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Molecular Polymorphisms Associated with Host Range in the Highly Conserved Genomes of Burrowing Nematodes, *Radopholus* spp.

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Six polymorphic bands of DNA were amplified from purified *Radopholus citrophilus* genomic DNA from one strain of each of the sibling species *R. citrophilus* and *R. similis* in random amplified polymorphic DNA analyses involving 380 single 10-base primers. Four of these polymorphic DNA fragments were successfully cloned and amplified through subsequent use of primers designed to complement the terminal sequences of the polymorphic DNA. Results of ensuing studies using mini-prepped DNA from 14 burrowing nematode strains collected from Florida, Hawaii, and Central America, characterized for their ability to parasitize citrus, indicated that a 2.4-kb fragment appeared to be associated with citrus parasitism in burrowing nematode populations from Florida. However, a fragment of comparable size was also detected in *R. citrophilus* from Hawaii and from burrowing nematode populations collected from Belize and Puerto Rico. Overall, findings suggest that the genome organization of the burrowing nematode sibling species *R. citrophilus* and *R. similis* is highly conserved. This remarkable genetic similarity should facilitate identification of genetic sequence related to important phenotypes such as citrus parasitism. Detection of *R. citrophilus*–specific DNA fragments in burrowing nematodes collected from Belize and Puerto Rico suggests that *R. citrophilus* is resident in some Central American countries.

Additional keywords: polymerase chain reaction, sequence characterized amplified region (SCAR), sequence tag site (STS), quarantine.

Burrowing nematodes, *Radopholus* spp., are migratory, endophytoparasitic nematodes that are prevalent in many tropical and subtropical regions throughout the world. They are highly destructive parasites and damage a wide range of plants by extensively wounding cortical tissues as they feed in roots (DuCharme 1959; Ford et al. 1960). *Radopholus* spp. are considered to be among the 10 most damaging plant-parasitic nematodes worldwide (Sasser and Freckman 1987). Their effect on citrus and banana is well documented. *Radopholus similis* has been detected in numerous countries throughout the tropics and subtropics (Gowen and Queneherve 1990) causing blackhead toppling disease or toppling disease of banana (Loos and Loos 1960) and slow wilt and yellows of black pepper (Van der Vecht 1950). Although citrus is present in most countries where burrowing nematodes are commonly associated with damage to banana, they only cause spreading decline of citrus in Florida (Suit and DuCharme 1953; Holdeman 1986).

Burrowing nematodes that attack citrus in Florida are morphologically indistinguishable from those that attack banana worldwide and thus had been considered to be the citrus race of *R. similis* (DuCharme and Birchfield 1956). However, the taxonomic status of the citrus race was elevated to sibling species status as *R. citrophilus* (Huettel et al. 1984b) on the basis of biochemical, physiological, and karyotypic differences that distinguished the citrus and banana races of *R. similis* (Huettel and Dickson 1981a, 1981b; Huettel et al. 1982, 1983a, 1983b, 1984a). More recently, minor morphological differences in the tail region in males between *R. similis* and *R. citrophilus* were identified (Huettel and Yaegashi 1988). Although Siddiqi (1986) classified the sibling species as subspecies, *R. similis* subsp. *similis* and *R. similis* subsp. *citrophilus,* federal and state regulatory agency personnel disregarded both taxonomic classifications and maintained the taxonomic status of these nematodes as races of *R. similis* "sensu lato" (Holdeman 1986) because a rapid and sensitive method to selectively identify burrowing nematodes that attack citrus has not been developed to support regulatory efforts.

Since burrowing nematodes that attack citrus have only been detected in Florida, federal and state quarantines are in place to prevent the dissemination of burrowing nematodes from Florida to other citrus production areas. Thus, detection of burrowing nematodes in a plant shipment or at a nursery site results in actions that prevent distribution of the crop to

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select markets/sites on the basis that burrowing nematodes may attack citrus.

Attempts to generate immunoassays to discriminate between the two sibling species were unsuccessful (D. T. Kaplan, unpublished data). Although glycoproteins have proven useful in the identification of plant varieties (Niedz et al. 1991), only three putative differences were detected between *R. similis* and *R. citrophilus* nematode homogenates (Kaplan and Gottwald 1992), emphasizing the close relationship of the two sibling species and negating glycoprotein profiles as a plausible means of burrowing nematode identification.

