A Population Genetics Study of *Discula Destructiva*, the Causal Agent of Dogwood Anthracnose

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DEDICATION

I dedicate this to my LORD GOD in heaven for blessing me with the opportunity and the abilities to perform this research, to my mother for her love and support, and to my father who is with me in spirit.

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ABSTRACT

Flowering dogwood (*Cornus florida* L.) is prized as an ornamental and contributes millions of dollars to the economy through tourism and sales each year. Dogwood anthracnose, caused by *Discula destructiva* Redlin, was observed in the late 1970s on the east and west coasts of the United States and by 1991 had quickly spread throughout much of the native ranges of *C. florida* and *C. nuttalli*. The objectives of this study were to investigate the genetic diversity and population structure of *D. destructiva* using simple sequence repeats (SSRs), and to test the cross-transferability of these markers to other *Discula* species. Fifty SSRs were developed from the sequenced genome of a *D. destructiva* isolate and used to evaluate 93 fungal isolates from 14 locations collected between 1989 and 2000. The data set was clone corrected (n = 69) and analyzed using polymorphic only (n = 40) and polymorphic and monomorphic SSRs (n = 47). Haploid genetic diversity was very low for both data sets (Hexp = 0.18 and 0.21). Bayesian clustering and distance analyses identified four and five genetic clusters that correspond to two major geographic areas, the eastern United States and the Pacific Northwest, and two time periods when the isolates were collected, Pre-1993 and Post-1993. Linkage disequilibrium was present in all subpopulations, which strongly suggested that the fungus was only reproducing asexually. A population bottleneck was statistically indicated in all populations, and was probably the result of the limited number of founding individuals on both coasts. These results support the hypothesis that *D. destructiva* is an exotic pathogen with separate introductions on the east and west coasts of North America. The cross-transferability test revealed that genomic DNA from 19 isolates of five other *Discula* species and two isolates of *Melanconis* spp. amplified with 17 of 47 primer pairs. These primers may be useful for investigating the genetic diversity and population structure of these *Discula* species.

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1. Introduction

Cornus florida L. (eastern flowering dogwood) and *C. nuttallii* Aud. (western flowering dogwood) are tree species in the big-bracted group of dogwoods (Eyde, 1988) and common understory trees of the North American deciduous eastern and western forests, respectively. *Cornus florida* is native and widespread throughout eastern North America from southeastern Ontario, Canada in the north, to as far west as eastern Texas (Call *et al*., 2016), whereas *C. nuttallii* is endemic to western North America and ranges from southwestern British Columbia to southern California with disjunct populations in northern Idaho and southern California (Keir *et al*., 2011). *Cornus florida* is a major component of eastern hardwood forests (Boring *et al*., 1981) and plays an important role in cycling calcium in the soils (Thomas, 1969). Dogwood trees provide food for numerous species of birds and mammals (Eyde, 1988; Mitchell *et al*., 1988) and are highly prized ornamental trees because of their attractive springtime bract display, red or yellow berries, and attractive fall foliage. Dogwoods are celebrated in major tourist attractions throughout the eastern United States (U.S.) (Redlin, 1991), which contribute to numerous local economies. Dogwood nursery sales in the U.S. exceeded $27 million in 2014 with more than $6.5 million from the Tennessee nursery industry (USDA, 2014).

Most foliar diseases of dogwoods are easily noticed in the landscape; however, they generally cause cosmetic damage and not tree mortality. Spot anthracnose is caused by the fungus *Elsinoe corni* and is an early season disfiguring disease affecting mostly landscape plantings in full sun early in the season. The disease is characterized by very small, uniform, round spots on the bracts and leaves, which may coalesce when heavily infected (Anderson *et al.*, 1994; Hagan and Mullen, 1999; Hansen, 2009). Powdery mildew is a fungal disease caused by *Erysiphe pulchra* (Li *et al*., 2006) that appears as a whitish powder on leaves, buds, and young shoots. Infection can cause stunting and disfigurement of the leaves, eventually affecting the overall health of the host including reducing flower and fruit production (Hansen, 2009; McRitchie, 1994). Although powdery mildew can be damaging to dogwoods, the impact is not as severe as dogwood anthracnose, which can kill trees in landscapes and native forests in a relatively short period.

Dogwood anthracnose was first observed on *C. nuttallii* during 1976 in Washington State (Byther and Davidson, 1979), whereas the first documented incidence of the disease in the eastern U.S. on *C. florida*

was around 1977 in New York (Daughtrey and Hibben 1983; Hibben and Daughtrey, 1988). Thousands of flowering dogwood trees within New York and Connecticut were either killed or seriously damaged by what was then considered a new "mysterious" disease (Pirone, 1980).

The disease began to spread across the natural western range of *C. nuttallii* (Smith-Salogga, 1982) including a disjunct population in Idaho (Daughtrey and Hibben, 1994; Salogga and Ammirati, 1983). The spread of dogwood anthracnose in the eastern U.S. was rapid, affecting dogwoods in the Northeast and southward along the spine of the Appalachian Mountains. The disease reached Georgia by 1987, where it had already infected dogwood trees in 30,000 acres of wilderness (Anderson *et al.,* 1991). In 1988, it was determined that both *C. nuttallii* in the west and *C. florida* in the east were being affected by the same disease (Hibben and Daughtrey, 1988).

Dogwood anthracnose affects bracts, fruits, seeds, leaves, twigs, branches, and trunks (Britton *et al*., 1993; Daughtrey and Hibben, 1983). Dogwoods exposed to more sunlight typically show symptoms on the lower canopy, whereas shaded trees will often be more uniformly and severely affected. Leaf spots have an irregular shape and size, which may have a yellow border that becomes red- to- purple over time. The center of the leaf spot may be light brown or necrotic, which often abscises leaving a "shot hole" appearance. Leaf blotches are lesions that are seen at leaf tips or along margins, and are typically larger than leaf spots with red- to- purple margins. Leaf blight affects the entire leaf and results in necrosis; some blighted leaves may not abscise, but remain attached to the tree during the winter. Necrosis of the bracts presents as red- to- purple- to- brown spots or blotches. Shoot tips and twigs die from mycelium growing from the leaf into stems where it may then enter the main trunk. Annual cankers are sunken necrotic areas involving the bark and primarily affecting the phloem tissues (Holliday, 1992), and they may form on twigs, branches, or the main trunk. Canker formation on the trunk is especially problematic as they often coalesce and effectively girdle the tree. Epicormic buds along the lower portion of the trunk become activated by the loss of apical dominance caused by the death of apical meristems on the leader or other infected branches (Daughtrey *et al*., 1996).

Dogwood anthracnose can affect any size and/or age of flowering dogwood tree (Anderson *et al.*, 1991) and the impacts of the disease can be devastating. The high mortality rates have been well-documented in Catoctin Mountain Park, Maryland. A 1984 disease impact survey revealed that 33% of *C. florida* trees had been killed by dogwood anthracnose, and only 3% of trees were free of symptoms (Mielke and

Langdon, 1986). A 1994 survey revealed an overall death of 94% of the native dogwood trees in the park (Sherald *et al*., 1996). Surveys conducted in The Great Smoky Mountain National Park revealed that dogwood anthracnose had killed 25% of *C. florida* by 1992 and 75% by 1994 (Windham, 1995).

Environmental factors considered to increase the risk of disease severity include the following: drought stress (Erbaugh *et al*., 1994), shaded locations (Anderson *et al*., 1994; Chellemi and Britton, 1992; Erbaugh *et al*., 1994), sufficient rainfall to allow secondary infection cycles (Britton, 1993), high elevation, and near a water source (Anderson *et al*., 1994).

The imported Japanese or Chinese dogwood (*C. kousa* Hance) has been reported to exhibit resistance to dogwood anthracnose (Daughtrey *et al*., 1988). Infected *C. kousa* trees may exhibit small leaf spots, but shoot dieback is not as severe, and tree mortality rarely occurs (Holmes and Hibben, 1989; Santamour *et al*., 1989). The United States Department of Agriculture (USDA) currently lists *C. florida* as endangered in Maine, "exploitably vulnerable" in New York, and threatened in Vermont (USDA, NRCS, 2016).

The causal agent of dogwood anthracnose was once though to be *Gloeosporium* species in the west (Byther and Davidson, 1979) and *Colletotricum gloesporioides* in the east (Pirone, 1980). The fulfillment of Koch's postulates revealed *Discula* species as the cause (Smith-Salogga, 1982) and was later characterized as a new species named *Discula destructiva* (Redlin, 1991). The two types of *Discula* associated with dogwood anthracnose were called *Discula* Type I (*D. destructiva*) and Type II (*D*. species) (Hibben and Daughtrey, 1994; Trigiano *et al*., 1991).

Discula destructiva is a haploid, ascomycetous fungus in the order Diaporthales, suborder Gnomonineae, and the family Apiognomonia and is an important pathogen of *C. florida* and *C. nuttallii*. *Apiognomonia* is the genus for the sexual state of some other *Discula* spp., but a teleomorph has not been observed for *D. destructiva* despite attempts to find a sexual state on diseased materials or efforts to induce the sexual state in the laboratory (Redlin, 1991; Smith-Salogga, 1982). Because of the apparent lack of a sexual stage, Allen *et al*. (2016) have argued to conserve the genus *Discula* for dogwood anthracnose. Because of lack of evidence for sexual reproduction, the pathogen has been considered to reproduce only by asexual means.

Discula destructiva has several demonstrated modes of dispersal. Localized dispersal can be accomplished by splashing water in the form of rain or overhead irrigation (Britton *et al.*, 1993;

Hadziabdic *et al*., 2010). Animals and birds may be involved in long distance dispersal by transporting infected fruits and seeds (Britton *et al.*, 1993). Insects that encounter conidia may also play an important role in disseminating this pathogen (Colby *et al*., 1995). Humans also contribute to the spread of dogwood anthracnose by moving infected plants from one area to another, as in the case of infected *C. florida* found in 1995 being exported to the United Kingdom from the U.S. (EPPO, 1996). It has since been found in Germany (Stinzing and Lang, 2003), Italy (Tantardini *et al*., 2004), and Switzerland (Holdenrieder and Sieber, 2007).

Different scenarios have suggested that *D. destructiva* may be either an introduced pathogen or an indigenous species that has gone unnoticed until the late 1970s (Mielke and Langdon, 1986). Phylogenetic analyses of the internally transcribed spacer region (ITS) indicated that *D. destructiva* did not arise from a native population (Caetano-Annoles *et al*., 2001). A more recent study detected the presence of *D. destructiva* on *Cornus* species plant material collected from China and Japan, the hypothesized center of origin, where it may live as an endophyte (Miller *et al*., 2026). DNA amplification fingerprinting (DAF) (Trigiano *et al*., 1995) reported very little genetic variability among eastern and western *D. destructiva* isolates and supports the exotic invader hypothesis. The DAF method generates an abundance of data, but cannot be linked to specific genomic sites or loci. Furthermore, a single band in a gel may contain multiple amplicons, which may or may not be present among all the isolates. Analysis of arbitrary signatures from amplification profiles (ASAP) (Caetano-Annoles *et al*., 1996) found low genetic diversity among isolates, and a population structure supporting recent and separate introductions on the east and west coasts of North America. ASAP dissects DAF products by finding complimentary sites with hairpin primers within the amplicons and is capable of finding differences more related to sequences of amplicons instead of primer sites (Caetano-Anolles and Gresshoff, 1996). Banding profiles of double stranded RNA (dsRNA) indicated that eastern and western isolates of *D. destructiva* likely have separate origins (Yao *et al*., 1997). Amplified fragment length polymorphisms (AFLPs) (Zhang, 2002) also revealed two distinct groups representing isolates from the east and west coasts of North America, further supporting the hypothesis of separate introductions. This study also found a higher level of genetic diversity in isolates collected near New York City compared to isolates collected from the north and south, where the disease was said to have spread, thus supporting the hypothesis that New York may be the disease epicenter in the eastern U.S.

Microsatellites or simple sequence repeats (SSRs) are repeating units of nucleotides that occur throughout the genome of eukaryotic organisms. They can be useful as genetic markers because they facilitate greater detection of genetic diversity by using discrete or defined loci compared to arbitrary primers (Powell *et al*., 1996), and therefore allow the evaluation of the same genomic sequences among isolates. This method relies on differences in allelic sizes as measured in base pairs (bp), but cannot distinguish differences between or among multiple sequences. SSRs provide high sensitivity, polymorphism, good reproducibility, require relatively small amounts of genomic DNA, and often cross-transfer to other species within a genus. They will be useful in this study because they enable a more reliable interpretation of the population diversity and structure of *D. destructiva* than the arbitrary primer methods previously used.

The aim of this study is to further investigate the population genetics of *D. destructiva* using SSRs. Therefore, the specific objectives of this study were the following: 1) to develop and test SSRs to analyze the genetic diversity and population structure of *D. destructiva* isolates collected across North America during different time periods; and 2) to test the cross-transferability of these markers to other *Discula* species.

