

**Genetic characterization and population structure of *Erysiphe pulchra*
the causal agent of powdery mildew on *Cornus florida* in the eastern
United States**

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Master of Science
Degree
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DEDICATION

I would like to dedicate this work to my loving wife, Lindsey Wyman, for her patience and steadfast support.

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I am sincerely grateful to my advisor, Dr. Robert N. Trigiano, for the opportunities I have had under his mentorship and guidance. Thank you for investing in my future.

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ABSTRACT

Cornus florida L. (flowering dogwood) is a deciduous understory tree endemic to the eastern United States. Known for its attractive bracts, *C. florida* is very popular for its ornamental qualities. After 1995, dogwood powdery mildew caused by *Erysiphe pulchra* Cooke & Peck reached epidemic levels throughout the *C. florida* growing region. Initially, both sexual and asexual stages of *E. pulchra* were regularly observed, but in recent years, the teleomorph has not been seen as often. Fifteen microsatellite loci were used to analyze the genetic diversity and population structure of 174 *E. pulchra* samples from 10 eastern states. The results of this study indicated low genetic diversity, lack of definitive population structure, and significant linkage disequilibrium within the sampled population. Evidence of a recent bottleneck was also observed. Our results suggest that *E. pulchra* has become clonal in eastern United States and may be an exotic plant pathogen to North America.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	1
Flowering dogwood	2
Dogwood powdery mildew	4
CHAPTER 2: GENETIC CHARACTERIZATION OF ERYSIPHE PULCHRA REVEALS LOW DIVERSITY IN THE PATHOGEN OF CORNUS FLORIDA.....	8
Abstract	9
Introduction	10
Materials and Methods	13
Sample collection and DNA extraction	13
Microsatellite development and selection	14
Genetic diversity	15
Population structure	16
Demography.....	17
Results	18
Microsatellite development and selection.....	18
Genetic diversity indices	18
Population structure	20
Demography.....	21
Discussion	21
REFERENCES	29
APPENDICES	37
Appendix I: Tables and figures	38
Appendix II: R code used in statistical analysis	60
VITA	68

LIST OF TABLES

TABLE 1. ORIGIN OF THE 174 SAMPLES OF <i>ERYSIPHE PULCHRA</i> ASSIGNED TO TWO SEPARATE SETS OF SUBPOPULATIONS USED IN SUBSEQUENT ANALYSIS	39
TABLE 2. FIFTEEN MICROSATELLITE LOCI USED TO EXAMINE THE GENETIC DIVERSITY AND POPULATION STRUCTURE OF <i>ERYSIPHE PULCHRA</i> SAMPLES FROM THE EASTERN UNITED STATES	48
TABLE 3. GENETIC DIVERSITY INDICES OF 8 SUBPOPULATIONS OF <i>ERYSIPHE PULCHRA</i> USING 15 MICROSATELLITE LOCI.....	50
TABLE 4. GENETIC DIVERSITY INDICES OF 2 SUBPOPULATIONS OF <i>ERYSIPHE PULCHRA</i> USING 15 MICROSATELLITE LOCI.....	51
TABLE 5. PAIRWISE POPULATION MATRIX OF NEI'S GENETIC DISTANCE OF <i>ERYSIPHE PULCHRA</i> UTILIZING THE CLONE CORRECTED 8-SUBPOPULATION DATA SET	52
TABLE 6. ANALYSIS OF MOLECULAR VARIANCE (AMOVA) FOR <i>ERYSIPHE PULCHRA</i> ACROSS 15 MICROSATELLITE LOCI FOR THE A. 8-SUBPOPULATION DATA SET GROUPED AS ONE HIERARCHAL GROUP AND B. 2-SUBPOPULATION DATA SET GROUPED AS ONE HIERARCHAL CLUSTER	53
TABLE 7. BOTTLENECK ANALYSES FOR <i>ERYSIPHE PULCHRA</i> SAMPLES IN THE A. 8-SUBPOPULATION DATA SET GROUPED AS ONE CLUSTER AND B. 2-SUBPOPULATION DATA SET SEPARATED AS TWO POPULATIONS (NORTH AND SOUTH) USING 15 MICROSATELLITE LOCI	54

LIST OF FIGURES

FIGURE 1. LOCATION OF THE 174 SAMPLES OF <i>ERYSIPHE PULCHRA</i> COLLECTED FROM 10 STATES IN THE EASTERN UNITED STATES. SAMPLES WERE ASSIGNED TO EIGHT ARBITRARY SUBPOPULATIONS BASED ON RELATIVE GEOGRAPHICAL PROXIMITY	55
FIGURE 2. MANTEL TEST OF <i>ERYSIPHE PULCHRA</i> SAMPLES FROM THE EASTERN UNITED STATES ARBITRARILY ASSIGNED TO EIGHT SUBPOPULATIONS. THE ANALYSIS USED 100,000 PERMUTATIONS AND SIGNIFICANCE OF $P < 0.001$	56
FIGURE 3. BAYESIAN CLUSTERING PROBABILITIES FOR <i>ERYSIPHE PULCHRA</i> SAMPLES IN EIGHT ARBITRARY SUBPOPULATIONS FROM THE EASTERN UNITED STATES, UTILIZING INSTRUCT (A) AND STRUCTURE (B). USING THE DEVIANCE INFORMATION CRITERION CALCULATIONS, INSTRUCT INDICATED THE HIGHEST PROBABILITY OF $K=1$ (A). EVANNO'S METHOD, UTILIZED BY STRUCTURE, CALCULATED THE OPTIMUM VALUE OF $K=2$ (B).....	57
FIGURE 4. STRUCTURE BAR GRAPH INDICATING TWO GENETIC CLUSTERS ($\Delta K=2$) OF <i>ERYSIPHE PULCHRA</i> SAMPLES FROM THE EASTERN UNITED STATES ORGANIZED AS (A) EIGHT AND (B) TWO ARBITRARY SUBPOPULATIONS AND ANALYZED UTILIZING 15 MICROSATELLITE LOCI. EACH BAR REPRESENTS THE PROBABILITY OF AN INDIVIDUAL SAMPLE BELONGING TO A DESIGNATED CLUSTER (REPRESENTED BY DIFFERENT COLORS).....	58
FIGURE 5. PRINCIPLE COORDINATED ANALYSIS (PCOA) OF <i>ERYSIPHE PULCHRA</i> SAMPLES ORGANIZED AS (A) EIGHT AND (B) TWO ARBITRARY SUBPOPULATIONS FROM THE EASTERN UNITED STATES, USING 15 MICROSATELLITE LOCI. AXES 1 AND 2 EXPLAIN THE MAJORITY OF OBSERVED VARIATION	59

CHAPTER 1

INTRODUCTION

Flowering dogwood

Cornus florida L., commonly known as flowering dogwood, is one of the most popular of roughly 65 species of dogwoods (*Cornus* sp.) within the *Cornaceae* (Brockman et al. 2002; Caetano-Anollés et al. 1999; Halls 1977; Reed 2004). Endemic to eastern and southeastern United States (US), *C. florida* can naturally be found as north as New York and southern Michigan, with its southernmost US borders in northern Florida and eastern Texas (Fowells 1965; Harlow et al. 1979; Mitchell et al. 1988). Isolated populations of *C. florida* are also found in the mountains of eastern Mexico (Fowells 1965; Harlow et al. 1979; Harrar and Harrar 1962; Mitchell et al. 1988). *Cornus florida* can grow in a wide variety of climatic conditions, withstanding temperatures as low as -34°C during the winter months of its northern limits and above 38°C in the summer months of the south (Fowells 1965; Mitchell et al. 1988).

Cornus florida is a deciduous understory tree regularly found throughout young forests of mixed pine-hardwoods in eastern US (Halls 1977; Harlow et al. 1979; Johnson 1961). Most commonly found along forest edges and on south and west facing slopes, *C. florida* grows well under the shade of a forest canopy (Bruun 1980; Harrar and Harrar 1962). Maximum photosynthesis of *C. florida* can take place in limited sunlight making it well adapted to shade (Fowells 1965; Halls 1977; Mitchell et al. 1988). The growing season of *C. florida* ranges between 160 days in southern Michigan to 300 days in northern Florida (Dirr 2009; Fowells 1965). In the northern growing regions *C. florida* may only grow to be the size of a shrub, whereas in the southernmost

regions of growth it can reach upwards of 12 m in height (Dirr 2009; Fowells 1965; Mitchell et al. 1988).

The showy bracts for which *C. florida* is known extend under 20-30 unobtrusive flowers (Caetano-Anollés et al. 1999; Reed 2004). *Cornus florida* flowers open over a 2 to 3 week period between March and June depending on where it is grown (Forest 1948; Reed 2004). The bracts of *C. florida* can be a creamy white, pink, or occasionally red (Johnson 1961; Reed 2004; Wadl et al. 2011). *Cornus florida* is an obligate out-crossing species due to its incompatibility for self-pollination (Kaveriappa et al. 1997; Reed 2004; Wadl et al. 2011). The flowers of *C. florida* are predominately pollinated by andrenidae and halictidae bees, as well as other generalist insects (Reed 2004; Sork et al. 2005; Wadl et al. 2011). *Cornus florida* trees reach maturity and fruit for the first time typically at 6 years of age (Spinner and Ostrom 1945). The ovoid shaped fruit of *C. florida* is fleshy, scarlet in color and approximately 1.25 cm long by 0.64 cm wide (Fowells 1965; Mitchell et al. 1988).

The fruits of *C. florida* known as drupes, are high in calcium and other necessary nutrients (Halls 1977; Mitchell et al. 1988). The high fat content of *C. florida* drupes is among the highest of any available food in the forests where it grows (Halls 1977; Halls and Epps Jr 1969). The abundant nutrients available in the drupes, leaves and bark of *C. florida*, make it a vital food source for much of the wildlife in these young forests (Gill and Healy 1974; Halls 1977; Mitchell et al. 1988). *Cornus florida* drupes are consumed by many song birds, wild turkey and small mammals, whereas whitetail deer and other

larger mammals eat the foliage and bark (Blair et al. 1983; Gill and Healy 1974; Halls and Epps Jr 1969; Stiles 1980).

Prized for its attractive bracts, scarlet colored drupes and appealing autumn foliage, *C. florida* is one of the most popular ornamental trees in eastern US (Dirr 2009; Orton 1993; Wadl et al. 2011; Wang et al. 2008). Sales of *C. florida* in the US exceeded 27 million dollars in 2014, with the majority of sales (25%) coming from the state of Tennessee (USDA 2015). Many cultivars of *C. florida* have been developed in the US, selecting for desirable traits such as bract color (Caetano-Anollés et al. 1999; Li et al. 2009; Witte 1995) and resistance to disease (Windham et al. 2007; Windham et al. 1998; Windham et al. 2002b, a). Until the introduction of the exotic dogwood anthracnose causing pathogen *Discula destructiva* Redlin (Mantooth et al. 2017; Redlin 1991) and dogwood powdery mildew caused by *Erysiphe pulchra* Cooke & Peck (Britton 1994; Hagan and Mullen 1997), disease management costs to produce *C. florida* were relatively low (Li et al. 2009). Management cost have increased substantially since the introduction of the aforementioned pathogens throughout eastern US (Li et al. 2009).

Dogwood powdery mildew

Powdery mildew was first reported on *C. florida* in 1887 in Illinois, US (Burrill and Earle 1887). This first report of dogwood powdery mildew was of the pathogen *Phyllactinia guttata*, having been identified by its ascocarp on *C. florida* leaves (Burrill and Earle 1887). Dogwood powdery mildew, however, wasn't commonly reported on *C. florida* until after the advent of *E. pulchra* in the mid 1990s in southern US (Britton 1994; Hagan

et al. 1998; Hagan and Mullen 1997; Li et al. 2009). Later studies by Klein et al. (1998) suggest that *P. guttata* doesn't infect *C. florida* and that association between the two occurred after the spread of the *P. guttata* ascocarp from *C. amomum*.