Molecular characterization of the burrowing nematode genome should facilitate the development of sensitive and accurate diagnostics as well as provide insight into genome organization. The purpose of this study was to identify molecular polymorphisms between the *R. similis* and *R. citrophilus* genomes and to determine if primers developed to selectively amplify these polymorphisms could be used to distinguish these species.

RESULTS

In order to identify potential differences between the two genomes of the burrowing nematode sibling species, a random amplified polymorphic DNA (RAPD) analysis was performed. Although thousands of bands were amplified from the purified genomic DNA from the two burrowing nematode strains by standard RAPD–polymerase chain reaction (PCR) using 380 different single 10-base primers, only six polymorphic bands were identified in ethidium bromide–stained agarose gels. All six of the polymorphisms were unique to *R. citrophilus* strain DK2 (Table 1). We made no further effort to evaluate amplification products of similar size from the two burrowing nematodes to verify that they had the same DNA sequence (i.e., no restriction mapping or sequence analysis was performed). In order to further evaluate the polymorphic bands obtained from *R. citrophilus* DNA, the bands were excised from gels and cloned into pT7Blue vector. We obtained clones of four of the fragments. The terminal ends of the clones were sequenced in order to design primers specific to each fragment. Primer sets were designed that either included the original 10-base primer sequence on either end (sequence characterized amplified regions, SCAR) or that were internal to the original primer sequence (sequence tag sites, STS) (Kesseli et al. 1992). Four primer sets yielded amplification patterns that differed for *R. citrophilus* and *R. similis* DNA (Fig. 1; Table 1).

To verify that polymorphic amplification products were not the result of differential PCR efficiency, DNA blots were prepared and probed with the original cloned fragments. These hybridizations generally revealed the presence of additional amplification products that were not visible with ethidium bromide staining. Primers 101 and 102 (Table 1) amplified a single 2.4-kb product from *R. citrophilus* that was visible with ethidium bromide staining (Fig. 1A, lanes 5 and 7), but no product was visible from *R. similis* (Fig. 1A, lanes 6 and 8). This fragment was cloned and hybridized to blots of PCR amplification products using either OPA11 (Fig. 1B, lanes 1 to 4) or primers 101 and 102 and template DNA from *R. citrophilus* and *R. similis* (Fig. 1B, lanes 5 to 8). These experiments revealed a second product of approximately 1.8 kb from *R. citrophilus*, but still no products were amplified from *R. similis* (Fig. 1B, lanes 5 and 7 versus lanes 6 and 8). Primers 103 and 104 (Table 1) amplified a single 1.2-kb fragment from *R. citrophilus* that was visible in ethidium bromide–stained agarose gels (Fig. 1C, lanes 5 and 7). Hybridization experiments revealed a band of similar size had also been amplified from *R. similis* (Fig. 1D lanes 5 to 8). These bands were not sequenced or restriction mapped, however, to ascertain the extent of homology. Hybridization also identified a 0.8-kb band that was amplified selectively from *R. citrophilus* (Fig. 1D, lanes 5 and 7). The primers 105 and 106 (Table 1) amplified two bands (1.0 and 0.8 kb) from *R. citrophilus* DNA (Fig. 1E, lanes 5 and 7), but only a single 0.8-kb band from *R. similis* (Fig. 1E, lanes 6 and 8). Hybridization experiments did not identify additional bands (Fig. 1F, lanes 5

Table 1. The commercial 10-base primers used to amplify each of the six unique random amplified polymorphic DNAs (RAPD), the size of each RAPD observed in ethidium bromide–stained agarose gels, the designation assigned to each RAPD, the designation for primers complementary to the terminal ends of the RAPDs and their sequence, and the sizes of their amplification products

