ıertson, D. Ammons, M. Klass, and E. Genomic organization of major sperm and pseudogenes in the nematode *Cae-* ırnal of Molecular Biology 199:1–13.
ı., and M. Klass. 1982. The location of ɜrm protein in *Caenorhabditis elegans* ıermatocytes. Developmental Biology

ꞁENBANK ACCESSION

ık accession numbers for the sequences is paper are as follows: *msp-1* (λ13.1.1), bp); *msp-2* (λ16.1.1), L24500 (860 bp), '.1.1), L24501 (984 bp).

Journal of Nematology 25(4):555–563. 1993.

Separation of Three Species of *Ditylenchus* and Some Host Races of *D. dipsaci* by Restriction Fragment Length Polymorphism

KAREN R. WENDT,[1] THIERRY C. VRAIN,[2] AND JOHN M. WEBSTER[1]

Abstract: This study examined the ribosomal cistron of *Ditylenchus destructor*, *D. myceliophagus* and seven host races of *D. dipsaci* from different geographic locations. The three species showed restriction fragment length polymorphisms (RFLPs) in the ribosomal cistron, the 18S rDNA gene, and the ribosomal internal transcribed spacer (ITS). Southern blot analysis with a 7.5-kb ribosomal cistron probe differentiated the five host races of *D. dipsaci* examined. Polymerase chain reaction (PCR) amplification of the ITS, followed by digestion with some restriction endonucleases (but not others), produced restriction fragments diagnostic of the giant race. Because the PCR product from *D. myceliophagus* and the host races of *D. dipsaci* was about 900 base pairs and the ITS size in *D. destructor* populations was 1,200 base pairs, mixtures of populations could be detected by PCR amplification. ITS fragments differentiated between *D. dipsaci* and *Aphelenchoides rhyntium* in mixed populations. This study establishes the feasibility of differentiation of the host races of *D. dipsaci* by probing Southern blots with the whole ribosomal cistron.

Key words: *Ditylenchus destructor*, *D. dipsaci*, *D. myceliophagus*, DNA, host race, internal transcribed spacer, nematode, polymerase chain reaction, restriction fragment length polymorphism, ribosomal DNA, sibling species.

Nematodes belonging to the genus *Ditylenchus* Filipjev are difficult to identify to species because of highly similar morphology and considerable intraspecific biological variation. Morphological differentiation of *Ditylenchus* is based mainly on tail shape and size, relative length of the stylet and post-vulval sac, and the number of cuticular lateral lines (10). However, these characters vary according to nematode developmental stage, culture medium, and temperature (2). The conclusion from a recent taxonomic review of the genus *Ditylenchus* was that identification is difficult and preparation of a workable key is almost impossible (3). Therefore, a sensitive and reliable technique is needed to differentiate species of *Ditylenchus*.

Ditylenchus dipsaci (Kühn) Filipjev, the stem nematode, is a migratory endoparasite of over 500 species of angiosperms (24). The main method of control of *D. dipsaci* is crop rotation, but the presence of morphologically indistinguishable races with different host preferences makes rotation difficult. Moreover, the presence of mixtures of nematodes, e.g., *Aphelenchoides ritzemabosi* and *D. dipsaci* in alfalfa fields, may confound diagnosis. A practical technique for rapidly and reliably identifying the host races of *D. dipsaci* is not available. Except for a larger "giant" race on field beans (13), the 30 host races of *D. dipsaci* (15) are separated on the basis of host preference. The races exhibit varying degrees of reproductive isolation, such as partial or complete reproductive incompatibility (6,21), and a wide range of chromosome numbers, from 2n = 24 to 56 (1).

Although protein electrophoresis separated species of *Ditylenchus* (5), attempts to separate races of *D. dipsaci* by esterase or catalase profiles (7,14) or by polyclonal antibodies developed against the surface antigens (11,28) failed. Palmer et al. (19) generated monoclonal antibodies (MAbs) against the oat race but discovered in an ELISA test that the MAbs were specific to only the original oat race isolate, not to all isolates of that race. Analysis of DNA is a more direct measure of variability than is protein analysis, and the application of

Received for publication 25 March 1993.
[1] Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, British Columbia V5A 1S6, Canada.
[2] Agriculture Canada, Research Station, 6660 N.W. Marine Drive, Vancouver, British Columbia V6T 1X2, Canada.
We are especially grateful to R. V. Anderson, G. Caubel, K. B. Eriksson, F. Gommers, D. J. Hooper, A. E. MacGuidwin, and R. Peaden for providing nematode cultures. We acknowledge financial support for the study from the Natural Sciences and Engineering Research Council of Canada.

such molecular analyses recently provided precise identification of three species of *Ditylenchus* but did not differentiate host races of *D. dipsaci* (18).

Ribosomal DNA has proven to be a particularly interesting and useful region for investigating reproductively isolated nematode populations at all taxonomic levels (9,27,29). The multiple copy, tandemly repeated, ribosomal gene cluster contains a wide spectrum of extremely conserved to highly heterogeneous sequence. The DNA sequences of the ribosomal DNA encoding 18S, 5.8S, and 26S genes are more conserved than the internal transcribed spacer (ITS) or intergenic spacer (IGS) region. Thus, depending on the organism, regions can be identified that differentiate a range of taxa from the level of genera to that of subspecific populations (12). Although Southern hybridization is a reasonably sensitive method for surveying the fine and gross organization of the ribosomal gene, it has associated disadvantages; for example, it may require a large amount of DNA or radioactive techniques for visualization (4). Polymerase chain reaction (PCR) amplification of specific DNA fragments overcomes these disadvantages (17).