2. Materials and Methods

2.1. Fungal Isolates

Ninety-three isolates of *Discula destructiva* collected from 1989-2000 across eastern and western North America were used in this study. Nineteen isolates of *D. destructiva* were obtained from a collection at Rutgers University (New Brunswick, New Jersey, USA) and fifty isolates were supplied from a collection at the University of Tennessee (UTK; Knoxville, Tennessee, USA)(Table 1). Genomic DNA of 24 other *D. destructiva* isolates as well as genomic DNA from isolates of five other *Discula* species were available from the UTK collection (Table 1). Stock cultures of *D. destructiva* isolates were maintained on potato dextrose agar (PDA; SIGMA-ALDRICH, St. Louis, Missouri, USA) for 10-14 days at room temperature (ca. 20 °C) before being used to inoculate medium to grow mycelia for DNA isolation. Stock cultures were transferred to fresh medium every two months.

2.2. DNA Extraction

Small pieces (ca. 5 mm^2) of mycelium were harvested from the periphery of 14-day-old fungal colonies and used to inoculate sterilized cellophane (BIO-RAD Laboratories, Hercules, California, USA) (Mattman and Farkas, 1958) placed on PDA contained in 60-mm Petri dishes. Cultures were grown at room temperature for 14 days. Fungal mycelia were flash frozen in liquid nitrogen and ground into a fine powder with a sterile mortar and pestle. Genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol with the following modifications: 1.5% of the total volume of polyvinylpyrrolidone (PVP) was added to the lysis buffer (AP1), and the initial incubation time was increased to 20 min. Genomic DNA was quantified using the NanoDrop ND-1000 Ultraviolet-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA) and stored at -20 °C until needed for amplification.

2.3. Verification of Discula destructiva Using Internally Transcribed Spacer Region (ITS)

The 66 living isolates from the UTK and RU collections were verified as *D. destructiva* by amplifying the ITS region of fungal ribosomal DNA using the polymerase chain reaction (PCR). Each 30 µl reaction

contained the following: 6 ng genomic DNA template, 1.5 µl of 5 µM each of ITS1 (5' - TCCGTAGGTGAACCTGCGG - 3') and ITS4 (5' - TCCTCCGCTTATTGATATGC - 3') (White *et al*., 1990), 3 µl of 25 mM $MgCL_2$, 3 µl of 10X PCR Gold Buffer (Applied Biosystems, Austin, Texas, USA), 1.5 µl dimethyl sulfoxide (DMSO; Fisher Scientific, Pittsburgh, Pennsylvania, USA), 0.24 µl of 5 U/µl AmpliTaq Gold® DNA polymerase (Applied Biosystems), 3 µl of 2.5 mM deoxynucleoside triphosphate (dNTPs), and 10.26 µl sterile distilled water. The thermocycler (Eppendorf AG, Hamburg, Germany) was programmed with initial denaturation of 95 °C for 3 min followed by 35 cycles of 95 °C for 1 min, 50 °C for 30 s, 72 °C for 80 s, and a final extension at 72 °C for 10 min. Five µl of ITS PCR products were electrophoresed through a 2% low melting point agarose gel in 1X TAE buffer for 1 h using 100 v/cm^2, stained with ethidium bromide, and visualized using a 2000 Gel Documentation System (BIO-RAD). A 100 bp DNA molecular weight ladder (Promega, Madison, Wisconsin, USA) was used for size comparison. PCR amplicons were purified using Qiagen QIAquick purification kit (Qiagen, Germany) according to the manufacturer's instructions. The purified DNA was quantified using NanoDrop ND-1000 ultraviolet-Vis Spectrophotometer and sequenced at the UTK Genomics Core (http://mbrf.utk.edu/). Fungal isolates were confirmed as *D. destructiva* by comparing the ITS sequences to known sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) available from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). The ITS sequences of the 66 isolates in live culture were aligned using BIOEDIT v7.2.5 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The 24 pre-1993 *D. destructiva* isolates (DNA only) and 21 *Discula* species were previously confirmed using the same procedure except that the primers ITS1 and IST2 (White *et al*., 1990) were used (unpublished data).

2.4. Microsatellite Primers

Discula destructiva type isolate MD235 was used for genome sequencing. Paired-end DNA library (Illumina) was made according to manufacturer's instructions and sequenced to produce 74.9 million 101 bp x 2 read pairs. The reads were filtered by requiring a minimal quality score of 20 in at least 70% of the bases. Filtered reads were assembled using SOAPdenovo2 with kmer=53. The final assembly was 49 Mb, with scaffold N50 126.6 kb (111 scaffolds) and contig N50 33,311 bp (429 contigs). Microsatellite markers (SSRs) were identified with a custom Perl script (https://github.com/statonlab/disculaSSRs). Di-, tri-, and tetra- nucleotide perfect repeats were only reported if they met the following criteria: di-

nucleotide motifs with 8-40 repeats, tri-nucleotide motifs with 7-30 repeats, and tetra-nucleotide motifs with 6-20 copies. Sequences were masked for low complexity regions with DUSTMASKER (Morgulis *et al*., 2006) and primers designed for repeats using PRIMER3 v2.3.6 (Untergasser *et al*., 2012). The following parameters were altered from the Primer3 default settings: maximum primer size was 27, primer product size ranged between 100 and 200 bp, primer minimum and maximum temperature was 55 ˚C and 65 ˚C, respectively, primer minimum GC percentage was between 40 and 60, the maximum allowable length of a mononucleotide repeat (mx poly-x) was set to 3, and primer GC clamp was set to 2. Primer pairs for 50 microsatellite loci (Table 2) with annealing temperatures between 59-63 °C were designed and manufactured by Integrated DNA Technologies (Corralville, Iowa, USA). Initially four isolates were randomly chosen from the UTK collection and used to screen primers for the ability to amplify alleles. A 10 µl amplification reaction contained the following: 1 µl of 0.25-2 ng/µl genomic DNA template, 1 µl of 2.5 µM of each primer pair, 1 µl of 25 mM $MgCl_2$, 1 µl of 10X PCR Gold buffer (Applied Biosystems), 0.5 µl DMSO, 0.1 µl of 5 U**/**µl AmpliTaq Gold® DNA polymerase, 1 µl (2.5 mM) dNTP's, and 4.4 µl sterile, distilled water. Reactions were completed in an automatic thermocycler under the following conditions: initial denaturation at 96 °C for 4 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were analyzed on the QIAxcel Capillary Electrophoresis System (QIAGEN, Valencia, California, USA) using an internal 25-bp size standard to provide raw allele length data. Forty-seven primer pairs that produced unambiguous products were selected for the study, and used to amplify DNA of the 93 *D. destructiva* isolates and other *Discula s*pecies isolates. Those reactions that did not produce a product were repeated at least once before assuming that the loci of these isolates either were a null allele or considered missing data for the analyses.

2.5. Data Analysis

FLEXIBIN v2 (Amos *et al*., 2007) was used to automatically bin raw allele length data into allelic classes. A ±2-3 bp standard error range was used to aid in determining the allele size classes. These binned allele sizes were used in all subsequent analyses.

Identical multilocus *D. destructiva* haplotypes were identified using POPPR v2.1.1 (Kamvar *et al*., 2014, 2015) a package for R v3.3.1 (R Core Team, 2016). All isolates used in this study were grouped into the

following six populations based on geographic location and time of collection: pre-1993 North, pre-1993 South, pre-1993 West, post-1993 North, post-1993 South, and post-1993 West North America. The Microsoft Excel macro GenAlEx v6.5 (Peakall and Smouse, 2006, 2012) and POPPR were used to calculate measures of genetic diversity including the number of multilocus haplotypes (MLG), average number of alleles per locus (Na), the average number of effective alleles per population (Ne), the number of private alleles (Pa), and Shannon-Wiener index of MLG diversity (H), eveness (E.5), Nei's genotypic diversity that is corrected for sample size (Hexp), pairwise population ϕ_{PT} (analogous to standardized *F*st for haploid data), and geneflow. Genetic distance was visualized by principal coordinate analysis (PCoA) and a dendrogram was created in POPPR using Bruvo's method (Bruvo *et al*., 2004). Bruvo's distance was utilized to construct and visualize Unweighted Pair Group Method using Arithmetic Averages (UPGMA) algorithms using 1,000 bootstraps with support values greater than 70%. Unlike Nei's genetic distance, Bruvo's distance is based on genetic distance between individuals, not populations (Kamvar *et al*., 2014). Genetic clustering and population structure were analyzed with STRUCTURE v2.3.4 (Pritchard *et al*., 2000) using a Bayesian Monte Carlo Markov Chain (MCMC) clustering method. The burn-in period was 500,000 with 500,000 MCMC repetitions completed after burn-in and there were 20 iterations of K = 1-15. STRUCTURE HARVESTER web v0.6.94 (Earl and von Holdt, 2012) was utilized to estimate the optimum value of K (genetic clusters) using Evanno *et al*. (2005) method and visualized using POPHELPER (Francis, 2016) package in R. Genetic differentiation was calculated using an analysis of molecular variance (AMOVA) performed with ARLEQUIN v3.5.2 (Excoffier and Lischer, 2010). Four variance partitions were used for the AMOVA including the following: all subpopulations grouped as one hierarchical group, partitioned based on two time periods (pre-1993 and post-1993), partitioned based on two geographic regions (eastern and western North America) and partitioned based on three host tree species (*C. florida*, *C. nuttallii*, and *C. kousa*). The program BOTTLENECK v1.2.02 (Cornuet and Luikart, 1996) was used to evaluate if a recent population bottleneck or expansion had occurred, and groups were based on the number of genetic clusters as defined by STRUCTURE. The data was coded as a diploid for analysis in BOTTLENECK and the following three statistical tests were performed: sign test, standardized differences test, and Wilcoxon sign-rank test. Three mutation models were used: infinite allele model (I.A.A.), stepwise mutation model (S.M.M.), and two phase model (T.P.M.). The standardized index of association ($\overline{r}_d$) (Agapow and Burt, 2001) was estimated using 10, 000 permutations, and the null hypothesis $\overline{r}_d = 0$ (linkage equilibrium) was tested in POPPR.

2.6. Cross-transfer of loci from Discula destructiva to other Discula specie

Cross-transferability of primers from *D. destructiva* to isolates of *D. umbinella* (n = 15), *D. quercina* (n = 2), *D. campestris* (n = 1), *D. faxina* (n = 1), and *Melanconis* species (n = 2) was evaluated using the same amplification protocol previously described. *Discula destructiva* isolate AS4 was used as a positive control for comparison of amplicon sizes. Data were binned and simply listed as base pair products; no statistical analyses were performed on the loci cross-transfer data for the other *Discula* species.

3. Results

3.1. ITS Sequences

A single band of about 550 bp was amplified using ITS1 and ITS4 primers for the previously uncharacterized 69 isolates of *D. destructiva* from RU and UTK (Fig. 1). The amplicon sequences were matched with 0.0 E-value and at least 99% identity to *D. destructiva* isolates in the NCBI database Accession Number DQ323528.1. Sequences of the amplicons exhibited very little variation and the isolates were confirmed as *D. destructiva*. The DNA of pre-1993 isolates from the UTK collection was previously confirmed as *D. destructiva* in a similar manner except ITS1 and ITS2 were used. Confirmation of this DNA was not completed with ITS1 and ITS4 because the paucity of genomic DNA available for these isolates. Sequencing of the ITS region of *Discula*-like or Type II isolates with ITS1 and ITS4 demonstrated that NC2 and VA17b were *Melanconis* species.

3.2. Microsatellite Primers

Forty-seven of the 50 primer pairs successfully amplified DNA and consistently produced a single product. Three primers failed either to provide consistent amplification of a single band or amplification from a sufficient number of isolates required for data analyses.

Binning the length (bp) of amplicons produced by the primers yielded distinct allelic classes. All amplicons except those amplified by primer pairs DD05, DD13, and DD17 were within the expected or calculated bp range $\pm$ 2 bp. Forty of the 47 loci were polymorphic and the mean number of alleles per locus was 2.2 and ranged from 1 to 5. The differences in the size range of alleles in loci were small, typically less than 10 bp, but seven loci had ranges equal to or greater than 10 bp (Table 2).

3.3. Data Analyses

The following clonal multilocus haplotypes were identified: 11 clones were identified in the post-1993 north, 12 in the post-1993 south, and one in the west, resulting in the exclusion of 24 individuals from the original 93 and only 69 unique haplotypes were used in all of the subsequent analyses. Analyses

were conducted for the clone corrected data set (n = 69) with: i) 47 SSRs which include both polymorphic (n = 40) and monomorphic (n = 7), and ii) with only the 40 polymorphic SSRs.

The 69 isolates included in the study were assigned to one of six subpopulations based on common geographical region (north, south, and west) and year of isolation (pre-1993 and post-1993). The mean number of alleles (Na) per subpopulation using 47 SSRs ranged from 1.21 in the post-1993 west to 1.68 in the pre-1993 west (Table 3). The mean number of effective alleles (Ne) per subpopulation ranged from 1.13 in the post-1993 west subpopulation to 1.37 in the pre-1993 west population. Shannon-Weiner index of multilocus genotype (MLG) diversity (H) ranged from 1.79 in both the pre-1993 north and pre-1993 west, to 3.37 in the post-1993 west. Genotypic diversity corrected for sample size (Hexp) ranged from 0.09 in the post-1993 west to 0.26 in the pre-1993 west and a mean total for all subpopulations was 0.18. Evenness (E.5) was 1 and a total of 15 private alleles (Pa) were discovered in five of the six designated subpopulations and included: two in the pre-1993 south, west, and post-1993 north, six in the post-1993 south, and three in the post-1993 west subpopulations. No private alleles were detected in the pre-1993 north subpopulation.