Erysiphe pulchra is one of the 873 obligate biotrophic fungal species from the *Erysiphaceae* or powdery mildew family (Braun and Cook 2012; Takamatsu et al. 1999; Takamatsu et al. 2015). *Erysiphe pulchra* has been identified to cause powdery mildew in flowering dogwood (*C. florida*), kousa dogwood (*C. kousa*) and pacific dogwood (*C. nuttallii*), all members of the big-bract dogwoods (Li et al. 2009; Windham et al. 2005). Powdery white mycelium, conidia, and conidiophores on the top surface of *C. florida* leaves are characteristic signs of *E. pulchra* infection. Symptoms of *E. pulchra* caused powdery mildew include red pigmentation of infected leaves, curling and/or necrosis of young leaves, and yellow to brown blotching on leaf surface (Klein et al. 1998; Li et al. 2009; Williamson and Blake 1999; Windham 1996). Stunted growth of *C. florida* and decreased flower and subsequent fruit production are also symptoms of powdery mildew caused by *E. pulchra* (Klein et al. 1998; Li et al. 2009; Williamson and Blake 1999).

Erysiphe pulchra reproduces both asexually and sexually through the development of conidia and ascospores, respectively (Daughtrey and Hagan 2001; Hagan and Mullen 1997; Mmbaga 2002). The ascocarp (chasmothecia) is thought to be the primary means of overwinter for *E. pulchra* in middle Tennessee and subsequently the primary source of inoculum the following season (Li et al. 2009; Mmbaga 2002; Mmbaga 2000). The *E. pulchra* chasmothecium can range from

approximately 75 to 128 μm in diameter with branched appendages and range in color from yellow to black depending on maturity (Klein et al. 1998; Li et al. 2009; Smith 1999). Chasmothecia are primarily detected on the abaxial surface of *C. florida* leaves and may contain upwards of 30 single celled ascospores (Li et al. 2009; Mmbaga 2002; Mmbaga 2000).

The anamorph of *E. pulchra* (*Oidium* sp.) forms highly vacuolated conidia which are ovoid in shape and approximately 28 x 14 μm in size (Li et al. 2009; Smith 1999). Early spring epidemics of *E. pulchra* have been observed to be polycyclic due to the concurrent infection of *C. florida* leaves by both ascospores and conidia (Li et al. 2009; Mmbaga 2002). Powdery mildew caused by *E. pulchra* has been observed to begin in late May to June (Li et al. 2009; Mmbaga 2002). When environmental conditions are favorable for *E. pulchra*, powdery mildew can spread very fast, producing large amounts of windborne conidia within days of initial infection (Li et al. 2009; Li et al. 2006). In recent years, however the teleomorph of *E. pulchra* has been observed less frequently (personal observation).

Before the present study, very little was known about the genetic diversity and population structure of *E. pulchra*. A study by Mmbaga et al. (2004) analyzed the internal transcribed spacer (ITS) region of *E. pulchra* DNA and found no differences among Tennessee and New York samples of *E. pulchra* on *C. florida* (GenBank accession no. AY224136). The samples analyzed in this study were also 100% identical to samples of *E. pulchra* on *C. kousa* in Japan (GenBank accession no. AB015935) (Mmbaga et al. 2004). These identical ITS sequences between all *E. pulchra* samples in

this study lead Mmbaga et al. (2004) to hypothesized that *E. pulchra* could be an exotic pathogen to *C. florida* in eastern US.

The research presented here utilized microsatellite loci to study the genetic diversity and population structure of *E. pulchra* in the eastern US. Microsatellites also known as simple sequence repeats (SSR) are species specific sequences of DNA that are tandem repeats of 1-6 nucleotide base pairs (Jarne and Lagoda 1996; Moges et al. 2016). These sequences are flanked by highly conserved regions, but are prone to strand slippage mutations and can be used to analyze diversity in a given species (Jarne and Lagoda 1996; Moges et al. 2016; Selkoe and Toonen 2006). With the apparent scarceness of the *E. pulchra* teleomorph, the hypothesis of the present study was that *E. pulchra* has become clonal in the eastern US due to predominant asexual reproduction.

CHAPTER 2
**GENETIC CHARACTERIZATION OF *ERYSIPHE PULCHRA* REVEALS
LOW DIVERSITY IN THE PATHOGEN OF *CORNUS FLORIDA***

A version of this chapter was originally submitted to the journal *Plant Disease* in 2018 by: Christopher R. Wyman, Denita Hadziabdic, Sarah L. Boggess, Timothy A. Rinehart, Alan S. Windham, Phillip A. Wadl, and Robert N. Trigiano

Wyman CR, Hadziabdic D, Boggess SL, Rinehart TA, Windham AS, Wadle PA, and Trigiano RN. "Genetic characterization of *Erysiphe pulchra* reveals low diversity in the pathogen of *Cornus florida*." *Plant Disease*

My primary contributions to this paper include (1) collection of some of the *Erysiphe pulchra* samples on *Cornus florida* leaves (2) lab work and data analysis for the project; (3) literature review and interpretation thereof; (4) majority of the writing associated with the paper; and (5) working with co-authors to incorporate edits and suggestions

Abstract

Cornus florida (flowering dogwood) is a popular understory tree endemic to the eastern hardwood forests of the United States (US). In 1996, dogwood powdery mildew caused by *Erysiphe pulchra*, an obligate, biotrophic fungus of large bracted dogwoods, reached epidemic levels throughout the *C. florida* growing region. In the late 1990s, both sexual and asexual stages of *E. pulchra* were regularly observed, but thereafter the teleomorph has been found less frequently. We examined the genetic diversity and population structure of 174 *E. pulchra* samples growing on *C. florida* leaves using 15 microsatellite loci. Clone correction analysis reduced the sample size to 97 multilocus haplotypes. Our study indicated both low genetic diversity and lack of definitive structure within the population sampled in eastern US. Significant linkage disequilibrium was also observed in seven of eight arbitrary geographically defined subpopulations, implying that predominant means of reproduction was asexually via conidia. This study also showed evidence of a recent population bottleneck among the sampled population. The results of our study inferred a high probability that *E. pulchra* has become clonal since its

advent in 1995, and lends support to the hypothesis that is an exotic pathogen to North America.

Introduction

Cornus florida L., flowering dogwood, is a deciduous understory tree endemic to the eastern United States (US) (Boring et al. 1981; Halls 1977; Johnson 1961). The drupes of *C. florida* are high in fat, calcium, and other nutrients making it a valuable source of food for wildlife (Gill and Healy 1974; Halls 1977; Mitchell et al. 1988). Known for its attractive bracts, drupes and appealing autumn foliage, *C. florida* is highly valued for its ornamental qualities (Johnson 1961). In 2014, US nursery sales of *C. florida* cultivars and those produced from seed sources surpassed \$27 million with Tennessee accounting for about 25 % of sales (USDA 2015).

Until the advent of *Discula destructiva* Redlin, the causal agent of dogwood anthracnose, in the 1970s (Redlin 1991) and *Erysiphe pulchra* Cooke & Peck, dogwood powdery mildew in 1993 and 1994 (Britton 1994; Hagan and Mullen 1997), disease management costs for nurseries to produce *C. florida* trees were estimated to be about \$49/acre/year. Disease management costs have increased considerably since the occurrence of these diseases (~\$800/acre/year), causing many small nurseries to end *C. florida* production (Li et al. 2009).

A powdery mildew disease on *C. florida* caused by *Phyllactinia guttata* was first reported in Illinois, US in 1887 (Burrill and Earle 1887). Reports of powdery mildew on *C. florida*

were, however, rare until 1993 and 1994 when powdery mildew caused by *E. pulchra* occurred statewide in Georgia and Alabama, respectively (Britton 1994; Hagan et al. 1998; Hagan and Mullen 1997; Li et al. 2009). In subsequent years, powdery mildew has regularly been observed throughout the *C. florida* growing region (Daughtrey and Hagan 2001; Hagan and Mullen 1997) .

Erysiphe pulchra is an obligate, biotrophic fungus that causes powdery mildew of three big-bracted dogwoods (*C. kousa*, *C. florida*, and *C. nuttallii*) and can be identified by the characteristic signs of white mycelium, conidiophores and conidia on the adaxial surface of leaves (Li et al. 2009; Li et al. 2007; Smith 1999; Windham et al. 2005). Disease symptoms include stunted growth of trees, necrosis of young leaves, red pigmentation near infected areas in leaves, and decrease flower and subsequent fruit production (Klein et al. 1998; Williamson and Blake 1999; Windham 1996). *Erysiphe pulchra* reproduces both sexually via conidia and sexually through the development of ascospores (Daughtrey and Hagan 2001; Hagan and Mullen 1997; Mmbaga 2002). Ascospores were thought to be the primary source of inoculum each year and airborne conidia acted as a secondary inoculum source (Mmbaga 2002). Both teleomorph and anamorph stages were observed when *E. pulchra* reached epidemic levels in the eastern US in 1995 (Li et al. 2009; Mmbaga 2002; Williamson and Blake 1999). However, in recent years, the sexual stage has been observed less frequently (personal observation). It is uncertain whether or not *E. pulchra* in the eastern US has become clonal because of reproducing primarily via asexual conidia.

Population genetics can be used as a vital tool for better utilization of control methods for various plant pathogens (McDonald and Linde 2002; McDonald and McDermott 1993). The study of population genetics has been utilized to locate the probable center of origin for pathogens such as *Phytophthora infestans* (Goss et al. 2014; Grünwald and Flier 2005), *Mycosphaerella graminicola* (Stukenbrock et al. 2006) and *P. ramorum* (Goss et al. 2009; Goss et al. 2011; Kamvar et al. 2015a). Population genetics also supports understanding genetic patterns of emergence and primary means of reproduction of a given pathogen (Goss et al. 2009; Goss et al. 2014; Grünwald et al. 2017).

Microsatellite loci or simple sequence repeats (SSR) are tandem repeats of 1-6 nucleotide base pairs that are flanked by highly conserved regions, distributed throughout most eukaryotic genomes (Jarne and Lagoda 1996; Moges et al. 2016; Selkoe and Toonen 2006), and have become widely used DNA markers in population studies (Benali et al. 2011; Selkoe and Toonen 2006). These regions typically have high mutation rates, and thus, can be potentially utilized to differentiate and identify individuals in the species of interest (Jarne and Lagoda 1996; Moges et al. 2016; Selkoe and Toonen 2006).

There is currently limited information on the genetic diversity and population structure of *E. pulchra* (Mmbaga et al. 2000). Because of the apparent infrequency of sexual reproduction, the hypothesis of this study was that *E. pulchra* reproduces most

prominently via conidia and has become essentially a clonal organism in the eastern US. To test this hypothesis, the objective of this study was to use species-specific microsatellite loci to characterize the genetic diversity and population structure of in the eastern US.

Materials and Methods

Sample collection and DNA extraction

One hundred and seventy-four samples of *E. pulchra* collected from fresh leaves of *C. florida* were used in this study (Table 1). Infected leaf samples were flash frozen in liquid nitrogen for 5 min then homogenized with a Bead Mill 24 (Fisher Scientific Company, Hampton, New Hampshire, US). The tissue homogenization step was repeated twice with a 5 min interval in liquid nitrogen between the steps. DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, California, US) with the following modifications to the manufacturer's protocol: 2% soluble polyvinylpyrrolidone (PVP) was dissolved in the lysis buffer (AP1); the initial incubation time in AP1 at 65°C was increased to 20 min with samples vortexed every 2 min; and the elution step performed with 50 µl of elution buffer (AE) two times. Extracted genomic DNA (gDNA) was quantified with the ND1000 Ultraviolet-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, US) and stored at -20°C until used for analyses.