				Product size using corresponding primers	
Operon primer	**Estimated size of RAPD**	**RAPD designation**	**Primers to corresponding RAPDs**	**DK2**	**DK6**
OP-A11	2,400[a]	DK#1	DK#101: CAATCGCCGTTAATTGCTGTGTTT	2,400	
			DK#102: CAATCGCCGTAGAATGCCATCATC	1,800[b]	
OPB08	1,800	DK#2	NA[c]	NA	NA
OP-K02	1,200	DK#3	DK#103: AATCGCCGTTAATTGCTGTGTTT	2,000[b]	
				1,200	1,200[b]
			DK#104: CAATCGCCGTAGAATGCCATCATC	1,100[b]	
				800[b]	
OP-M02	1,400	DK#4	DK#107: ACAACGCCTCAAAGAATGAACGTT	1,400	900
			DK#108: ACAACGGCCTCAATACACGCATCCA		
OP-O10	1,100	DK#5	DK#105:TTCCTTTTGTAAACTTGATCGGTCG	1,000	
				800	800
			DK#106: TCCTTTTGTAAACTTGATCGGTCG		
OP-T12	2,500	DK#6	NA	NA	NA

[a] Estimated size in base pairs.
[b] Polymorphisms detected only by DNA hybridization.
[c] Not applicable.

