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A Microsatellite Marker for Studying the Ecology and Diversity of Fungal Endophytes (*Epichloë* spp.) in Grasses

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Randomly amplified polymorphic DNA fingerprinting, which is based on PCR with arbitrary 10-nucleotide primers, were used to analyze genetic diversity among isolates of the endophytic ascomycete *Epichloë typhina*, which were collected at a single field site from a population of one of its hosts, the grass *Bromus erectus*. One of the polymorphic randomly amplified polymorphic DNA PCR products occurred in all isolates as single bands with different but closely related sizes. Two of the size variants of this product were cloned and sequenced, and they were found to represent the same DNA sequence, except for a stretch of tandem repeats of the trinucleotide AAG · TTC, which differed in size, consisting of 8 and 18 repeats, respectively. Tandem repeats of this type are called microsatellites. Oligonucleotides were synthesized corresponding to portions of the sequence flanking the microsatellite and were used for PCR amplification of the loci from the genomic DNAs of different *Epichloë* isolates. A single PCR product was found for most isolates, indicating that the sequence represented a single genetic locus. Five alleles that could clearly be distinguished in size were found in a population of 91 field isolates. PCR with $(AAC)_8$ and $(AAG)_8$ as primers yielded a number of amplified bands from genomic DNA of *Epichloë* isolates, indicating that these types of microsatellites occur frequently in the genome of this fungus. A survey of all fungal DNA sequences currently deposited in the DNA sequence databases of EMBL and GenBank revealed that microsatellites of different repeating units are widespread in fungi. Our findings indicate that microsatellite-containing loci may be used as molecular markers for population studies of *Epichloë* species and many other unrelated fungi.

Microsatellites, which are also known as short tandem repeats or simple sequence length polymorphisms, are stretches of tandem mono-, di-, tri-, and tetranucleotide repeats of varying lengths (1, 21). Such sequences are widely dispersed in eukaryotic genomes (1, 7, 21), including those of fungi (15); they are also present but less frequent in prokaryotic genomes. Oligonucleotides corresponding to microsatellites have widely been used as fingerprinting probes to identify RFLP polymorphisms among DNA samples from different individuals (1, 15), as illustrated by the recent use of labeled $(CAT)_5$ to fingerprint restricted DNAs of various fungi (4). The loci containing microsatellites themselves are also ideal markers for studies of gene flow and genetic variation because they are often highly polymorphic, containing a wide range of repeats, and because they usually undergo Mendelian inheritance. When amplified by PCR with specific flanking primers, different alleles of a microsatellite-containing locus can readily be identified by their different sizes. Applications for microsatellite markers include genome mapping, paternity and kinship analyses, clone or strain identification, and population genetic studies including estimations of population size and assignment of inbreeding coefficients. Compared with randomly amplified polymorphic DNA (RAPD) PCR, restriction fragment length polymorphism, and isozyme markers, microsatellite markers can be analyzed with considerably less material, even if it is old or partially degraded. For studies of plant-associated fungi, microsatellite markers have the added advantage that the analysis can be performed directly in planta if the flanking primers are sufficiently specific.

Microsatellite loci have been used extensively to characterize mammalian genomes (1, 20, 21) and have received growing attention as markers for plant genetics (13, 24). To our knowledge, microsatellite loci have thus far been used in only three cases for studies of fungi, and in all of these cases they were used for fungal strain identification (11, 12, 18).

Our long-term goal is to use molecular markers to study the biodiversity and population genetics of the grass endophyte *Epichloë typhina* (*Clavicipitaceae*, *Ascomycotina*) at several field sites in the Jura mountains of Switzerland, where it frequently colonizes *Bromus erectus*, the dominant grass species. The population genetics of *Epichloë* species has recently received much attention (3, 19). These fungi grow asymptomatically and act as mutualistic symbionts during the vegetative phase of their grass hosts, producing alkaloids that protect them from herbivores; however, they later sterilize the hosts by producing reproductive stromata in place of the developing inflorescences (3, 19). For population biologists, such pathogens, which are termed "castrators" (2), are highly interesting in an evolutionary dimension since they fix the genotype of the host and thus may increase their medium-term chances of persistence but at the same time compromise the genetic diversity of the host and thus their chances of survival in the long term. A group of closely related fungi, represented by the genus *Acremonium*, have evolved into a completely nonpathogenic lifestyle (3, 19). They grow asymptomatically as mutualistic symbionts throughout their life in their host grass; they do not affect its sexual reproduction but grow into its inflorescences and thus are clonally transmitted through seeds to the next host generation (3). In this case, the fungi increase their medium-term chances of persistence through perfect adaptation to the life cycle of their host, but they may compromise their own genetic diversity and thereby their chances of survival in the long term. Interestingly, a molecular analysis of tubulin genes in *Acremonium* strains recently revealed that

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these fungi may not be completely asexual but may diversify by sexual hybridization with *Epichloë* species (17, 22).

Here, we describe the characterization of a polymorphic microsatellite-containing locus in *E. typhina* which was initially identified by RAPD PCR, the development of a specific PCR test to characterize this locus, and the analysis of this locus in a natural field population of fungi.