Microsatellite development and selection

A library of 237 microsatellite markers was developed by de novo sequencing using Ion Torrent technology (Unpublished data). Twenty-eight microsatellite primer pairs were used to screen and identify polymorphisms using 93 of the samples in a 96 well plate. Both a positive control (a sample that amplified with all primer pairs) and negative control (sterile distilled water substituted for gDNA) were used in all PCR reactions to ensure validity of our data. Ten µl PCR reactions were assembled to test microsatellite loci, and in subsequent data collections, contained the following: 10 ng gDNA of both the host and pathogen, 2.5 µM for each forward and reverse primers, 2X AccuStart II PCR ToughMix (QuantaBio, Beverly, Massachusetts, USA), and sterile distilled water. The reaction cycles were completed in a Mastercycler Pro automatic thermal cycler (Eppendorf Biotech Company, Hamburg, Germany) under the following conditions: 3 min of initial denaturation at 94°C, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min, with a final extension before the final holding temperature at 4°C.

Amplicons were analyzed with a Qlaxcel Capillary Electrophoresis System (Qiagen, Valencia, California, US) utilizing a 15/600 bp internal alignment marker and scored using a 25-bp DNA size marker to obtain raw allele length data. Fifteen of the twenty-eight microsatellite loci tested were polymorphic, and subsequently used to amplify gDNA from the remaining *E. pulchra* samples in the study (Table 2). All subsequent PCR reactions were completed in 96-well plates containing both aforementioned positive and negative controls. PCR reactions that did not produce products were

repeated at least two times before being considered missing data or null alleles. Samples with more than 50% missing data were eliminated from further analyses.

Genetic diversity

Raw allele length data were binned into statistically similar allelic classes using FLEXIBIN v2 (Amos et al. 2007). The binned allele data were used in all subsequent analyses. Samples were organized into the following two sets of subpopulations: as eight arbitrary subpopulations based on relative geographical proximity (Fig. 1), and as two subpopulations representing those obtained from north of the Tennessee-Kentucky border (North) and those from south of the Tennessee-Kentucky border (South). Data were clone corrected using POPPR v2 (Kamvar et al. 2015b; Kamvar et al. 2014), a package for R v3.3.1 (Team 2013), to remove identical multilocus haplotypes within each subpopulation. In order to prevent biasing analyses towards clonality, all subsequent analyses were performed with clone corrected data (Grünwald et al. 2017; McDonald 1997; Tsui et al. 2012).

POPPR v2 was used to compute various genetic diversity indices including the Shannon-Weiner index of diversity (H), a diversity index that takes in to account both richness and evenness of the given species (Shannon 2001), and Nei's genotypic diversity (Nei 1978) across all loci and sample sites for both sets of subpopulations. Number of private alleles and allelic richness also were calculated through the use of POPPR v2. The standard index of association ($\bar{r}_d$), a measurement of multilocus linkage

disequilibrium (Agapow and Burt 2001), was calculated in POPPR v2 using 10,000 permutations for both sets of r_d subpopulations. Pairwise Nei's genetic distance (Nei 1978) of both subpopulations data sets were estimated using the R package adegenet v2.1.0 (Jombart 2008). The R package ade4 v1.7-8 (Dray and Dufour 2007) was used to calculate and visualize a Mantel test (Mantel 1967) using 100,000 permutations to compare the relationship between genetic and geographic distance of individuals in both subpopulation sets.

Population structure

Bayesian clustering analysis of the 8-subpopulation set was completed using STRUCTURE v2.3.4, which uses a Monte Carlo Markov Chain (MCMC) method to group individuals into definitive population clusters (ΔK) (Pritchard et al. 2000; Tsui et al. 2012). The parameters used in the STRUCTURE analysis were as follows: a burn-in period of 200,000 with 600,000 MCMC repetitions, 20 iterations, and the number of cluster ΔK analyzed 1-10. The optimum value of ΔK was estimated using STRUCTURE HARVESTER web v0.6.94 (Earl and vonHoldt 2012) utilizing Evanno's method (Evanno et al. 2005). POPHELPER (Francis 2016) was used to visualize the STRUCTURE results.

Bayesian clustering analysis was also examined using the program InStruct (Gao et al. 2007). The program InStruct eliminates the assumption of Hardy-Weinberg equilibrium within clusters and is suggested for analyzing highly clonal populations (Gao et al.

2007). The parameters used in the InStruct Bayesian analysis were as follows: 10 MCMC chains for each value of K (1-10) with a burn-in period of 500,000 and 20 iterations of thinning interval (Dean et al. 2015). The optimum value of K was evaluated using the deviance information criterion (DIC) as outlined by Gao et al. (2011).

POPPR v2 was used to calculate Bruvo's genetic distance (Bruvo et al. 2004) between all individuals, which was subsequently visualized through principal coordinates analysis (PCoA) through the R program adegenet v2.1.0. An analysis of molecular variance (AMOVA) was evaluated for both subpopulation data sets using POPPR v2 with samples grouped as one hierarchical cluster per instruct result.

Demography

Both Sign and Wilcoxon signed-ranks significance tests were applied in the program BOTTLENECK v1.2.02 (Cornuet and Luikart 1996) to assess if a recent expansion or bottleneck in the population had occurred (Piry et al. 1999). The Sign test has a null hypothesis of equal probability of positive and negative differences between observed and expected heterozygosity (Cornuet and Luikart 1996), whereas the Wilcoxon signed-ranks test is a one-tailed test with a null hypothesis of no significant excess of heterozygosity (Luikart et al. 1998; Piry et al. 1999). The set of 8-subpopulations were grouped as one cluster, per InStruct result (Mantooth et al. 2017; Tsui et al. 2012), and was coded as diploid for this analysis (Tsui et al. 2012). The following three models were used to determine if the 15 loci used remained in mutation-drift equilibrium:

stepwise mutation model (S.M.M), infinite allele model (I.A.A), and two-phase model (T.P.M.) (Piry et al. 1999). BOTTLENECK v1.2.02 was also used to analyze the 2-subpopulation data set grouped as two separate populations, with the aforementioned models and parameters.

Results

Microsatellite development and selection

Five of the 28 loci evaluated were monomorphic and therefore removed from further analyses. A threshold percentage of samples amplified was set (70%). Fifteen of the twenty-three remaining loci amplified DNA from >70% of the samples and were retained in the analyses (Table 2). Genetic diversity of the 15 loci ranged from 0.22 (EP054) to 0.60 (EP030) (Table 2). The number of alleles identified after allelic binning varied between 2 (EP027, EP042, EP055, EP060, and EP066) and 4 (EP039 and EP041) (Table 2). The average allelic richness of the 8-subpopulation set was 2.7 (Table 3) and 3.6 for the 2-subpopulation set (Table 4).

Genetic diversity indices

Data was analyzed using two different approaches when grouping samples into subpopulations: 1) as eight arbitrary subpopulations based on relative geographical proximity, and 2) as two subpopulations representing sample from north and south of the Tennessee-Kentucky border, respectively. Within the 8-subpopulation data set 48 individuals were identified not to be MLG, which reduced the original sample size from

174 to 126 (Table 3). Clone correction of the 2-subpopulation data set reduced the original sample size from 174 to 108 individuals. Ninety-seven multilocus haplotypes remained in both subpopulation data sets post-clone correction (Tables 3 and 4).

The Shannon-Weiner index of diversity (H) of the 8-subpopulations ranged from 2.08 to 3.47 (Table 3). Within the 2-subpopulation data set, H was 3.93 for the north and 4.04 for the south subpopulations (Table 4). The 8-subpopulation data set contained 3 private alleles (P_a), found within 3 of the subpopulations (Table 3), whereas the 2-subpopulation set contained 22 P_a (Table 4). The genetic diversity (H_{exp}) adjusted for average sample size of the 8-subpopulation data set was 0.45, and ranged from 0.19 to 0.5 (Table 3). The average H_{exp} was 0.43 in the 2-subpopulation dataset (Table 4).

The total $\bar{r}_d$ of both the 8 and 2-subpopulation data sets differed significantly from 0 and indicated asexual or clonal reproduction (Tables 3 and 4). The Kentucky subpopulation within the 8-subpopulation data set (Pop 4), however, did not significantly differ from 0 and may suggest sexual reproduction in this subpopulation (Table 3).

The pairwise population matrix of Nei's genetic distance for the 8-subpopulation data set estimated the highest genetic distance between the Mississippi and southwest Virginia subpopulations (Pop 1 and Pop 5) at 0.423, whereas the lowest genetic distance of 0.022 was calculated between the Mississippi and Knoxville, Tennessee subpopulations (Pop 1 and Pop3) (Table 5). Nei's genetic distance for the 2-

subpopulation data set was 0.099. The Mantel test was used to determine correlation between genetic distance and geographical distance. Significant positive correlation between genetic and geographical distance was calculated (Fig. 2).

Population structure

STRUCTURE, which uses Bayesian clustering analysis, estimated an optimum ΔK of 2 that indicated two clusters within the 8-subpopulation set (Fig. 3a). Subpopulations 1, 2, 3, and 8 were highly admixed between the two clusters, whereas subpopulations 4, 5, 6, and 7 clustered together with low admixture (Fig. 4a). In the 2-subpopulation data set both subpopulations were admixed (Fig. 4b). InStruct Bayesian clustering analysis of the 8-subpopulation set calculated the most probable ΔK to be 1, and indicated a single population cluster of the pathogen (Fig. 3b). One continuous cluster, without definite structure was indicated by principle coordinate analysis (PCoA) of both subpopulation data sets, which supported the InStruct result (Fig. 5). Both Bayesian clustering analyses were used on the 2-subpopulation data set with the same outcomes, respectively (InStruct data not shown).

Analysis of molecular variance (AMOVA) of the 8-subpopulation data set calculated that the majority of genetic variation was within subpopulations (81.39%), with 18.61% of variation among subpopulations (Table 6). The AMOVA of the 2-subpopulation data set estimated variation within subpopulations to be 90.23% and among the 2-

subpopulations 9.77% (Table 6). Differentiation among subpopulations in both data sets was high and moderate, respectively (Table 6).

Demography

Analysis of both subpopulation data sets with BOTTLENECK v1.2.02 using both Sign and Wilcoxon tests indicated a significant excess of genetic diversity or heterozygosity in relation to the number of alleles present in the grouped data. The evidence suggested that recent bottleneck had occurred (Table 7).

Discussion

The results of our study indicated low genetic diversity, absence of population structure, and significant correlation between geographical and genetic distance among sampled *E. pulchra* individuals. Previous to this study, limited information was available on the genetic diversity of *E. pulchra*. With sexual reproduction less commonly observed, our hypothesis for the study was that *E. pulchra* has become clonal due to predominant reproduction via asexually produced conidia in the eastern US.

Similar to observations of other clonally reproducing phytopathogens (Kamvar et al. 2015a; Lehner et al. 2015; Mantooth et al. 2017), genetic diversity among subpopulations of *E. pulchra* was low, lending support to the hypothesis that *E. pulchra* was a clonal pathogen. Nei's genotypic diversity (H_{exp}) of all eight subpopulations was moderately low, however it was lowest in northern subpopulations with the exception of

New Jersey (Pop8). Powdery mildew fungal pathogens produce conidia that can be wind-dispersed over long distances (McDonald and Linde 2002). Barley powdery mildew conidia are wind-dispersed from the United Kingdom to Denmark, a distance of approximately 500 miles or 800 km (Hermansen et al. 1978; Parks et al. 2009). The limited genetic diversity of the northern *E. pulchra* subpopulations may suggest that conidia are the predominate means of inoculum dispersed by prevailing winds in the spring from the south towards the north, similar to *Peronospora tabacina* (Aylor 1999; Aylor et al. 1982), and *Puccinia triticina* (Hamilton and Stakman 1967; Kolmer 2005). *Erysiphe pulchra* conidia have a latent period of 7 days (Li et al. 2005), which could allow for rapid dispersion of the pathogen locally and to a more distant geographic range in a single season (Aylor 2003). The higher diversity observed in New Jersey samples can likely be explained as an effect of the higher sample size of Pop8.