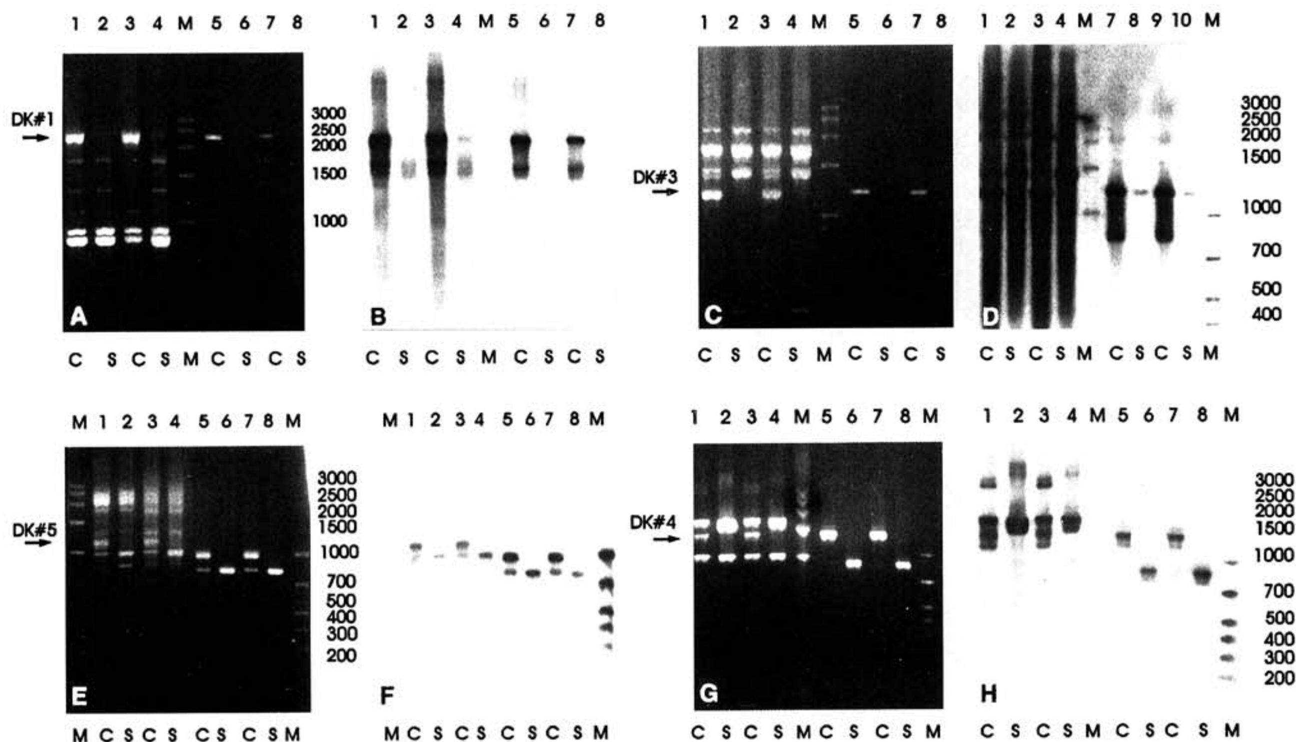

Fig 1. Amplification products from *Radopholus citrophilus* (DK2) and *R. similis* (DK6) using 25 ng of cesium purified genomic DNA per reaction. **A,** Lanes 1 to 4, random amplified polymorphic DNA (RAPD) profiles for primer OP-A11; lanes 5 to 8, amplification products for primers DK#101 and DK#102. **B,** Blot of **A** probed with digoxigenin (DIG)–labeled DK#1. **C,** Lanes 1 to 4, RAPD profiles for primer OP-K02; lanes 5 to 8, amplification products for primers DK#103 and DK#104. **D,** Blot of **C** probed with DIG-labeled DK#3. **E,** Lanes 1 to 4, RAPD profiles for primer OP-O10; lanes 5 to 8, amplification products for primers DK#105 and DK#106. **F,** Blot of **E** probed with DIG-labeled DK#5. **G,** Lanes 1 to 4, RAPD profiles for primer OP-M02; lanes 5 to 8, amplification products for primers DK#107 and DK#108. **H,** Blot of **F** probed with DIG-labeled DK#4. Marker (M) = mixture of regularly spaced bands of linear double-stranded DNA (DNA Molecular Weight Marker XII; Boehringer Mannheim Corp., Indianapolis, IN). C = DK2; S = DK6.

Table 2. Detection of burrowing nematode strains in test plant roots 30 days after inoculation

Nematode	Origin	Sour orange	Rough lemon	Tomato
DK1	Florida	+	+	+
DK2	Florida	+	+	+
DK3	Hawaii	–	–	+
DK4	Florida	+	+	+
DK5	Florida	+	+	+
DK6	Florida	–	–	+
DK7	Florida	–	–	+
DK8	Florida	+	+	+
DK9	Belize	–	–	+
DK10	Costa Rica	–	–	+
DK11	N. Costa Rica	–	–	+
DK12	Costa Rica	–	–	+
DK13	Puerto Rico	–	–	+
DK14	Puerto Rico	–	–	+

[a] + = Median values range from 100 to 1,200 nematodes per plant with burrowing nematodes detected in >95% of test plants.
[b] – = Median values range from 0 to 30 nematodes per plant with burrowing nematodes detected in <5% of the test plants.

to 8); the upper band appeared to be specific to *R. citrophilus*. Primers 107 and 108 (Table 1) designed to complement the termini of the 1.4-kb RAPD amplification product from OPM02 (DK4) (Fig. 1G, lanes 1 and 3) yielded amplification products of different sizes from *R. citrophilus* (1.4 kb) and *R. similis* (0.9 kb) (Fig. 1G, lanes 5 to 8). Hybridizations revealed that these products were specific to the respective nematode strains (Fig. 1H, lanes 5 to 8). As mentioned, we were not successful in designing primers for two of the six polymorphic DNA bands because we were not able to clone them. These are listed in Table 1 (1.8 and 2.5 kb).

To further evaluate the relationship of primers that selectively amplified polymorphic bands in *R. citrophilus* to citrus parasitism, multiple *Radopholus* populations were assayed for the ability to parasitize citrus (Table 2). Primers 101 and 102 amplified the predicted 2.4-kb product from mini-prepped DNA from each of the Florida burrowing nematode strains that reproduced on citrus (Fig. 2A and B, DK1, 2, 4, 5, and 8). A product of similar size was also amplified from the *R. citrophilus* population from Hawaii (DK3) (Fig. 2A and B, DK3) that does not parasitize citrus (Huettel et al. 1986). Amplification of the fragment from burrowing nematode strains collected from Puerto Rico and Belize (Fig. 2C and D, DK9 and 14) suggests that these isolates are also *R. citrophilus*. The other primer sets were not further evaluated in this experiment and bands of similar size were not sequenced or subjected to restriction mapping to ascertain the extent of sequence homology among them.

DISCUSSION

The limited number of polymorphic bands obtained from the RAPD screen of the two nematode sibling species suggests a high degree of genome conservation between these nematodes. Although it has been reported that these two sibling species have different chromosome numbers (*R. citrophi-*