The objective of this study was to determine whether some of the homologous and heterologous regions of the ribosomal gene cluster, namely the whole rDNA cistron, the 18S gene, and the ITS, could separate three species of *Ditylenchus* and nine races of *D. dipsaci*.

Materials and Methods

The nematode isolates used in this study originated from North America, Europe, and Africa from cultivated and noncultivated plant hosts (Table 1). Some populations were derived directly from the field and others had been in culture for a number of years. *Aphelenchoides rhyntium* (APH) was maintained on *Botrytis cinerea* culture in the laboratory.

Extraction and restriction of DNA: Nematodes were washed from the lids of fungal plates or extracted from nematode-infested dried or fresh plant material in a Baermann funnel, and the nematodes were screened from the aqueous suspension with a 30 μm-pore-d sieve. The nematode DNA was extracted (29). Initially, Southern blots of digested *Ditylenchus* genomic DNA were probed with the whole ribosomal cistron and then reprobed with a portion of the ribosomal gene. Although there was not enough DNA from some *D. dipsaci* populations to perform Southern hybridizations, all populations were amplified by PCR and subsequently digested with restriction enzymes. Genomic or PCR-amplified DNA samples (0.5 to 1.0 μg) were digested by restriction endonucleases with four, five, or six-base recogni-

TABLE 1. Origin of the *Ditylenchus* populations used in this study.

	Code	Host	Origin	Source
D. myceliophagus	MYC	Mushroom	Germany	*Rhizoctonia cerealis*
D. destructor	UK	*Solanum tuberosum*	Ireland	*R. cerealis*
	WIS	*S. tuberosum*	U.S. (Wisconsin)	Excised corn roots
D. dipsaci				
alfalfa	ALF	*Medicago sativa*	U.S. (Washington)	Greenhouse alfalfa plants
alfalfa	FAL	*M. sativa*	France	Alfalfa callus
beet	BET	*Beta vulgaris*	France (Alsace)	Greenhouse faba beans
giant	FGI	*Vicia faba*	France (Le Rheu)	Greenhouse faba beans
giant	GIA	*V. faba*	England (Hertfordshire)	Dried zucchini marrows
potato	POT	*S. tuberosum*	Netherlands	Dried potatoes
red clover	RCL	*Trifolium pratense*	France (Domagne)	Greenhouse red clover plants
teasel	TEA	*Dipsacus fullonum*	England (Somerset)	Dried teasel stems
tulip	TUL	*Tulipa gesneriana*	Netherlands	Dried tulip bulbs

ecies of *Ditylenchus* and nine
saci.

RIALS AND METHODS

›de isolates used in this study
›m North America, Europe,
om cultivated and nonculti-
›sts (Table 1). Some popula-
rived directly from the field
d been in culture for a num-
phelenchoides rhyntium (APH)
ed on *Botrytis cinerea* culture
ory.
nd restriction of DNA: Nema-
ished from the lids of fungal
tracted from nematode-
l or fresh plant material in a
unnel, and the nematodes
d from the aqueous suspen-
μm-pore-d sieve. The nema-
as extracted (29). Initially,
ts of digested *Ditylenchus* ge-
were probed with the whole
tron and then reprobed with
he ribosomal gene. Although
enough DNA from some *D.*
ations to perform Southern
s, all populations were ampli-
and subsequently digested
ion enzymes. Genomic or
d DNA samples (0.5 to 1.0
ested by restriction endonu-
our, five, or six-base recogni-

	Source
	Rhizoctonia cerealis
	R. cerealis
	Excised corn roots
	Greenhouse alfalfa plants
	Alfalfa callus
	Greenhouse faba beans
	Greenhouse faba beans
ire)	Dried zucchini marrows
	Dried potatoes
	Greenhouse red clover plants
	Dried teasel stems
	Dried tulip bulbs

tion sites, according to the manufacturers' recommended procedures (Bethesda Research Laboratories [BRL], Burlington, Ontario; Boehringer Mannheim, Germany; Pharmacia, Baie d'Urfé, Quebec). DNA samples were electrophoresed in horizontal gels (16) of 0.7 to 1.5% agarose, depending on the size of the fragments. Fragment sizes were subsequently estimated with a regression line of size standards and electrophoretic migration. We used a 1-kb ladder (Pharmacia) as size standards.

Southern blots: Two probes (29) were used in Southern blots. The 7.5-kb ribosomal gene fragment from the *Bursaphalenchus xylophilus* Ibaraki isolate (pBx2) comprises the ribosomal cistron from the 5′ end of the 18S coding unit to the 3′ end of the 28S coding unit. The 18S probe, from *B. mucronatus* (pBm3), is a 1.1-kb fragment that contains the entire 18S subunit coding region. The DNA probes used for Southern blots were ^{32}P-labeled using random oligonucleotide primers (8).

From 1.0 to 2.0 μg of Rsa I-digested genomic DNA was electrophoresed and Southern blotted to nylon filters (16). Filters were hybridized to denatured rDNA probe labelled with [^{32}P]dATP at 62 C in 5 × SSPE (1 × SSPE = 0.18 M NaCl, 10 mM ($Na_{1.5}$) PO_4, 1 mM Na_2EDTA, pH 7.4), 5 × Denhardts (1 × Denhardt's = 0.02% w/v bovine serum albumin, Ficoll 400 and polyvinylpyrrolidone 40) and 2.0% SDS (sodium dodecyl sulfate). The filters were washed four times at 62 C in 2 × SSPE and 0.2% SDS.