The standard index of association ($\bar{r}_d$) revealed significant linkage disequilibrium in both subpopulation data sets, which also supported the hypothesis of predominantly asexual reproduction. Within the 8-subpopulation data set, however, in one subpopulation linkage disequilibrium was not detected, suggesting sexual reproduction may be occurring there. However, that should be interpreted with caution because the apparent indication of sexual recombination may be an artifact that small sample sizes and percent missing data can have on the $\bar{r}_d$ of a subpopulation (Kamvar 2016). However sexual recombination in Pop 4 cannot be ruled out. The subpopulation in which this occurred had more than 50% missing data for 3 of the 15 loci. The criteria used to form

the 8 arbitrary subpopulations may also have an effect on the accuracy of the result. When clone corrected and analyzed as a 2-subpopulation data set (with larger sample sizes), significant linkage disequilibrium was evident in both subpopulations. The Mantel test revealed a significant positive correlation between genetic and geographic distances. This positive correlation is indicative of isolation by distance, which would take place in a wind-dispersed conidia scenario (Barrès et al. 2008). The correlation of genetic and geographical distance also suggests a limit to where pathogen overwinter occurs, presumably the southern states, which then act as an origin for epidemics in northern states annually. If *E. pulchra* were to overwinter in all subpopulations, correlations between genetic and geographical distance would be limited because of long distance dispersal, where overtime, genetic structure would be more pronounced (Barrès et al. 2008; Kamvar et al. 2015a). If this were the case, we would expect to see population structure by location similar to that of *E. graminis* f. sp. *hordei* (Brown 1994; Brown et al. 1991) and *Blumeria graminis* f. sp. *tritici* (Parks et al. 2009), which was not evident in our results. Nei's genetic diversity results indicated high gene flow between all subpopulations, conceivably as a consequence of wind-dispersal over a large geographical range (Aylor et al. 1982; Barrès et al. 2008).

The main difference between our results and that of other clonal or predominately asexual reproducing phytopathogens was the lack of definitive genetic structure of the *E. pulchra* population. Many other primarily asexually reproducing plant pathogens, such as *P. ramorum* (Ivors et al. 2006; Kamvar et al. 2015a), *Sclerotinia sclerotiorum*

(Kamvar et al. 2017; Lehner et al. 2015), and *Discula destructiva* (Mantooth et al. 2017), have exhibited population structure, whereas *E. pulchra* populations lacked definitive structure. This difference in structure may be indicative of how *E. pulchra* has been dispersed as compared to the aforementioned pathogens. The population structure of *D. destructiva* was a result of multiple introductions of the exotic pathogen to both the eastern and western US (Mantooth et al. 2017). The lack of definitive population structure found in our results may suggest a narrow introduction of the pathogen with limited genetic diversity to eastern US (Barrès et al. 2008; Goss et al. 2009).

InStruct, a Bayesian clustering program that excludes Hardy-Weinberg equilibrium assumptions of no genetic drift, no gene flow, no mutations, random mating and no natural selection (Gao et al. 2007; Hartl 1988) was used. Instruct indicated one cluster as the most probable population structure of *E. pulchra*. When population structure was analyzed with STRUCTURE utilizing Evanno's method, a ΔK of 2 was indicated to be the most probable structure. However, Evanno's method utilized by STRUCTURE Harvester in determining the optimum value of ΔK cannot mathematically calculate a ΔK of 1 (Evanno et al. 2005). The PCoA results for the 8-subpopulation data set indicated a lack of grouping which added support to the InStruct result. Both PCoA and InStruct results supported the hypothesis of the clonal nature of *E. pulchra* in the eastern United States.

AMOVA results of the 8-subpopulation data set indicated high genetic differentiation among subpopulations. Genetic differentiation differs from genetic diversity in that it measures the difference in allele frequencies among different subpopulations, rather than the individual variation within a subpopulation (Barrès et al. 2008; Gregorius 1987). High genetic differentiation among subpopulations supports the scenario of airborne dissemination from the south northward in the spring. If *E. pulchra* epidemics in the north are caused by wind dispersed conidia from the south annually, it would result in a divergence of allelic frequencies due to isolation by distance (Boileau et al. 1992; Carlier et al. 1996; Rivas et al. 2004).

Powdery mildew pathogens have the following three possible means of overwintering: in a dormant stage (chasmothecia containing asci with ascospores), as mycelium in dormant buds of the host, or in climates where both host and pathogen grow throughout the year (Brown and Hovmøller 2002; Mmbaga 2002). A study of the winter survival of *E. pulchra* in middle Tennessee indicated the chasmothecia or sexually produced structure to be the predominant means for overwintering (Mmbaga 2002). In this study, chasmothecia were counted over a three-year period and abundance was limited by the formation of chasmothecia later in the season. Their study suggested that environmental conditions may affect either the host or pathogen and possibly restricts chasmothecia development (Mmbaga 2002). It is possible that formation of chasmothecia and ascospores is dependent upon different mating types (Debuchy et al. 2010; Mmbaga 2002) and the current environment has become less supportive for one

of the mating types, which would be similar to formation of *P. infestans* oospores in the US. (Goodwin et al. 1995).

The results of our study suggest that *E. pulchra* had essentially become clonal in the eastern US due to predominantly asexual reproduction. Although structures associated with the teleomorph are thought to be the main means of overwinter for *E. pulchra* (Mmbaga 2002), our results indicated significant linkage disequilibrium, little genetic diversity, and lack of definitive population structure. This may be indicative of the teleomorph becoming less common and overwintering may also take place by other means. The lack of genetic diversity in the eastern US population may also be the result of introduction of a limited number of genetically dissimilar individuals (Barrès et al. 2008; Goss et al. 2009). If very few genetically novel individuals were introduced to the eastern US, sexual reproduction would do little to increase genetic diversity as has been seen in other exotic plant pathogens (Barrès et al. 2008; Giraud 2004; Goss et al. 2009; Raboin et al. 2007). The results of our study indicated that a recent bottleneck has occurred, which also supports the likelihood of *E. pulchra* being an exotic pathogen introduced to the eastern US.

Dogwood powdery mildew was first reported on *C. florida* by Burrill and Earle (1887) and was ascribed to *P. guttata* by chasmothecium morphology. Klein et al. (1998) reported that *P. guttata* does not infect *C. florida* and that the chasmothecia most likely became associated with *C. florida* post-dissemination from *C. amomum*. Dogwood

powdery mildew on *C. florida* was not a common until the mid-1990s (Daughtrey and Hagan 2001; Hagan et al. 1998; Hagan and Mullen 1997). Mmbaga et al. (2004) amplified the ITS regions of *E. pulchra* samples in Tennessee and found a 99% match between their samples (GenBank accession no. AY224136) and an *E. pulchra* sample from *C. kousa* in Japan (GenBank accession no. AB015935) (Mmbaga et al. 2004; Takamatsu et al. 1999). This result led to the hypothesis that the rapid spread of *E. pulchra* in the eastern US was possibly due to an introduction of *E. pulchra* on infected *C. kousa* imported to breed cultivars of dogwood resistant to *D. destructiva* (Mmbaga et al. 2004). *C. kousa* trees are generally resistant to *E. pulchra*, but may develop on seedlings (Li et al. 2009; Li et al. 2007; Windham 1996) or shoots from dormant epicormic shoots (personal observation). *Erysiphe pulchra* was first reported in China in 2012 infecting *C. kousa* subsp. *chinensis* and identified by sequencing the ITS region (GenBank accession no. KF601334) and matched the sample of *C. kousa* from Japan (Bai et al. 2014; Takamatsu et al. 1999). The low genetic diversity, clonality, and bottleneck found in *E. pulchra* in the eastern US indicates an exotic origin of the pathogen (Goss et al. 2009) and supports the Mmbaga et al. (2004) hypothesis that *E. pulchra* is an introduced pathogen, most likely from Japan or other locations in Asia where *C. kousa* is grown. Further research into the possible spread of *E. pulchra* from Asia to the eastern US is warranted and should include more *E. pulchra* samples from *C. florida*, *C. nuttallii* from western US (Windham et al. 2005), and Italy (Garibaldi et al. 2009).

The low genetic diversity, lack of population structure, and presence of linkage disequilibrium supports our hypothesis that *E. pulchra* in the eastern US has become clonal in nature due to predominantly reproducing asexually. Our results also infer that *E. pulchra* is most likely an exotic pathogen to *C. florida* in the eastern US, however more research is needed to conclude the origin of introduction. The results of our study also indicate a high probability that *E. pulchra* overwinters in the south, conceivably as the sexual structure or chasmothecia, with long distance wind dispersal to the north by prevailing winds.

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APPENDICES

Appendix I: Tables and Figures

Table 1. Origin of the 174 samples of *Erysiphe pulchra* assigned to two separate sets of subpopulations used in subsequent analysis

Sample	Collection site	Collection date	8 Subpopulation set ^a	2 Subpopulation set ^b
A2	Poplarville, MS	3-Sep-13	Pop 1	S
A3	Poplarville, MS	3-Sep-13	Pop 1	S
A4	Poplarville, MS	3-Sep-13	Pop 1	S
D1	Poplarville, MS	3-Sep-13	Pop 1	S
D2	Poplarville, MS	3-Sep-13	Pop 1	S
D3	Poplarville, MS	4-Sep-13	Pop 1	S
D4	Poplarville, MS	4-Sep-13	Pop 1	S
D5	Poplarville, MS	4-Sep-13	Pop 1	S
D6	Poplarville, MS	4-Sep-13	Pop 1	S
DesotoT1	Brooklyn, MS	16-Jun-16	Pop 1	S
DeSotoT2	Brooklyn, MS	16-Jun-16	Pop 1	S
DesotoT3	Brooklyn, MS	16-Jun-16	Pop 1	S
DeSotoT4	Brooklyn, MS	16-Jun-16	Pop 1	S
PopT1	Poplarville, MS	16-Jun-16	Pop 1	S
PopT2	Poplarville, MS	16-Jun-16	Pop 1	S
PopT3	Poplarville, MS	16-Jun-16	Pop 1	S
GA_DAL_1	Dalton, GA	5-Sep-16	Pop 2	S
GA_FRS_1	Forsyth, GA	5-Sep-16	Pop 2	S
GA_MCD_1	McDonough, GA	5-Sep-16	Pop 2	S
Nash1	Nashville, TN	22-May-13	Pop 2	S
Nash10	Nashville, TN	22-May-13	Pop 2	S

Table 1. continued

Sample	Collection site	Collection date	8 Subpopulation set^a	2 Subpopulation set^b
Nash11	Nashville, TN	22-May-13	Pop 2	S
Nash12	Nashville, TN	22-May-13	Pop 2	S
Nash2	Nashville, TN	22-May-13	Pop 2	S
Nash3	Nashville, TN	22-May-13	Pop 2	S
Nash4	Nashville, TN	22-May-13	Pop 2	S
Nash5	Nashville, TN	22-May-13	Pop 2	S
Nash6	Nashville, TN	22-May-13	Pop 2	S
Nash7	Nashville, TN	22-May-13	Pop 2	S
Nash8	Nashville, TN	22-May-13	Pop 2	S
Nash9	Nashville, TN	22-May-13	Pop 2	S
PMnash	Nashville, TN	21-Jun-16	Pop 2	S
TN_CKV_1	Cookeville, TN	21-Jun-16	Pop 2	S
TN_CRS_1	Crossville, TN	21-Jun-16	Pop 2	S
TN_LAN_1	Lancaster, TN	21-Jun-16	Pop 2	S
TN_MUR_1	Murfesboro, TN	21-Jun-16	Pop 2	S
TN_MUR_2	Murfesboro, TN	28-Jun-16	Pop 2	S
TN_MUR_3	Murfesboro, TN	28-Jun-16	Pop 2	S
TN_MUR_4	Murfesboro, TN	28-Jun-16	Pop 2	S
AppSpringBH1	Maryville, TN	10-Jun-16	Pop 3	S
AppSpringBH2	Maryville, TN	10-Jun-16	Pop 3	S
ChChiefPMMary	Maryville, TN	21-Jun-16	Pop 3	S
TN_SEQ_1	Knoxville, TN	8-Jul-15	Pop 3	S