Moreover, we report the identification of additional potential microsatellites in *Epichloë* species by PCR with trinucleotide repeat motifs of 24 bases as primers. This method, which has recently been used to study diverse genomes of plants and animals (6), can be used profitably with genomic DNA from fungi. It provides a quick test for establishing the likelihood of finding useful microsatellite markers in a particular organism before embarking on library screens, and it allows one to choose the best probes for screening genomic libraries.

We have also screened the fungal sequences deposited in the GenBank and EMBL DNA sequence databases for the occurrence of mono-, di-, and trinucleotide repeat motifs, and we report the relative abundance of different microsatellite repeats in fungi.

MATERIALS AND METHODS

Biological materials. All biological materials were collected from Nenzlinger Weide, a species-rich limestone grassland in the Swiss Jura mountains. Stromata of *E. typhina* were collected separately from 66 infected *B. erectus* plants at the field site. Stromata were peeled off the plant tillers with a sterilized razor blade. An additional 25 *Epichloë* strains were isolated from vegetative samples of *B. erectus* collected at the field site, according to published procedures (9). All fungal strains were maintained on potato dextrose agar and were grown in liquid culture (10). Fungal material was freeze-dried prior to DNA extraction.

DNA extraction. Fungal DNA was prepared according to the method described by Zolan and Pukkila (28). DNA was measured by Hoechst dye staining and then by fluorography with the TKO 100 fluorometer (Hoeffer Scientific Instruments, San Francisco, Calif.).

Identification of DNA polymorphisms by PCR. Strain-specific DNA fingerprints of the genomes of different *Epichloë* strains were generated by RAPD PCR with RAPD primer T-14 (AATGCCGCAG, Operon Technologies Inc., Alameda, Calif.) according to the method described by Williams et al. (26). A specific fingerprint of the fungal DNA was visualized following electrophoresis in 1.5% (wt/vol) agarose, in TAE buffer (0.04 M Tris acetate, 1.0 mM EDTA), and staining with ethidium bromide. A subset of the fungal strains were tested by PCR with $(AAC)_8$ and $(AAG)_8$ repeat primers. In this case, the annealing temperature was 45°C.

Cloning and sequencing of alleles of a microsatellite-containing locus. Putative alleles 62A and 62B generated by RAPD PCR were isolated after preparative electrophoresis in 1.5% (wt/vol) SeaKem GTG agarose (FMC Bioproducts, Rockland, Maine)–TAE using the Geneclean II kit (Bio 101 Inc., La Jolla, Calif.). The isolated bands were inserted into the pCR II cloning vector (Invitrogen, San Diego, Calif.) by TA cloning and were transformed into *Escherichia coli* bacteria. Recombinant plasmid DNA was isolated by the alkaline lysis method (16). Single-stranded DNA was prepared and sequenced by using the Sequenase version 1.0 DNA sequencing kit (U.S. Biochemical, Cleveland, Ohio).

Nucleotide sequence accession numbers. The sequences of the *Epichloë* microsatellite loci and flanking DNA have been deposited in the EMBL database (accession number X91791).

PCR primers. PCR primers were designed to complement the DNA sequence flanking the microsatellite region of alleles 62A and 62B according to the criteria described by Innis and Gelfand (8). The sequences of the forward and reverse primers are 5′-CGC ACA ATA CGT CAG CTA GGA ATG-3′ and 5′-CCT GAA TCA ACT TTG CTA TCA GGC-3′, respectively.

Detection of a sequence containing a fungal microsatellite. Specific primers were used to amplify the microsatellite-containing loci from *Epichloë* stromata and from each fungal isolate grown in culture. Amplification reactions were performed in volumes of 25 μl containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 0.1% (wt/vol) Triton X-100, 200 μM each dATP, dCTP, dGTP, and dTTP (Pharmacia, Uppsala, Sweden), 0.2 μM each primer, 50 ng of genomic DNA, and 0.25 U of Super *Taq* DNA polymerase (Stehelin & Cie, Basel, Switzerland). Amplification was performed in a DNA Thermal Cycler (microtiter plate format; MJ Research, Inc., Watertown, Mass.) programmed for 30 cycles consisting of 1 min at 94°C, 2 min at 65°C, and 3 min at 72°C and then a final extension for 10 min 72°C. The PCR products were fractionated in 2.0% (wt/vol) agarose, in 0.5× TBE buffer (0.045 M Tris-borate, 0.001 M EDTA), or in 3% (wt/vol) Metaphor (FMC Bioproducts) agarose in TAE buffer, and were detected by staining with ethidium bromide.

