

Use of (CAT)₅ as a DNA Fingerprinting Probe for Fungi

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We thank Paul Zambino for originally suggesting the (CAT)₅ sequence and Brad Hillman for the synthesis of the probe. David Rizzo provided most of the *Heterobasidion annosum* isolates used in this study and determined the isozyme and mating type alleles. Jim Worrall provided the remaining *H. annosum* isolates. Wendy Zimmerman provided the *Ophiostoma piliferum* isolates.

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ABSTRACT

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A new DNA fingerprinting probe, (CAT)₅, was evaluated for its ability to detect variation in higher fungi. The probe, which apparently detects variable number tandem repeat (VNTR) loci, was shown to be highly sensitive to genetic variation. The DNA of each of 30 single-basidiospore progeny of a dikaryon of *Heterobasidion annosum* exhibited a unique fingerprint after *Pst*I restriction, electrophoresis, and in-gel hybridization with the oligonucleotide. Twelve of 28 scored bands were heterozygous in the parent dikaryon. Six bands segregated in the expected 1:1 ratio, and the remaining six exhibited skewed segregation ratios. Two new bands were observed among the progeny but not the parent dikaryon, suggesting a high mutation rate (>0.3%), consistent with other reported VNTR loci. A few new bands were also observed among single-ascospore progeny

of a heterokaryotic isolate of *Ophiostoma piliferum*, a blue-stain fungus. The probe was useful in delineating genotypes (fingerprinting), in identifying infraspecific variants, and, potentially, in quantifying genetic variation. Each tested field isolate of *H. annosum* displayed a unique fingerprint, except for certain isolates from adjacent trees. Somatic incompatibility tests supported the conclusion that isolates with identical fingerprints were derived from clonal spread of the pathogen over many years through the root systems of adjacent trees. Several fingerprint bands were specific to either of the two host-specialized (on pine and fir) groups of *H. annosum* in North America. Likewise, several bands were unique to isolates of either of two host-specialized varieties (var. *wagneri* and var. *ponderosum*) of *Leptographium wagneri*, an asexual pathogen native to western North America on Pinaceae. More polymorphisms were seen with the (CAT)₅ probe than in 21 isozyme markers used in an earlier study.

Restriction fragment length polymorphisms (RFLPs) of DNA have been utilized in an increasing number of applications, including paternity studies, forensic investigations, and analysis of genetic diversity within and between populations. Using probes capable of hybridizing to multiple bands, Jeffreys et al (19) were the first to use the term "DNA fingerprint" to describe unique RFLP patterns of individuals. Many of the probes used to detect DNA fingerprints hybridized to variable number tandem repeat (VNTR) loci that are located in minisatellite DNA regions interspersed throughout the genome (17-20). Probes have been developed to detect fingerprints in a number of fungi (2,6,11,12,15,21-25,28-30,35,37,39,40). Typically, the utilized probes were obtained by isolating, cloning, and screening highly repetitive genomic DNA sequences from the species being studied.

A common characteristic of VNTR probes is the presence of a core sequence that is tandemly repeated numerous times throughout the length of the probe (19,20). On the basis of this information, Ali et al (1) synthesized a number of short oligonucleotide probes consisting of tandem repeats of a core sequence. These probes were effective in detecting RFLPs in humans and

have since been shown to detect RFLPs in a wide variety of organisms (5,27,31,33,36,38). Synthetic oligonucleotide probes have advantages over cloned genomic DNA probes in that they work with a wide variety of organisms, the core sequence and length can be easily changed, and they are relatively inexpensive to manufacture (1). In addition, less costly in-gel hybridization techniques can be utilized with the synthetic oligonucleotide probes (34). However, interpretation of the number of loci and assignment of alleles can be difficult for data obtained from multi-locus probes.

Although synthetic oligonucleotides have been used as probes in the study of genetic variation in phytopathogenic fungi (29,38), their potential has not been fully explored. We developed a new 15-bp oligonucleotide, (CAT)₅, as a DNA fingerprinting probe (9). This sequence was based on observations of tandem CAT repeats in repetitive DNA sequences in yeast (40). The purposes of this work were to examine the heritability of bands detected with (CAT)₅ and to test the utility of (CAT)₅ in detecting genetic diversity within and between fungal populations and closely related taxa.

MATERIALS AND METHODS

Isolates. All the isolates used are deposited in the culture collection of the junior author. Single-basidiospore progeny (homo-

karyotic strains) were obtained from an in vitro-produced basidiome of a dikaryotic *Heterobasidion annosum* (Fr.:Fr.) Bref. isolate by the techniques described by Worrall and Harrington (42). The New Hampshire population of *H. annosum*, which included isolate B832, was from a stand of predominantly red pine (*Pinus resinosa*) on the campus of the University of New Hampshire, Durham. The population of *H. annosum* from New York originated from plantations of Scots pine (*P. sylvestris*) and Norway spruce (*Picea abies*) at a site in Syracuse, New York. Although *H. annosum* is capable of clonal spread to adjacent trees, each isolate was tested by the technique of Worrall and Harrington (42) for somatic incompatibility to confirm that it was a unique genotype or clone. Host and location of the California isolates of *H. annosum* from fir and pine were listed elsewhere (14). Single-ascospore strains were obtained from perithecia of a heterokaryotic isolate of *Ophiostoma piliferum* (Fr.:Fr.) Syd. & P. Syd., which originated from pine chips in South Carolina. The host and location of the *Leptographium wageneri* (Kendrick) M. J. Wingfield isolates were listed elsewhere (43).

