: Primers specific for *P. scribneri* (p*Ps*) and *P. penetrans* (p*Pp*) *msp* sequences were designed (Fig. 2). In addition, a probe sequence, mspG, was identified (Fig. 2) that was highly complementary to all known *msp* genes. This probe served as a positive control to assay for the presence of *msp* DNA in amplification products. To determine whether p*Ps* and p*Pp* would selectively amplify species-specific *msp* gene fragments, a second PCR reaction using the first PCR product as templates was performed. PCR products obtained from *P. scribneri* and both *P. penetrans* isolates (OR and MD) using mspF and mspR primers were diluted 1:1,000. One microliter of diluted PCR product was added to separate 50-µl PCR reactions. Each separate template was subjected to thermocycling with the following primer sets: 1) primer mspF and mspR, 2) primer mspR and pPp, and 3) primers mspR and pPs. These PCR reactions were placed in the thermocycler and the following cycling parameters were applied: denaturing step at 94°C for 2 minutes, 30 amplification cycles at 94°C for 30 seconds followed by 50°C for 15 seconds and 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. The PCR reaction products were analyzed by electrophoresis as previously described.

Dot-blot hybridization of PCR products to species-specific probes: Radiolabeled oligonucleotide probes p*Pp*, p*Ps* and a plant-parasitic nematode specific primer, mspG (Fig. 2), were end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase, (PNK) (USB, Cleveland, OH), 10 pmoles of oligonucleotide, 20 pmoles [γ-^{32}P]ATP at 6,000 Ci/mmol, 5 units PNK in a total reaction volume of 50 µl PNK buffer (50 mM Tris pH 7.6, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol). The reaction mix was incubated at 37°C for 1 hour. Five microliters of spin-column purified *msp* PCR product (ca. 25 ng) were denatured and pipetted

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                                                                       [------------
                MSP F ---->                   MSP G ---->              pPs and pPp
1  Ps-1         GAAGGTCGTCTTCAACGCACCccgttcgatGACAAGCACACCTACTACATGAAG gttagttaatttaa
2  Ps-2                            ................................... GTCAGTTAATTTAA
3  Ps-3                            .........................t......... gtcagttaatttaa
4  Ps-4                            ..................t.t.............. gtcagttaatttaa
5  PpOR1                                  ...c........................ gtaaggacacaact
6  PpOR2                            .........c........................ GTAAGGATAAGGAC
7  PpMD3                           ........t.......................... gtaaggacaaaact
8  PpMD4                           .t........c.....a.................. gtaaggacacaact
9  PpMD5                               ......c........................ gtaaggacaaaact
10 PpMD6                           ..........c........................ gtaaggacaaaact

              -----------------INTRON---------------------------------------]
                ---->
1  Ps-1         aattaaaatttccaaatcaaataataaa----------------------tccctttttcccacag atc
2  Ps-2         CATTAAAATTTCCaaatcaaataataaa-----------------------tcccttttcccacag ...
3  Ps-3         aattaaaatttccaaatcaaata------------------------------tctttttccacag ..t
4  Ps-4         cattaaaatttccaaatctattaataaa----------------------tcccctttttccgcag ...
5  PpOR1        ctcttctcttaaat-cataatcttgctaatgccattcc--------cattcccctccatattccag ...
6  PpOR2        AAAACTCtcttctcataaaatatatttcagtgctaatactaataacattccctttcaatttcacag ...
7  PpMD3        ctcttcacataaaaat-acatattcctaataccatccc--------cattccccttcaaattccag ...
8  PpMD4        ctcttctcttaaat-cataatcttgctaatgaaatca---------cattcccctccatattccag ...
9  PpMD5        cttttctcttaaat-cataatcttgctaatgaaattacaata---cattcccctccatattccag ...
10 PpMD6        ctcttctcataaaatcataatcttgctaatgccattcc-------cattcccctttcaattcccag ...

1  Ps-1         atcaactccggcggtcaccgcatcggcttcgccttcaaaacgaccaacccgcaacgtctgaacatggacc
2  Ps-2         ......................................................................
3  Ps-3         .c.........a..a.......................................................
4  Ps-4         ......................................................................
5  PpOR1        ...........a..a........t..............g..a............................
6  PpOR2        ...........a..a........t..............g..a..............c.............
7  PpMD3        ..ac.......a..a........t..............g..a..............c.............
8  PpMD4        ...........
9  PpMD5        ...........
10 PpMD6        ...........