For the 18S Southern hybridizations, the nylon filters previously hybridized with the rDNA probe were stripped of the probe by washing twice for 5 minutes in a solution of 1.5 M NaCl and 0.5 M NaOH at room temperature (ca 20 C) and neutralizing for 30–60 minutes in a solution of 1.0 M ammonium acetate and 0.02 M NaOH, before hybridization with the 18S probe.

Polymerase chain reaction: The ITS region (including ITS 1, ITS 2, and the 5.8S subunit) in 100–200 ng of genomic DNA from each population was amplified with kits, according to the manufacturers' recommendations (Perkin-Elmer Cetus, Eden Prairie, MN; Promega, Madison, WI). The primers used were two universal ITS primers, each 21 base pairs long, developed from *Xiphinema bricolensis* (27); one primer with a sequence starting 171 base pairs from the 3′ end of the 18S ribosomal subunit, and the other primer 80 base pairs into the 5′ end of the 26S gene.

Negative controls, consisting of the mixture without template DNA but brought to the same volume, were run with each amplification. Reaction profiles were as follows: 1.5 minutes at 96 C, 30 seconds at 50 C, 4 minutes at 72 C; 40 cycles of 45 seconds at 96 C, 30 seconds at 50 C, 4 minutes at 72 C and a final cycle of 45 seconds at 96 C, 30 seconds at 50 C, and 10 minutes at 72 C. The PCR thermocycler used was the Twin Block System EC Cycler (Ericomp). The PCR products from several amplification runs were pooled for digests.

RESULTS

Differentiation of Ditylenchus species: All three nematode species displayed a unique pattern of fragments when Southern hybridizations were probed with the complete ribosomal cistron (Fig. 1). Only a limited amount of DNA was available from *D. destructor* (WIS), *D. dipsaci* (BET, FAL, FGI, RCL), and *A. rhyntium* (APH) populations, and these generated fainter fragment patterns than others. When the described Southern transfer of the digested DNA of *D. myceliophagus, D. destructor* (UK), and *D. dipsaci* teasel (TEA) was reprobed with the *B. mucronatus* fragment containing most of the 18S rDNA gene, *D. dipsaci* showed a unique pattern of fragments, but identical patterns occurred for *D. myceliophagus* and *D. destructor* (data not shown).

Amplification by PCR of DNA from *D. myceliophagus* and all populations of *D. dipsaci* generated a single ITS fragment of about 0.9 kb, whereas the PCR product of *D. destructor* isolates was about 1.2 kb (Fig. 2A). A PCR amplification of a mixed sam-

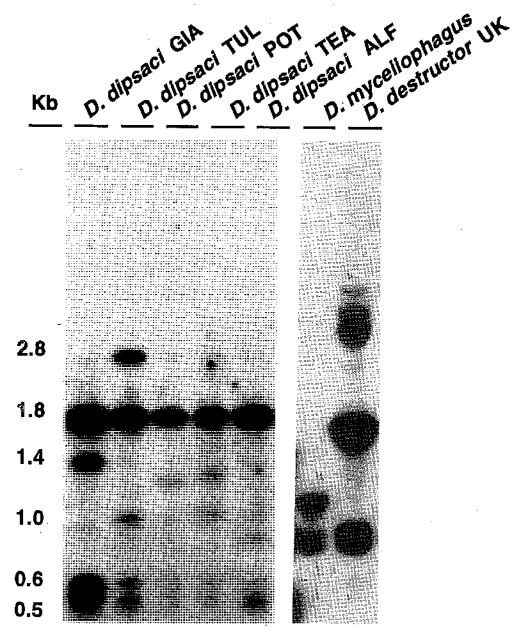


FIG. 1. Autoradiograph of a Southern transfer of Rsa I-digested genomic DNA from *Ditylenchus myceliophagus, D. destructor,* and five host races of *D. dipsaci* probed with the 7.5-kb rDNA cistron (from *Bursaphelenchus xylophilus*) under moderately stringent conditions. A longer autoradiographic exposure was performed for *D. dipsaci* (12 hours) than for *D. myceliophagus* and *D. destructor* (8 hours). Populations abbreviated as in Table 1.

ple of *D. dipsaci* (POT) and *D. destructor* (UK) DNA generated respective bands of 0.9 and 1.2 kb, (Fig. 2B). Similarly, PCR amplification of a mixture of *D. dipsaci* (ALF) and *A. rhyntium* (APH) DNA produced two bands corresponding to each genus in the mixture (Fig. 2B).

All enzymes that restricted the ITS fragment provided different patterns for each of the three species of *Ditylenchus* (Figs. 3–6, Table 2). In general, few bands were shared among the species, and the three species were distinguished readily from each other by their RFLPs.

Differentiation of host races of D. dipsaci: Each of five populations of *D. dipsaci* produced unique fragment patterns when hybridized to a heterologous rDNA cistron (Fig. 1). Three bands at 1.8, 0.6, and 0.5 kb characterized all *D. dipsaci* populations. Some band sizes (e.g., 2.8 kb, 1.2 kb, and 1.0 kb) were shared between some populations, and others were unique to one *D. dipsaci* isolate examined (e.g., 1.4 kb for the giant race and 0.8 kb for the alfalfa race). When reprobed with the 18S probe, the fragment patterns were identical for four of the *D. dipsaci* host races (ALF, POT, TEA, and POT) and could be distinguished from the giant race pattern by an extra band at 1.4 kb (data not shown).