Isozyme markers and mating type alleles. Isozyme markers for esterase (EST, EC 3.1.1.1) and glucosephosphate isomerase (GPI, EC 5.3.1.9) were determined for 30 single-basidiospore progeny of *H. annosum* isolate B832 by the methods of Zambino and

Harrington (43), except that buffer systems A and D were used for EST and buffer systems D and E were used for GPI. Determination of mating type alleles among the progeny was done by pairing isolates on malt extract agar and looking for clamp connections, as described by Worrall and Harrington (42).

DNA extraction. Isolates were grown in 25 ml of liquid medium (2% malt extract and 0.2% yeast extract) in 125-ml Erlenmeyer flasks at room temperature (20–25 C) and lighting for 2–3 wk prior to harvesting for DNA extraction. Mycelial mats were collected by vacuum filtration on Whatman #54 filter paper (Whatman Paper, Maidstone, England) and dried for 10 min by placing them between paper towels. Dried mats were ground to a fine powder in liquid nitrogen with a mortar and pestle.

DNA was extracted by a modification of the method of Della-porta et al (8). Two milliliters of extraction buffer (100 mM Tris, pH 8.0; 50 mM EDTA, pH 8.0; 500 mM NaCl; 1.25% sodium dodecyl sulfate [SDS]; 10 mM β -mercaptoethanol; 4 mM spermidine; 1 mM spermine; and 1 mM phenylmethylsulfonyl fluoride) at 65 C was added per gram of mycelial mat, and the mixture was stored at –20 C until all isolates were ready to be processed. The mixtures were incubated at 65 C for 30 min followed by the addition of 40% (v/v) 5 M potassium acetate. The mixtures were stored at –20 C for 30 min followed by centrifugation at