The diversity metrics were similar when using only the 40 polymorphic loci. The mean number of alleles (Na) per subpopulation ranged from 1.25 in the post-1993 west to 1.80 in the pre-1993 west (Table 4). The mean number of effective alleles (Ne) per subpopulation ranged from 1.16 in the post-1993 west and 1.43 in the pre-1993 west. Genotypic diversity corrected for sample size (Hexp) ranged from 0.11 in the post-1993 west to 0.31 in the pre-1993 west with a mean total of 0.21 across all subpopulations. Shannon-Wiener indix of MLG diversity (H), evenness (E.5), and private alleles (Pa) were the same as when calculated with 47 loci.

The analysis of molecular variance (AMOVA) was performed using four different analyses in which subpopulations were i) combined in one hierarchical group, ii) two time periods (pre-1993 and post-1993), iii) two geographic regions (east and west), and iv) three hosts (*C. florida*, *C. nuttallii*, and *C. kousa*). The 47 SSRs with subpopulations as one hierarchical group indicated that 57.76% of the genetic variation was attributed to variations among populations and 42.24% within populations ($p < 0.001$), and an *F*st of 0.58 (Table 5). When partitioned based on time (pre-1993 and post-1993) 34.31% of variation was attributed to variations among populations but the results were insignificant ($p = 0.1$). However, 29.19% of variation was found among individuals within populations, 36.50% within

individuals ($p < 0.001$). The variance among groups relative to the total variance (*F*ct) was 0.34, the variance among subpopulations within groups (*F*sc) was 0.44, and the variance among subpopulations relative to to the total variance (*F*st) was 0.64. The third AMOVA including 47 SSRs was partitioned based on two geographic regions (eastern and western North America) and attributed 25.40% variation among populations but was not significant (p =0.6). A total of 38.47% of variation was found among individuals within populations, and 36.13% within individuals (p <0.001) (*F*ct = 0.25, *F*sc = 0.51, and *F*st = 0.64). When partitioned by host, 32.17% and 67.83% of the variation was attributed among populations and within populations, respectively (Table 5).

The analysis of molecular variance (AMOVA) for the 40 SSRs with subpopulations as one hierarchical group indicated that 58.47% of the variation was attributed among populations, 41.53% within populations (*F*st = 0.58) (Table 6). The AMOVA based on two time periods (pre-1993 and post-1993) attributed 33.76% of variation among populations (p = 0.9), 30.26% among individuals within populations, 35.98% within individuals ($p < 0.001$) (*F*ct = 0.33, *F*sc = 0.46, and *F*st = 0.64). The AMOVA partitioned based on two geographic regions (eastern and western North America) indicated that 26.53% of variation was attributed among populations (p =0.13), 38.21% among individuals within populations, 35.26% within individuals (p <0.001) (*F*ct = 0.26, *F*sc = 0.52, and *F*st = 0.64). The AMOVA partitioned by host attributed 37.04% of variation among populations and 62.96% of variation within populations (Table 6).

Pairwise population differentiation (ϕ_{PT}) using 47 SSRs (Table 7) and 40 SSRs (Table 8) indicated significant ($p < 0.05$) subpopulation differentiation except between the north and south subpopulations, and ranged from 0.45 – 0.77. Low levels of gene flow were found between all subpopulations using 47 loci (Table 9) and 40 loci (Table 10). The lowest amount of gene flow was found between the post-1993 west and pre-1993 south subpopulations (0.15) and the post-1993 west and pre-1993 north (0.16) subpopulation. The highest was detected between pre-1993 west and pre-1993 north subpopulations (0.62). Pairwise population *F*st was calculated using 47 SSRs and 40 SRRS and the results indicated that the largest differentiation was found among post-1993 west and pre-1993 south (0.77) and pre-1993 north (0.75) subpopulations. The smallest amount of differentiation as indicated by pairwise population *F*st, was found among pre- and post-1993 north and south subpopulation.

Principle coordinates analysis (PCoA) using 47 SSRs (Fig. 2) generated the same results as PCoA using 40 SSRs (Fig. 3) and indicated four distinct clusters in which the first two axes explained 52.92% of the observed variation. The pre-1993 and post-1993 west subpopulations clustered separately, whereas the post-1993 north and south subpopulations grouped together as did the pre-1993 north and south populations. One isolate from the pre-1993 western population clustered with the pre-1993 north and south group.

The UPGMA dendrograms calculated using 47 loci (Fig. 4) and 40 polymorphic loci (Fig. 5) both revealed the same pattern as shown by the PCoA. The pre-1993 west isolates formed a distinct cluster and isolates from the post-1993 west group formed a cluster. The post-1993 north and south isolates formed a single cluster and the pre-1993 north and south isolates formed a cluster. All genetic clusters were supported with bootstrap values over 72.

Genetic clustering analyses performed by STRUCTURE indicated four genetic clusters (K = 4) based on time and region of collection when using 47 SSRs (Fig. 6). The isolates from the pre-1993 north and south subpopulations formed a cluster with little admixture; the post-1993 north and south subpopulations each formed a cluster and were admixed with each other, the post-1993 west subpopulation formed a relatively homogenous cluster, and the pre-1993 west subpopulation was admixed with the post-1993 west and pre-1993 north and south clusters, with one individual assigned to the cluster shared by the pre-1993 north and south subpopulations (Fig. 7). When K = 5 was used with 47 SSRs, the post-1993 north and south subpopulations did not cluster together but formed two separate clusters (Fig. 8). Clustering analyses using 40 SSRs indicated the presence of five genetic clusters (K =5) (Fig. 9) in which the clustering assignment was the same as 47 SSRs at K = 5 (Fig. 10). When K = 4 was used with 40 SSRs the clustering assignment of subpopulations was the same as 47 SSRs at K = 4 (Fig. 11). PCoA and UPGMA results support four genetic clusters.

The program BOTTLENECK was used to determine if a recent population bottleneck or expansion occurred. The Sign tests (Tables 11 and 12), the Wilcoxon test and the standardized differences tests applied to all three mutation models, showed significant genetic diversity excess ($p < 0.01$) with 47 loci and with 40 polymorphic loci in all four genetic clusters, which indicated that a recent population bottleneck has occurred. The mode-shift indicator showed allele frequency distortion for all genetic clusters.

Linkage disequilibrium was tested and alleles were determined to be linked across loci ($p < 0.05$) in all six designated subpopulations (Fig. 12 and 13). The standardized index of association ($\overline{r}_d$) using 47 loci and 40 polymorphic loci, differed significantly from zero, supporting the hypothesis that *D. destructiva* reproduces asexually via conidia (Tables 3 and 4).

Nineteen of forty-seven primer pairs were capable of supporting DNA amplification of isolates from other *Discula* species (Table 13), although primer pair DD09 produced multiple products. An average of 6.6 loci were amplified from *D. umbrinella* isolates from *Quercus robur* (pedunculate oak) and *Fagus sylvatica* (beech), whereas only 4 loci amplified from *D. umbrinella* isolates from *Castanea* (*Ct*.) *sativa* (chestnut). An average of 2.8 loci were amplified from isolates of *D*. *querinca* from *Q. garryana* (Oregon white oak) and *C. florida, D. fraxinea* from *Fraxinus americana* (white ash), and *D. campestris* from *Acer saccharum* (sugar maple). Four primer pairs amplified loci from NC2, a *Melanconis* spp., whereas with another *Melanconis* isolate, VA17b, no loci were amplified. A mean of 5.6 *Discula* species isolates was amplified by the 19 *D. destructiva* primers. The highest number of *Discula* species isolates that *D. destructiva* primers cross-transferred to, was 14 (DD36), whereas there were several primers (DD02, DD09, DD15, DD37, and DD42) that only amplified DNA from one other *Discula* species isolate. The size of amplicons from *Discula* species isolates was generally similar to that of the *D. destructiva* isolate AS4 except those from primer pair DD32, which were almost 1.5 times larger than the amplicon from AS4 (Table 13).

4. Discussion

This SSR-based study analyzed the genetic diverisity and population structure of *D. destructiva* isolates and found the presence of low genetic diversity, low gene flow, subpopulation differentiation between geographic regions and two time periods, a recent population bottleneck, and asexual reproduction. There was very little difference between analyses performed with 47 SSRs and 40 polymorphic SSRs.

The low genetic diversity found among *D. destructiva* isolates in this study agrees with previous studies that utilized DAF (Trigiano *et al*., 1995), ASAP (Caetano-Annoles *et al.*, 1996), and AFLPs (Zhang and Blackwell, 2002). The current assessment of diversity among isolates supports the hypothesis that *D. destructiva* is an exotic species, which was recently introduced into North America (Trigiano *et al*., 1995). Low genetic diversity is commonly found in introduced species because of founder effects in which a small number of representatives or even a single clone of the organism becomes the founder(s) of a population in the new area. Higher genetic diversity would be expected if this species were native to North America (Maruyama and Furest, 1985; Nei *et al*., 1975).

The population structure of *D. destructiva* is best explained by subpopulations delimited by geographic regions and times of collection. Genetic clusters defined by time of collection (pre- and post-1993) can be explained by multiple introductions of the pathogen that occurred on each coast. Distinct genetic clusters and population differentiation separates eastern and western isolates, which corroborates the findings of other studies that used ASAP (Caetano-Annoles *et al*., 1996), AFLPs (Zhang and Blackwell, 2002), and dsRNA banding profiles (Yao *et al*., 1997), and supports the scenario of separate introductions on the east coast and west coast. The lack of gene flow between east and west coast isolates indicates that *D. destructiva* did not spread from eastern North America to western North America, or vice versa. However, a single pre-1993 western isolate, (M10 from UTK), clustered with the post-1993 eastern group, which suggests the possibility of movement between coasts. The introduction of dogwood anthracnose into Missouri and Kansas was reported to have occurred due to the movement of infected nursery stock (Daughtrey *et al*., 1996), demonstrating that trade has resulted in transporting dogwood anthracnose within North America.

The low genetic diversity found among *D. destructiva* isolates in previous studies (Caetano-Annoles *et al.*, 1996; 2001; Trigiano *et al*., 1995) has suggested that a population bottleneck, or a significant

reduction in population size, may have occurred. The observed genetic diversity (H_o) was compared to expected genetic diversity under mutation drift equilibrium (H_{EQ}). Excess genetic diversity was indicated because H_o was significantly greater than H_{EQ}, which supports the contention that the population has recently undergone a bottleneck. The distortion uncovered by the mode-shift indicator occurs when fewer alleles are present at low frequency, and shifted into intermediate frequency allele classes, which is characteristic for recent population bottlenecks (Luikart *et al*., 1998). These results support the hypothesis of a founder effect in which limited genetic diversity in a population is due to a small number of representatives from a more diverse native population being introduced into a new geographical area (Maruyama and Furest, 1985; Nei *et al*., 1975).

The standardized index of association ($\overline{r}_d$) can determine if populations reproduce asexually or sexually by examining multilocus linkage disequilibrium. Significant linkage disequilibrium ($p < 0.05$) was present among *D. destructiva* isolates due to linkage among loci indicating asexual reproduction (Kamvar et al., 2014). The low genetic diversity and asexual reproduction of *D. destructiva* is indicative of an introduced exotic pathogen (Goss, 2009).

The UPGMA strongly supported the divergence of *D. destructiva* from other *Discula* species and found similar genetic relationships to those revealed by previous studies in which eastern and western *D. destructiva* isolates were separated from each other (Caetano-Annoles *et al*., 1996; Zhang and Blackwell, 2002) and from other *Discula* species (Caetano-Annoles *et al*., 2001).

Human activity, especially globalized trade, provides a pathway for the introduction of potential pathogens and pests that can have devastating ecological, social, and financial impacts (Boyd *et al.*, 2013; Grunwald, 2012; McNeely, 2006). As international trade increases, so does the importation of new pathogens and pests into new areas and onto new hosts (Fisher *et al.*, 2012). Introduced forest pathogens account for the loss of $2.1 billion in forest products each year in the U.S., although the true environmental costs cannot be measured (Pimentel *et al.*, 2000; 2001). One of the five major drivers for the loss of biodiversity and changes in ecosystem services is invasive exotic species (MEA, 2005). Native trees are threatened mostly by introduced organisms, fungi in particular (Boyd *et al.*, 2013; Fisher *et al.*, 2012). Plant diseases can be so devastating that the impacts cannot only influence biodiversity and ecosystems, but human history as well, as in the case of the Irish potato famine caused by *Phytophthora infestans*, which resulted in millions of people either being forced to migrate or die from starvation (May

and Ristaino, 2004; McNeely, 2006). Understanding the pathways that foreign pathogens, like *D. destructiva*, are introduced is vital to the prevention of future invasions and potentially devastating consequences (Grunwald, 2012).