1  Ps-1         cgcccaatggagtgcttgacccgaaggaatccatcaacattgccatctcctgcgatgccttcgattctgc
2  Ps-2         ......................................................................
3  Ps-3         ...................................t................................t.
4  Ps-4         ......................................................................
5  PpOR1        .c..a...........c...............................................cg.a..
6  PpOR2        ....a...........c...............................................cg.a..
7  PpMD3        ....a...........c...............................................cg.a..

1  Ps-1         aaccgaggccaccaacaacgaccgcgtcaccgtggaatggaccaacacaccggagggagcagccaagcaa
2  Ps-2         ......................................................................
3  Ps-3         .......c..tt..........................................................
4  Ps-4         ......................................................................
5  PpOR1        t.....a..g..............t.......................c...................g.
6  PpOR2        t.....a..g..............t.......................c..a..................
7  PpMD3        t.....a.gg..............t.......................c...................g.

                            <---- PRIMER MSP R
1  Ps-1         ttccgtcgcgaaTGGTTCCARGGWGATGGAATGG
2  Ps-2         ...................
3  Ps-3         ....................
4  Ps-4         ....................
5  PpOR1        .....a...
6  PpOR2        .....c...
7  PpMD3        ..a..a..............
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FIG. 2. Nucleotide sequence of *msp* gene fragments from *Pratylenchus scribneri* and *P. penetrans* strains OR and MD and (*P. scribneri*, sequences 1–4; *P. penetrans* OR, sequences 5 and 6; and *P. penetrans* MD, sequences 7–10). Primers mspF, mspG, and mspR are shown in bold on the Ps-1 sequence and identified above the sequence. Primers pPs (*P. scribneri* specific) and pPp (*P. penetrans* specific) are shown in bold on sequences Ps-1 and PpOR-2, respectively. Dots indicate identity with the Ps-1 sequence. Blank spaces indicate that no sequence was determined. For several genes, e.g., Pp MD 4–6, only the intron and immediate flanking regions were sequenced. Intron sequences of each gene are shown in full. Dashes indicate gaps inserted for alignment purposes. The introns were aligned for all available genes from each species. Introns are not readily alignable between species; the global intron alignment should not be given great credence.

onto a nylon filter and cross-linked by irradiation with UV light for 3 minutes. Replicate filters were subjected to a prehybridization step by adding 6× SSC (20× SSC is 3 M NaCl/0.3 M sodium citrate, pH 7.0) with 0.5% SDS, 5× Denhardt's reagent, 100 μg/ml denatured fragmented salmon sperm DNA and then incubated at 37°C for 1 hour. One filter was hybridized with ^{32}P-labeled p*Ps* and the other with ^{32}P-labeled p*Pp* probe for 3 hours at 50°C. Filters were subsequently washed in 2× SSC, 0.5% SDS for 5 minutes at room temperature; 2× SSC, 0.1% SDS for 15 minutes at room temperature; and 0.1× SSC, 0.5% SDS for 30 minutes at 37°C. A final series of three washes was performed with 0.1× SSC and 0.5% SDS for 30 minutes each at 50°C. The filters were placed on X-ray film with an intensifying screen for 12 hours at −80°C and then developed.

Filters were rinsed three times with 2× SSC heated to 100°C for 15 minutes to strip the radioactive probes. Filters were then re-hybridized as above with mspG to demonstrate that all dots contained *msp* gene DNA.

Results

Amplification reactions utilizing mspF and mspR with genomic DNA present in crude lysates can be routinely used to amplify *msp* gene fragments. *Msp* genes from free-living nematodes in the genera *Caenorhabditis, Rhabditis* and *Pelodera,* as well as several species of plant-parasitic nematodes, have all been readily amplified with primers mspF and mspR (Setterquist et al., unpubl.) Therefore, it is likely that these primers will amplify *msp* DNA from a large number of nematode genera.