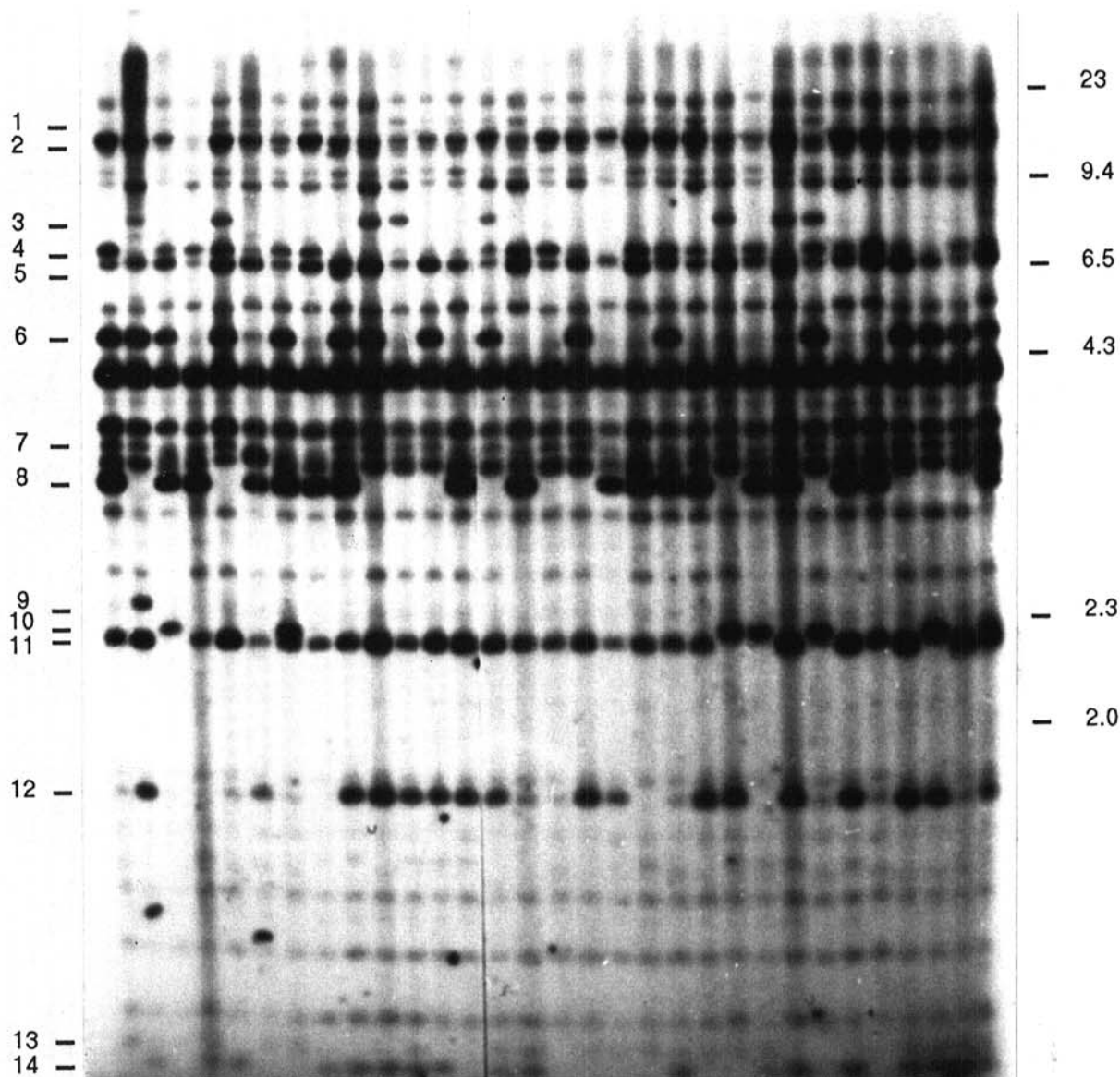


Fig. 1. DNA fingerprint patterns detected by $(CAT)_5$ in 30 *Heterobasidion annosum* single-basidiospore isolates from a dikaryon (far right lane). Numbers on the left designate heterozygous bands and new bands (bands 5 and 9). Approximate migration distance and fragment sizes (kb) of lambda DNA digested with *Hind*III are indicated on the right.

25,000 g for 20 min. The supernatant was mixed with 0.58 volumes of ice-cold isopropanol and placed at -20°C for 30 min. The resulting DNA precipitate was collected by centrifugation at 20,000 g for 10 min. Pelleted DNA was resuspended in 700 μl of TE (10 mM Tris and 1 mM EDTA, pH 8.0) at 37°C for 2–4 h and transferred into 1.5-ml microcentrifuge tubes. The tubes were centrifuged at 15,000 g for 10 min to remove insoluble debris prior to DNA precipitation. Seventy-five microliters of 3 M sodium acetate was added to each sample in addition to 500 μl of ice-cold isopropanol. The samples were gently mixed and incubated at room temperature for 2–4 h. Following centrifugation for 2 min at 15,000 g, DNA pellets were washed with 1 ml of 70% ethyl alcohol for 10 min and then air dried and resuspended in 330 μl of TE. DNA was extracted once with phenol-chloroform-isoamyl alcohol (25:24:1) followed by two or three extractions with chloroform-isoamyl alcohol (24:1). After precipitation, the DNA was resuspended in 100–300 μl of sterile double-distilled water at 37°C for 2–4 h prior to quantification.

DNA was quantified with a TKO-100 minifluorometer (Hofer Scientific Instruments, San Francisco, CA) as instructed by the manufacturer. For each sample, 10 μg of DNA was restricted with *Pst*I (5 U of enzyme per microgram of DNA) for 16 h at 37°C in a total reaction volume of 100 μl . Restrictions were stopped by the addition of 2 μl of 0.5 M EDTA (pH 8.0) per reaction.

One percent agarose gels (20 cm \times 25 cm \times 5 mm) were cast with 250 ml of 1.25 \times Tris-borate electrophoresis buffer (0.089 M Tris, 0.089 M boric acid, and 0.002 M EDTA, pH 8.45). Molecular biology-grade agaroses from several sources (Fisher Scientific, Pittsburgh, PA; VWR Scientific, Philadelphia, PA; and FMC, Rockland, ME) were used with no significant differences observed. However, for repeated probing of the gel, a high-strength agarose such as SeaKem Gold (FMC) was preferred. Gels were loaded with a mixture containing 2–10 μg of DNA in gel loading buffer type III (26). Gels were run at 35 V for 40 h at room temperature with a 1- to 2-mm buffer overlay on top of the gels. After the gels were photographed, they were dried for 45 min at room temperature and then for 60 min at 50°C under vacuum.

Hybridization. The dried gels were placed in covered plastic boxes (25 \times 35 cm) for the following steps. Prehybridizations, hybridizations, and washings were done at 42°C with shaking (50 rpm). The gels were incubated in 250 ml of denaturing solution (0.5 M NaOH and 1.5 M NaCl) for 45 min, incubated in 250 ml of neutralizing solution (1.0 M Tris, pH 8.0, and 1.5 M NaCl) for 45 min, and rinsed in sterile double-distilled water for 20 min. The gels were dried under vacuum for 20 min at 50°C prior to prehybridization in 50 ml of Church's (7) hybridization buffer (250 mM $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$, pH 7.4, 7% SDS, 1 mM EDTA, and 1% bovine serum albumin) and prehybridized for a minimum of 1 h.