An exotic fungus may not exhibit virulence on its native host, but when it comes in contact with a new host, the fungus can become extremely virulent because of the lack of host resistance that develops over time through coevolution (Boyd *et al*., 2013). This could be the case with *D. destructiva* and the native dogwood species of North America (Miller *et al.,* 2016). Understanding how a new disease spreads is important to implementing preventive measures and control strategies (Boyd *et al*., 2013). Dogwood anthracnose is an example of how an introduced fungal pathogen can spread quickly over the hosts' geographic range and reach epidemic proportions. Analyzing the population structure and genetic diversity of a pathogen helps to uncover the history of the pathogen, allowing insight into its origin, how it entered the new area, and potential consequences (Vercauteren *et al*., 2010).

Redlin (1991) provided the scientific description of *D. destructiva*, which was previously known as *Discula* Type I. He noted that some fungi isolated from diseased tissues resembled *D. destructiva*, but were sufficiently different morphologically that they could not be assigned to this new species, and they were referred to as *Discula* Type II (Daughtrey and Hibben, 1994; Trigiano *et al*., 1991). Additionally, all *D. destructiva* isolates produced polyphenol oxidase, whereas the morphological variants did not (Trigiano *et al*., 1991). Sequencing of the ITS region with primers 1 and 4 or 1 and 2 (White *et al*., 1990) confirmed that the Type I isolates were *D. destructiva*, whereas the two Type II isolates, NC-2 and VA17b, were not *D. destructiva*, but most likely *Melanconis* spp., which are pathogens of birch and closely related to some other *Discula* species (Castelbury *et al*., 2002; Zhang and Blackwell, 2001). Both genera occupy the same clade in the Diaporthales, but some *Discula* species, including *D. destructiva*, are classified in the Gnomoniaceae, whereas, *Melanconis* spp. are placed in a sister family, the Melanonidaceae (Castelbury *et al*., 2002). A BLAST search using the ITS sequences from other *Discula* species included in this study confirmed their identity as *Discula*.

The test for cross-transferability of the microsatellite primer pairs revealed shared loci between *D. destructiva* and other *Discula* species indicating they were related and thus allowing their relationships to be analyzed (Dawson, *et al*., 2010; Moore *et al*., 1991). *Discula destructiva* was most related to *D. umbrinella* isolates from oak and beech, followed by *D. umbrinella* from chestnut, *D. querinca*, *D.*

fraxinea, and *D. campestris*, with the *Melanconis* spp. being more distantly related. This relationship is similar to that described in a previous study (Zhang and Blackwell, 2001), but *D. umbrinella* was not included in their analyses.

The 47 microsatellite primer pairs used in this study successfully amplified loci in *D. destructiva* and in other *Discula* species. Sufficient allelic data was produced by these primers to analyze genetic diversity, population structure, and linkage disequilibrium of *D. destructiva* isolates. *Discula destructiva* has been considered an introduced pathogen because of the sudden appearance of dogwood anthracnose near ports of entry on both the east and west coasts of North America, which rapidly spread across the distribution of native dogwoods and caused significant losses of trees. The results of this SSR based population genetics study support this scenario and provide a new evolutionary perspective that may be valuable in future analyses and models. Future population studies involving Asian specimens could elucidate the origin of *D. destructiva* and provide novel insight into the spread and distribution of this invasive pathogen..

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Appendix

Table 1. Isolates of *Discula destructiva* and *Discula* species included in this study.

Discula species	Culture No.	Collection[a]	Host species	Location[b]	Location/Year[c]
D. destructiva	AL151	UTK	*C. florida*	AL	S/1992
	AS1	UTK	*C. florida*	TN	S/2000
	AS11	UTK	*C. florida*	TN	S/2000
	AS111	UTK	*C. florida*	TN	S/2000
	AS12	UTK	*C. florida*	TN	S/2000
	AS14	UTK	*C. florida*	TN	S/2000
	AS15a[d]	UTK	*C. florida*	TN	S/2000
	AS15b[d]	UTK	*C. florida*	TN	S/2000
	AS18a	UTK	*C. florida*	TN	S/2000
	AS18b	UTK	*C. florida*	TN	S/2000
	AS22	UTK	*C. florida*	TN	S/2000
	AS32	UTK	*C. florida*	TN	S/2000
	AS37	UTK	*C. florida*	TN	S/2000
	AS39	UTK	*C. florida*	TN	S/2000
	AS4	UTK	*C. florida*	AL	S/2000
	AS51	UTK	*C. florida*	TN	S/2000
	AS58	UTK	*C. florida*	TN	S/2000
	AS85[d]	UTK	*C. florida*	TN	S/2000
	AT4	UTK	*C. florida*	VA	N/Pre-1993
	BC	UTK	*C. nuttallii*	British Columbia	W/Pre-1993
	BT4[d]	UTK	*C. florida*	TN	S/1991
	CORNU2	UTK	*C. nuttallii*	OR	W/Pre-1993
	E3c	UTK	*C. florida*	PA	N/1990
	E11[d]	UTK	*C. florida*	TN	S/1991
	E87[d]	UTK	*C. florida*	TN	S/1993
	E90[d]	UTK	*C. florida*	PA	N/1990
	E114[d]	UTK	*C. florida*	TN	S/1993

Table 1. continued.

Discula species	Culture No.	Collection[a]	Host species	Location[b]	Location/Year[c]
	E118	UTK	*C. florida*	TN	S/1989
	E124	UTK	*C. florida*	PA	N/1990
	E128	UTK	*C. florida*	PA	N/1990
	E145[d]	UTK	*C. florida*	TN	S/1991
	E170	UTK	*C. florida*	TN	S/1991
	GA1	UTK	*C. florida*	GA	S/1989
	GR325	UTK	*C. florida*	NY	N/Pre-1993
	MDRR1	RU	*C. florida*	MD	N/Pre-1993
	M10	UTK	*C. nuttallii*	OR	W/Pre-1993
	M10b	UTK	*C. nuttallii*	OR	W/Pre-1993
	M18a	UTK	*C. nuttallii*	OR	W/Pre-1993
	M28e	UTK	*C. nuttallii*	OR	W/Pre-1993
	MA18	UTK	*C. kousa*	MA	N/1989
	MAF2	RU	*C. florida*	MA	N/1989
	MAK2	RU	*C. kousa*	MA	N/1989
	MAK5	RU	*C. kousa*	MA	N/1989
	MDDR9	RU	*C. florida*	MD	N/Pre-1993
	MD1	UTK	*C. florida*	MD	N/1990
	MD1	RU	*C. florida*	MD	N/1999
	MDRR1[d]	RU	*C. florida*	MD	N/1999
	MDRR2	RU	*C. florida*	MD	N/1999
	MDRR4[d]	RU	*C. florida*	MD	N/1999
	NJ135[d]	UTK	*C. florida*	NJ	N/Pre-1993
	PA5	RU	*C. florida*	PA	N/1989
	PA129[d]	UTK	*C. florida*	PA	N/Pre-1993
	PA-031013-3	RU	*C. florida*	PA	N/Pre-1993
	PA-031013-5[d]	RU	*C. florida*	PA	N/Pre-1993

Table 1. continued.

Discula species	Culture No.	Collection[a]	Host species	Location[b]	Location/Year[c]
	PA-0308203	RU	*C. florida*	PA	N/Pre-1993
	PA-0308205	RU	*C. florida*	PA	N/Pre-1993
	PA-5	RU	*C. florida*	PA	N/1989
	RI10[d]	UTK	*C. florida*	TN	S/1991
	RI16	UTK	*C. florida*	TN	S/1991
	RI18[d]	UTK	*C. florida*	TN	S/1991
	RI20	UTK	*C. florida*	TN	S/1991
	RI21	UTK	*C. florida*	TN	S/1991
	RI5[d]	UTK	*C. florida*	TN	S/1991
	RI9	UTK	*C. florida*	TN	S/1991
	SC101	UTK	*C. florida*	SC	S/Pre-1993
	SC106	UTK	*C. florida*	SC	S/1990
	SG11	UTK	*C. florida*	TN	S/1991
	SG15	UTK	*C. florida*	TN	S/1991
	SG21	UTK	*C. florida*	TN	S/1991
	SG23	UTK	*C. florida*	TN	S/1991
	SG6	UTK	*C. florida*	TN	S/1991
	SG8	UTK	*C. florida*	TN	S/Pre-1993
	Shadow1	UTK	*C. florida*	TN	S/1991
	Shadow2	UTK	*C. florida*	TN	S/1991
	Sig.Mtn.1	UTK	*C. florida*	TN	S/1991
	TN1	UTK	*C. florida*	TN	S/1989
	TN12	UTK	*C. florida*	TN	S/Pre-1993
	TN14	UTK	*C. florida*	TN	S/Pre-1993
	TN15	UTK	*C. florida*	TN	S/Pre-1993
	TN16	UTK	*C. florida*	TN	S/Pre-1993
	TN22	UTK	*C. florida*	TN	S/Pre-1993

Table 1. continued.

Discula species	Culture No.	Collection[a]	Host species	Location[b]	Location/Year[c]
	TN24	UTK	*C. florida*	TN	S/Pre-1993
	TN8	UTK	*C. florida*	TN	S/1989
	Univ. of South2	UTK	*C. florida*	TN	S/1991
	VA161	UTK	*C. florida*	VA	N/Pre-1993
	W64	UTK	*C. nuttallii*	OR	W/1994
	W94[d]	UTK	*C. nuttallii*	WA	W/1994
	W133	UTK	*C. nuttallii*	CA	W/Pre-1993
	W145	UTK	*C. nuttallii*	CA	W/1994
	W162	UTK	*C. nuttallii*	CA	W/1994
	WAP2	RU	*C. nuttallii*	WA	W/1990
	WAP272	RU	*C. nuttallii*	WA	W/1990
	WAP31	RU	*C. nuttallii*	WA	W/1989
	WAP51	RU	*C. nuttallii*	WA	W/Pre-1993
D. umbrinella	67	OP	*Quercus robur*	Switzerland	Pre-1993
	115	OP	*Castanea* (Ct.) *sativa*	Switzerland	Pre-1993
	116	OP	*Ct. sativa*	Switzerland	Pre-1993
	221	OP	*Fagus sylvatica*	Switzerland	Pre-1993
	319	OP	*Q. robur*	Switzerland	Pre-1993
	324	OP	*Q. robur*	Switzerland	Pre-1993
	416	OP	*Ct. sativa*	Switzerland	Pre-1993
	427	OP	*Ct. sativa*	Switzerland	Pre-1993
	510	OP	*F. sylvatica*	Switzerland	Pre-1993
	518	OP	*F. sylvatica*	Switzerland	Pre-1993
	611	OP	*Q. robur*	Switzerland	Pre-1993
	617	OP	*Q. robur*	Switzerland	Pre-1993

Table 1. continued.

Discula species	Culture No.	Collection[a]	Host species	Location[b]	Location/Year[c]
	P4	OP	*F. sylvatica*	Poland	Pre-1993
	LT135	OP	*F. sylvatica*	France	Pre-1993
	LTO68	OP	*Q. robur*	Great Britian	Pre-1993
D. querinca	DQB	OP	*Q. garryana*	Switzerland	Pre-1993
	LOMtA	UT	*C. florida*	TN	1992
D. campestris	DCAMP	GS	*Acer saccharum*	WI	1989
D. fraxinea	89-32-2	GS	*Fraxinus Americana*	PA	1993
Discula-like fungus (Type II)[e]					
	VA17B	UT	*C. florida*	VA	1991
	NC2	UT	*C. florida*	NC	1991

[a] Collections from University of Tennessee, Knoxville (UTK); Rutgers University (RU); and received from Dr. Orlando Petrini (OP) Microbiology Institute and Department of Forest and Wood Sciences, s Federal Institute of Technology, ETH-Zentrum, Zurich, Switzerland (current address: POLE Pharma Consulting, Ticino, Switzerland), and Dr. Glen Stanosz (GS), University of Wisconsin, Madison.

[b] Locations use United States postal state designations unless otherwise noted.

[c] Location: S = South and N = North in Eastern United States, and W = Western North America. If year of isolation is unknown, then it is labeled as pre-1993.

[d] Determined to be clones and eliminated from final analyses.

[e] Identified as a species of *Melanconis*.

Table 2. GenBank accession numbers, primer sequences, expected allele sizes (base pairs), number of alleles, and allelic ranges (bp) of 47 SSRs used to assess genetic diversity of *Discula destructiva* isolates and transfer to other *Discula* species.