Nucleotide sequences of *msp* gene fragments were obtained for two different *P. penetrans* OR genes, one *P. penetrans* MD gene, and four *P. scribneri* genes (Fig. 2). The total number of *msp* genes present in each organism has not been estimated. The coding regions are highly conserved and can be easily aligned with previously known *msp* sequences. The introns from each species can also be readily aligned and have obvious sequence identity. Intergeneric alignment of putative intron sequences, however, is difficult since the degree of nucleotide sequence similarity is low. For example, the first 27 bases of the intron in the four aligned *P. scribneri* sequences exhibit 92.6% sequence similarity, while the consensus sequence from this region shares only 29.6% identity with the same region from the *P. penetrans* MD sequence. Therefore, it was straightforward to use the intron sequences to select species-specific sequences (Fig. 2).

Specific amplification of Pratylenchus MSP genes: The results of a second round of PCR using primer mspR with either primer p*Pp* or p*Ps* in the presence of PCR template from the two geographical isolates of *P. penetrans* and *P. scribneri* are shown in Fig. 3. Primer p*Ps* produces only an *msp* band for *P. scribneri,* whereas p*Pp* produces an amplification product *msp* band for both the OR and MD strains of *P. penetrans* (lanes 2 and 3).

Dot-blot hybridization of specific probe to

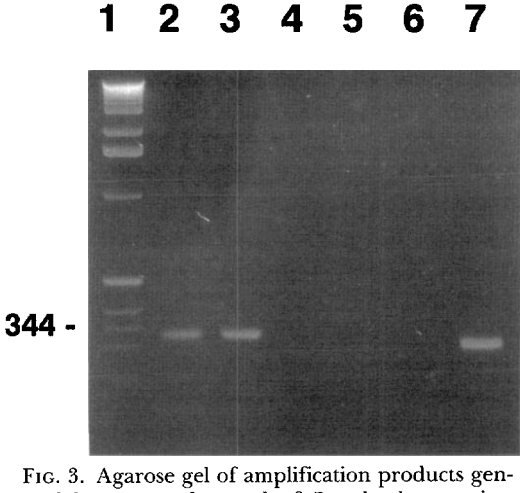

Fig. 3. Agarose gel of amplification products generated by a second round of *Pratylenchus* species-specific PCR. PCR reactions were performed using primer mspR with either primer pPp or pPs in the presence of *msp* PCR DNA from two geographical isolates of *P. penetrans* and *P. scribneri.* Lane 1—molecular weight markers; lane 2—*P. penetrans* OR (primer set: mspR/pPp); lane 3—*P. penetrans* MD (mspR/pPp); lane 4—*P. scribneri* (mspR/pPp); lane 5—*P. penetrans* OR (mspR/pPs); lane 6—*P. penetrans* MD (mspR/pPs); lane 7—*P. scribneri* (mspR/pPs).

Pratylenchus msp PCR products: As is apparent from the autoradiogram (Fig. 4), the *P. penetrans*-specific primer hybridized only to the *P. penetrans msp* PCR product and the *P. scribneri*-specific primer hybridized only to the *P. scribneri msp* PCR product. In each case, the PCR DNA hybridized to the positive control probe, mspG, confirming the presence of *msp* DNA on each filter.

Discussion

The *msp* gene amplification technique described here allows one to distinguish two *Pratylenchus* species, *P. scribneri* and *P. penetrans,* by the selective amplification of a specific fragment using a single species-specific primer or by hybridization to a species-specific probe. The approach is potentially useful as it likely can be used to distinguish nematode genera in most cases and, in some cases, related nematode species as well.

The success of the approach derives from the following favorable characteristics of the *msp* gene family: (i) the genes that encode the MSP protein are frequently multi-copy and thus provide a greater number of targets for PCR; (ii) the *msp* gene is relatively short, and nearly complete sequence data can be obtained with only two primers; (iii) mspF and mspR may permit amplification from a wide variety of divergent nematodes; (iv) amplified *msp* DNA has been found to include a putative intron in at least one *msp* gene for every nematode species examined to date; and (v) the variability of the putative intron regions is such that they provide probe target sequence segments that