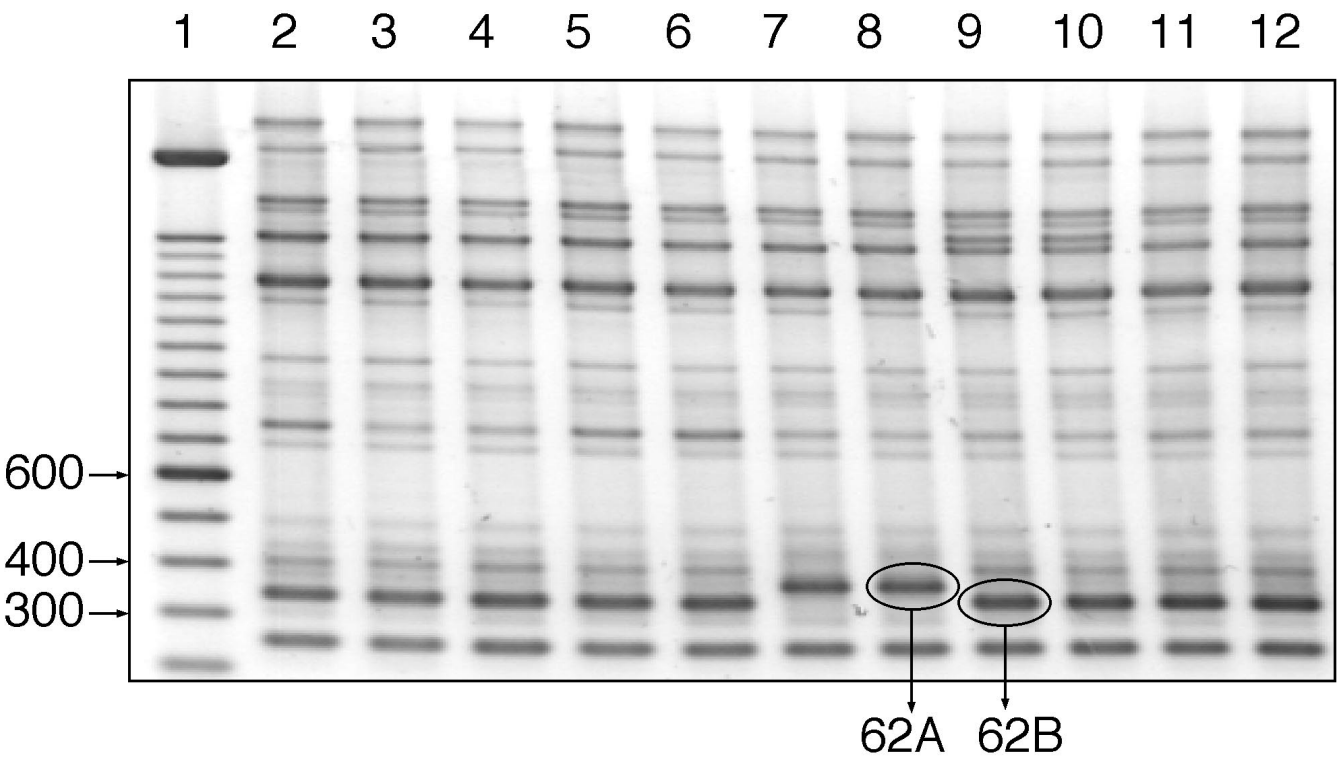

FIG. 1. Agarose gel electrophoresis of *Epichloë* RAPD bands produced by primer T-14. Lanes: 1, molecular size markers (100-bp ladder with the 300-, 400-, and 600-bp markers indicated in the left margin); 2 to 12, amplification products from 11 of 91 different *Epichloë* isolates from a natural field population. The bands designated 62A and 62B are putative alleles of the same genetic locus.