Approximately 50 ng of the 15-mer oligonucleotide (CAT)₅ was end labeled with 100 μCi of ^{32}P -dCTP (Dupont/NEN, Boston,

MA) using terminal deoxynucleotidyl transferase (Promega Co., Madison, WI) according to the manufacturer's protocol. The reaction was stopped by adding 2 μl of 0.5 M EDTA and 28 μl of TE. The unincorporated nucleotides were removed by spin column chromatography with Sephadex G25-80 (Sigma Chemical Co., St. Louis, MO). The purified probe was then added directly to the hybridization buffer. Hybridization was carried out for 16–18 h.

Gels were washed twice for 45 min with 250 ml of 6 \times SSC (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium pyrophosphate and twice for 45 min with 250 ml of 5 \times SSC, 0.1% sodium pyrophosphate. Gels were blotted dry for 10 min on Whatman 3 MM paper, wrapped in plastic wrap, and placed in autoradiography cassettes with two Dupont/NEN Cronex Lightning Plus intensifying screens. The gels were exposed to Kodak X-omat AR film (Kodak, Rochester, NY) for 2–7 days at -20°C prior to film development. Gels were reprobed up to four times with no loss of band intensity. Prior to reprobing, the gels were denatured, neutralized, and rinsed as described above.

Data analysis. Consistent with previous work (5,31) in which multilocus fingerprinting probes were used, the presence or absence of a band of particular molecular weight was scored as two alleles at a single locus. Only distinct and reproducible bands were scored. Band intensity was consistent among DNA samples from an individual fungal strain, and only rarely was variation found in relative intensity of a particular band among different strains.

For analysis of single-spore progeny of *H. annosum*, only bands of similar intensity and molecular weight on a single gel were considered to be identical. Chi-square analysis was used to test segregation ratios of individual bands in the 30 progeny. The program RI Plant Manager v2.4, written by Dr. Kenneth Manly (Roswell Park Cancer Institute, Buffalo, NY), was used to determine linkage in addition to chi-square analysis. Frequency of generation of new alleles was determined by dividing the number of new alleles by the total number of possible alleles, i.e., total number of alleles in the parent dikaryon multiplied by the number of progeny scored.

RESULTS

Genetics of (CAT)₅ markers. A series of crosses resulting in synthesized dikaryons and single-spore progeny was carried out to investigate the behavior of the loci detected by (CAT)₅ and to determine whether they could be used as heritable genetic markers. Bands hybridizing with the (CAT)₅ probe were heritable. Twenty-eight bands were identified with the (CAT)₅ probe in *Pst*I restrictions of DNA from a dikaryon of *H. annosum* (isolate B832). These same bands were found in the 30 single-basidiospore (homokaryotic) progeny of that dikaryon (Fig. 1). On the basis of the RFLPs of the single-spore progeny, 16 of the putative loci were homozygous; i.e., they occurred in each of the 30 progeny. Segregation of alleles of six of the 12 putatively heterozygous loci (bands 2, 4, 6, 8, 12, and 13) was not significantly different from a 1:1 ratio as determined by chi-square analysis (Table 1). Two others (bands 5 and 9) did not appear in the parent dikaryon and were considered new alleles; band 5 occurred in two progeny and band 9 in one. The dikaryon was heterozygous at the mating type locus (alleles A₇ and A₈) and the isozyme loci PGI-1 and EST-2. The alleles for these three loci segregated independently and in a 1:1 ratio as determined by chi-square analysis. Fingerprint bands 10 and 11 showed skewed segregation but appeared to be linked ($P < 0.01$), and only one progeny showed the presence of both bands. No other linkage was detected among the remaining heterozygous bands, the mating type locus, or isozyme loci.

Single-basidiospore strains (homokaryons) of *H. annosum* were mated in vitro to synthesize dikaryons. The synthesized dikaryons with clamp connections contained the full complement of bands from each homokaryon (Fig. 2), although for some bands there was a difference in signal intensity between the dikaryon and the original homokaryons.

TABLE 1. Segregation of bands detected by (CAT)₅ in single-spore progeny (homokaryons) of *Heterobasidion annosum*

Band number	Observed ratio ^a	χ^2 ^b
1	23:7	8.53*
2	17:13	0.53
3	8:22	6.53*
4	20:10	3.33
6	15:15	0.00
7	26:4	16.13*
8	17:13	0.53
10	6:24	10.80*
11	25:5	13.33*
12	16:14	0.13
13	19:11	2.13
14	21:9	4.8*

^a Presence:absence of band found among the 30 progeny.

^b χ^2 value for significant difference from expected 1:1 segregation ratio. Asterisk denotes divergence of the observed ratio from the expected ratio at $P < 0.05$.