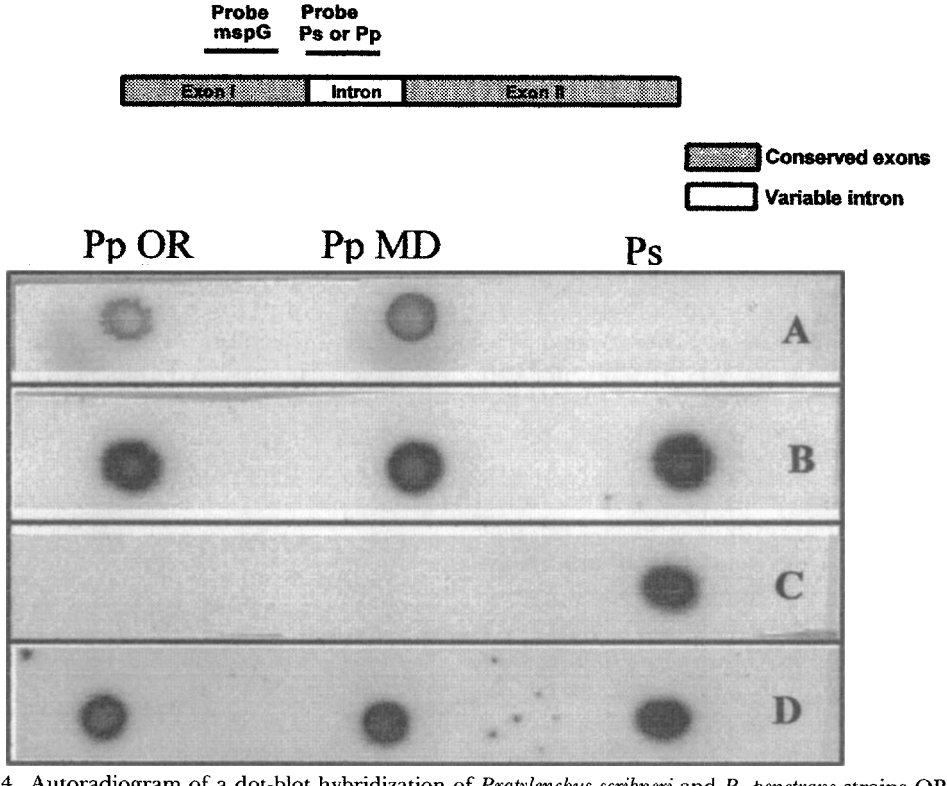

Fig. 4. Autoradiogram of a dot-blot hybridization of *Pratylenchus scribneri* and *P. penetrans* strains OR and MD *msp* PCR products. PCR products from each nematode strain (PpOR, PpMD, and Ps) were bound to nylon strips. Strip A was probed with an oligonucleotide specific to a *P. penetrans* intron. The probe was removed and re-probed with mspG as a positive control, which is shown as strip B. Strip C was probed with an oligonucleotide specific to a *P. scribneri* intron. The probe was removed and the strip was re-probed with mspG as a positive control, which is shown as strip D.

can differentiate nematode genera and, in at least some cases, species within the same genus.

Many of our presumptions concerning genus and species-specific primers based on the putative intron sequences relied on data acquired in the sequencing of a significant number of *msp* genes in many free-living nematodes. In all nematodes examined to date, there appears to be little conservation of *msp* introns between genera or what are known to be widely diverged species. However, in instances where two species are closely related, the introns may not be of sufficient diversity to provide species-specific primers. For example, in *Globodera rostochiensis* and *G. pallida* the available intron sequences are similar and differ by only 3 to 4 bases (8).

Within the genome of a single species, each *msp* gene typically has a unique intron. It is especially important to understand this point when designing primers or hybridization probes. Thus, it would appear, based just on the sequence information shown in Fig. 2, that probe *Pp* would detect the OR strain to the exclusion of the MD strain. This is in fact not true, as shown in Fig. 4. One explanation is that only a small number of genes have been characterized from each strain and, moreover, they may not be homologues. Thus, it was assumed during probe design that the Pp probe target was actually in both the MD and OR strains even though the intron containing it was only sequenced in the OR case. This assumption was supported by the results presented here. Moreover, these results emphasize the fact that one need not sequence all *msp* genes within a species to identify suitable probe targets. An alternative probe based on a portion of the PpOR1 intron sequence that is also present in Pp MD4 gave similar results (unpubl.).