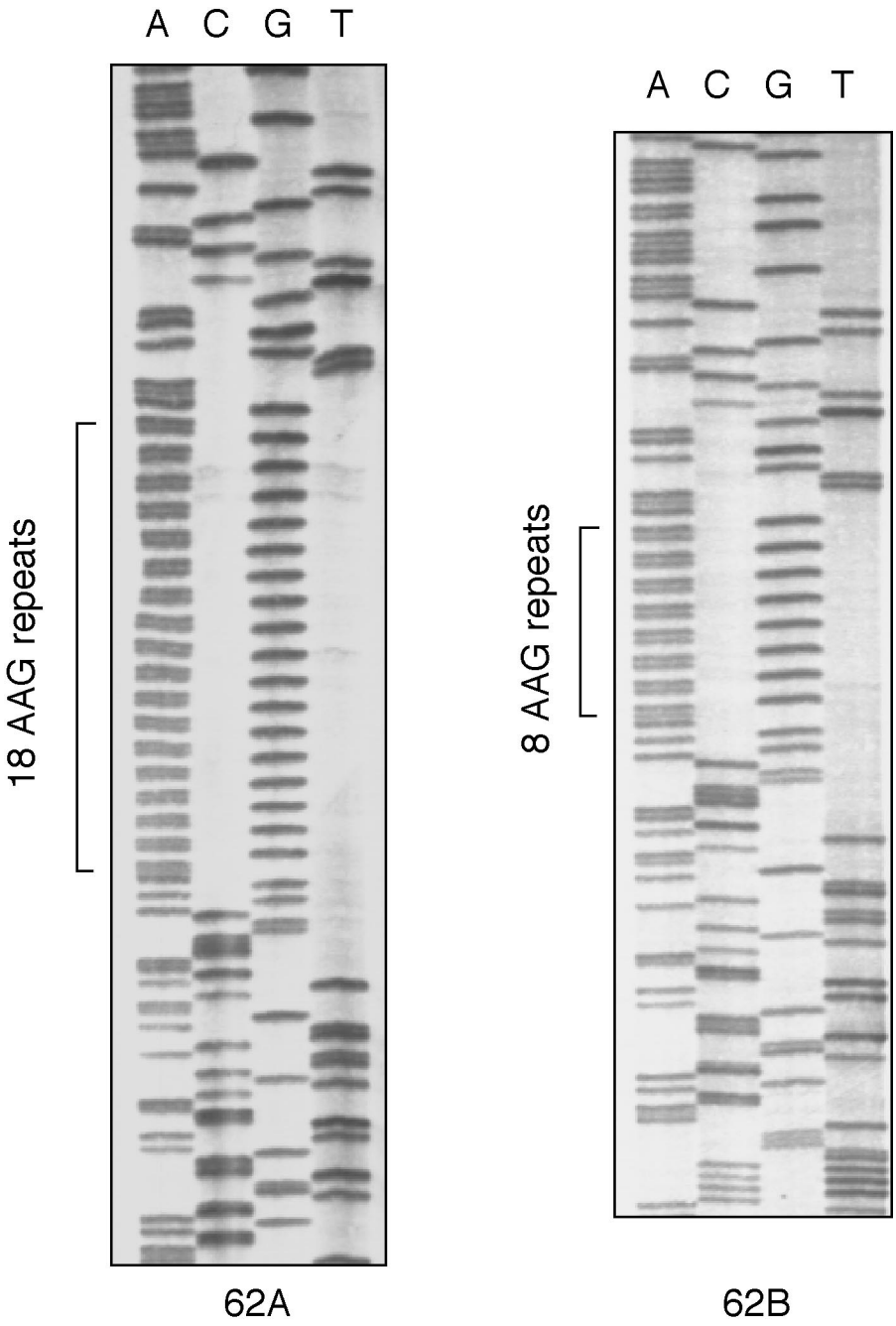

FIG. 2. Sections of sequencing gels of recombinant clones representing bands 62A and 62B (highlighted in Fig. 1). The polymorphic portions of the clones containing either 8 or 18 AAG repeats (microsatellites) are marked. The DNA sequences immediately flanking the microsatellite are identical. The sequence parts outside the sections shown are also identical.

Sequence searches. The programs BLAST and FASTA of the University of Wisconsin Genetics Computer Group (5) were used to search all fungal sequences deposited in the GenBank and EMBL DNA sequence databases. The *Epichloë* microsatellite-containing allele was included in the BLAST search. All possible mono-, di-, and trinucleotide repeat combinations were included in the FASTA searches. Permutated and complementary repeats were searched and recorded simultaneously. Complementary stretches of 20 bp or more were recorded for the FASTA search (25).

RESULTS

Identification of DNA polymorphisms among isolates of *E. typhina* by RAPD PCR. DNA fingerprints of *Epichloë* strains isolated from infected *B. erectus* were generated by RAPD PCR by using Operon primer T-14. All DNA samples gave reproducible fingerprint patterns of 14 to 18 bands (Fig. 1). The banding patterns were consistent in duplicates within a single run and in subsequent runs. The majority of the RAPD bands were the same size in the various fungal isolates (as expected for individuals of the same species). Some individual-specific polymorphisms were observed. Two polymorphic bands were closely related in size and appeared to segregate in the different fungal isolates tested (Fig. 1). These bands were, therefore, thought to be alleles of a single genetic locus.

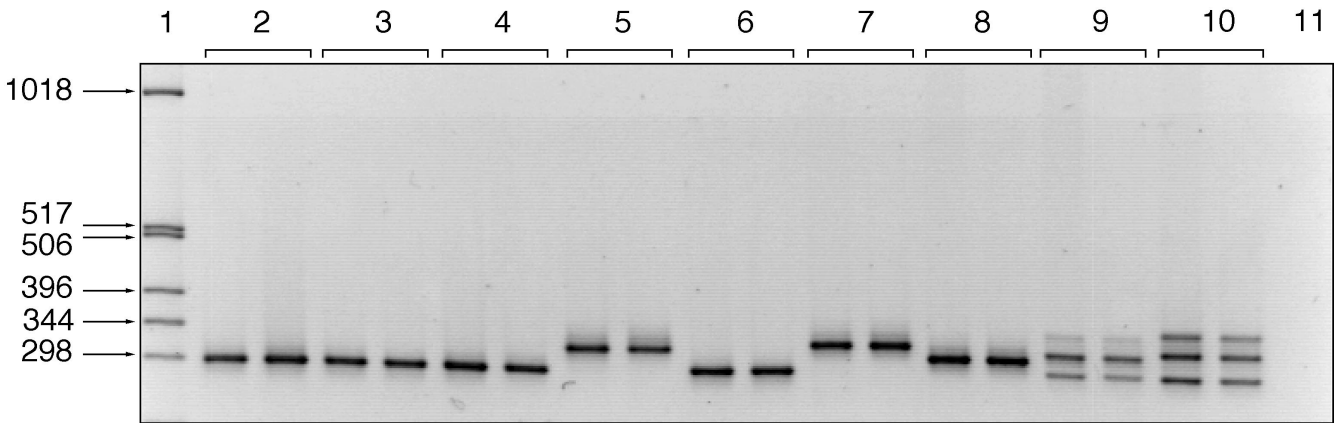

FIG. 3. Amplification of *Epichloë* genomic DNA with specific primers. Lanes: 1, molecular size markers (sizes of bands in base pairs, indicated in the left margin); 2 to 10, duplicate amplification products resulting from PCR of nine *Epichloë* isolates; 11, a control amplification without DNA.