Table 1. continued

Sample	Collection site	Collection date	8 Subpopulation set^a	2 Subpopulation set^b
TN_SEQ_10	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_11	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_12	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_13	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_15	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_16	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_17	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_18	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_19	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_2	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_20	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_21	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_22	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_23	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_24	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_25	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_3	Knoxville, TN	8-Jul-15	Pop 3	S
TN_SEQ_4	Knoxville, TN	8-Jul-15	Pop 3	S
Hosta_Garden	Knoxville, TN	16-Jun-16	Pop 3	S
DogPMAg	Knoxville, TN	27-May-16	Pop 3	S
TN_KNX_1.1	Knoxville, TN	6-Jun-16	Pop 3	S
TN_KNX_1.2	Knoxville, TN	16-Jun-16	Pop 3	S

Table 1. continued

Sample	Collection site	Collection date	8 Subpopulation set ^a	2 Subpopulation set ^b
TN_KNX_1.3	Knoxville, TN	16-May-16	Pop 3	S
TN_KNX_4	Knoxville, TN	22-Jul-16	Pop 3	S
TN_KNX_3	Knoxville, TN	9-Jun-16	Pop 3	S
VP523A	Knoxville, TN	5-Jul-12	Pop 3	S
VP523B	Knoxville, TN	5-Jul-12	Pop 3	S
VP524	Knoxville, TN	5-Jul-12	Pop 3	S
VP533A	Knoxville, TN	5-Jul-12	Pop 3	S
VP533BB	Knoxville, TN	5-Jul-12	Pop 3	S
VP536	Knoxville, TN	5-Jul-12	Pop 3	S
VP609B	Knoxville, TN	5-Jul-12	Pop 3	S
VP612	Knoxville, TN	5-Jul-12	Pop 3	S
VP625A	Knoxville, TN	5-Jul-12	Pop 3	S
VP625B	Knoxville, TN	5-Jul-12	Pop 3	S
VP700	Knoxville, TN	5-Jul-12	Pop 3	S
VPP525	Knoxville, TN	5-Jul-12	Pop 3	S
VPP525A	Knoxville, TN	5-Jul-12	Pop 3	S
KY_WILL_1.2	Williamsburg, KY	11-Aug-16	Pop 4	S
TN_CLIN_1	Clinton, TN	11-Aug-16	Pop 4	S
TN_JELL_1	Jellico, TN	11-Aug-16	Pop 4	S
TN_JELL_2	Jellico, TN	11-Aug-16	Pop 4	S
TN_POW_1	Powell, TN	11-Aug-16	Pop 4	S
KY_BRE_1	Berea, KY	11-Aug-16	Pop 4	N

Table 1. continued

Sample	Collection site	Collection date	8 Subpopulation set^a	2 Subpopulation set^b
KY_BRE_2	Berea, KY	11-Aug-16	Pop 4	N
KY_COR_1	Corbin, KY	11-Aug-16	Pop 4	N
KY_FLO_1.1	Florence, KY	11-Aug-16	Pop 4	N
KY_GRT_1	Georgetown, KY	11-Aug-16	Pop 4	N
KY_GRT_2	Georgetown, KY	11-Aug-16	Pop 4	N
KY_LEX_1.1	Lexington, KY	11-Aug-16	Pop 4	N
KY_LEX_1.2	Lexington, KY	11-Aug-16	Pop 4	N
KY_LON_1.1	London, KY	11-Aug-16	Pop 4	N
KY_LON_1.2	London, KY	11-Aug-16	Pop 4	N
KY_LON_2	London, KY	11-Aug-16	Pop 4	N
KY_MTV_1.1	Mount Vernon, KY	11-Aug-16	Pop 4	N
KY_MTV_1.2	Mount Vernon, KY	11-Aug-16	Pop 4	N
KY_STP_1	Cumberland Falls, KY	11-Aug-16	Pop 4	N
OH_COM_1	Covington, OH	11-Aug-16	Pop 4	N
VA_MAC_1	Maces Springs, VA	13-Aug-16	Pop 5	N
VA_MAR_1.2	Marion, VA	13-Aug-16	Pop 5	N
VA_MAR_1.3	Marion, VA	13-Aug-16	Pop 5	N
VA_MAR_2	Marion, VA	13-Aug-16	Pop 5	N
VA_RAD_1	Radford, VA	13-Aug-16	Pop 5	N
VA_WHY_1	Wytheville, VA	13-Aug-16	Pop 5	N
VA_ARC_1	Arcadia, VA	13-Aug-16	Pop 5	N
VA_BLK_1	Blacksburg, VA	13-Aug-16	Pop 5	N

Table 1. continued

Sample	Collection site	Collection date	8 Subpopulation set^a	2 Subpopulation set^b
VA_ROA_1	Roanoke, VA	13-Aug-16	Pop 5	N
OH_CAM_1	Cambridge, OH	12-Aug-16	Pop 6	N
OH_CAM_2	Cambridge, OH	12-Aug-16	Pop 6	N
OH_CAM_3	Cambridge, OH	12-Aug-16	Pop 6	N
OH_ZAN_1.1	Zanesville, OH	12-Aug-16	Pop 6	N
OH_ZAN_1.2	Zanesville, OH	12-Aug-16	Pop 6	N
OH_ZAN_2	Zanesville, OH	12-Aug-16	Pop 6	N
OH_ZAN_3	Zanesville, OH	12-Aug-16	Pop 6	N
OH_ZAN_4	Zanesville, OH	12-Aug-16	Pop 6	N
OH_ZAN_5	Zanesville, OH	12-Aug-16	Pop 6	N
OH_ZAN_6.1	Zanesville, OH	12-Aug-16	Pop 6	N
OH_ZAN_6.2	Zanesville, OH	12-Aug-16	Pop 6	N
OH_BEV_1	Belle Valley, OH	12-Aug-16	Pop 6	N
OH_MAR_1	Marietta, OH	12-Aug-16	Pop 6	N
WV_GCR_1	Cairo, WV	12-Aug-16	Pop 6	N
WV_PAR_1	Parkersburg, WV	12-Aug-16	Pop 6	N
WV_PAR_2	Parkersburg, WV	12-Aug-16	Pop 6	N
WV_PAR_2.2	Parkersburg, WV	12-Aug-16	Pop 6	N
KY_CLA_1	Clarksburg, WV	12-Aug-16	Pop 6	N
KY_MGT_2.1	Morgantown, WV	12-Aug-16	Pop 6	N
KY_MGT_2.2	Morgantown, WV	12-Aug-16	Pop 6	N
KY_RACC_1	Bristol, WV	12-Aug-16	Pop 6	N

Table 1. continued

Sample	Collection site	Collection date	8 Subpopulation set^a	2 Subpopulation set^b
WV_MGT_1	Morgantown, WV	12-Aug-16	Pop 6	N
MD_WIL_1	Williamsport, MD	13-Aug-16	Pop 6	N
VA_BV_1	Buena Vista, VA	13-Aug-16	Pop 7	N
VA_FHS_1	Fishersville, VA	13-Aug-16	Pop 7	N
VA_HAR_1	Harrisonburg, VA	13-Aug-16	Pop 7	N
VA_HAR_2	Harrisonburg, VA	13-Aug-16	Pop 7	N
VA_HAR_3	Harrisonburg, VA	13-Aug-16	Pop 7	N
VA_NEM_1	New Market, VA	13-Aug-16	Pop 7	N
VA_NEM_2	New Market, VA	13-Aug-16	Pop 7	N
VA_WDS_1	Woodstock, VA	13-Aug-16	Pop 7	N
PA_CAR_1	Carlisle, PA	13-Aug-16	Pop 7	N
PA_CHA_1	Chambersburg, PA	13-Aug-16	Pop 7	N
PA_GRC_1	Greencastle, PA	13-Aug-16	Pop 7	N
PA_SHIP_1	Shippensburg, PA	13-Aug-16	Pop 7	N
VA_WIN_1	Winchester, VA	13-Aug-16	Pop 7	N
VA_WIN_2	Winchester, VA	13-Aug-16	Pop 7	N
VA_WIN_3.1	Winchester, VA	13-Aug-16	Pop 7	N
VA_WIN_3.2	Winchester, VA	13-Aug-16	Pop 7	N
VA_WIN_4	Winchester, VA	13-Aug-16	Pop 7	N
RU10	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU11	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU12	New Brunswick, NJ	1-Jul-15	Pop 8	N

Table 1. continued

Sample	Collection site	Collection date	8 Subpopulation set ^a	2 Subpopulation set ^b
RU13	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU14	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU15	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU16	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU18	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU20	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU21	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU23	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU24	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU25	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU26	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU27	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU28	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU29	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU3	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU30	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU31	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU32	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU33	New Brunswick, NJ	1-Jul-15	Pop 8	N

Table 1. continued

Sample	Collection site	Collection date	8 Subpopulation set^a	2 Subpopulation set^b
RU5	New Brunswick, NJ	1-Jul-15	Pop 8	N
RU7	New Brunswick, NJ	1-Jul-15	Pop 8	N

^a Eight arbitrary subpopulations based on geographical juxtaposition

^b Two subpopulations: N = north of the Tennessee-Kentucky border; S = south of the Tennessee-Kentucky border

Table 2. Fifteen microsatellite loci used to examine the genetic diversity and population structure of *Erysiphe pulchra* samples from the eastern United States

GenBank Accession No.	Locus	Forward and Reverse Primers (5'-3')	Repeat Motif	No. of Alleles	Allele Range (bp)	He _a
MG913149	EP027	F:AAAACGCACGACTTACCTACC R:TGGAGTCTTCACCATCAACTC	(CCGCG) ₃	2	177-182	0.47
MG913150	EP030	F:TGCTATCACCTCGTACTTTGC R:CGCTCAACAAAGAAATGAGTC	(GTC) ₄	3	156-161	0.6
MG913151	EP039	F:GCTGTAAGACGGGAGATTTTC R:TCTCTCCTACAGTACCGATTGAA	(CGAG) ₃	4	144-154	0.54
MG913152	EP041	F:ATAGGAGGAGCAACCAAAATG R:GCATCCTTTTCCACAAGATTT	(ATAA) ₃	4	203-213	0.54
MG913153	EP042	F:TTTGGATGAGCTGTTGTTGTT R:AAGATGCTGGTACAAGATTGG	(TTA) ₄	2	151-153	0.42
MG913154	EP043	F:GGCAGTGTTCTCCATTTATCA R:TTTGAACCAGAATTTGAACCA	(ATTCTA) ₃	3	145-153	0.19
MG913155	EP051	F:ATACTAAAGGATCCCCGAAGG R:GCTAGCCCTCCTTAATAGTCTCT	(GTAT) ₃	3	148-154	0.51
MG913156	EP052	F:CGCTTTAATGCTAGGATTTTG R:AACAAGAGATTTCTTGGCCTA	(AAAT) ₃	3	138-143	0.42
MG913157	EP054	F:GTCTCTTTACAAGGGCTCCAC R:CCCGAGTACCAAGGTAAATGT	(TCA) ₄	3	130-134	0.42
MG913158	EP055	F:AAGAGCCACTTGTCCTTTCTG R:ATGTTTCTTGCAGTTTGTTCG	(TTTA) ₃	2	146-149	0.43

Table 2. continued

GenBank Accession No.	Locus	Forward and Reverse Primers (5'-3')	Repeat Motif	No. of Alleles	Allele Range (bp)	He _a
MG913159	EP056	F:GCAAGATAGCAGAGAAAGCAA R:TACTGGGATCCTGATTCTTCC	(CAAC) ₃	3	151-157	0.48
MG913160	EP058	F:CCTTCGCTTTTTACGTTATCC R:GAGCACCTACGGACCCTAC	(AAAT) ₃	3	149-155	0.51
MG913161	EP060	F:TAGAGCTCCTTCAATCACCTG R:GGAAGACTTCGGAGTGACAGT	(CAT) ₄	2	148-150	0.46
MG913162	EP064	F:ACAAAACCAAGTGGAACAACA R:GGGTGTGTTGATGTCGTTAAG	(ACA) ₄	3	151-157	0.22
MG913163	EP066	F:CTGGGGGAGGACTATTGTCTA R:CGGGTAGAAAGGGTGATTAAG	(TTTC) ₃	2	147-150	0.47

^aH_{exp}- Nei's genotypic diversity

Table 3. Genetic diversity indices of 8 subpopulations of *Erysiphe pulchra* using 15 microsatellite loci.