Locus	Forward (F) and Reverse (R) Primers (5'-3')	Repeat motif	Expected size (bp)	No. of alleles	Allele range (bp)
DD01	F:GGAAGTACTCAGCCTCAGCC	$(CAT)_{17}$	179	3	174-268
	R:GAGGTCTGGTGGTGTTGAGG				
DD02	F:GTTGAGTCGAGTGGTGGAGG	$(GTG)_8$	160	1	162
	R:GATTGCCCTCACCCTCATCC				
DD03	F:TGCTTGTGTTGTCCGAATGC	$(TGC)_{11}$	142	2	140-144
	R:GGCACATAGACGCGCTATCG				
DD04	F:AATAGGTGCTCAGTTGGCGG	$(TGC)_8$	151	1	150
	R:AACGTCGACAGCCTTCTACG				
DD05	F:TTAAAGGATCGCATCGTGGC	$(TGC)_{13}$	196	4	115-170
	R:AACTCAAATGCAGCAAGCCG				
DD06	F:CATGATCATCTTCGCCGTCG	$(CCA)_8$	193	1	197
	R:CAGGTTGAGCGCATGATGC				
DD07	F:ATCCCGGCTATGCTCTTTCG	$(TTG)_8$	185	2	185-188
	R:AGGAGGATGGTGGCAATAGC				
DD08	F:CTAGCTCAGGATCAGCGACG	$(GAG)_7$	135	2	136-139
	R:AGGAGTACGAACGTGAACGC				
DD09	F:CCTGGCACTCTCTCGATTTCC	$(CCG)_8$	160	1	161
	R:ATGATCATGACCGGTCTCGC				
DD10	F:TCCTCATCCATTCGTTGCGG	$(TGT)_7$	155	2	149-154
	R:GCTCAAAGTACATCAACAGACTCC				
DD11	F:CATCCTCGACTCTGATGGCC	$(CCA)_7$	100	1	102
	R:CGGTGCCATGACATAACTGC				

Table 2. continued.

Locus	Forward (F) and Reverse (R) Primers (5'-3')	Repeat motif	Expected size (bp)	No. of alleles	Allele range (bp)
DD12	F:ATCAAGAGTCCACCCACTCG	$(GGT)_7$	162	2	161-164
	R:TGGACAGATTGGGTGAGTGC				
DD13	F:CGACCAGAACCATGACCACC	$(CCT)_9$	173	3	178-186
	R:CCTTCTCTGGTCTACCTGGC				
DD14	F:ACAGTTGAGGTCGTAAGCGG	$(TGG)_8$	194	2	191-196
	R:AGCAGCTCCAGAAGAACACC				
DD15	F:CACTCAACAACAGCGACACC	$(GCA)_9$	148	3	129-153
	R:TGCGGAGTGTCATATGAGGC				
DD16	F:AAACCCAAACGACAATGCCG	$(CAC)_8$	192	2	192-197
	R:ATTAGCTGGGCCGCTGTTGG				
DD17	F:TCTTGCGCGGTAATGTCTCC	$(CTA)_{16}$	198	4	162-182
	R:ACCTGTAAACAAGATGAACGCC				
DD18	F:GTTGCGCCGTTTGTAGTGC	$(CAG)_{15}$	175	2	164-176
	R:CTTCGAATCGCACGTCTTCG				
DD19	F:GTGCTGCTGTTGACTTGTGG	$(TGC)_7$	166	2	163-166
	R:GTAGCCTCTCCGAATCAGCC				
DD20	F:TTTGCTGAAGGAGGTACGGC	$(TGG)_{11}$	148	2	146-149
	R:GCATCAGCATCAGCATCAGC				
DD22	F:CTTGGTGGGCTGTTTGTGC	$(GGT)_7$	191	2	192-197
	R:AGACGACAACACCAGCATCG				
DD23	F:AGAAGCTGTCAAGAGGCACC	$(ACC)_{12}$	197	4	184-200
	R:AAGCGGAGTTCTGAGACAGG				
DD24	F:CAGCTTACAACAGGTCAAGGC	$(AGC)_7$	187	5	176-193
	R:GTAGAGAGAGGATCCTGCGG				

Table 2. continued.

Locus	Forward (F) and Reverse (R) Primers (5'-3')	Repeat motif	Expected size (bp)	No. of alleles	Allele range (bp)
DD25	F:TCAAACTCAGACTCGCTGCC	$(GAG)_7$	181	3	179-185
	R:GAACCAGGTCTCCAAGCAGC				
DD26	F:AGTCGCTCTTCTCAACGAGG	$(CAG)_9$	185	3	179-189
	R:CTGAATTGAGCAGCGGCAGC				
DD27	F:GTCTCTGACTGAACCTCCGC	$(ATT)_9$	158	2	150-156
	R:GAAGGACGATGCTCTCTCGG				
DD28	F:AATAAACAGCACACACCGCC	$(AGA)_9$	185	3	176-185
	R:ATTGTTTGGTTGATGGCGGG				
DD29	F:CGATGCCAGCGGTTTAAACG	$(AGC)_7$	124	2	123-126
	R:GCAGACGCTCATGATTTCCC				
DD30	G F:CATTGTACTCAGAGGCCCG	$(TGC)_7$	200	2	199-203
	R:CAGATCAACAACTGCCAGGC				
DD31	G F:TGAAGACGGTTGACTGTGC	$(AG)_{10}$	165	2	163-167
	R:CATTGAGAATCTGCTGCACCG				
DD32	F:TCACATGAAGGAGACGAGCG	$(AG)_9$	160	2	157-160
	R:CTCTTTCACCACCTCCTCCC				
DD33	F:CCAAGGGTAGATGGTCAAGCC	$(TC)_{11}$	187	2	184-187
	R:AGGAAGCAAAGGGAGGATTGG				
DD34	F:GAAAGACACTGCACAAGCCG	$(GT)_9$	197	2	197-200
	R:CCTGGAGAGCAGAACAGTGG				
DD35	F:ATACCAGCTTCAGCCCATGG	$(AC)_{11}$	193	2	193-196
	R:CTCTGTGTTGGTGTATGCGC				
DD36	F:TACACTCACCAAGCATCGCC	$(TC)_{12}$	196	2	195-200
	R:AGGCCTGGTAAGCAAGTTGG				

Table 2. continued.

Locus	Forward (F) and Reverse (R) Primers (5'-3')	Repeat motif	Expected size (bp)	No. of alleles	Allele range (bp)
DD37	F:GTCACCAGGAATAGGACCGC	$(AC)_{15}$	159	2	160-163
	R:GGATGACCTGGGACTCTTGC				
DD39	F:TTTGATCATTCTCGGCCGCC	$(CT)_9$	127	1	127
	R:AGAGTCATCGCATGGTTCCG				
DD40	F:AGGCTTGCCTAATCGAAGCG	$(TA)_8$	159	2	159-164
	R:ACAAAGGAGCTGCTTGTAGC				
DD41	F:AGACCTTGATCGGAGACAGC	$(TC)_{10}$	136	1	136
	R:CATGATTGGCTTTCGGCTGG				
DD42	F:AATGGATTCCTGTCGCTCGG	$(TA)_{18}$	149	3	146-152
	R:GAGCGAGCGAGTGCATATCC				
DD43	F:TTCCACCATGCAATGCAACC	$(AC)_{12}$	110	2	108-113
	R:GAAGAGCCCGAGTTGTTTGC				
DD44	F:CGAACGTTGCTGTATGTGCC	$(AG)_{10}$	137	2	136-141
	R:AGTGTTCCGAGTTGTACCGG				
DD45	F:TCCTAGGCACTTTGATGGCG	$(CA)_8$	142	3	140-148
	R:CTCGGTCAACGGCATAGTGG				
DD47	F:CTTGTTGGGTTGCGAAACGG	$(TG)_{10}$	187	2	188-193
	R:GGCCGACTGTGATATCACCG				
DD48	F:AGGTATGCTCACTATGGCGCT	$(GA)_{13}$	169	2	167-172
	R:TGATGGTGTTCTCGGTCCG				
DD49	F:ATGTCAAGGTGAGTCAGGCG	$(TC)_{11}$	154	3	154-160
	R:CAATGGCTTCCACCAATGGC				
DD50	F:ATGGCTGTCATGCACAATCC	$(TA)_8$	177	2	175-178
	R:TGTTGTCGTGGATGGATGGG				

Table 3. Diversity metrics of 69 *Discula destructiva* isolates from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993) using 47 SSRs.

Subpopulation	N[a]	MLG[b]	Na[c]	Ne[d]	H[e]	E.5[f]	Pa[g]	Hexp[h]	$\overline{r}_d$[i]	*p* value
Pre-1993 North	6	6	1.45	1.32	1.79	1.00	0	0.21	0.05	$p = 0.013$
Pre-1993 South	12	12	1.49	1.26	2.48	1.00	2	0.17	0.05	$p = 0.016$
Pre-1993 West	6	6	1.68	1.37	1.79	1.00	2	0.26	0.21	$p < 0.001$
Post-1993 North	19	14	1.43	1.29	2.64	1.00	2	0.17	0.24	$p < 0.001$
Post-1993 South	41	29	1.53	1.24	3.37	1.00	6	0.15	0.16	$p < 0.001$
Post-1993 West	9	8	1.21	1.13	2.08	1.00	3	0.09	0.08	$p = 0.027$

[a] N - total number of isolates.

[b] MLG - number of multi locus genotypes observed after clone correction.

[c] Na - number of different alleles.

[d] Ne - number of effective alleles.

[e] H - Shannon-Wiener index of MLG diversity.

[f] E.5 - evenness.

[g] Pa - number of private alleles in each population.

[h] Hexp - Nei's genotypic diversity corrected for sample size.

[i] $\overline{r}_d$ - standardized index of association.

Table 4. Diversity metrics of 69 *Discula destructiva* isolates from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993)using 40 SSRs.

Subpopulation	N[a]	MLG[b]	Na[c]	Ne[d]	H[e]	E.5[f]	Pa[g]	Hexp[h]	$\overline{r}_d$[i]	*p* value
Pre-1993 North	6	6	1.53	1.37	1.79	1.00	0	0.25	0.05	$p = 0.018$
Pre-1993 South	12	12	1.58	1.31	2.48	1.00	2	0.20	0.05	$p = 0.016$
Pre-1993 West	6	6	1.80	1.43	1.79	1.00	2	0.31	0.21	$p < 0.001$
Post-1993 North	19	14	1.50	1.34	2.64	1.00	2	0.20	0.24	$p < 0.001$
Post-1993 South	41	29	1.63	1.28	3.37	1.00	6	0.17	0.16	$p < 0.001$
Post-1993 West	9	8	1.25	1.16	2.08	1.00	3	0.11	0.08	$p = 0.032$

[a] N - total number of isolates.

[b] MLG - number of multi locus genotypes observed after clone correction.

[c] Na - number of different alleles.

[d] Ne - number of effective alleles.

[e] H - Shannon-Wiener index of MLG diversity.

[f] E.5 - evenness.

[g] Pa - number of private alleles in each population.

[h] Hexp - Nei's genotypic diversity corrected for sample size.

[i] $\overline{r}_d$ - standardized index of association.

Table 5. Analysis of molecular variance (AMOVA) for 69 *Discula destructiva* isolates across 47 SSRs. Three analyses were conducted: the first included all subpopulations as one hierarchical group (i), the second was based on two time periods- pre-1993 and post-1993 (ii), partitioned based on two geographic regions- eastern and western North America (iii), and partitioned based on host- *C. florida, C. nuttallii,* and *C. kousa*.

Variance Partition	Degrees of Freedom	Sum of Squares	Variance Component	% of Variation	*p* value
(i)					
Among populations	5	243.99	4.41	58.47	$p < 0.001$
Within populations	63	197.155	3.13	41.53	$p < 0.001$
Total	68	441.15	7.54		
*F*st = 0.58					
(ii)					
Among populations	1	130.21	2.94	33.76	$p = 0.09$
Among individuals within populations	4	113.78	2.63	30.26	$p < 0.001$
Within individuals	63	197.16	3.13	35.98	$p < 0.001$
Total	68	441.15	8.7		
*F*ct = 0.33, *F*sc = 0.46, *F*st = 0.64					
(iii)					
Among populations	1	88.58	2.35	26.53	$p = 0.13$
Among individuals within populations	4	155.41	3.39	38.21	$p < 0.001$
Within individuals	63	197.16	3.13	35.26	$p < 0.001$
Total	68	441.15	8.88		
*F*ct = 0.26, *F*sc = 0.52, *F*st = 0.64					

Table 5. continued.

Variance Partition	Degrees of Freedom	Sum of Squares	Variance Component	% of Variation	*p* value
(iv)					
Among populations	2	72.91	2.46	32.17	$p < 0.001$
Within populations	66	342.67	5.19	67.83	$p < 0.001$
Total	68	415.59	7.65		
*F*st = 0.32					

[a] *F*st - the variance among subpopulations relative to the total variance.

[b] *F*ct - the variance among groups relative to the total variance.

[c] *F*sc - the variance among subpopulations within groups.

Table 6. Analysis of molecular variance (AMOVA) for 69 *Discula destructiva* isolates across 40 SSRs. Three analyses were conducted: the first included all subpopulations as one hierarchical group (i), the second was based on two time periods- pre-1993 and post-1993 (ii), partitioned based on two geographic regions- eastern and western North America (iii), and partitioned based on host- *C. florida, C. nuttallii,* and *C. kousa*.

Variance Partition	Degrees of Freedom	Sum of Squares	Variance Component	% of Variation	*p* value
(i)					
Among populations	5	243.99	4.41	58.47	$p < 0.001$
Within populations	63	197.155	3.13	41.53	$p < 0.001$
Total	68	441.15	7.54		
*F*st = 0.58					
(ii)					
Among populations	1	130.21	2.94	33.76	$p = 0.09$
Among individuals within populations	4	113.78	2.63	30.26	$p < 0.001$
Within individuals	63	197.16	3.13	35.98	$p < 0.001$
Total	68	441.15	8.7		
*F*ct = 0.33, *F*sc = 0.46, *F*st = 0.64					
(iii)					
Among populations	1	88.58	2.35	26.53	$p = 0.13$
Among individuals within populations	4	155.41	3.39	38.21	$p < 0.001$
Within individuals	63	197.16	3.13	35.26	$p < 0.001$
Total	68	441.15	8.88		
*F*ct = 0.26, *F*sc = 0.52, *F*st = 0.64					

Table 6. continued.