Cloning and sequencing of two microsatellite-containing alleles and design of specific primers. Two of the polymorphic bands (named 62A and 62B) identified by RAPD PCR (Fig. 1) were purified and cloned. Sequencing of multiple recombinant clones from each band revealed that the bands were alleles and that the alleles were identical except for a stretch of tandem trinucleotide repeats (AAG · TTC). The 62A allele is 370 bp in length and contains a stretch of 18 AAG · TTC repeats, whereas the 62B allele is 340 bp in length and contains a stretch of 8 AAG · TTC repeats (Fig. 2). The difference in the number of repeats accounts for the different sizes observed on the RAPD PCR gels. Homology searches in the EMBL and GenBank DNA sequence databases revealed no significant matches for the sequences flanking the microsatellite. We do not know at present whether all or part of this sequence is expressed. Sequence data from the microsatellite-containing alleles were used to design specific PCR primers complementary to regions flanking the microsatellite loci.

Screening of *B. erectus* endophytes by PCR. By using the allele-specific PCR primers, the microsatellite-containing locus was invariably amplified with DNA isolates prepared from 66 individual *Epichloë* stromata taken at the field site from *B. erectus* and from 25 additional endophyte strains isolated from *B. erectus* leaves and grown in culture. Amplification was reproducible with the same allele size resulting from subsequent amplifications from the same strain. The allele sizes correlated with band sizes observed after RAPD PCR (Operon primer T-14) with the same strains. At least five unique PCR product size classes were identified by standard agarose gel electrophoresis (Fig. 3). The large allele corresponding to clone 62A was present in 25.3% of the *Epichloë* population, three medium-size alleles were present in 6% of the population, and the small allele corresponding to clone 62B was present in 65.9% of the population. Two samples tested (2.2% of the population) contained three allele sizes (Fig. 3, lanes 9 and 10). Control PCRs without DNA (Fig. 3) or with DNA samples from unrelated fungi or from *B. erectus* (not shown) did not yield any amplification products.

TABLE 1. Abundance of microsatellites with sizes of 20 bp or more in fungal genomic sequences currently deposited in the GenBank and EMBL databases

Repeated nucleotide(s) (permutation[s]; complementary sequence[s])	No. of microsatellites
AT (TA)	263
A (T)	160
AAT (ATA, TAA; TTA, TAT, ATT)	50
AAC (ACA, CAA; TTG, TGT, GTT)	22
AAG (AGA, GAA; TTC, TCT, CTT)	21
ATC (TCA, CAT; TAG, AGT, GTA)	19
AC (CA; TG, GT)	17
AG (GA; TC, CT)	15
AGC (GCA, CAG; TCG, CGT, GTC)	14
CCA (CAC, ACC; GGT, GTG, TGG)	11
C (G)	10
CCT (CTC; TCC; GGA, GAG, AGG)	9
ACG (CGA, GAC; TGC, GCT, CTG)	8
ACT (CTA, TAC; TGA, GAT, ATG)	5
CCG (CGC, GCC; GGC, GCG, CGG)	2
CG (GC)	0
Total	626

Screening of genomic *Epichloë* DNA by PCR with trinucleotide repeats as primers. PCR amplifications were performed using oligonucleotides corresponding to the most frequent microsatellite sequences found in fungal database searches as primers. $(AAG)_8$ and $(AAC)_8$ primers produced multiple bands in PCRs of different *Epichloë* isolates, indicating that these sequences are prevalent in the endophyte genome. Reproducible, isolate-specific bands ranging from 300 to 1,800 bp in length were observed with both primers. Between 10 and 12 bands were distinguishable with the $(AAC)_8$ primer (Fig. 4, upper panel). Between 8 and 10 bands were distinguishable with the $(AAG)_8$ primer (Fig. 4, lower panel). PCR using $(AT)_{12}$ and $(AAT)_8$ yielded smears rather than a banding pattern. The consistency of banding patterns seen with the different fungal isolates indicates that these sequences are consistently represented in the *Epichloë* population. It is probable that many of these sequences have variable lengths at a given locus, as shown in detail for the locus represented by alleles 62A and 62B (Fig. 1 to 3).