Subpopulatio n	N	MLG	H	Pa	H _{exp}	A _r	$\bar{r}_d$	$\bar{r}_d$ P-value
Pop 1	16	14	2.64	0	0.46	2.82	0.21	<0.01
Pop 2	23	15	2.71	1	0.5	2.91	0.18	<0.01
Pop 3	42	32	3.47	0	0.42	2.72	0.2	<0.01
Pop 4	20	16	2.77	0	0.19	2.34	0.01	NS ^a
Pop 5	9	8	2.08	0	0.29	2.55	0.27	<0.01
Pop 6	22	17	2.83	0	0.33	2.58	0.2	<0.01
Pop 7	18	11	2.4	1	0.31	2.57	0.27	<0.01
Pop 8	24	13	2.56	1	0.47	2.82	0.28	<0.01
Total/Average	174	97		3	0.45		0.17	<0.01

N- Number of samples; MLG- Number of multilocus genotypes post-clone correction; H- Shannon-Weiner index of diversity; Pa- Number of private alleles in each population; H_{exp}- Nei's genotypic diversity adjusted for sample size; A_r- Allelic richness; $\bar{r}_d$ - Standard index of association; ^aNS- Not significant

Table 4. Genetic diversity indices of 2 subpopulations of *Erysiphe pulchra* using 15 microsatellite loci.

Subpopulation	N	MLG	H	Pa	H_{exp}	A_r	$\bar{r}_d$	$\bar{r}_d$ P-value
South	86	57	4.04	18	0.46	3.57	0.16	<0.01
North	88	51	3.93	4	0.41	3.56	0.19	<0.01
Total/Average	174	97		22	0.44		0.16	<0.01

N-Number of samples; MLG- Number of multilocus genotypes post-clone correction; H- Shannon-Weiner index of diversity; Pa- Number of private alleles in each population; H_{exp}- Nei's genotypic diversity adjusted for sample size; A_r- Allelic richness; $\bar{r}_d$ - Standard index of association

Table 5. Pairwise population matrix of Nei's unbiased genetic distance of *Erysiphe pulchra* utilizing the clone corrected 8-subpopulation data set.

Subpopulations	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8
Pop 1	–							
Pop 2	0.061	–						
Pop 3	0.022	0.044	–					
Pop 4	0.399	0.189	0.341	–				
Pop 5	0.423	0.214	0.37	0.025	–			
Pop 6	0.352	0.176	0.304	0.027	0.025	–		
Pop 7	0.358	0.146	0.313	0.034	0.057	0.037	–	
Pop 8	0.038	0.037	0.027	0.363	0.379	0.323	0.309	–

Table 6. Analysis of molecular variance (AMOVA) for *Erysiphe pulchra* across 15 microsatellite loci for the A. 8-subpopulation data set grouped as one hierarchical group and B. 2-subpopulation data set grouped as one hierarchical cluster.

A. Eight Subpopulations

Source of Variation	df	Sum of Squares	Variance Components	% of Variation	P value
Among Subpopulations	7	85.37	0.61861 Va	18.61	<i>P</i> < 0.001
Within Subpopulations	118	319.30	2.70601 Vb	81.39	
Total	125	404.68	3.32462		
$\Phi_{st}^a = 0.19$					

B. Two Subpopulations

Source of Variation	df	Sum of Squares	Variance Components	% of Variation	<i>P</i> value
Between 2-Subpopulations	1	21.26	0.33712 Va	9.77	<i>P</i> < 0.001
Within 2-Subpopulations	106	329.99	3.11319 Vb	90.23	
Total	107	351.26	3.45031		
Φ _{st} ^a = 0.10					

^a Φ_{st} – Phenotypic differentiation index or the variance among subpopulations in relation to the total variance.

Table 7. Bottleneck analyses for *Erysiphe pulchra* samples in the A. 8-subpopulation data set grouped as one cluster and B. 2-subpopulation data set separated as two populations (north and south) using 15 microsatellite loci.

A.

Mutation model (excess/deficit) ^a					
Genetic Cluster	I.A.M	T.P.M.	S.M.M.	Mode-shift ^b	P-value
1	15/0	15/0	15/0	Shifted	<i>P</i> <0.001

B.

Mutation model (excess/deficit) ^a					
Genetic Cluster	I.A.M	T.P.M.	S.M.M.	Mode-shift ^b	P-value
South	15/0	15/0	15/0	Shifted	<i>P</i> <0.001
North	15/0	15/0	15/0	Shifted	<i>P</i> <0.001

I.A.M.- infinite allele model, T.P.M.- two-phase mutation model, S.M.M.- stepwise mutation model

^a(Excess/deficit) designate the number of loci exhibiting either excess or deficit of gene diversity under mutation-drift equilibrium

^bA shifted mode is expected in subpopulations which had a recent bottleneck

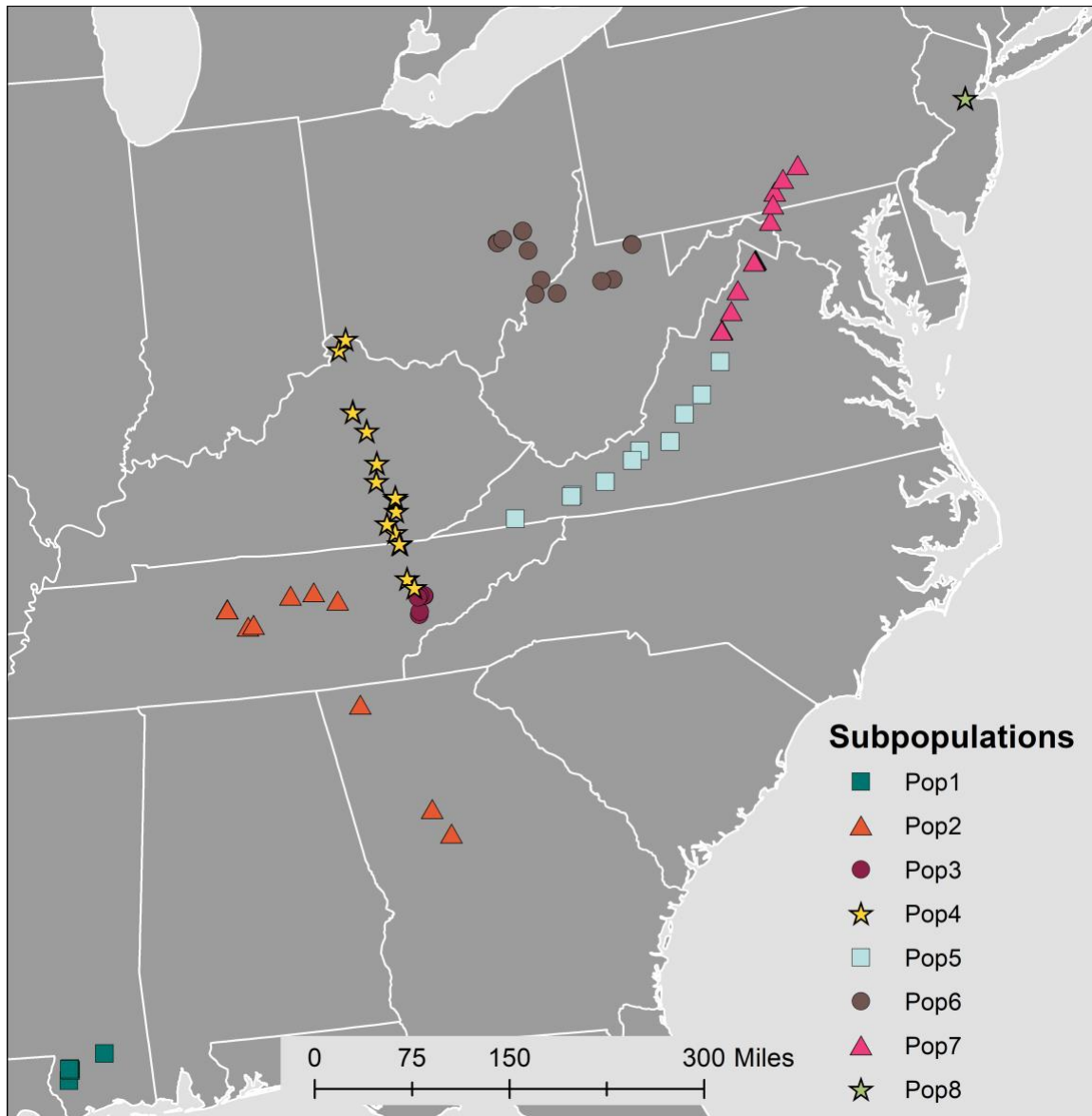


Fig 1. Location of the 174 samples of *Erysiphe pulchra* collected from 10 states in the eastern United States. Samples were assigned to eight arbitrary subpopulations based on relative geographical proximity.

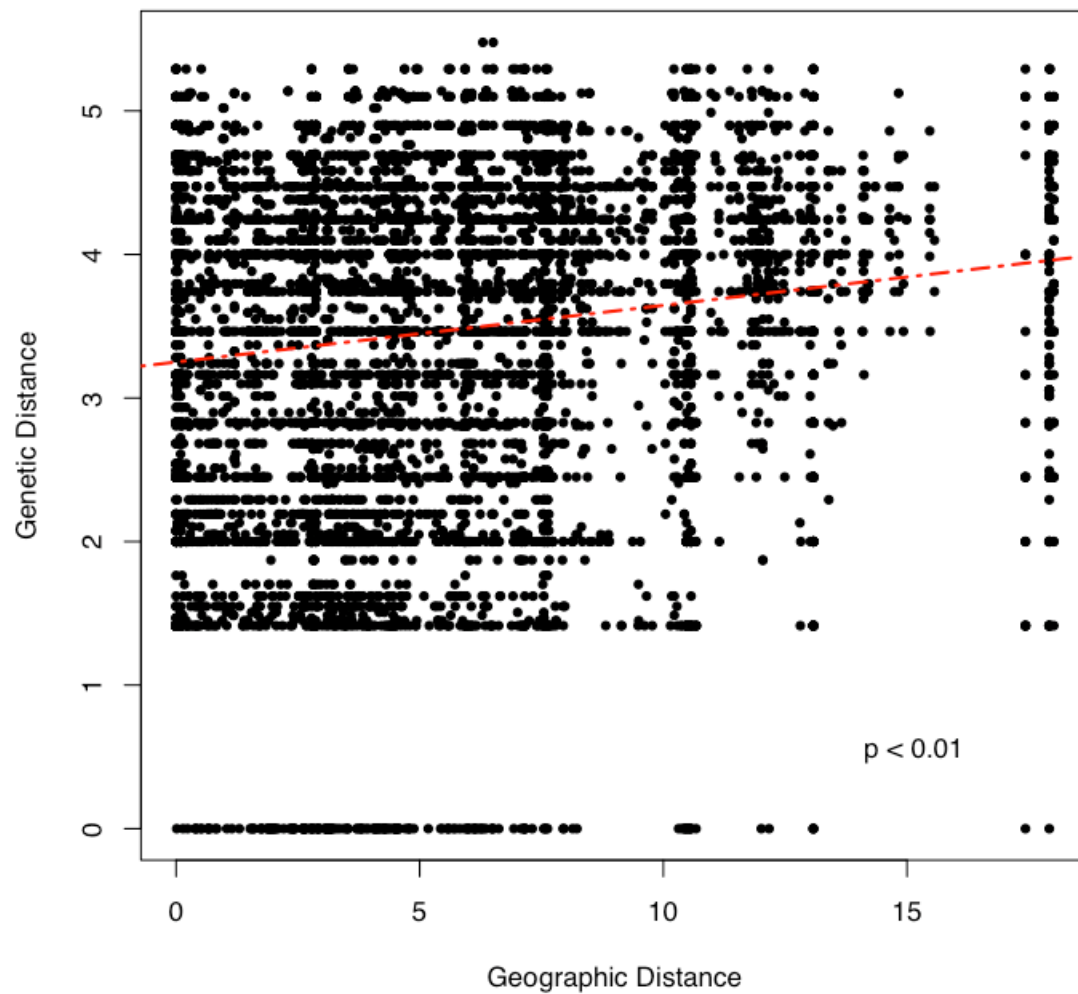


Fig 2. Mantel test of *Erysiphe pulchra* samples from the eastern United States arbitrarily assigned to eight subpopulations. The analysis used 100,000 permutations and significance of $P < 0.001$. Geographic distance is in log or km value?