Variance Partition	Degrees of Freedom	Sum of Squares	Variance Component	% of Variation	*p* value
(iv)					
Among populations	2	91.93	3.19	37.04	$p < 0.001$
Within populations	66	358.19	5.43	62.96	$p < 0.001$
Total	68	450.12	8.62		
*F*st = 0.37					

[a] *F*st - the variance among subpopulations relative to the total variance.

[b] *F*ct - the variance among groups relative to the total variance.

[c] *F*sc - the variance among subpopulations within groups.

Table 7. Pairwise population differentiation (ϕ_{PT}) (analogous to standardized *F*st for haploid data) for 69 *Discula destructiva* isolates from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993)using 47 SSRs.

	Pre-1993 North	Pre-1993 South	Pre-1993 West	Post-1993 North	Post-1993 South	Post-1993 West
Pre-1993 North	-	0.40	0.01	0.00	0.00	0.00
Pre-1993 South	0.00	-	0.00	0.00	0.00	0.00
Pre-1993 West	0.45	0.50	-	0.00	0.00	0.00
Post-1993 North	0.45	0.52	0.56	-	0.36	0.00
Post-1993 South	0.56	0.59	0.66	0.00	-	0.00
Post-1993 West	0.75	0.77	0.59	0.64	0.64	-

*F*st values below diagonal and probability based on 10,000 permutations is shown above diagonal.

Table 8. Pairwise population differentiation (ϕ_{PT}) (analogous to standardized *F*st for haploid data) for 69 *Discula destructiva* isolates from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993)using 40 SSRs.

	Pre-1993 North	Pre-1993 South	Pre-1993 West	Post-1993 North	Post-1993 South	Post-1993 West
Pre-1993 North	-	0.40	0.01	0.00	0.00	0.00
Pre-1993 South	0.00	-	0.00	0.00	0.00	0.00
Pre-1993 West	0.45	0.50	-	0.00	0.00	0.00
Post-1993 North	0.45	0.52	0.56	-	0.35	0.00
Post-1993 South	0.56	0.59	0.66	0.00	-	0.00
Post-1993 West	0.75	0.77	0.59	0.64	0.64	-

*F*st values below diagonal and probability based on 10,000 permutations is shown above diagonal.

Table 9. Pairwise gene flow values for 69 *Discula destructiva isolates* from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993)using 47 SSRs.

	Pre-1993 North	Pre-1993 South	Pre-1993 West	Post-1993 North	Post-1993 South	Post-1993 West
Pre-1993 North	-					
Pre-1993 South	0.00	-				
Pre-1993 West	0.62	0.51	-			
Post-1993 North	0.60	0.47	0.39	-		
Post-1993 South	0.40	0.35	0.26	0.00	-	
Post-1993 West	0.16	0.15	0.34	0.28	0.29	-

Table 10. Pairwise gene flow values for 69 *Discula destructiva* isolates from three geographic regions (north, south, and west)and two time periods (pre-1993 and post-1993)using 40 SSRs.

	Pre-1993 North	Pre-1993 South	Pre-1993 West	Post-1993 North	Post-1993 South	Post-1993 West
Pre-1993 North	0.00					
Pre-1993 South	0.00	0.00				
Pre-1993 West	0.62	0.51	0.00			
Post-1993 North	0.61	0.47	0.39	0.00		
Post-1993 South	0.40	0.35	0.26	0.00	0.00	
Post-1993 West	0.16	0.15	0.34	0.28	0.29	0.00

Table 11. Bottleneck determination by sign tests for 69 *Discula destructiva* isolates using 47 SSRs and grouped by the four genetic clusters as described by STRUCTURE.

	Mutation models[a]			
Cluster	I.A.M	T.P.M.	S.M.M	*p* value
Pre-1993 East[b]	47/0[c]	47/0	46/1	$p < 0.01$
Pre-1993 West	45/2	45/2	43/4	$p < 0.01$
Post-1993 East[b]	47/0	47/0	45/2	$p < 0.01$
Post-1993 West	46/0	46/0	46/0	$p < 0.01$

[a] I.A.M. = infinite allele model; T.P.M. = two-phase mutation models; S.M.M. = stepwise mutation model.

[b] East includes both north and south subpopulations.

[c] Number of alleles in excess/deficit.

Table 12. Bottleneck determination by sign tests for 69 *Discula destructiva* isolates using 40 SSRs and grouped by the four genetic clusters as described by STRUCTURE.

	Mutation models[a]			
Cluster	I.A.M.	T.P.M.	S.M.M.	*p* value
Pre-1993 East[b]	40/0[c]	40/0	39/1	$p < 0.01$
Pre-1993 West	38/2	38/2	36/4	$p < 0.01$
Post-1993 East[b]	40/0	40/0	38/2	$p < 0.01$
Post-1993 West	39/0	39/0	39/0	$p < 0.01$

[a] I.A.M. = infinite allele model; T.P.M. = two-phase mutation models; S.M.M. = stepwise mutation model.

[b] East includes both north and south subpopulations.

[c] Number of alleles in excess/deficit.

Table 13. Cross-transfer of loci in *Discula destructiva* to isolates of other *Discula* or *Discula*-like species.

	Primer[a]									
	DD02	DD04	DD05	DD06	DD08	DD09[c]	DD13	DD15	DD22	DD25
Species/isolate	Amplicon Size[b]									
Discula umbrinella										
67	--[b]	154	--	--	136	--	185	--	--	162
115	--	154	--	--	--	--	--	--	--	--
116	--	--	--	204	136	--	--	--	--	--
221	--	--	--	--	136	--	--	163	--	162
319	--	--	148	--	139	--	--	--	--	162
324	--	152	--	--		--	--	192	--	162
416	--	152	--	--	136	--	--	--	--	--
427	--	--	--	--	143	--	--	--	--	--
510	--	--	--	202	--	--	--	--	--	162
518	--	--	--	--	--	--	--	147	--	162
611	--	--	--	171	--	--	--	173	--	162
DU617	--	152	--	--	--	--	--	--	--	162
DUP4	--	--	--	--	--	--	--	--	--	162
LT135	--	--	--	--	--	--	--	--	166	162
LT068	--	--	--	--	--	--	--	163	--	162
D. quercina										
DQB	161	150	--	--	--	--	--	--	--	--
LoMTA	--	--	166	--	139	--	--	--	--	--
D. campestris	--	--	--	--	--	--	--	--	--	--
D. fraxinea	--	--	--	--	136	--	--	--	--	--
Melanconis										
VA17b	--	--	--	--	--	--	--	--	--	--
NC2	161	--	--	--	--	--	--	--	--	181
D. destructiva										

Table 13. continued.

	Primer[a]									
	DD02	DD04	DD05	DD06	DD08	DD09[c]	DD13	DD15	DD22	DD25
Species/isolate	Amplicon Size[b]									
AS4 (control)	161	150	166	197	136	162	185	147	193	181

	Primer[a]								
	DD26	DD30	DD32	DD36	DD37	DD42	DD47	DD48	DD49
Species/isolate	Amplicon Size[b]								
Discula umbrinella									
67	169	--	382	190	163	--	--	--	--
115	--	205	--	--	--	--	188	--	159
116	--	205	--	--	--	--	188	--	159
221	169	205	382	190	--	--	--	--	164
319	169	--	382	190	--	--	--	--	164
324	169	--	382	190	--	--	--	--	164
416	--	--	--	--	--	--	--	--	157
427	--	205	--	--	--	--	188	--	157
510	169	--	382	144	--	--	196	--	164
518	169	--	382	144	--	--	--	172	164
611	169	--	382	190	--	--	--	--	164
DU617	169	--	382	197	--	--	--	--	164
DUP4	169	--	382	190	--	--	--	--	164
LT135	169	--	382	--	--	--	--	--	164
LT068	180	--	382	190	--	--	--	--	--
D. querinca									
DQB	--	--	--	245	--	--	--	--	--
LoMTA	--	--	--	--	--	--	--	--	--

Table 13. continued.

	Primer[a]								
	DD26	DD30	DD32	DD36	DD37	DD42	DD47	DD48	DD49
D. campestris	--	--	--	190	--	--	--	163	--
D. fraxinea	--	--	--	199	--	--	--	--	--
Melanconis spp.									
VA17b	--	--	--	--	--	--	--	--	--
NC2	--	--	--	190	--	143	--	--	--
D. destructiva									
AS4 (control)	186	201	161	197	163	150	192	172	157

[a] Primer codes are located in Table 1.

[b] Numbers equal amplicon size in base pairs; -- = no amplification.

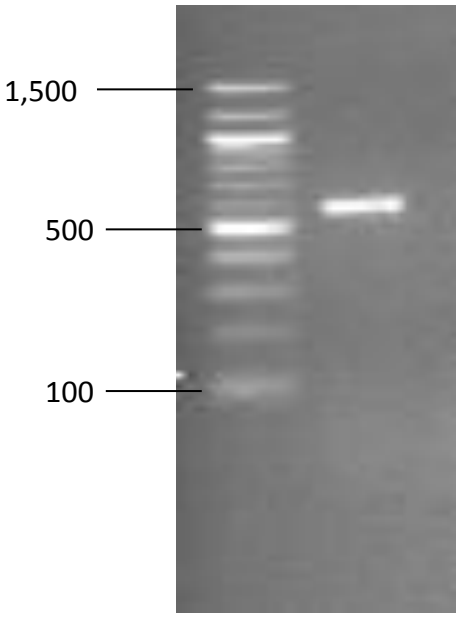

Figure 1. Internally transcribed spacer region of approximately 550 base pairs as visualized in a 2% agarose gel.

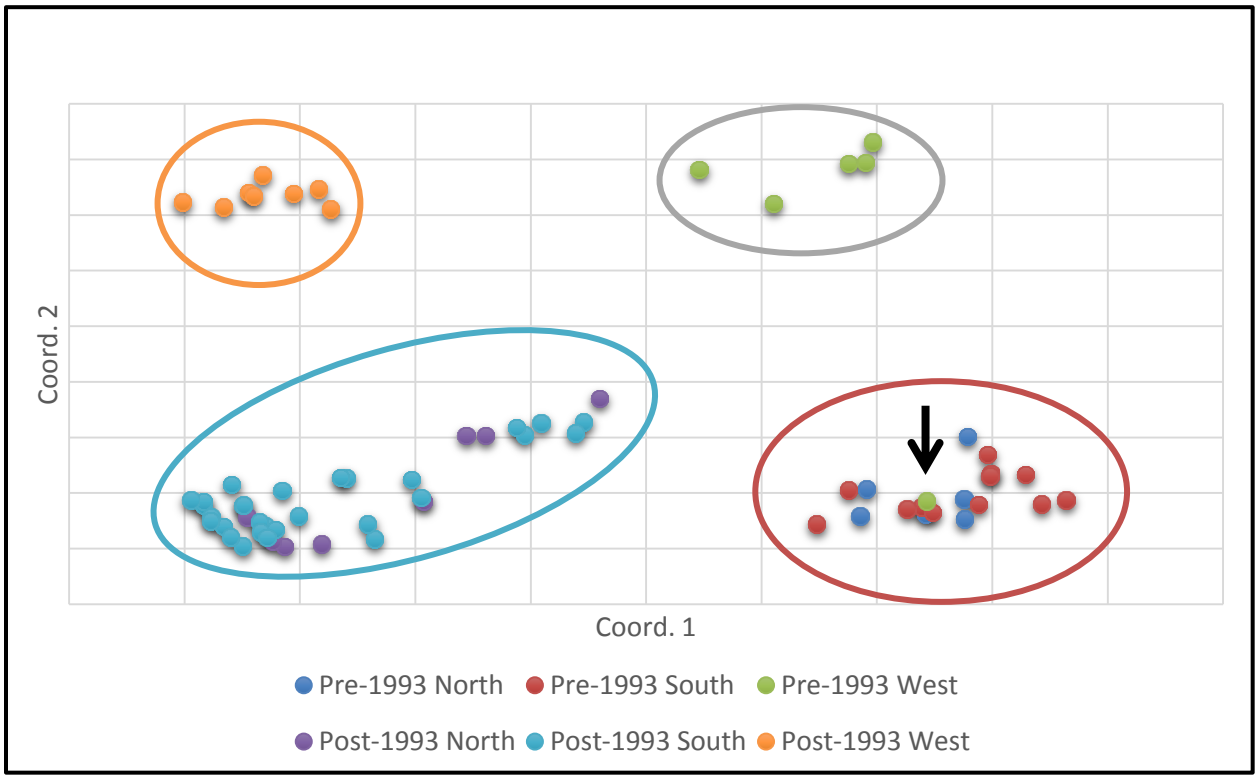

Figure 2. Principle coordinates analysis (PCoA) observed among 69 *Discula destructiva* isolates from three geographic regions and two time periods using 47 SSRs. The first two axes explain 52.92% of the observed variation.