The inheritance of (CAT)₅ markers in single-ascospore (homokaryotic) progeny of a heterokaryotic strain (C482) of *O. piliferum* was also examined (Fig. 3). As in the *H. annosum* progeny, the *O. piliferum* progeny examined had most of the bands from the parent heterokaryon. The parent heterokaryon was apparently heterozygous in 16 of 28 bands scored. The relative intensity of a few bands in the progeny was stronger than in the parent. Each of the six progeny had a unique fingerprint.

Genetic diversity in populations. Small populations of *H. annosum*, *L. wagneri* var. *wagneri*, and *L. w. ponderosum* (T. C. Harrington & F. W. Cobb) T. C. Harrington & F. W. Cobb were investigated to determine whether the loci detected by (CAT)₅ would be useful in detecting genetic diversity within and between fungal populations.

Fingerprints of *H. annosum* dikaryons were highly variable, and each clone tested had a unique fingerprint. Fifty-one bands were found in a sampling of 12 isolates from two populations, and no two isolates had identical fingerprints (Fig. 4).

The mitotic stability of the fingerprints was examined by comparing isolates of a large, established clone. Isolates of *H. annosum* from eight juxtaposed trees in a New Hampshire pine stand were shown to be of a single somatic incompatibility group; i.e., the eight isolates were thought to have arisen from a single clone of the pathogen that was spreading along and in the roots of adjacent trees. The eight clonal isolates had identical DNA fingerprints as detected by (CAT)₅ (Fig. 5).

Intraspecific variants could be identified with the (CAT)₅ probe. Fingerprints of *H. annosum* isolates from New Hampshire and New York, which had been identified as the pine group of the host-specialized pathogen (14), were compared with three isolates of the pine group and three isolates of the fir group from California (Fig. 6). No two isolates had common fingerprints. One major band (approximately 4.2 kb) was found in all isolates, but other major bands were unique to either the pine or fir isolates. Many of the major bands were found in all pine isolates.

Taxonomic varieties of an asexual pathogen were also distinguished by the (CAT)₅ probe. DNA fingerprints were determined for eight isolates of *L. wagneri* varieties *wagneri* and *ponderosum*. All but two isolates (lanes 3 and 4) of *L. w. wagneri* had unique fingerprint patterns (Fig. 7). The two varieties had distinct fingerprint patterns characterized by a series of three wide-spaced double bands (bands 1, 4, and 5) observed in var. *wagneri* and a series of two narrow-spaced double bands (bands 2 and 3) observed in var. *ponderosum* as well as a number of characteristic single bands. Two isolates of *L. w. pseudotsugae* T. C. Harrington & F. W. Cobb had fingerprints similar to those of the isolates of var. *ponderosum* but had additional variety-specific bands (not shown). Markers detected by (CAT)₅ were more variable than markers detected by isozyme analysis (43).

DISCUSSION

The oligonucleotide (CAT)₅ has been shown to be a useful fingerprinting probe for higher fungi. The ability to reproducibly detect a large number of highly stable but hypervariable loci in a variety of fungi makes this probe highly versatile in studies of fungal ecology, population biology, and taxonomy. In the sexually outcrossing organisms tested, every isolate had a unique fingerprint, and even homokaryotic progeny from an individual heterokaryon or cross had unique fingerprints. The (CAT)₅ markers were more sensitive to variation than were isozyme markers or heterokaryon compatibility and corresponded well with differences in somatic incompatibility.

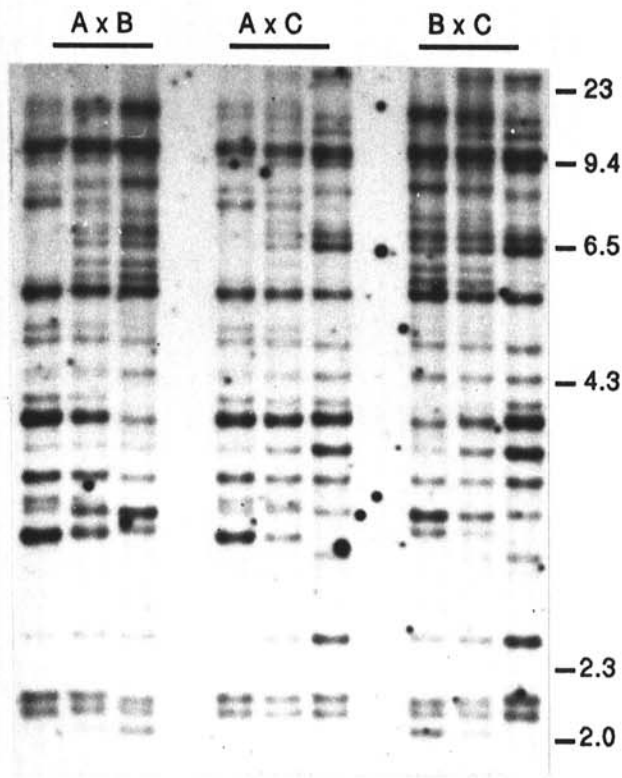


Fig. 2. DNA fingerprint patterns detected by (CAT)₅ in three synthesized *Heterobasidion annosum* dikaryons. Dikaryons are flanked by the parental homokaryons. Three parental homokaryons (A, B, and C) were used to synthesize the dikaryons. Approximate migration distance and fragment sizes (kb) of lambda DNA digested with *Hind*III are indicated on the right.