In order to assess the variability in the PCR-amplified ribosomal spacer region, initially a battery of 14 enzymes was used to digest the ITS fragment of *D. dipsaci* TEA and ALF. The results of the restriction analysis demonstrated similar banding patterns between these two races (Table 2). The ITS of all the *D. dipsaci* races including the giant races was digested with seven enzymes, namely, Dde I, Hae III, Hinc II, Hinf I, Hpa II, Pst I, and Rsa I. Digestion of the ITS of each host race, except the two populations of the giant race, produced patterns identical with those of the teasel and alfalfa races. Although not all enzymes, e.g., Pst I and Dde I, showed differences between the giant race and the other *D. dipsaci* races (Fig. 3), four of the seven enzymes tested (Hae III, Hpa II, Hinf I, and Rsa I) generated patterns unique to this giant race (GIA), and different from those characteristic of all other populations of *D. dipsaci,* as well as from the other two *Ditylenchus* species tested (Figs. 4–6). Not all gels are shown, but all fragment sizes generated by the 14 restriction enzymes are tabulated (Table 2).

DISCUSSION

This investigation expands on an earlier study in which Palmer et al. (18) probed Southern hybridizations with random DNA fragments isolated from two libraries, one from *D. myceliophagus* and the other from *D. dipsaci* oat race. Their probes could differentiate between these two species but not define unknown species of *Ditylenchus.*

In the initial Southern blot experiments in the present study, the 7.5-kb rDNA probe derived from *Bursaphelenchus* revealed sequence variation between each species of *Ditylenchus* and between host

examined (e.g., 1.4 kb for the
d 0.8 kb for the alfalfa race).
ɔed with the 18S probe, the
terns were identical for four
ɔsaci host races (ALF, POT,
'OT) and could be distin-
the giant race pattern by an
1.4 kb (data not shown).
ɔ assess the variability in the
ed ribosomal spacer region,
tery of 14 enzymes was used
ITS fragment of *D. dipsaci*
F. The results of the restric-
lemonstrated similar banding
een these two races (Table 2).
l the *D. dipsaci* races including
s was digested with seven en-
y, Dde I, Hae III, Hinc II,
I, Pst I, and Rsa I. Digestion
each host race, except the two
of the giant race, produced
tical with those of the teasel
aces. Although not all en-
st I and Dde I, showed dif-
een the giant race and the
ci races (Fig. 3), four of the
es tested (Hae III, Hpa II,
Rsa I) generated patterns
giant race (GIA), and differ-
e characteristic of all other
f *D. dipsaci*, as well as from
ɔ *Ditylenchus* species tested
ɔt all gels are shown, but all
generated by the 14 restric-
are tabulated (Table 2).

DISCUSSION

gation expands on an earlier
ı Palmer et al. (18) probed
bridizations with random
ts isolated from two librar-
D. myceliophagus and the
). *dipsaci* oat race. Their
differentiate between these
t not define unknown spe-
hus.
Southern blot experiments
t study, the 7.5-kb rDNA
from *Bursaphelenchus* re-
ce variation between each
ylenchus and between host