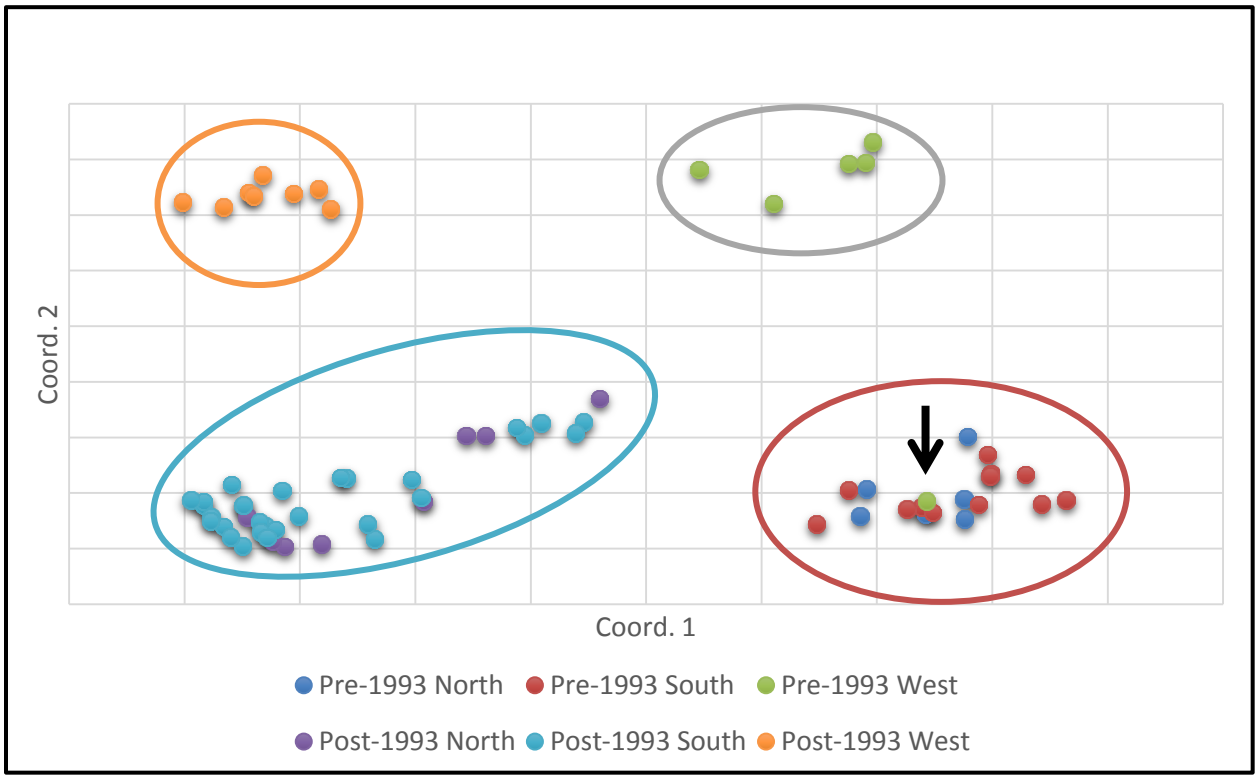

Figure 3. Principle coordinates analysis (PCoA) observed among 69 *Discula destructiva* isolates from three geographic regions and two time periods using 40 SSRs. The first two axes explain 52.92% of the observed variation.

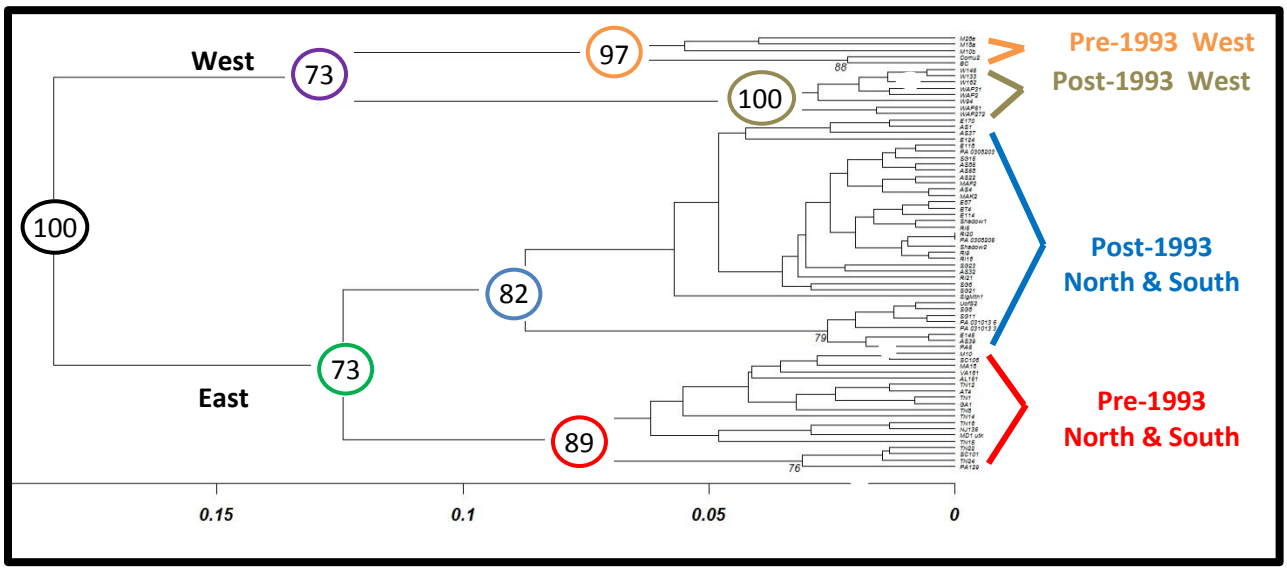

Figure 4. UPGMA dendrogram of 69 *Discula destructiva* isolates collected from three geographic regions and two time periods, using 47 SSRs revealing four distinct groups.

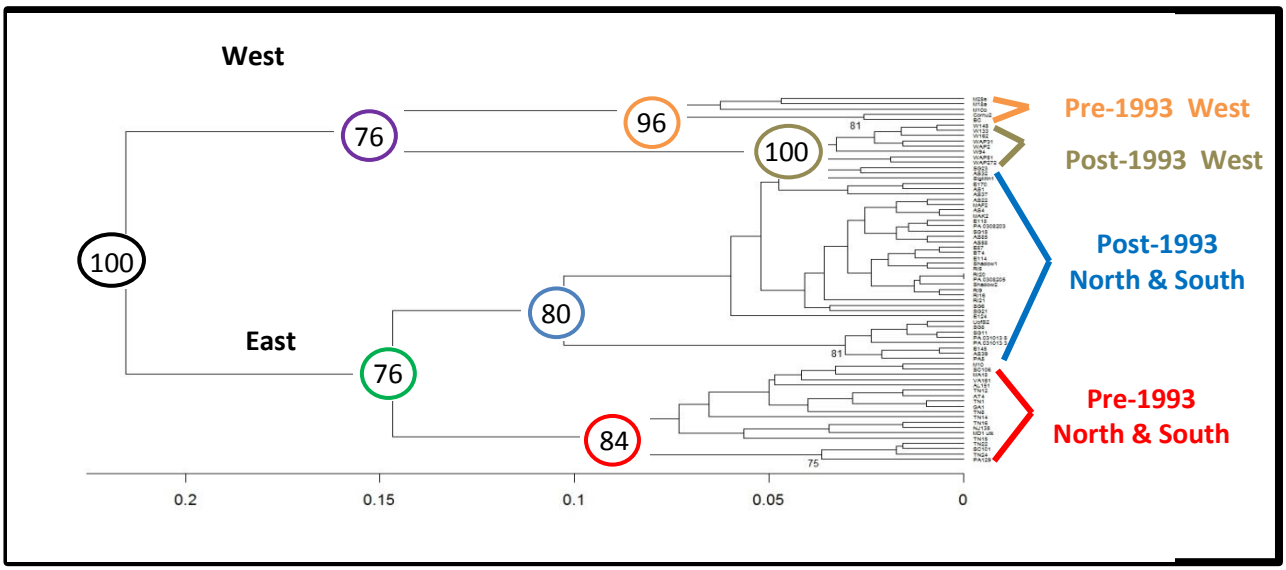

Figure 5. UPGMA dendrogram of 69 *Discula destructiva* isolates collected from three geographic regions and two time periods, using 40 SSRs revealing four distinct groups.

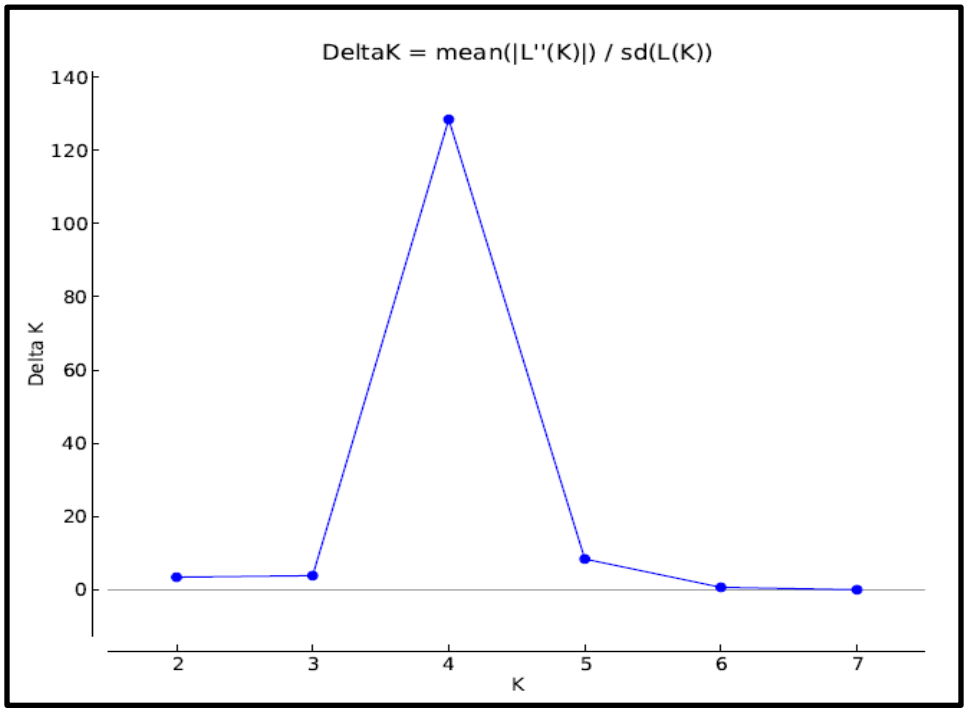

Figure 6. Maximum ΔK at K = 4 for 69 *Discula destructiva* isolates using 47 SSRs.

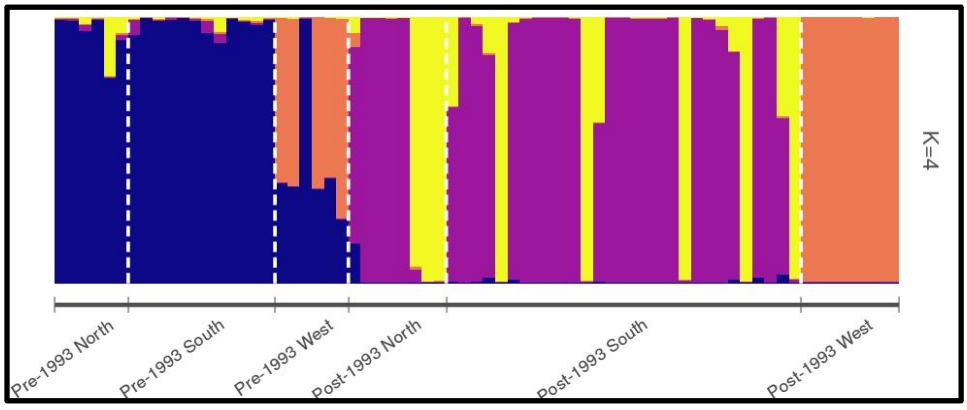

Figure 7. Structure bar graph representing four genetic clusters of 69 *Discula destructiva* isolates from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993) using 47 SSRs.

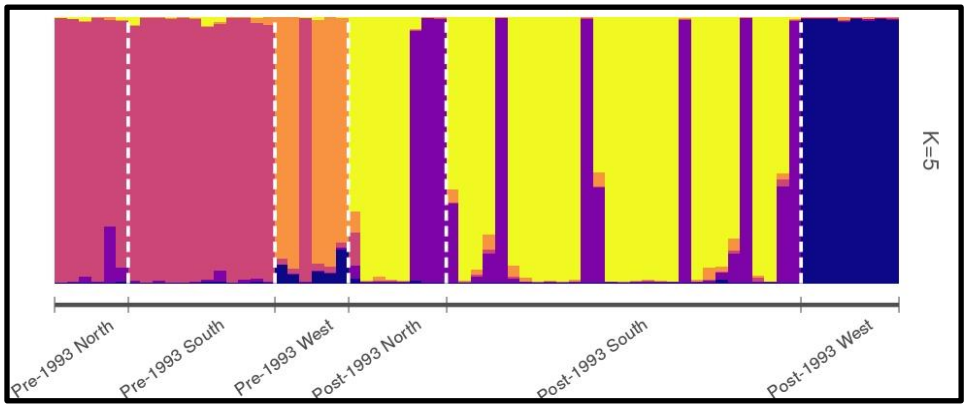

Figure 8. Structure bar graph representing five genetic clusters of 69 *Discula destructiva* isolates from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993) using 47 SSRs.