The amplification of *msp* DNA from a single isolate by primers mspF and mspR provides a plentiful source of target that can be tested with multiple species-specific primers in a second PCR reaction. Identification is predicated on the presence or absence of a distinct PCR band. Diagnosis based upon the absence of a character could be problematic without proper controls, since the absence of a band in the PCR reaction may simply indicate a failed reaction. Use of a general primer set (e.g., mspF and mspR) included in a multiplex PCR reaction with a third specific primer (mspG) can serve as a suitable positive control. The use of dot-blot hybridizations to *msp* PCR DNA with appropriate controls may provide a simple and convenient way to identify nematodes. Alternatively, it might be possible to develop a colorimetric reverse dot-blot containing oligonucleotide intron probes immobilized onto a nylon membrane strip (25).

A major limitation of any approach based on hybridization to a PCR-amplified gene is the availability of sequence data. Probes that can make the desired distinction must be designed. Thus, a target sequence that is found in all members of a taxonomic group, e.g., genus, but not in other groupings of the same taxonomic rank is ideal for determination at that taxonomic rank. In contrast, a target sequence that is too variable or too conserved may be problematic. This difficulty can be partially overcome by the choice of hybridization conditions; in general, however, the solution is acquisition of suitable sequence information. In the present case, we have demonstrated that *msp* gene sequence information can be rapidly collected and that species-specific discrimination can be obtained.

Literature Cited

1. Ammons, D. R. 1990. An evolutionary study of the MSP multigene family: A model system of study. Ph. D. thesis, University of Houston, Houston, TX.

2. Barker, K. R. 1991. Rotation and cropping systems for nematode control: The North Carolina experience-introduction. Journal of Nematology 23: 342–343.

3. Beckenbach, K., M. J. Smith, and J. M. Webster. 1992. Taxonomic affinities and intra- and interspecific variation in *Bursaphelenchus* spp. as determined by polymerase chain reaction. Journal of Nematology 24:140–147.

4. Bennett, K. L., and S. Ward. 1986. Neither a germ-line specific nor several somatically expressed

genes are lost or rearranged during embryonic chromatin diminution in the nematode *Ascaris lumbricoides* var. *suum*. Developmental Biology 118:141–147.

5. Castagnone-Sereno, P., C. Piotte, P. Abad, M. Bongiovanni, and A. Dalmasso. 1991. Isolation of a repeated DNA probe showing polymorphism among *Meloidogyne incognita* populations. Journal of Nematology 23:316–320.

6. Caswell-Chen, E. P., V. M. Williamson, and F. F. Wu. 1992. Random amplified polymorphic DNA analysis of *Heterodera cruciferae* and *H. schachtii* populations. Journal of Nematology 24:343–351.

7. Cenis, J. L. 1993. Identification of four major *Meloidogyne* spp. by random amplified polymophic DNA(RAPD-PCR). Phytopathology 83:76–78.

8. Chen, R. 1992. The genomic organization of the major sperm protein gene family in the plant-parasitic nematode *Globodera rostochiensis* and *Globodera pallida*. M.S. thesis, Central Michigan University, Mt. Pleasant, MI.

9. Curran, J., M. A. McClure, and J. M. Webster. 1986. Genotypic differentiation of *Meloidogyne* populations by detection of restriction fragment length difference in total DNA. Journal of Nematology 18:83–86.

10. Esbenshade, P. R., and A. C. Triantaphyllou. 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. Journal of Nematology 22:10–15.

11. Ferris, V. R., J. M. Ferris, J. Faghihi, and A. Ireholm. 1994. Comparison of isolates of *Heterodera avenae* using 2-D PAGE protein pattern and ribosomal DNA. Journal of Nematology 26:144–151.

12. Gárate, T., M. P. Robinson, M. R. Chacón, and R. M. E. Parkhouse. 1991. Characterization of species and races of the genus *Meloidogyne* by DNA restriction enzyme analysis. Journal of Nematology 23:414–420.