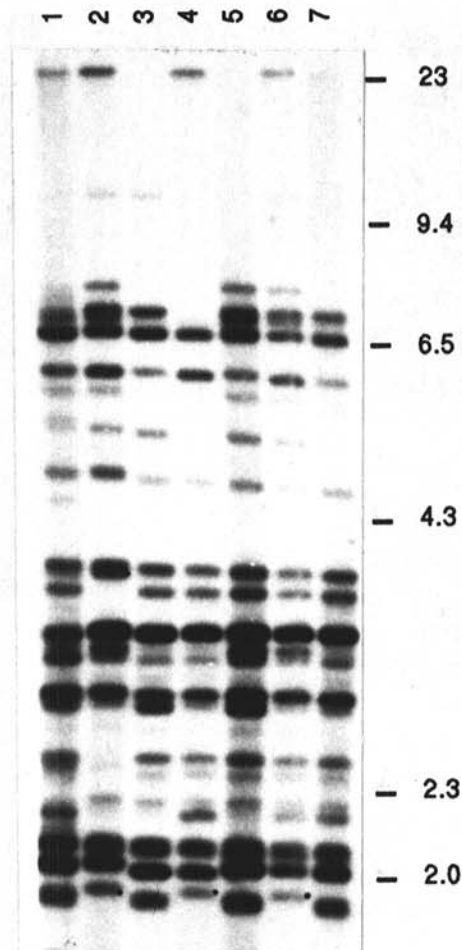


Fig. 3. Comparisons of DNA fingerprint patterns detected by (CAT)₅ in *Ophiostoma piliferum* single-ascospore (homokaryotic) progeny. Lane 1 is the parent heterokaryon (C482); lanes 2-7 are single-ascospore progeny. Approximate migration distance and fragment sizes (kb) of lambda DNA digested with *Hind*III are indicated on the right.

Genetic characteristics. The alleles detected by (CAT)₅ were clearly heritable. This was demonstrated by the analyses of progeny from a self-fertile heterokaryon of *O. piliferum* and a dikaryon of *H. annosum*. Further, synthesized dikaryons of *H. annosum* showed bands of the parent homokaryons.

After meiosis, the loci identified with the (CAT)₅ probe showed some unusual genetic characteristics and were similar in some respects to other identified VNTR loci. In a dikaryon of *H. annosum*, mating type alleles and isozyme markers segregated in the expected 1:1 ratio among the haploid homokaryotic progeny, but only six of 12 heterozygous bands detected by (CAT)₅ segregated in the expected 1:1 ratio. The remaining six bands exhibited skewed segregation ratios. Segregation distortion has been reported for other loci in fungi (10,11,21). The high proportion of loci that did not segregate in a 1:1 ratio suggests that unequal crossing over of DNA at meiosis, which has been previously observed at VNTR loci (3), may be occurring.

Two bands found among the single-spore progeny of *H. annosum* but not detected in the parent dikaryon were considered to be newly generated alleles. Repeated analysis from two separate DNA isolations confirmed that the bands were not experimental artifacts. The observed generation rate of new alleles of 0.0036 with (CAT)₅ is much higher than the average forward mutation rate reported in fungi (approximately 10⁻⁶ per gene) but falls within the range of published rates for other VNTR alleles: 0.0014 (18), 0.0068 (4), and 0.05, the highest mutation rate of any described VNTR locus (41). The mechanism responsible for the generation of new alleles at VNTR loci is unknown, but Jeffreys et al (18) postulated that interallelic, unequal crossing over through meiosis or mitosis may play a role.

Although the (CAT)₅ markers had a high mutation rate through meiosis and there have been reports of somatic instability at VNTR loci (4), the (CAT)₅ markers appeared to be stable through mitosis. Eight clonal isolates of *H. annosum* had identical fingerprints.