Sequence searches. Homology searches of fungal sequences in the GenBank and EMBL DNA sequence databases were made with all possible mono-, di-, and trinucleotide motifs, as was described for plants by Wang et al. (24). Poly(A) repeats corresponding to mRNA sequences in the database were removed from the analysis. A total of 626 microsatellite alleles (Table 1) were found in sequences of 31 fungal species (Table 2). These included 501 microsatellite alleles found in *Saccharomyces cerevisiae*. The most abundant repeats in fungi were

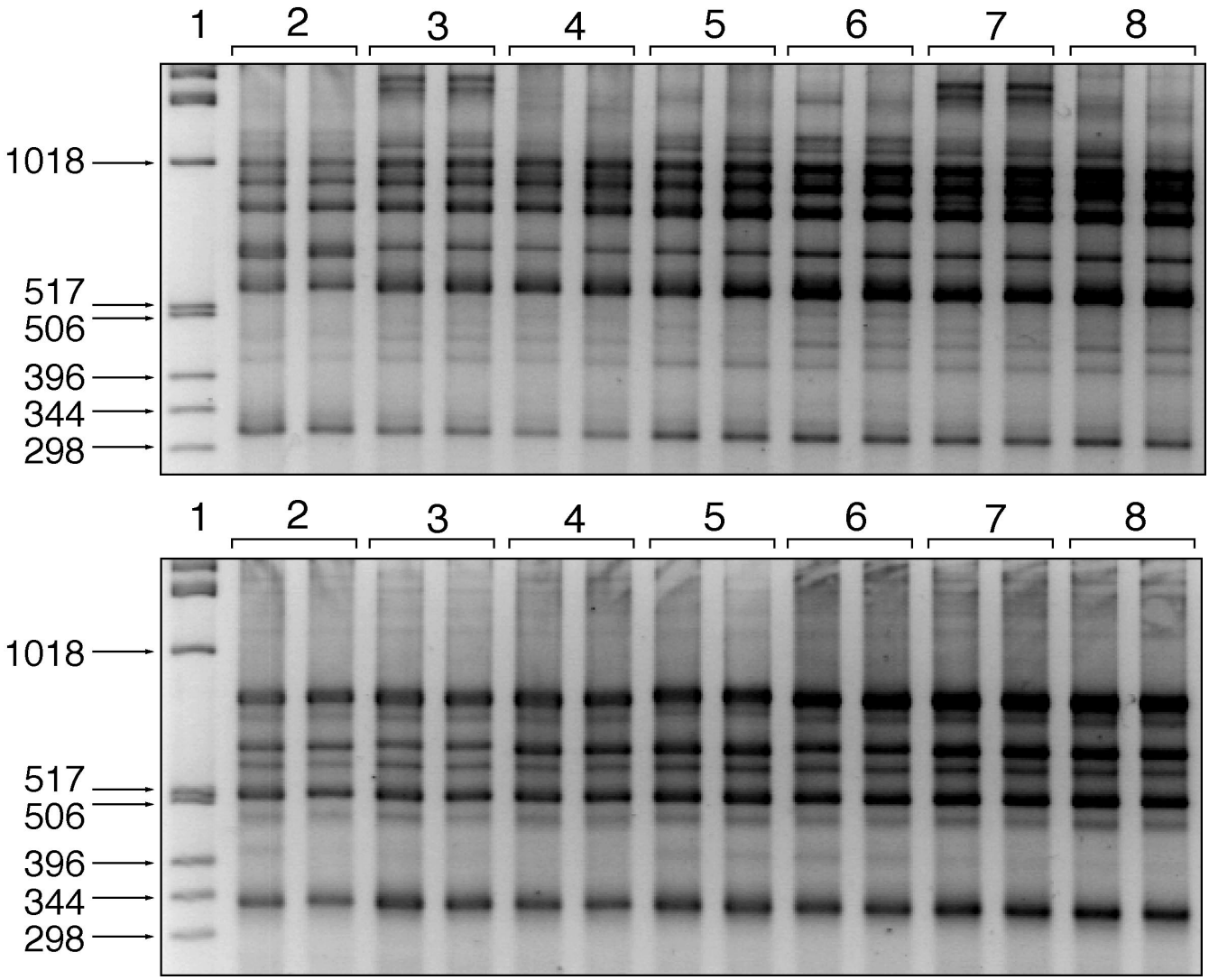

FIG. 4. Agarose gel electrophoresis of PCR bands produced on amplification of *Epichloë* DNA with 24-mer trinucleotide repeats as primers. (Upper panel) AAC_8 primer. (Lower panel) AAG_8 primer. Lanes: 1, molecular size markers (sizes of bands in base pairs, indicated in the left margin); 2 to 8, duplicate amplification products resulting from PCR of seven *Epichloë* isolates.

$(AT)_n$ followed by $(A)_n$, $(AAT)_n$, $(AAC)_n$, $(AAG)_n$, $(ATC)_n$, $(AC)_n$, $(AG)_n$, $(AGC)_n$, $(CCA)_n$, $(C)_n$, $(CCT)_n$, $(ACG)_n$, $(ACT)_n$, and $(CCG)_n$. No $(CG)_n$ repeats were found.

DISCUSSION

We describe a new method for identification of microsatellite-containing loci within genomic DNA. This method involves sequence analysis of segregating, polymorphic RAPD bands which are closely related in size. In our system, two such bands were sequenced and were shown to contain trinucleotide repeats of varying lengths. Repeat length differences corresponded precisely to size differences of polymorphic bands seen by RAPD fingerprinting.