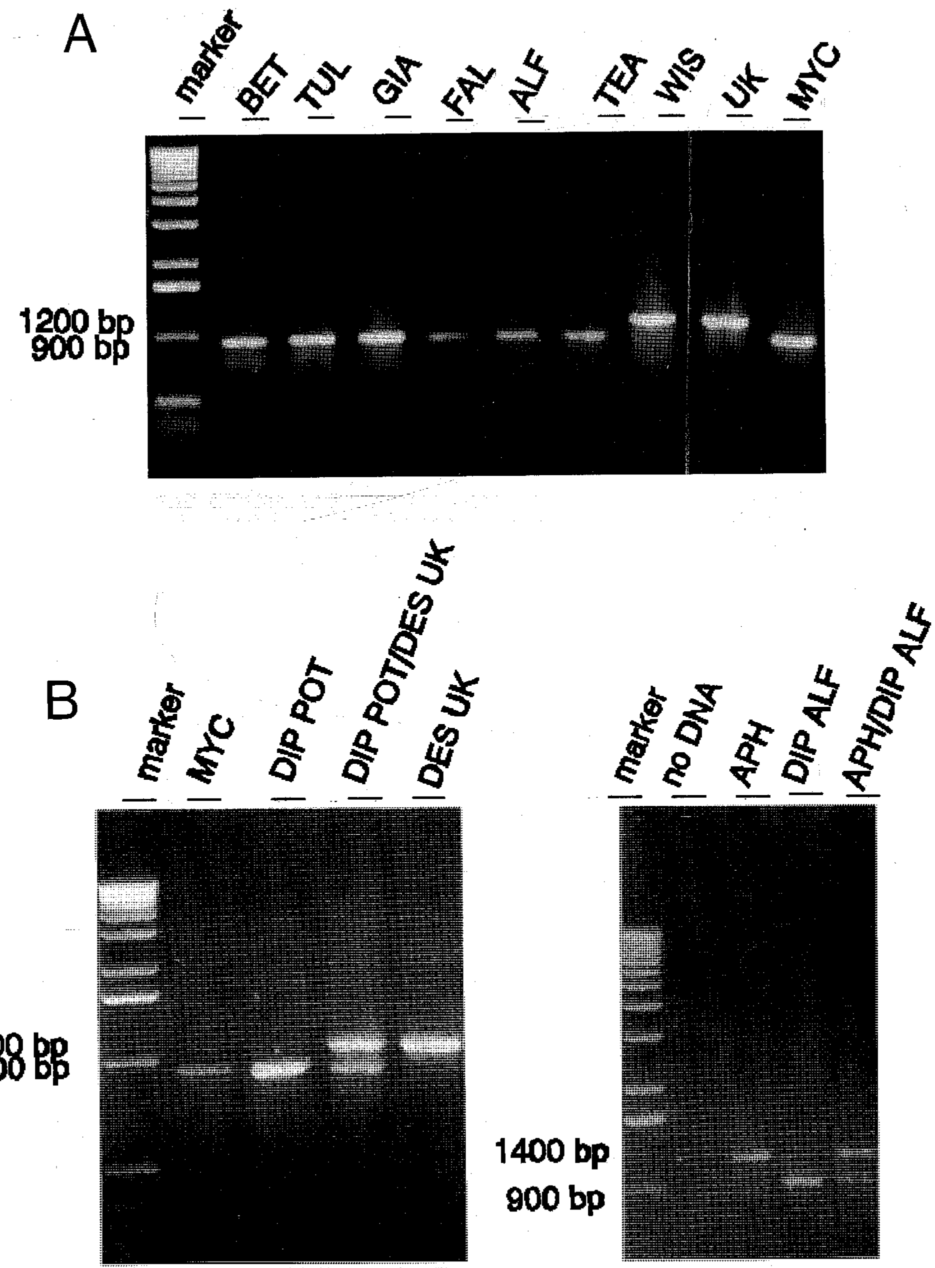


FIG. 2. A) Agarose gel of the internal transcribed spacer (ITS) amplified by polymerase chain reaction (PCR) from *Ditylenchus myceliophagus* (MYC), *D. destructor* (WIS, UK), and host races (BET, TUL, GIA, FAL, ALF, TEA) within *D. dipsaci*. B) Two agarose gels showing the ITS fragments resulting from PCR amplification of DNA from *D. myceliophagus* (MYC), *D. dipsaci* (DIP POT), *D. dipsaci* and *D. destructor* (DES UK) DNA mixed together, *D. destructor* (DES UK), water (no DNA), *Aphelenchoides rhyntium* (APH), *D. dipsaci* (DIP ALF), and *A. rhyntium* and *D. dipsaci* mixed. Populations abbreviated as in Table 1. Marker = 1 kb ladder.

races of *D. dipsaci* within the ribosomal cistron. However, closer examination using only the 18S portion of the ribosomal probe failed to distinguish between the three species and between the five host races (except for the giant race). This failure is not unexpected, because this 18S gene is one of the most conserved regions in the ribosomal gene cluster (12).

Each of the three species of *Ditylenchus*

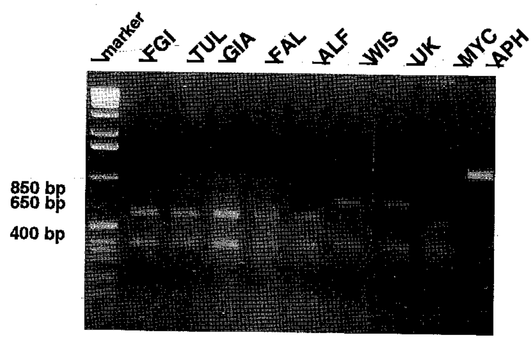


FIG. 3. Agarose gel of the PCR-amplified internal transcribed spacer (ITS) of *Ditylenchus myceliophagus* (MYC), *D. destructor* (WIS, UK), host races within *D. dipsaci* (abbreviated as in Table 1), and *Aphelenchoides rhyntium* (APH) digested with the restriction enzyme Pst I. Populations of *D. dipsaci* abbreviated as in Table 1. Marker = 1 kb.

were readily separated from each other and *A. rhyntium* by PCR amplification of the ITS (including the 5.8S gene) and restriction with an appropriate enzyme. Restriction of the ITS fragment produced four groups: *D. myceliophagus* (MYC), two populations of *D. destructor* (WIS and UK), the giant race of *D. dipsaci*, and the other diploid host races of *D. dipsaci*.

The ITS of the ribosomal cistron in *Ditylenchus* (and *A. rhyntium*) is an appropriate region for species separation but does not differentiate the host races. The host races of *D. dipsaci* were also closely related in another study (18), in which a *D. dipsaci* oat race probe in dot blot experiments did not differentiate the races. In our study, restriction digests of the ITS with 14 different enzymes yielded identical restriction patterns for all but one of the examined races. The polyploid giant race had characteristic patterns of fragments in four (Rsa I, Hpa II, Hae III, Hinf I) of seven enzymic digests.

Sturhan (22,23) suggested that the giant race is not a mere host race but should be considered a sibling species, because it did not produce fertile progeny when crossed with one of the diploid races. However, in another study, fertile F_1 progeny resulting from such crosses were observed (15). Because polyploidy is suspected of playing an important role in the cytogenic evolution of some amphimictic groups of nematodes (26), the hypothesis that the giant race is a sibling species is strengthened. The rDNA sequence heterogeneity presented here also supports this sibling species status.