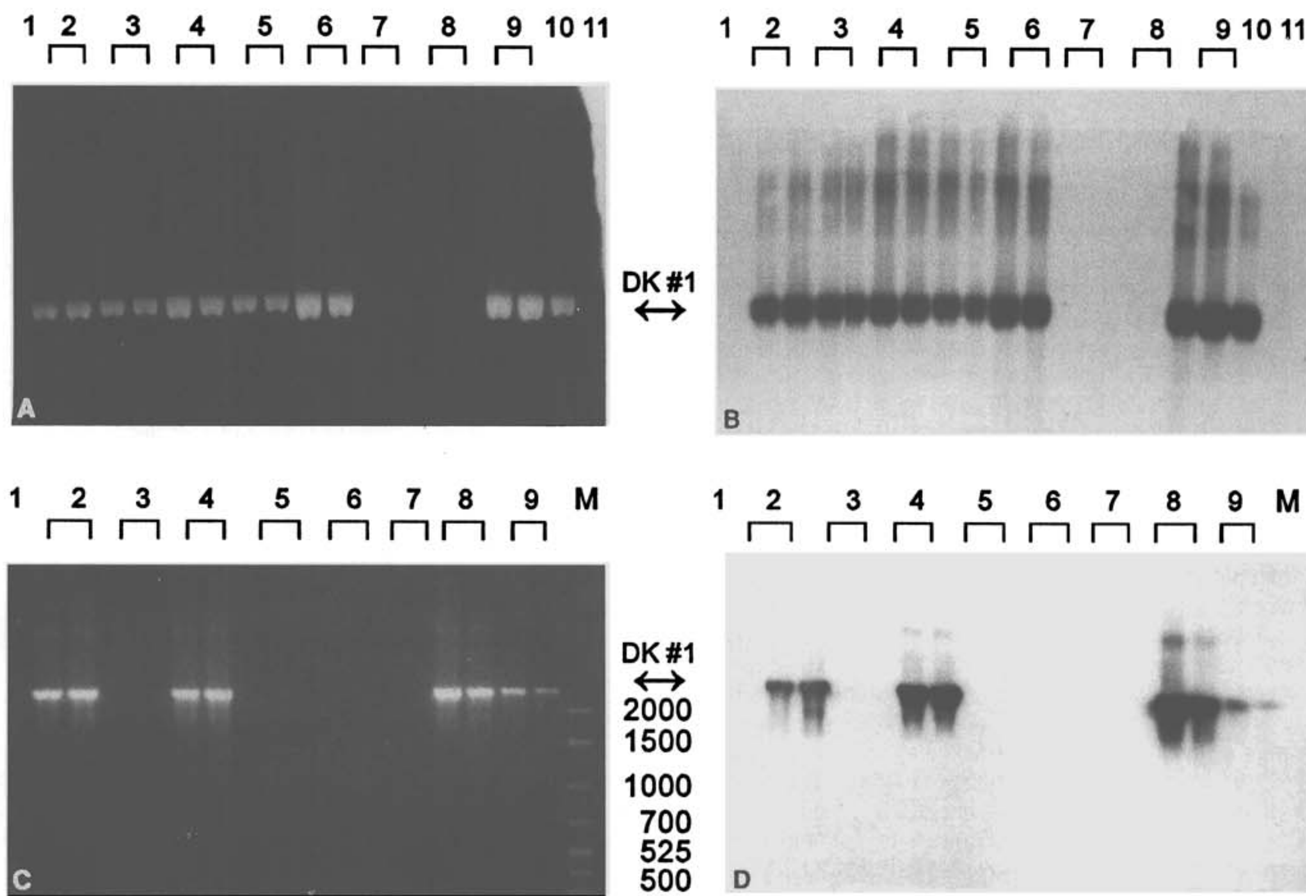

Fig 2. Amplification of DK#1 from miniprep DNA from 14 burrowing nematode strains (DK1 to 14) using primers DK#101 and DK#102 (Table 1). **A** and **C,** Ethidium bromide–stained agarose gels. **B** and **D,** DNA blots of **A** and **C,** respectively, probed with digoxigenin-labeled DK#1. **A** and **B,** lane 1, primers DK#101 and and DK#102 only (no DNA template added to polymerase chain reaction mix); lane 2, duplicate reactions for DK1; lane 3, duplicate reactions for DK2; lane 4, duplicate reactions for DK3; lane 5, duplicate reactions for DK4; lane 6, duplicate reactions for DK5; lane 7, duplicate reactions for DK6; lane 8, duplicate reactions for DK7; lane 9, duplicate reactions for DK8; lane 10, cesium purified genomic DNA DK2; lane 11, cesium purified genomic DNA DK6. **C** and **D,** Lane 1, primers DK101 and 102 only; lane 2, duplicate reactions for DK2; lane 3, duplicate reactions for DK6; lane 4, duplicate reactions for DK9; lane 5, duplicate reactions for DK10; lane 6, duplicate reactions for DK11; lane 7, duplicate reactions for DK12; lane 8, duplicate reactions for DK13; lane 9, duplicate reactions for DK14. Marker (M) = mixture of regularly spaced bands of linear double-stranded DNA (BioMarker EXT, Bio Ventures, Inc., Murfreesboro, TN).