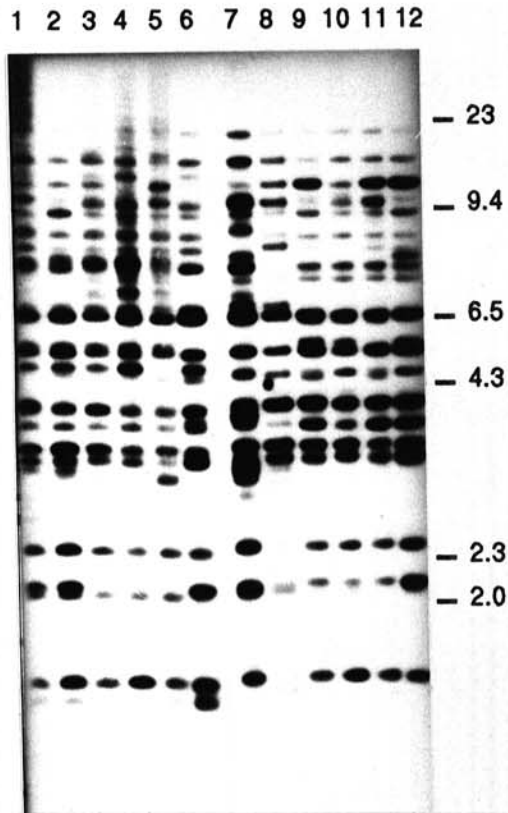


Fig. 4. DNA fingerprint patterns detected by (CAT)₅ in a *Heterobasidion annosum* population from New York (lanes 1-6) and a population from New Hampshire (lanes 7-12). Approximate migration distance and fragment sizes (kb) of lambda DNA digested with *Hind*III are indicated on the right.

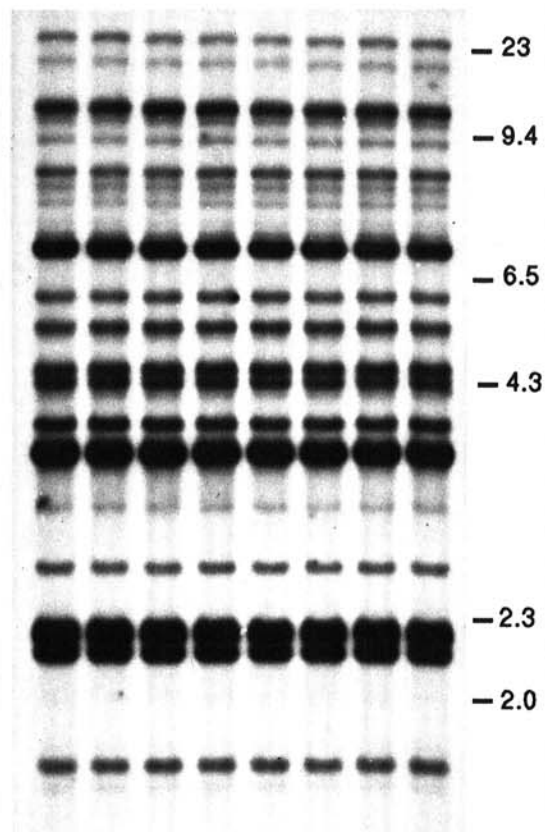


Fig. 5. Comparisons of DNA fingerprint patterns detected by (CAT)₅ in a single *Heterobasidion annosum* somatic incompatibility group from a New Hampshire population. Approximate migration distance and fragment sizes (kb) of lambda DNA digested with *Hind*III are indicated on the right.

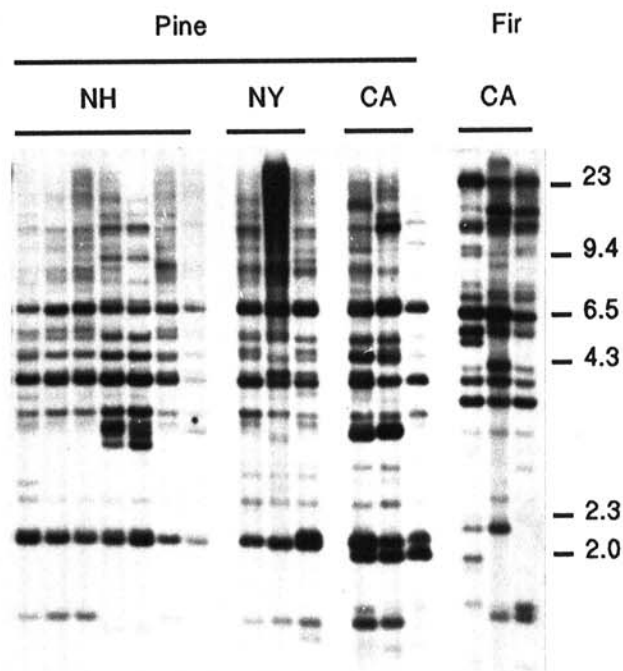


Fig. 6. Comparisons of DNA fingerprint patterns detected by (CAT)₅ in *Heterobasidion annosum* pine group isolates from New York, New Hampshire, and California with fir group isolates from California. Approximate migration distance and fragment sizes (kb) of lambda DNA digested with *Hind*III are indicated on the right.

This clone was found to have colonized trees over an area at least 10 m in diameter; and on the basis of the estimated spread rates for *H. annosum* in pine stands (16), this clone was probably established 10 yr or more previous to our sampling.

Applications. The (CAT)₅ probe proved useful for characterizing individual strains or genotypes for ecological studies, but potential was also seen for quantifying genetic variation within and among populations and for identifying infraspecific variants of fungi. This new probe appears to be more sensitive than isozymes (43) or heterokaryon incompatibility (44) for detecting fine levels of variation.