Mixtures of *D. dipsaci* and *A. ritzemabosi* often coexist in alfalfa (*Medicago sativa*) fields; indeed, the alfalfa samples collected in Wyoming showed typical damage from *D. dipsaci* and contained a mixture of these

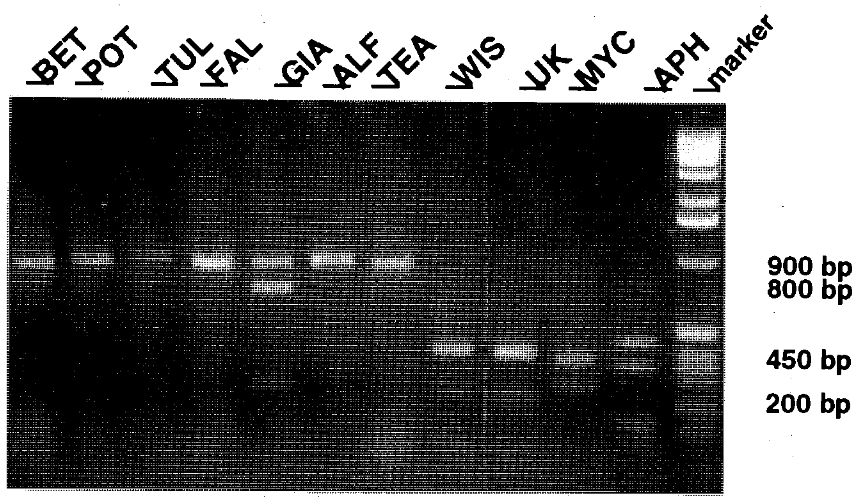
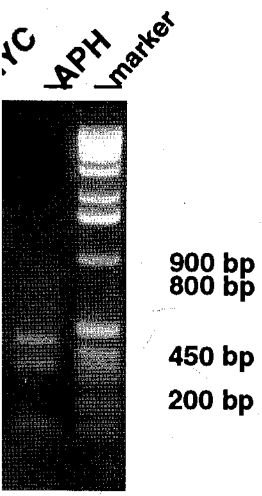


FIG. 4. Agarose gel of the PCR-amplified internal transcribed spacer (ITS) of *Ditylenchus myceliophagus* (MYC), *D. destructor* (WIS, UK), host races within *D. dipsaci* (abbreviated as in Table 1), and *Aphelenchoides rhyntium* (APH) digested with the restriction enzyme Hae III. The 0.9-kb fragment in GIA is a consequence of a partial digest. Marker = 1 kb ladder.

were also closely related in an- (18), in which a *D. dipsaci* oat n dot blot experiments did not e the races. In our study, re- ;ests of the ITS with 14 differ- s yielded identical restriction all but one of the examined polyploid giant race had char- atterns of fragments in four II, Hae III, Hinf I) of seven ests.
22,23) suggested that the giant mere host race but should be a sibling species, because it did fertile progeny when crossed the diploid races. However, in ly, fertile F_1 progeny resulting rosses were observed (15). Be- oidy is suspected of playing an ole in the cytogenic evolution phimictic groups of nematodes pothesis that the giant race is a es is strengthened. The rDNA eterogeneity presented here s this sibling species status.
of *D. dipsaci* and *A. ritzemabosi* st in alfalfa (*Medicago sativa*) d, the alfalfa samples collected showed typical damage from d contained a mixture of these



cer (ITS) of *Ditylenchus myceliophagus* ed as in Table 1), and *Aphelenchoides* fragment in GIA is a consequence of

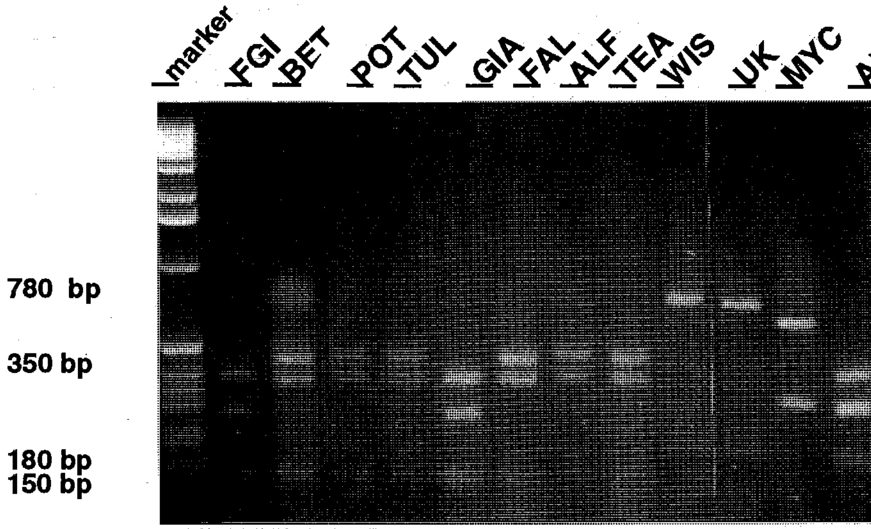


FIG. 5. Agarose gel of the PCR-amplified internal transcribed spacer (ITS) of *Ditylenchus myceliophagus* (MYC), *D. destructor* (WIS, UK), host races within *D. dipsaci* (abbreviated as in Table 1), and *Aphelenchoides rhyntium* (APH) digested with the restriction enzyme Hinf I. The faint bands with sizes larger than 350 kb in BET, GIA, FAL, and TEA are the result of a partial digest. Marker = 1 kb ladder.

two species (F. Gray, pers. comm.), which are relatively similar under low magnification. The ITS of *A. rhyntium*, a close relative of *A. ritzemabosi*, could be distinguished in a mixture of this species and *D. dipsaci*. Therefore, this method could be useful to confirm the morphological diagnosis of mixed or pure infestations of nematodes.