lus n = 5; *R. similis* n = 4) (Huettel and Dickson 1981b), the RAPD analysis indicates that the nematode genomes are highly similar. This is supported by recent reports from a RAPD analysis of *R. similis* populations from Sri Lanka. In those experiments, 85% of the 14 populations tested had identical profiles, even though they were from different geographic locations (Hahn et al. 1994). The apparent limited genetic variation among burrowing nematode populations on a worldwide basis contrasts with other plant-parasitic nematode species thus far examined (Caswell-Chen et al. 1992; Cennis 1993). Polymorphic DNA within a single species is readily detected for most plant parasitic nematodes. The reason for the apparent highly conserved nature of the *Radopholus* genome is unknown.

It has previously been suggested that the limited genetic variation observed in the *Radopholus* genome may be due to laboratory culture techniques (Hahn et al. 1994). We do not believe this to be the case, however. Some of the populations included in our study were in culture for a relatively short time (less than 3 months). It is more likely that genome organization is related to the reproductive mode, long-range nematode dissemination through vegetative plant propagation, wide host range, and the nematode's migratory parasitic habit.

Furthermore, *Radopholus* is bisexual and believed to reproduce predominantly via amphimixis but to be capable of parthenogenesis (Huettel and Dickson 1981a). Other nematode species that reproduce via parthenogenesis possess considerable genetic variation nonetheless (Cenis 1993; Xue et al. 1993). We believe that this factor probably only plays a minor role, if any, in the limited variation observed in *Radopholus*. It seems far more probable that short- and long-distance transport of *Radopholus*-infested plant material (Gowen and Queneherve 1990; Holdeman 1986; Huettel et al. 1986) has contributed to the apparent homogeneity. The migratory habit of the nematode (Suit and DuCharme 1953) combined with a wide host range (Gowen and Queneherve 1990) may also be a factor. *Radopholus* may also avoid selection pressure by moving away from a poor or nonhost plant. *Radopholus* spp. have probably evolved relatively recently and little time for accumulation of silent mutations has thus far passed. In light of

fixed differences in isozyme profiles between *R. similis* and *R. citrophilus* (Huettel et al. 1983a), the RAPD data suggest that *R. citrophilus* is probably a recent radiation (Moritz 1994) and that differences in karyotype (Huettel and Dickson 1981b) are not associated with unique genetic sequence.

In general, variation that is observed within the *Radopholus* genome appears to be correlated with ability to expand host range or with discernible morphological differences. Polymorphic bands observed in our RAPD analysis appeared to be related either to the ability to attack citrus or to previously reported biochemical differences between the burrowing nematode sibling species *R. citrophilus* and *R. similis* (Huettel et al. 1983a, 1983b). Likewise, in the analysis of Indonesian burrowing nematode populations, divergent RAPD patterns could be associated with citrus parasitism and/or with distinctive morphological traits (Hahn et al. 1994). Further, the presence of bands associated with citrus parasitism in *R. citrophilus* is an intriguing observation. These data suggest a high degree of relation between the presence of certain banding patterns and host range. If shifts in host range can be correlated to the presence of highly specific DNA markers, the high degree of genome conservation observed may facilitate the characterization of genetic loci controlling host range. We are currently expanding the range of populations tested to more precisely assess the level of genome conservation in *Radopholus* populations from distinct geographical regions.

One way to assess the significance of markers correlated to parasitism is through genetic crossing and segregation analysis. Although *Radopholus* can reproduce by facultative meiotic parthenogenesis (Huettel and Dickson 1981a), both interspecific (D. T. Kaplan, unpublished data) and intraspecific (Huettel et al. 1982) copulation have been observed. Although sexual recombination has not been documented in *Radopholus*, through selective mating schemes, it may be possible to observe the co-segregation of polymorphic bands (DNA markers) with host preference. The markers we have identified in this study may provide useful tools to identify potential genetic recombination events. Further, these studies may shed light on the mechanism(s) governing genome conservation in *Radopholus* and may also be used to clarify the taxonomic status of the sibling species.