Specific primers flanking the microsatellite-containing loci allow PCR amplification of single polymorphic bands from genomic DNA (14). Multiple alleles were observed following PCR amplification of genomic DNAs from 91 isolates from a single population of *Epichloë typhina* on *B. erectus*. Two of the fungal isolates tested carried multiple allele sizes. Stromatal DNA was used in one of these cases; thus, the multiple bands may have resulted from fertilization events between fungal genotypes differing in the size of their alleles. In the other case, DNA came from a culture of haploid fungal material grown out from the host plant. A third example of multiple alleles was observed when PCR was performed directly on infected plant material containing haploid fungal material (unpublished data). The amplification of multiple bands from haploid fungal material could be the result of multiple endophyte infections within a single host or of previous hybridization of multiple fungal strains within one host. This phenomenon has previously been observed between different endophyte species in *Festuca arundinacea* (17, 22) and is thought to be a driving force in the evolution of the taxon.

We used repeat motifs of 24 bases as primers to screen DNA samples by PCR for the presence of microsatellites. This method, which has been recently used for fingerprinting of diverse genomes from plants and vertebrates (6), differs from the random amplified microsatellite polymorphism PCR method (27), which uses repeat primers with 5′ and 3′ anchors

TABLE 2. Abundance of microsatellite-containing loci in different fungal sequences deposited in the EMBL or GenBank databases (listed and numbered in alphabetical order)

Species	No. of loci
Acremonium chrysogenum	2
Aspergillus nidulans	3
Aspergillus niger	3
Blastocladiella emersonii	2
Candida albicans	6
Candida boidinii	4
Candida maltosa	5
Candida tropicalis	6
Emericella nidulans	3
Erysiphe graminis	1
Geotrichum candidum	4
Histoplasma capsulatum	11
Kluyveromyces lactis	7
Kluyveromyces marxianus	3
Mucor racemosus	1
Neocallimastix frontalis	1
Neurospora crassa	12
Pachysolen tannophilus	1
Phycomyces blakesleeanus	4
Physarum polycephalum	10
Podospora anserina	3
Rhizopus niveus	6
Rhizopus oryzae	1
Saccharomyces cerevisiae	501
Saccharomyces douglasii	3
Saccharomyces exiguus	1
Schizosaccharomyces pombe	14
Trichoderma harzianum	3
Ustilago maydis	2
Yarrowia lipolytica	2
Zygosaccharomyces rouxii	1
Total	626

in combination with RAPD primers for identification of repeat loci, and from methods for the detection of specific microsatellites with unique primers. The primers and annealing conditions were chosen to ensure that only microsatellites of lengths sufficient to be polymorphic in any given population would be identified (25). This method scans the genome for repeat sequences which are within 2,000 bases and in opposite orientation of each other. Banding patterns resulting from PCR of *Epichloë* DNA using $(AAG)_8$ and $(AAC)_8$ as primers showed that these repeat motifs are present in the *Epichloë* genome on multiple occasions. This suggests that it should be possible to find other microsatellite regions for use in studies of this species.

Homology searches of fungal sequences deposited in DNA sequence databases revealed that microsatellite loci are widespread in fungi and that they are abundant enough for use as markers for fungal genetics. A total of 626 repeat motifs of 20 bp or longer were found in sequences from 31 fungal species. The large number (501) of repeats found in *S. cerevisiae* most likely reflects the active effort to sequence the yeast genome rather than a bias toward repeat formation in that species. Even when these sequences were removed from the analysis, 125 repeats from 30 fungal species remained. This number is comparable to the result of 130 microsatellite sequences found in 13 plant species and is deemed abundant enough to encourage use of these markers for plant genetics (24).

Repeat frequencies in plants and fungi are similar. The most abundant repeats found in plant (13, 24) and fungal (Table 1) sequences were $(AT)_n$ and then $(A)_n$. Intermediate abundances in both plants and fungi were found for $(AAT)_n$, $(AAC)_n$, $(AAG)_n$, $(AGC)_n$, $(AC)_n$, and $(AG)_n$ repeats. No $(CG)_n$ and only two $(CCG)_n$ motifs were found in fungi and neither were found in plants. Valle (23) described the high frequency of $(AT)_n$ repeats, as opposed to other dinucleotide repeats, on yeast chromosome III. Relative repeat frequencies found in mammalian database searches were considerably different from those seen with plants and fungi, with $(AC)_n$ being by far the most common dinucleotide repeat, followed by $(AG)_n$ and $(AT)_n$ (6). Again, $(CG)_n$ was shown to be very rare. $(CAG)_n$ was the most common trinucleotide repeat, followed by $(AAT)_n$ and $(TCC)_n$ (20). The scarcity of $(CG)_n$ and $(CCG)_n$ repeat motifs may reflect the fact that GC dinucleotides are unstable because they are susceptible to methylation and subsequent deamination to form TG (28).

The frequency of microsatellite loci in fungal genomes should encourage their use as markers for studies of fungi. Data from databank homology searches and/or repeat PCR should provide directions for designing probes to isolate clones from genomic libraries. Alternatively, microsatellite loci may be developed as described in this paper. Segregating polymorphic bands may be identified by RAPD PCR, cloned, and sequenced, and specific, microsatellite-flanking primers may be developed.