13. Hyman, B. C. 1988. Nematode mitochondrial DNA: Anomalies and applications. Journal of Nematology 20:523–531.

14. Jones, P., D. J. Ambler, and M. P. Robinson. 1988. The application of monoclonal antibodies to the diagnosis of plant pathogens and pests. Pp. 767–776 *in* Proceedings of the Brighton Crop Protection Conference-Pests and Diseases. Farnham: BCPC Publications.

15. Joyce, S. A., A. M. Burnell, and T. O. Powers. 1994. Characterization of *Heterorhabditis* isolates by PCR amplification of segments of mtDNA and rDNA genes. Journal of Nematology 26:260–270.

16. Kalinski, A., and R. N. Huettel. 1988. DNA restriction fragment length polymorphism in races of the soybean cyst nematode *Heterodera glycines*. Journal of Nematology 20:532–538.

17. Klass, M. R., S. Kinsley, and L. C. Lopez. 1984. Isolation and characterization of a sperm-specific gene family in the nematode *Caenorhabditis elegans*. Molecular and Cellular Biology 4:529–537.

18. Marchuk, D., M. Drumm, A. Saulino, and F. S. Collins. 1990. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. Nucleic Acids Research 19:1154.

19. Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich. 1986. Specific enzymatic amplification of DNA—*in vitro* polymerase chain reaction. Cold Spring Harbor Symposium 51:263–273.

20. Novitski, C. E., S. Brown, R. Chen, A. S. Corner, H. J. Atkinson, and M. J. McPherson. 1993. Major sperm protein genes from *Globodera rostochiensis*. Journal of Nematology 25:548–554.

21. Payan, L. A., and D. W. Dickson. 1990. Comparison of populations of *Pratylenchus brachyurus* based on isozyme phenotypes. Journal of Nematology 22:538–545.

22. Powers, T. O., and T. S. Harris. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. Journal of Nematology 25:1–6.

23. Powers, T. O., and L. J. Sandall. 1988. Estimation of genetic divergence in *Meloidogyne* mitochondrial DNA. Journal of Nematology 20:505–511.

24. Pinochet, P., J. L. Cenis, C. Fernández, M. Doucet, and J. Marull. 1994. Reproductive fitness and random amplified polymorphic DNA variation among isolates of *Pratylenchus vulnus*. Journal of Nematology 26:271–277.

25. Saiki, R. K., P. S. Walsh, C. H. Levenson, and H. A. Erlich. 1989: Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. Proceedings of the National Academy of Sciences USA 86:6230–6234.

26. Schnieder, T. 1993. The diagnostic antigen encoded by gene fragment D-nu-3-14: A major sperm protein of *Dictyocaulus viviparus*. International Journal of Parasitology 23:383–389.

27. Schots, A., F. J. Gommers, J. Bakker, and E. Egberts. 1990. Serological differentiation in plant-parasitic nematode species with polyclonal and monoclonal antibodies. Journal of Nematology 22:16–23.

28. Scott, A. L., J. Dinman, D. J. Sussman, and S. Ward. 1989. Major sperm protein and actin genes in free-living and parasitic nematodes. Parasitology 98: 471–478.

29. Scott, A. L., J. Dinman, D. J. Sussman, P. Yenbutr and S. Ward. 1989. Major sperm protein genes from *Onchocerca volvulus*. Molecular and Biochemical Parasitology 36:119–126.

30. Sepsenwol, S., H. Ris, and T. M. Roberts. 1989. A unique cytoskeleton associated with crawling in the amoeboid sperm of the nematode, *Ascaris suum*. J. Cell Biol. 108:55–66.

31. Setterquist, R. A., and G. E. Fox. 1995. *Dictyocaulus viviparus:* Nucleotide sequence of Dv3–14. International Journal of Parasitology 25:137–138.

32. Ward, S., D. J. Burke, J. E. Sulston, A. R. Coulson, D. G. Albertson, D. Ammons, M. Klass, and E. Hogan. 1988. Genomic organization of major sperm protein genes and pseudogenes in the nematode *Caenorhabditis elegans*. Journal of Molecular Biology 199: 1–13.

33. Williamson, V. M., E. P. Caswell-Chen, F. F. Wu, and D. Hanson. 1994. PCR for nematode identification. Pp. 119–127 *in* F. Lamberti, C. De Giorgi, and D. McK. Bird, eds. Advances in molecular plant nematology. New York: Plenum Press.