The markers developed in this study may also have practical applications. This research may contribute to the development of diagnostic tools that may afford regulatory agencies with a sensitive, accurate, reliable, and rapid method to identify *R. citrophilus*. The genetic data suggest that burrowing may not be restricted to Florida and Hawaii. Experiments are being conducted in tandem with bioassays (Kaplan 1994) to verify host range of *Radopholus* collected from throughout Florida, Central America, and Hawaii. Previous studies have identified polymorphic DNA markers that may be used to discriminate between species and races of sedentary endoparasitic nematodes (i.e., *Meloidogyne* spp.) (Williamson et al. 1994). We have extended these findings to a migratory form and have also shown a surprisingly high degree of genome conservation among widely dispersed populations.

Perhaps most significant, however, is the observation that some markers appear to be correlated with host range. Although we cannot yet determine how close the relationship is, the genetic tools developed in this study should enable us to precisely identify loci controlling parasitism in *Radopholus* species. We further intend to use these tools to attempt to isolate and characterize genes from *Radopholus* species that are important to parasitism.

MATERIALS AND METHODS

Nematode.

Single strains of the two burrowing nematode sibling species *R. citrophilus* DK2 and *R. similis* DK6 were maintained in carrot disk culture and extracted from culture by enzymatic maceration (Kaplan and Davis 1990). These strains were used for RAPD analysis and subsequent development of species-specific primers. Both DK2 and DK6 were originally isolated in Florida from citrus and banana, respectively. Analyses of karyotype, isozymes, total protein patterns, and host range were previously reported by Huettel et al. (1983a, 1983b, 1984a). In those studies, DK2 and DK6 were identified as Orlando I citrus and Orlando banana, respectively. In addition, DK2 has been routinely used in USDA-ARS citrus germ plasm evaluations for the past 14 years, reproducing well in roots of citrus rootstocks considered to be burrowing nematode susceptible, but unable to reproduce in roots of citrus rootstocks considered to be resistant to the burrowing nematode (Kaplan and O'Bannon 1985).

Fourteen burrowing nematode populations (Table 2) cultured on excised carrot disk were assayed to determine if tomato (*Lycopersicon esculentum* L.), rough lemon (*Citrus limon* (L.) Burm. f.), and sour orange (*Citrus aurantium* (L.)) were hosts using a laboratory bioassay (Kaplan 1994). Median values ≥30 nematodes per plant were considered hosts. These populations were subsequently used in a molecular analysis of the relative specificity of the primers developed to DK2-specific polymorphisms detected in RAPD analyses.

DNA extraction for RAPD analysis and development of sequence-specific primers.

The nucleic acid extraction procedure used for RAPD analyses and for development of primers specific to burrowing nematode specific polymorphisms for DK2 and DK6 was as follows. Nematodes (mixed life cycle stages) and eggs were ground in liquid nitrogen. The nematode homogenate was incubated in extraction buffer (0.5% sodium dodecyl sulfate, 10 mM Tris-HCl; pH 8.0, 100 mM NaCl; 25 mM EDTA; 0.1 mg proteinase K per ml) for 1 h at 65°C. Each sample was then phenol:chloroform extracted three times. Nematode DNA was precipitated by overnight incubation with 250 mM NaCl and 70% ethanol. The DNA pellet was washed twice in 70% ethanol and resuspended in TE (10 mM Tris, 1 mM EDTA) with 20 mg RNAase A per ml. After a 30-min incubation at 37°C, the suspension was phenol:chloroform extracted and the aqueous phase was collected. DNA was precipitated overnight with 10 M ammonium acetate and 100% ethanol and washed twice with 70% ethanol. The pelleted DNA was resuspended in TE and DNA concentration determined spectrophotometrically at 260 nm. Yields averaged 640 µg of nucleic acid per 1.0 million nematodes and eggs.

RAPD analyses.