The DNA fingerprints detected with a particular batch of synthesized (CAT)₅ were highly reproducible. We observed no differences in the fingerprints of individual isolates tested more than 10 times during a 3-yr period with both in-gel and Southern blot hybridizations. However, we observed minor variation in the DNA fingerprints obtained with an oligonucleotide synthesized from a source different from the original oligonucleotide used for these experiments. The minor variation is perhaps due to a single base error in synthesis or to differences in synthesis methods, which may have resulted in different hybridization specificities for the two probes.

Investigations with *H. annosum*, *O. piliferum*, and other ascomycetes such as *Ceratocystis* species (13) indicated that the (CAT)₅ probe was capable of detecting a high degree of genetic variability in sexually outcrossing fungi. Aside from the isolates of *H. anno-*

sum derived from the same clone, each tested dikaryon had a unique fingerprint. Isolates of the same somatic incompatibility group are mitotically derived and had identical fingerprints. Thus, the (CAT)₅ markers can be effectively used to distinguish sexual from asexual reproduction in *H. annosum*.

The (CAT)₅ probe was the most sensitive marker used to date to detect variation in the genetically depauperate asexual fungus *L. wageneri*. Isolates of a given variety of *L. wageneri* taken from a wide geographic area (hundreds of kilometers apart) have been shown to have the same isozyme electromorphs among 21 loci tested (43) and to be of the same heterokaryon compatibility group (44). However, unique fingerprints were found among all but one pair of the isolates of *L. wageneri* tested with (CAT)₅.

Although a limited number of isolates was used, the varieties of *L. wageneri* were clearly distinguished by their DNA fingerprints. This variation in fingerprints was consistent with the data from the more laborious isozyme analyses (43). Similarly, analysis of the fingerprint data indicate that the fir and pine groups of *H. annosum* can be easily distinguished by the (CAT)₅ probe. If a larger sample size were used, genetic diversity values within and between these two host-specialized, infraspecific groups based on the (CAT)₅ probe may prove as useful as values based on isozyme analysis in elucidating relationships (32). Certainly, the preliminary work necessary to identify a sufficient number of isozymes for such comparisons would be more time consuming than the application of the (CAT)₅ probe.

The results discussed here demonstrate the wide variety of potential applications of the (CAT)₅ fingerprinting probe. Although cloned DNA sequences have been shown to be effective (25,30,39), the use of synthetic oligonucleotide probes offers a number of advantages. Most notably, fingerprinting with the probe (CAT)₅ appears to be widely applicable to the higher fungi, eliminating the time-consuming process of developing and screening probes specific to the fungus to be studied. In addition, the small size of the (CAT)₅ probe makes it suitable for in-gel hybridizations, thus negating the need for transfer of the restricted DNA to membranes for hybridization.

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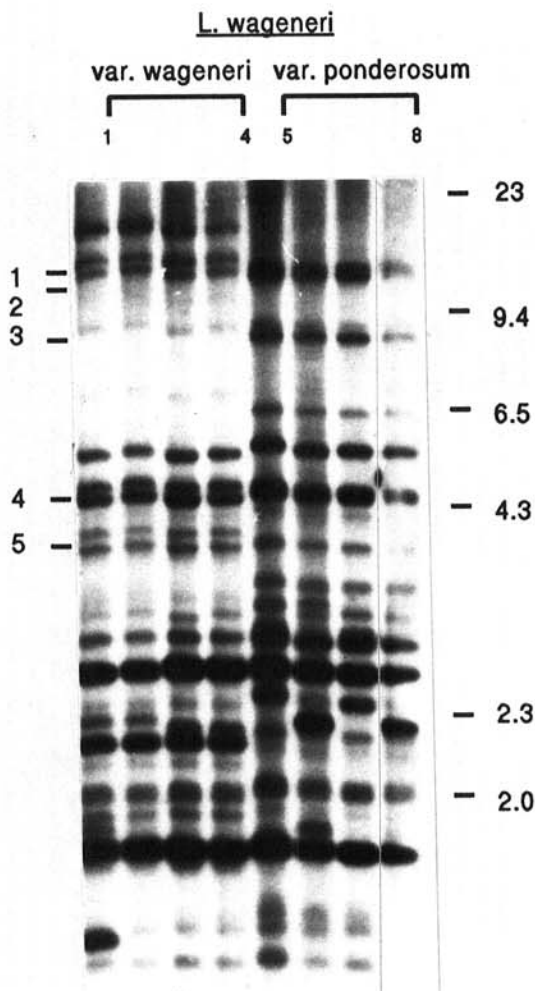


Fig. 7. DNA fingerprint patterns detected by (CAT)₅ in isolates of *Leptographium wageneri* var. *wageneri* (lanes 1-4) and var. *ponderosum* (lanes 5-8). Numbers on the left indicate doublet bands unique to var. *wageneri* (1, 4, and 5) and var. *ponderosum* (2 and 3). Approximate migration distance and fragment sizes (kb) of lambda DNA digested with *Hind*III are indicated on the right.

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