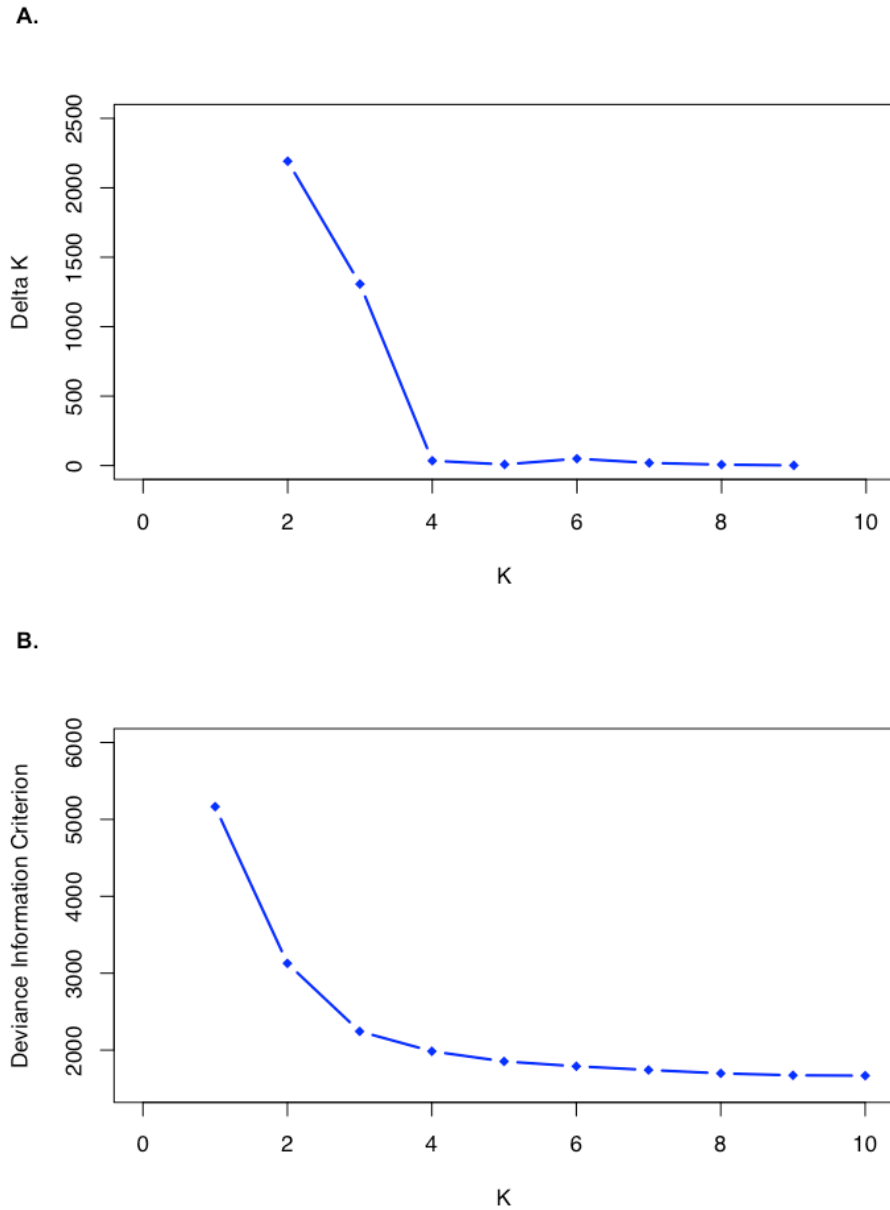


Fig 3. Bayesian clustering probabilities for *Erysiphe pulchra* samples in eight arbitrary subpopulations from the eastern United States, utilizing InStruct (A) and STRUCTURE (B). Using the deviance information criterion calculations, InStruct indicated the highest probability of K=1 (A). Evanno's method, utilized by STRUCTURE, calculated the optimum value of K=2 (B).

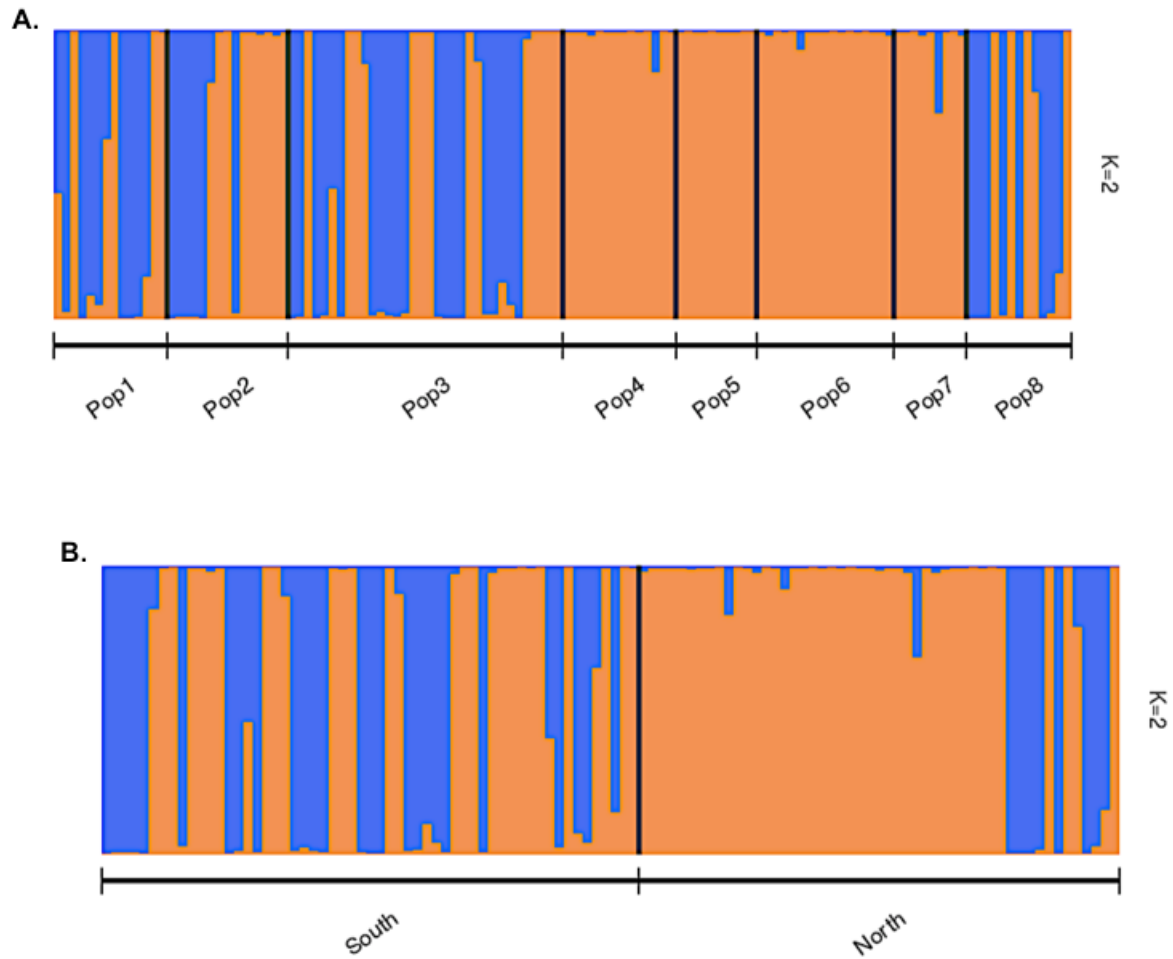


Fig 4. Structure bar graph indicating two genetic clusters ($\Delta K=2$) of *Erysiphe pulchra* samples from the eastern United States organized as (A) eight and (B) two arbitrary subpopulations and analyzed utilizing 15 microsatellite loci. Each bar represents the probability of an individual sample belonging to a designated cluster (represented by different colors).

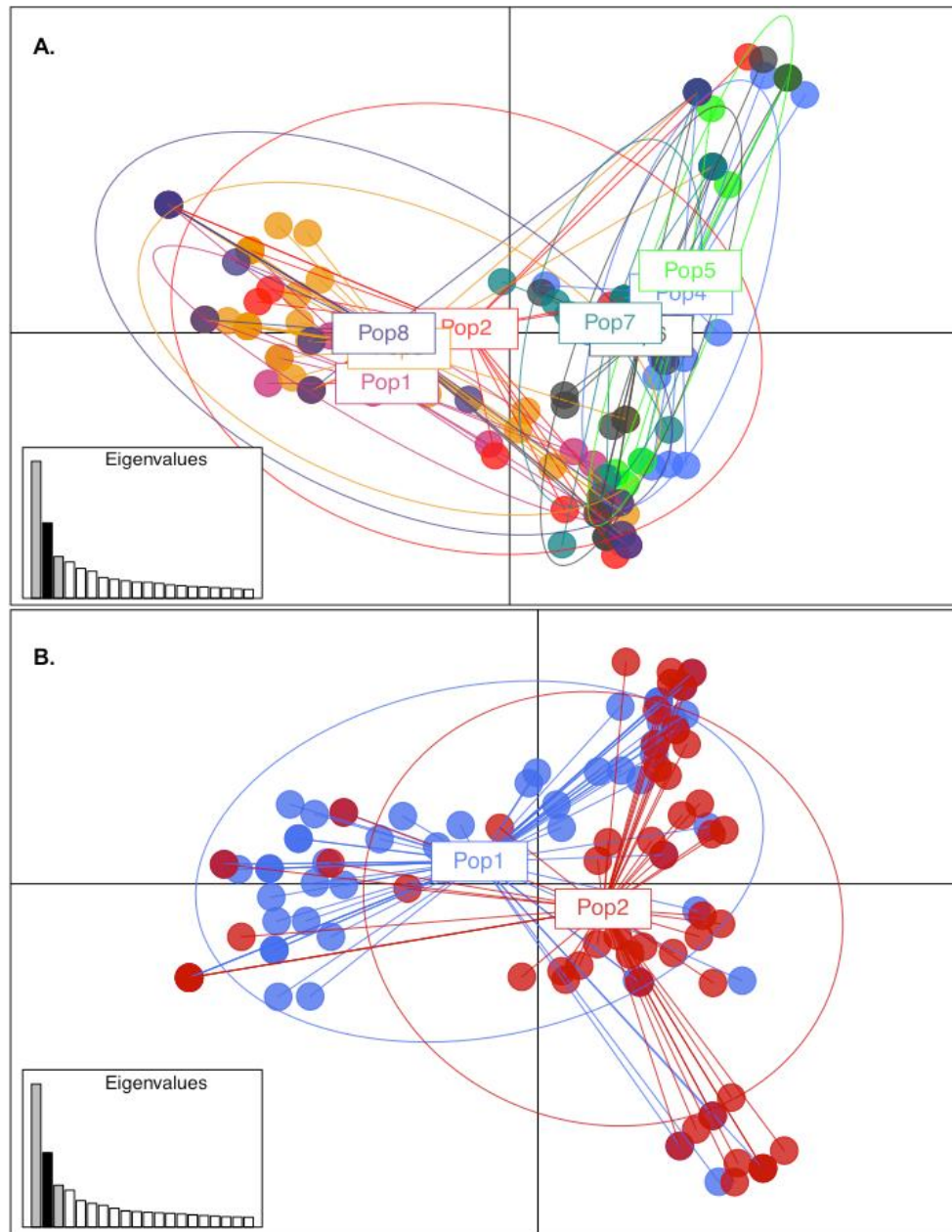


Fig 5. Principle coordinated analysis (PCoA) of *Erysiphe pulchra* samples organized as (A) eight and (B) two arbitrary subpopulations from the eastern United States, using 15 microsatellite loci. Axes 1 and 2 explain the majority of observed variation.