Nematode DNA was screened with 380 random-decamer primers (Primer Kits A-Q, T, and X; Operon Technologies, Alameda, CA) according to manufacturer's specifications

(Williams et al. 1990). Amplification reactions were performed in 25-μl reaction volumes containing 1.0 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, CT), 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 1.75 mM $MgCl_2$, 100 μM of each dNTP, 0.2 μM primer, and 25 ng of nematode DNA. PCR was performed in a Perkin-Elmer Cetus thermal cycler using a preheated block (94°C) for 45 cycles (94°C, 1 min; 35°C, 1 min; and 72°C, 2 min). The reaction tubes were subsequently held at 4°C until retrieval. Then, 15 μl of the reaction mixture was loaded into a 0.8% agarose gel and electrophoresed in 1× TBE (Tris, borate, EDTA; pH 8.0) at 110 V for 2 h. Amplification products were detected with ethidium bromide (10 mg/ml) on a UV transilluminator. Molecular weight markers were either pGEM (Promega, Madison, WI), lambda cut with *Pst*I, or BioMarker XT or EXT (Bio Ventures, Inc., Murfreesboro, TN). Two independent reactions for each nematode-primer combination were included in each run. Results were verified by running reactions on three separate batches of purified nematode DNA.

DNA cloning and sequencing.

DNA bands of interest were excised from the gels and recovered using a GENECLEAN kit (Bio-101, La Jolla, CA) according to manufacturer's protocols. The isolated DNA was ligated into pT7Blue and transformed into *Escherichia coli* strain NovaBlue. Transformants were identified by blue/white selection. Insertion was verified by an *Eco*RI/*Sal*I digest of miniprepped plasmid DNA. Double-stranded plasmid DNA template for sequence analysis was prepared by alkaline lysis minipreps. Sequencing reactions were performed with a Sequenase II kit (USB, Cleveland, OH) and [αS^{35}]dCTP, according to manufacturer's instructions, and gels were run on Long Ranger (J. T. Baker, Inc., Phillipsburg, NJ) at 50W. Sequence was read and analyzed with a Hitachi Digitizer and DNAsis software.

Primer design.

Primers were designed to DNA sequence of the terminal ends of the cloned RAPD fragments located inside the original Operon 10-mer sequence (Kesseli et al. 1992). Primer design and PCR reaction conditions were optimized using PC Gene software (Intelligenetics, Mountain View, CA). Primers that included the original Operon 10-mer sequence plus an additional 10 to 12 bases were also constructed (SCAR) when amplification products from STS primers did not discriminate between DK2 and DK6. Amplification reactions for STS and SCAR were performed in 25-μl reaction volumes containing 1.0 U of *Taq* polymerase (Perkin-Elmer), 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 2.56 mM $MgCl_2$, 130 μM of each dNTP, 0.2 μM primer, and 20 ng of nematode DNA. PCR for STS was performed using a preheated block (94°C) for 45 cycles (94°C, 1 min; 63°C, 1 min; and 72°C, 2 min) followed by a single 5-min extension at 72°C. The reaction tubes were subsequently held at 4°C until retrieval. PCR for SCAR was essentially the same except that the annealing temperature was reduced to 53°C for 1 min. Then, 15 μl of the reaction mixture was loaded into a 0.8% agarose gel and electrophoresed in 1× TBE (pH 8.0) at 110 V for 2 h. Amplification products were electrophoresed and observed as previously described.

Hybridization.

DNA blots were made from RAPD and corresponding STS and SCAR reactions as described by Maniatis et al. (1982). Flash Membrane (Stratagene, La Jolla, CA) was probed with the RAPD fragment used to generate the sequence specific primers. Probes were labeled with digoxigenin (DIG) and developed using the Genius Non radioactive Labeling Kit, (Boehringer Mannheim Corp., Indianapolis, IN). Cronex X-ray film (Dow, Wilmington, DE) was used to identify DIG-labeled reaction products.

Survey.

A molecular and biological survey was conducted using 14 burrowing nematode populations collected from Florida, Hawaii, and Central America. The populations and the locations where they were isolated are listed in Table 2. DNA was extracted from 14 burrowing nematode strains collected to test the specificity of the SCAR-specific primers 101 and 102 by grinding approximately 1,000 nematodes for 15 s in disposable micro-homogenizer tubes as described for extraction of plant genomic DNA (Edwards et al. 1991) with 10 μM dithreotol (DTT) added to the extraction buffer. PCR reaction conditions were those described above. Citrus parasitism for each population was estimated using a laboratory host-index system (Kaplan 1994).

Designation.

Nematode strains, primers, and cloned DNA were named according to the guidelines published for parasitic nematodes (Bird and Riddle 1994).

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