The occurrence of at least five different alleles at the microsatellite-containing locus described here in a single population of *E. typhina* demonstrates that this fungus is genetically diverse in nature. Using PCR with the allele-specific primers, we have also detected amplification products with different lengths in a wide variety of *Epichloë* isolates from various host plants from all over the world (unpublished data). Thus, the microsatellite marker will be useful for estimations of *Epichloë* diversity on local, regional, and global scales. Application of this and other microsatellite markers will allow us to estimate gene flow within and between *Epichloë* populations.

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REFERENCES

1. **Bruford, M. W., and R. K. Wayne.** 1993. Microsatellites and their application to population genetic studies. Curr. Opin. Genet. Dev. **3:**939–943.
2. **Burdon, J. J.** 1993. The structure of pathogen populations in natural plant communities. Annu. Rev. Ecol. Syst. **31:**305–323.
3. **Clay, K.** 1990. Fungal endophytes of grasses. Annu. Rev. Ecol. Syst. **21:**275–297.
4. **DeScenzo, R. A., and T. C. Harrington.** 1994. Use of $(CAT)_5$ as a DNA fingerprinting probe for fungi. Phytopathology **84:**534–540.
5. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
6. **Gupta, M., Y.-S. Chyi, J. Romero-Severson, and J. L. Owen.** 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theor. Appl. Genet. **89:**998–1006.
7. **Hamada, H.-J., M. G. Petrino, and T. Kakanuga.** 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc. Natl. Acad. Sci. USA **79:**6465–6469.
8. **Innis, M. A., and D. H. Gelfand.** 1990. Optimization of PCRs, p. 315–322. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, New York.
9. **Latch, G. C. M., and M. J. Christensen.** 1985. Artificial infection of grasses with endophytes. Ann. Appl. Biol. **107:**17–24.
10. **Leuchtmann, A.** 1994. Isozyme relationships of *Acremonium* endophytes from twelve *Festuca* species. Mycol. Res. **98:**25–33.
11. **Lieckfeldt, F., W. Meyer, and T. Boerner.** 1993. Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. J. Basic Microbiol. **33:**413–426.
12. **Meyer, W., T. G. Mitchell, F. Z. Fredman, and R. Vilgalys.** 1993. Hybridiza-

tion probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. J. Clin. Microbiol. **31:**2274–2280.
13. **Morgante, M., and A. M. Olivieri.** 1993. PCR-amplified microsatellites as markers in plant genetics. Plant J. **3:**175–182.
14. **Paran, I., and R. W. Michelmore.** 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. **88:**985–993.
15. **Rosewich, U. T., and B. A. McDonald.** 1994. DNA fingerprinting in fungi. Methods Mol. Cell Biol. **5:**41–48.
16. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
17. **Schardl, C. L., A. Leuchtmann, H.-F. Tsai, M. A. Collett, D. M. Watt, and D. B. Scott.** 1994. Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen, *Epichloë typhina*. Genetics **136:**1307–1317.
18. **Schoenian, G., O. Meusel, H. J. Tietz, W. Meyer, Y. Graeser, I. Tausch, W. Presber, and T. G. Mitchell.** 1993. Identification of clinical strains of *Candida albicans* by DNA fingerprinting with the polymerase chain reaction. Mycoses **36:**171–179.
19. **Siegel, M. R., G. C. M. Latch, and M. C. Johnson.** 1987. Fungal endophytes of grasses. Annu. Rev. Phytopathol. **25:**293–315.
20. **Stallings, R. L.** 1994. Distribution of trinucleotide microsatellites in different categories of mammalian genomic sequence: implications for human genetic disease. Genomics **21:**116–121.
21. **Tautz, D.** 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. **17:**6463–6471.
22. **Tsai, H.-F., J.-S. Liu, C. Staben, M. J. Christensen, G. C. M. Latch, M. R. Siegel, and C. L. Schardl.** 1994. Evolutionary diversification of fungal endophytes of tall fescue grass by hybridization with *Epichloë* species. Proc. Natl. Acad. Sci. USA **91:**2542–2546.
23. **Valle, G.** 1993. TA-repeat microsatellites are closely associated with ARS consensus sequences in yeast chromosome III. Yeast **9:**753–759.
24. **Wang, Z., J. L. Weber, G. Zhong, and S. D. Tanksley.** 1994. Survey of plant short tandem DNA repeats. Theor. Appl. Genet. **88:**1–6.
25. **Weber, J. L.** 1990. Informativeness of human $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ polymorphisms. Genomics **7:**524–530.
26. **Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:**6531–6535.
27. **Wu, K.-S., R. Jones, L. Danneberger, and P. A. Scolnik.** 1994. Detection of microsatellite polymorphisms without cloning. Nucleic Acids Res. **22:**3257–3258.
28. **Zolan, M. E., and P. J. Pukkila.** 1986. Inheritance of DNA methylation in *Coprinus cinereus*. Mol. Cell. Biol. **6:**195–200.