The unique patterns of fragments produced from restriction analysis of the ITS

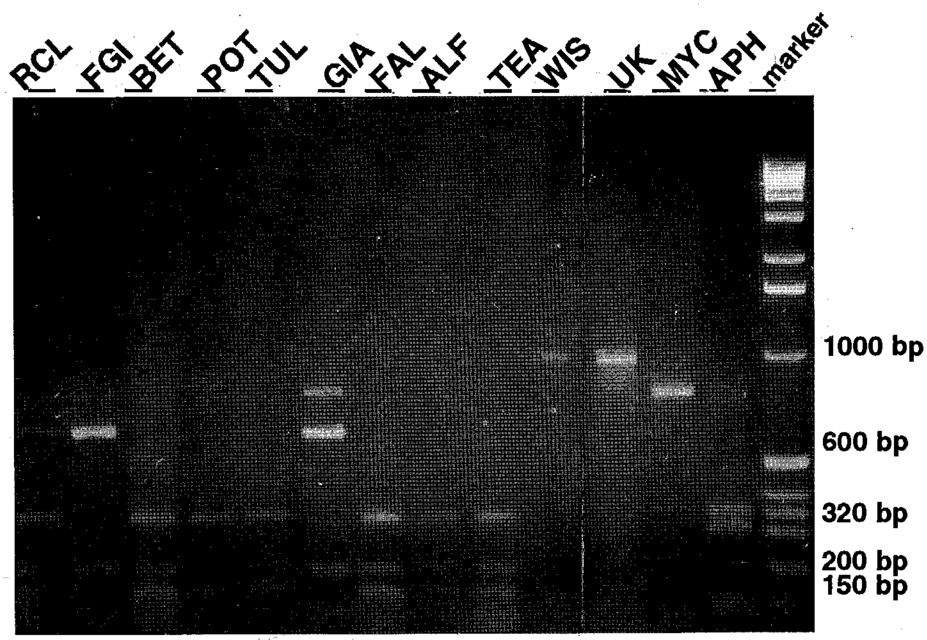


FIG. 6. Agarose gel of the PCR-amplified internal transcribed spacer (ITS) of *Ditylenchus myceliophagus* (MYC), *D. destructor* (WIS, UK), host races within *D. dipsaci* (abbreviated as in Table 1), and *Aphelenchoides rhyntium* (APH) digested with the restriction enzyme Hpa II. The 0.9-kb fragment in GIA is the result of a partial digest. Marker = 1 kb ladder.

TABLE 2. Fragment sizes (kb) resulting from digestion of the internal transcribed spacer of *Ditylenchus myceliophagus*, *D. destructor* (UK and WIS races), and host races and the giant race of *D. dipsaci* with 14 restriction enzymes.

Enzyme	*D. dipsaci* Host races	*D. dipsaci* Giant race	*D. destructor*	*D. myceliophagus*
Acc I		–†	1200	
	900			900
Alu I	900	–		900
			370	
			290	
BamH I		–	1000	
				900
	340			
	220			
	180			
Dde I			670	
			570	
	310	310		
				300
	290	290		
				250
	200	200		
				130
Dra I		–	1200	
				900
	340			
	250			
Hae III	900			
		800		
			450	450
		200		200
			170	
Hinc II			900	900
	800	800		
			250	
Hinf I			780	
				630
	440			
	350	350		
				310
			180	
	150	150		
Hpa II			1000	
				900
		600		
	320			
	200	200		
	180			
Nsi I		–	1200	
	900			900
Pst I			850	
	650	650		
				620
	400	400	400	400
Rsa I				900
			600	
		490		
	450	450		
	250		250	
			170	
	140			
Sau 3A		–	540	
				440
			400	
	340			
	260			
	200			
			180	
	110			
	100			100
TaqI		–	640	
	340			
				320
				260
	230			
			200	
				160
			150	
	130			

† A dash in the column indicates that this experiment was not performed.

of *D. myceliophagus, D. destructor,* and *D. dipsaci* are not confused by morphological similarities between species or geographic populations, or by host- and temperature-induced morphological differences within a species. Although the host races of *D. dipsaci* examined in this study generally originated from different geographic locations and different food sources, Southern hybridizations utilized herein revealed no differences, except for the giant race. The two populations of the giant race from England and France generated identical patterns of fragments with three enzymes tested (Pst I, Hinf I, Hpa 2). Similarly, U.S. and French isolates of the alfalfa race consistently showed the same ITS restriction patterns. In addition, because PCR amplification has been used successfully on preserved organisms (25), taxonomic features that are obscured by fixative techniques will not impede the molecular diagnosis of preserved museum specimens, although such diagnosis is destructive.

Analysis of the ribosomal cistron by

)3

'ontinued

dipsaci		
Giant race	*D. destructor*	*D. myceliophagus*
450		
	250	
	170	
–	540	
		440
	400	
	180	
		100
–	640	
		320
		260
	200	
		160
	150	

:olumn indicates that this experiment was

phagus, D. destructor, and *D.* ıt confused by morphological ɛtween species or geographic or by host- and temperature-phological differences within though the host races of *D.* ned in this study generally ɔm different geographic loca-'erent food sources, Southern s utilized herein revealed no xcept for the giant race. The ɪns of the giant race from En-ance generated identical pat-ɡments with three enzymes Hinf I, Hpa 2). Similarly, U.S. olates of the alfalfa race con-'ed the same ITS restriction ddition, because PCR ampli-ɛen used successfully on pre-sms (25), taxonomic features ured by fixative techniques le the molecular diagnosis of ɪseum specimens, although s is destructive.

f the ribosomal cistron by

Southern hybridizations and restriction analysis of fragments amplified by PCR indicates that *Ditylenchus* species and the host races of *D. dipsaci* can be identified using DNA techniques. The use of the ribosomal cistron provides a powerful tool for studying the systematics of unknown species, as well as for the identification of known species in a mixed population of host races in the nematode genus *Ditylenchus*.