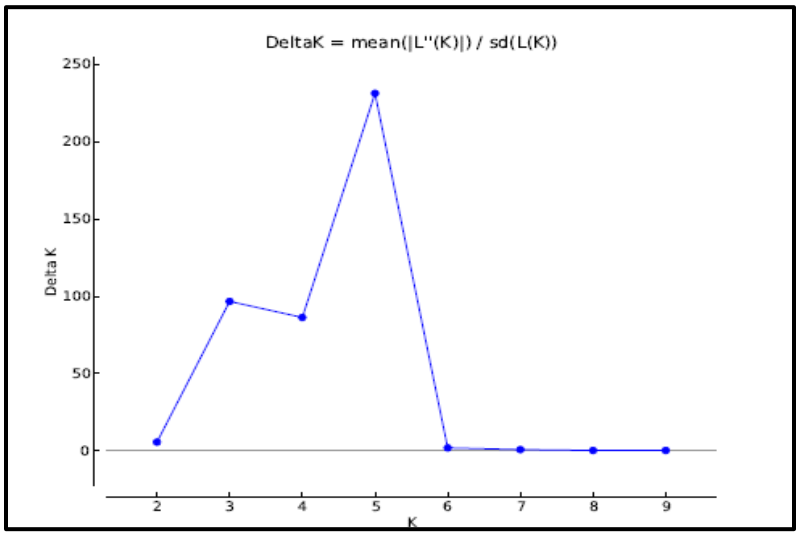

Figure 9. Maximum ΔK at K = 5 for 69 *Discula destructiva* isolates using 40 SSRs.

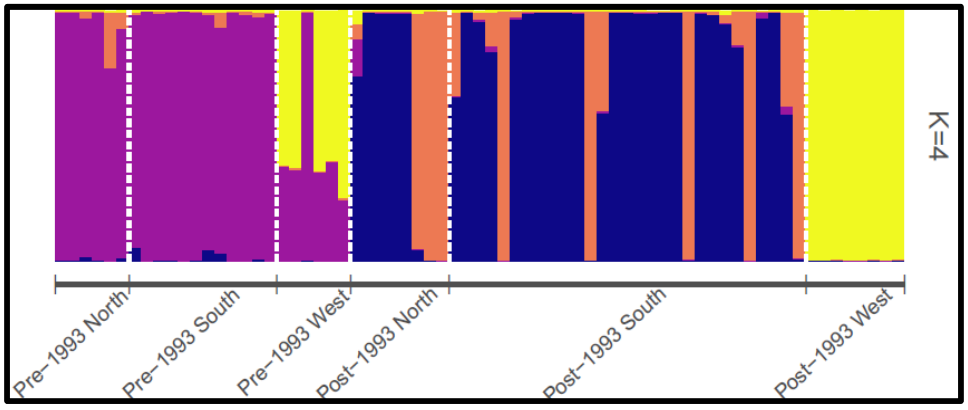

Figure 10. Structure bar graph representing four genetic clusters of 69 *Discula destructiva* isolates from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993) using 40 SSRs.

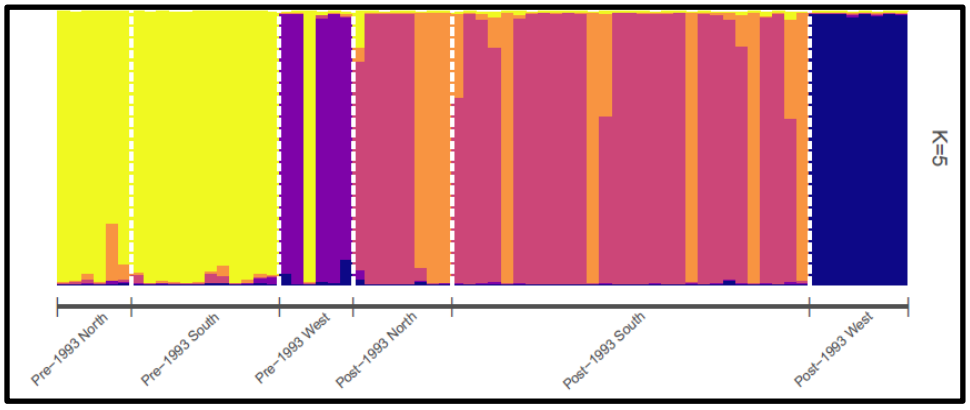

Figure 11. Structure bar graph representing five genetic clusters of 69 *Discula destructiva* isolates from three geographic regions (north, south, and west) and two time periods (pre-1993 and post-1993) using 40 SSRs.

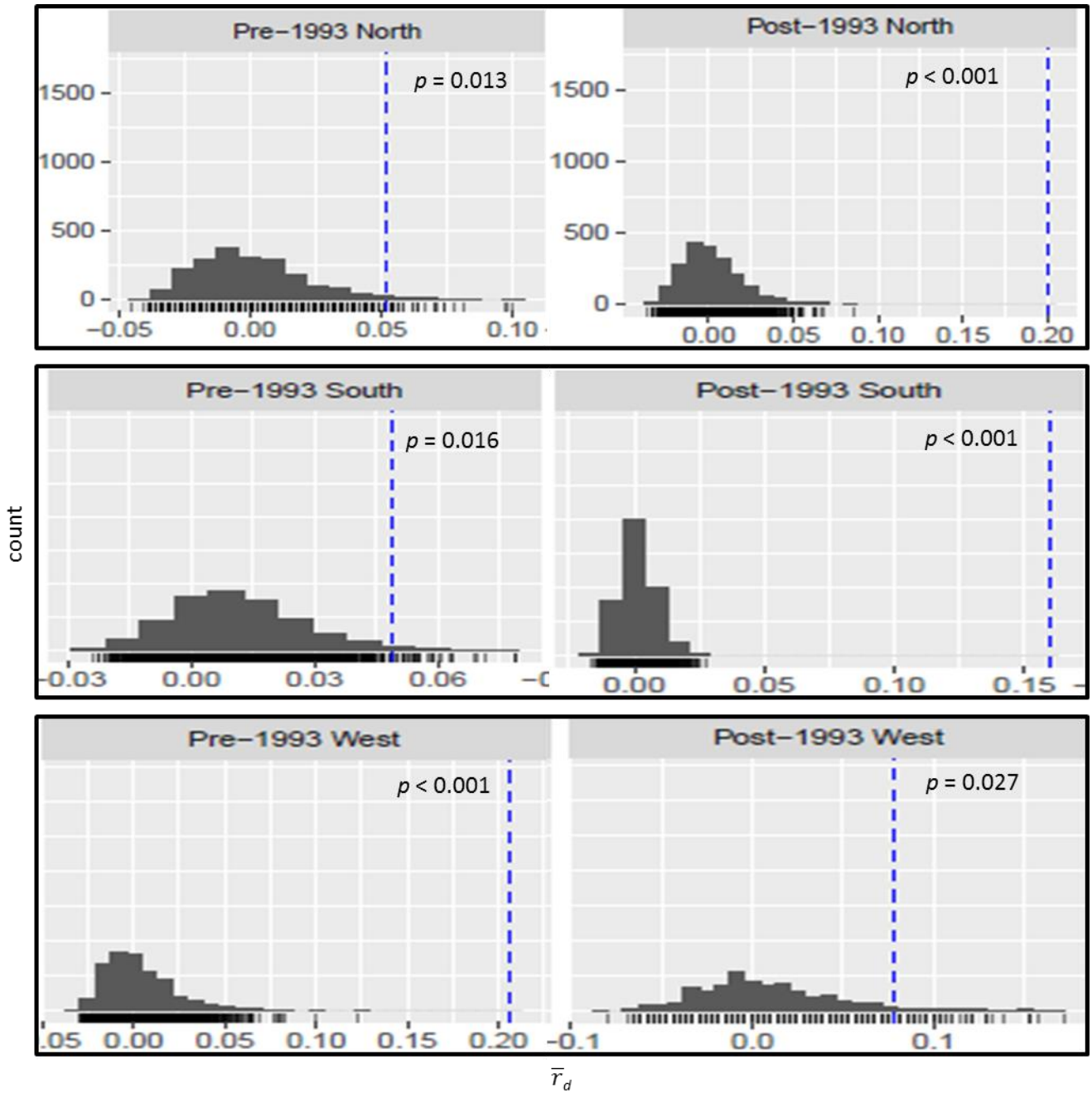

Figure 12. Visualization of linkage disequilibrium tests of 69 *Discula destructiva* isolates using 47 SSRs.

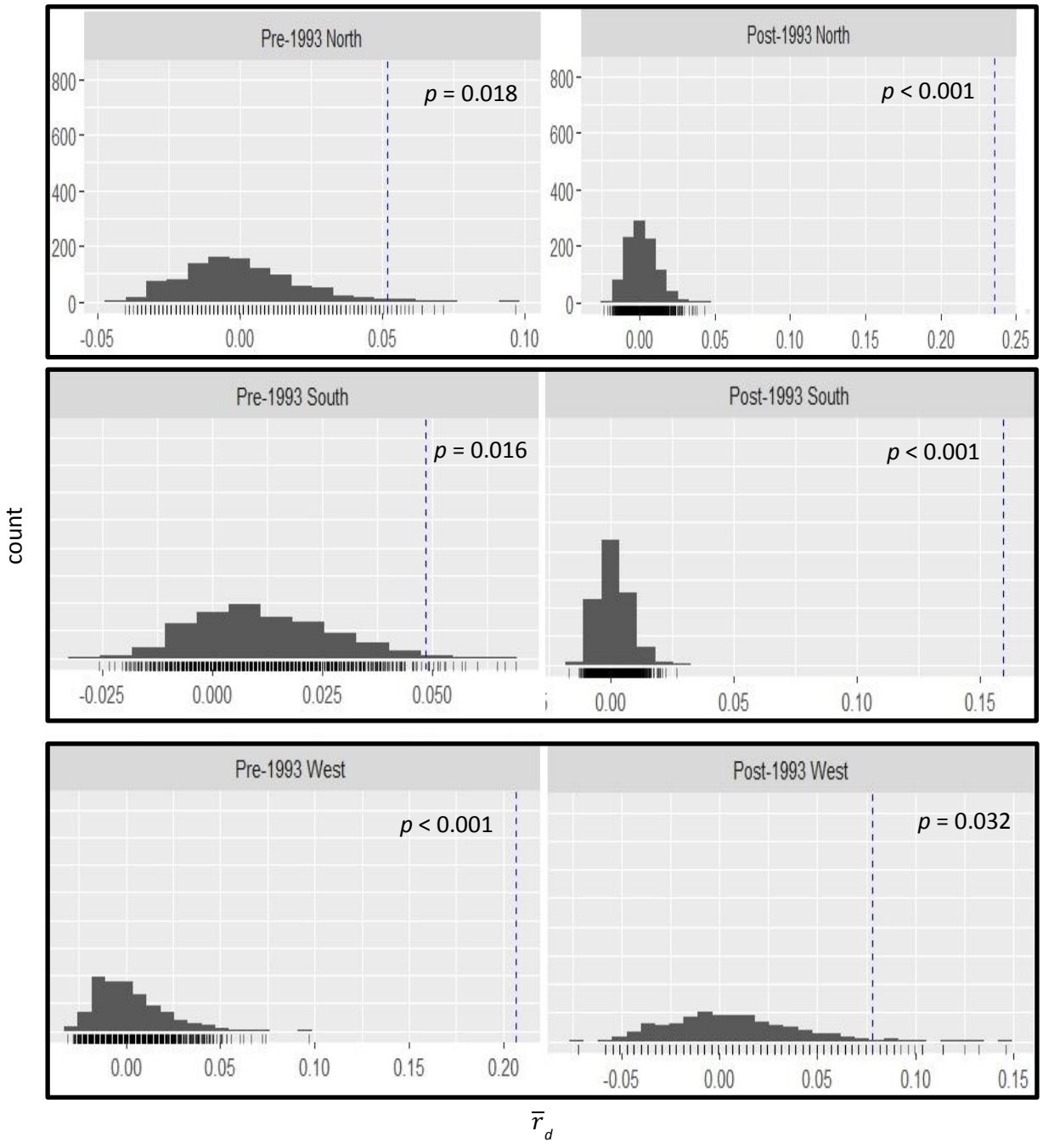

Figure 13. Visualization of linkage disequilibrium tests of 69 *Discula destructiva* isolates using 40 SSRs.

Vita

Kristie Mantooth graduated *summa cum laude* from the University of Tennessee at Knoxville in 2012. She was awarded a Bachelor of Science degree in Plant Science with a concentration in Plant Biotechnology and received the Top Collegiate Graduate Award in the College of Agriculture and Natural Resources. She then joined the Department of Entomology and Plant Pathology as a graduate student where she continued to pursue her educational goals and scientific interests.