Literature Cited

1. Barabashova, V. N. 1984. Polyploidy in stem nematodes of the *Ditylenchus dipsaci* complex (Nematoda Tylenchida). Vestnik Khar'kovskago Universiteta No 262, 76–78 (Abstracted in Helminthological Abstracts Series B 55:56).
2. Barraclough, R., and R. E. Blackith. 1962. Morphometric relationships in the genus *Ditylenchus*. Nematologica 8:51–58.
3. Brzeski, M. W. 1991. Review of the genus Ditylenchus Filipjev, 1936 (Nematoda: Anguinidae). Revue de Nématologie 14:9–59.
4. Curran, J. 1991. Application of DNA analysis to nematode taxonomy. Pp. 125–143 *in* W. R. Nickle, ed. Manual of agricultural nematology. New York: Marcel Dekker.
5. Dickson, D. W., D. Huisingh, and J. N. Sasser. 1971. Dehydrogenases, acid and alkaline phosphatases, and esterases for chemotaxonomy of selected *Meloidogyne, Ditylenchus, Heterodera* and *Aphelenchus* spp. Journal of Nematology 3:1–16.
6. Eriksson, K. B. 1974. Intraspecific variation in *Ditylenchus dipsaci* I. Compatibility tests with races. Nematologica 20:147–162.
7. Eriksson, K. B., and J. Granberg. 1969. Studies of *Ditylenchus dipsaci* races using electrophoresis in acrylamide gel. Nematologica 15:530–534.
8. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Analytical Biochemistry 132:6–13.
9. Ferris, V. R., J. M. Ferris, and J. Faghihi. 1993. Variation in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. Fundamental and Applied Nematology 16:177–184.
10. Fortuner, R. 1982. On the genus *Ditylenchus* Filipjev, 1936 (Nematoda: Tylenchida). Revue de Nématologie 5:17–38.
11. Gibbons, L. N., and G. S. Grandison. 1968. An assessment of serological procedures for the differentiation of biological races of *Ditylenchus dipsaci*. Nematologica 14:184–188.
12. Hillis, D. M., and M. T. Dixon. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. Quarterly Review of Biology 66:411–453.
13. Hooper, D. J. 1983. Observations on stem nematode, *Ditylenchus dipsaci,* attacking field beans, *Vicia faba*. Rothamsted Report, Part 2:239–260.
14. Hussey, R. S., and L. R. Krusberg. 1971. Disc-electrophoretic patterns of enzymes and soluble proteins of *Ditylenchus dipsaci* and *D. triformis*. Journal of Nematology 3:79–84.
15. Ladygina, N. M. 1988. Biological races; Caryotypes and hybridization of *Ditylenchus*. Pp. 101–126 *in* V. G. Gubina, ed. Nematodes of plants and soils: Genus *Ditylenchus*. Karachi, Pakistan: Saad Publications.
16. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
17. Mullis, K. B., and F. A. Faloona. 1987. Specific synthesis of DNA *in vitro* via a polymerase catalyzed chain reaction. Methods in Enzymology 155:335–350.
18. Palmer, H. M., H. J. Atkinson, and R. N. Perry. 1991. The use of DNA probes to identify *Ditylenchus dipsaci*. Revue de Nématologie 14:625–628.
19. Palmer, H. M., H. J. Atkinson, and R. N. Perry. 1992. Monoclonal antibodies (MAbs) specific to surface expressed antigens of *Ditylenchus dipsaci*. Fundamental and Applied Nematology 15:511–515.
20. Seinhorst, J. W. 1957. Some aspects of the biology and ecology of stem eelworms. Nematologica 11 (Supplement):355–361.
21. Sturhan, D. 1964. Interbreeding experiments with biological races of the stem eelworm (*Ditylenchus dipsaci*). Nematologica 10:328–334.
22. Sturhan, D. 1971. Biological races. Pp. 51–71 *in* B. M. Zuckerman, W. F. Mai and R. A. Rohde, eds. Plant parasitic nematodes, vol. 2. New York: Academic Press.
23. Sturhan, D. 1983. The use of the subspecies and the superspecies categories in nematode taxonomy. Pp. 42–53 *in* A. R. Stone, H. M. Platt, and L. F. Khalil, eds. Concepts in nematode systematics. London: Academic Press.
24. Sturhan, D., and M. W. Brzeski. 1991. Stem and bulb nematodes, *Ditylenchus* spp. Pp. 423–465 *in* W. R. Nickle, ed. Manual of agricultural nematology. New York: Marcel Dekker.
25. Thomas, W. K., S. Paabo, F. X. Villablanca, and A. C. Wilson. 1990. Spatial and temporal continuity of kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens. Journal of Molecular Evolution 31:101–112.
26. Triantaphyllou, A. C. 1984. Chromosomes in evolution of nematodes. Pp. 77–101 *in* A. K. Sharma and A. Sharma, eds. Chromosomes in evolution of eukaryotic groups, vol. 2. Boca Raton, FL: CRC Press.
27. Vrain, T. C., D. A. Wakarchuk, C. A. Lévesque, and R. I. Hamilton. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15:563–573.
28. Webster, J. M., and D. J. Hooper. 1968. Serological and morphological studies on the inter- and intraspecific differences of the plant-parasitic nematodes *Heterodera* and *Ditylenchus*. Parasitology 58:879–891.
29. Webster, J. M., R. V. Anderson, D. L. Baillie, K. Beckenbach, J. Curran, and T. Rutherford. 1990. DNA probes for differentiating isolates of the pinewood nematode species complex. Revue de Nématologie 13:255–263.