Appendix II: R Code Used in Statistical Analysis

```

library(poppr)
library(devtools)
library(adeigenet)
library(ape)

#### Reading GenAlEx file
EP<- read.genalex ("EP_8_174_15_Rinput_103017.csv", ploidy = 1, geo = FALSE,
region = FALSE, genclone = FALSE)
EP

##### Poppr Table #####
#### sample= is setting your permutations
EPpoppr<- poppr(EP, sample = 15000)
EPpoppr
write.table(EPpoppr, file = "EP_8_174_15_popprtable_103017.txt", sep = "\t")
summary(EP)

missingno(EP, type = "zero", cutoff = 0.05, quiet = FALSE, freq = FALSE)
mlg(EP, quiet = FALSE)

mlg.table(EP, sublist = "ALL", blacklist = NULL, mlgsub = NULL, bar = TRUE, total =
FALSE, quiet = FALSE)

EP

#####IBD#####
Dgen <- dist(EP$tab)
Dgen

##### Clone Correcting Data #####
clonecorrect(EP, strata = NA, combine = FALSE, keep = 1)
EPCC<-clonecorrect(EP, combine = FALSE, keep = 1)
EPCC

##### Shufflepop for ia #####
#first pop##
shufflepop(EPCC, method = 1)
method1<-popsub(EPCC, 1)
method1
ia(method1)

#second pop##
shufflepop(EPCC, method = 1)

```

```
method1<-popsub(EPCC, 2)
method1
ia(method1)
```

```
#3rd pop##
shufflepop(EPCC, method = 1)
method1<-popsub(EPCC, 3)
method1
ia(method1)
```

```
#4th pop##
shufflepop(EPCC, method = 1)
method1<-popsub(EPCC, 4)
method1
ia(method1)
```

```
#5th pop##
shufflepop(EPCC, method = 1)
method1<-popsub(EPCC, 5)
method1
ia(method1)
```

```
#6th pop##
shufflepop(EPCC, method = 1)
method1<-popsub(EPCC, 6)
method1
ia(method1)
```

```
shufflepop(EPCC, method = 1)
method1<-popsub(EPCC, 7)
method1
ia(method1)
```

```
shufflepop(EPCC, method = 1)
method1<-popsub(EPCC, 8)
method1
ia(method1)
```

```
mlg(EPCC, quiet = FALSE)
```

```
MLGCC<- mlg.table(EPCC, sublist = "ALL", blacklist = NULL, mlgsub = NULL, bar =
TRUE, total = FALSE, quiet = FALSE)
MLGCC
write.table(MLGCC, "EPCC_8_un_15_MLGCCtable_103017")
```

```

EPCC.vec <- mlg.vector(EPCC)
EPCC.vec

EPCCpoppr<- poppr(EPCC, sample = 10000)
EPCCpoppr
write.table(EPCCpoppr, "EPCCpopprTable_103017.txt", sep = "\t")

##### Private Alleles #####
EPCCpa<- private_alleles(EPCC, form = alleles ~Pop, report = "table", level =
"population", count.alleles = TRUE, drop = FALSE)
EPCCpa
write.table(EPCCpa, "EPCCpoppr_pa_Table_103017.txt", sep = "\t")
#Get raw number of private alleles per locus
pal<- private_alleles(EPCC, locus ~ Pop)
write.table(pal, file = "EPCCpoppr_pal_Table_103017.txt", sep = "\t")
#Get percentages
sweep(pal, 2, nAll(EPCC)[colnames(pal)], FUN = "/")

EPCCdiv <- locus_table(EPCC, info = TRUE)
EPCCdiv
EPnotCCdiv <- locus_table(EP, info = TRUE)
EPnotCCdiv
write.table(EPCCdiv, "EPCCdiv_table.txt", sep = "\t")
write.table(EPnotCCdiv, "EPdiv_table.txt", sep = "\t")
#### Difference between CC and not CC
locus_diff <- EPnotCCdiv - EPCCdiv
barplot(locus_diff[, "1-D"], ylab = "Change in Simpson's Index", xlab = "Locus", main =
"Comparison of Clone-Corrected vs. Uncorrected Data")

summary(EPCC)

##### Export to Genalex file#####
genind2genalex(EPCC, filename = "EPCC_8_174_15_Routput_103017.csv", quiet =
FALSE, pop = NULL, allstrata = TRUE, geo = FALSE, geodf = "xy", sep = ",", sequence
= FALSE)

EPCC.df <- genind2df(EPCC)
EPCC.df

write.table(EPCC.df, file = "EPCC_DF_Table_103017")

##### Calculate the Index pf Association and Standard Index off Association
ia(EPCC)
set.seed(1009)

```

```

EPCCia <- ia(popsb(EPCC), sample = 10000)

write.table(EPCCia, file = "EPCC_ia_Table_100217.txt", sep = "\t")

pair.ia(EPCC, quiet = FALSE, plot = TRUE, low = "blue", high = "red", limits = NULL,
index = "rbarD")

#### Bruvos Distance ####

EPCC_diss.dist <- diss.dist(EPCC)
EPCC_diss.dist

heatmap(as.matrix(EPCC_diss.dist), symm = TRUE)

Motif_length <- c(5, 3, 4, 4, 3, 6, 4, 4, 3, 4, 4, 4, 3, 3, 4)
Motif_length

EPCC_bruvo.dist <- bruvo.dist(EPCC, replen = Motif_length)
heatmap(as.matrix(EPCC_bruvo.dist), symm = TRUE)
attr(EPCC_bruvo.dist, "Labels")

EPCC_bruvo.dist.attr <- attr(EPCC_bruvo.dist, "labels")
attr(EPCC_bruvo.dist, "labels")
EPCC_bruvo.dist

#### Bruvo boot ####

EPCCDisTree <- bruvo.boot(EPCC, replen = Motif_length, cutoff = 75)
EPCCDisTree

cols <- rainbow(16)

plot.phylo(EPCCDisTree, cex = 0.50, font = 4, adj = 0, label.offset = 0.005)
nodelabels(EPCCDisTree$node.labels, adj = c(1.5, 1.2), frame = "none", cex = 0.7, font
= 4, xpd = TRUE)
axisPhylo(side = 1, cex = 0.55, font = 4)

#### minimum Spanning Network Bruvo (using Bruvo's distance) ####
set.seed(9005)

EPCC_bruvo.msn <- bruvo.msn(EPCC, replen = Motif_length, vertex.label.cex = 0.7,
vertex.label.dist = -0.5, palette = topo.colors(8))
EPCC_bruvo.msn

```

```

library(igraph)
plot(EPCC_bruvo.msn$graph,vertex.size = V(EPCC_bruvo.msn$graph)$size * 6,
vertex.label.cex = 0.75, vertex.label.dist = 1)
legend(1.55, 1, bty = "n", cex = 0.75, legend = EPCC_bruvo.msn$populations, title =
"Populations", fill = EPCC_bruvo.msn$colors, border = NULL)

### Minimum Spanning Network Poppr (using Distance matrix) ###
EPCC_diss.dist <- diss.dist(EPCC)
EPCC_diss.dist

set.seed(9005)
EPCC_poppr.msn <- poppr.msn(EPCC, EPCC_diss.dist, vertex.label = "inds", palette =
rainbow, gadj = 30, gweight = 2, wscale = TRUE, vertex.label.cex = 0.75,
vertex.label.dist = 0.8)

##### AMOVA #####
EPCC_AMOVA <- poppr.amova(EPCC, hier = ~Pop, clonecorrect = TRUE, within =
TRUE, dist = NULL, squared = TRUE, correction = "quasieuclid", filter = TRUE,
threshold = 0, missing = "zero", cutoff = 0.05, quiet = FALSE, method = "ade4", nperm =
99999)
EPCC_AMOVA

##### Nei's Distance by pop #####
EPCC_genpop <- genind2genpop(EPCC)
EPCC_genpop
EPCC.nei <- dist.genpop(EPCC_genpop, method = 1, diag = FALSE, upper = FALSE)
EPCC.nei

mydf.pop <- as.data.frame(as.matrix(EPCC.nei))
write.csv(mydf.pop, file = "EPCC_8_135_15_nei_13018.csv")

##### Nei's Distance by sample #####
EPCC.nei.dist <- nei.dist(EPCC, warning = TRUE)
EPCC.nei.dist
mydf <- as.data.frame(as.matrix(EPCC.nei.dist))
write.csv(mydf, file = "EPCC_16_135_15_nei.dist_81517.csv")

### PCoA ###
EPCC.eucl <- cailliez(EPCC_bruvo.dist)
EPCC.eucl
pcoa.EPCC <- dudi.pco(EPCC.eucl, row.w = "uniform", scanmf = FALSE, nf = 3, full =
FALSE, tol = 1e-07)

```

```

barplot(pcoa.EPCC$eig[1:50], main = "PCoA eigenvalues", col = heat.colors(50))

s.label(pcoa.EPCC$li)
title("PCoA of EP dataset 1-2")
add.scatter.eig(pcoa.EPCC$eig[1:27], 3,1,2)

s.class(pcoa.EPCC$li, pop(EPCC), xax = 1, yax = 2, csub = 1)
title("PCoA of Erysiphe pulchra populations\naxes 1-2")
add.scatter.eig(pca1$eig[1:20], nf = 3, xax = 2, yax = 2, posi = "bottomright")

col <- c("violetred3", "red", "orange2", "royalblue1", "green", "grey27", "turquoise4",
"slateblue4")
s.class(pcoa.EPCC$li, pop(EPCC), xax = 1, yax = 2, col = transp(col, 0.8), axesell =
FALSE, cstar = 1, cpoint = 4, grid = FALSE)
add.scatter.eig(pcoa.EPCC$eig[1:20], nf = 3, xax = 2, yax = 2, posi = "bottomleft")

##PCA##
sum(is.na(EPCC$tab))
X <- scaleGen(EPCC, NA.method = "mean")
X
class(X)
dim(X)
X[1:126,1:42]
pca1 <- dudi.pca(X, center = FALSE, scale = FALSE, scanf = FALSE, nf = 3)
barplot(pca1$eig[1:50], main = "PCA eigenvalues", col = heat.colors(50))
pca1

s.label(pca1$li)
title("PCA of EP dataset 1-2")
add.scatter.eig(pca1$eig[1:27], 3,1,2)

s.class(pca1$li, pop(EPCC), xax = 1, yax = 2, csub = 1)
title("PCA of Erysiphe pulchra populations\naxes 1-3")
add.scatter.eig(pca1$eig[1:20], nf = 3, xax = 2, yax = 3, posi = "bottomright")

col <- funky(10)
s.class(pca1$li, pop(EPCC), xax = 1, yax = 3, col = transp(col, 0.8), axesell = FALSE,
cstar = 1, cpoint = 4, grid = FALSE)
title("PCA of Erysiphe pulchra populations\naxes 1-3")
##### Monte-Carlo test #####

### IBD ###

```

```

EPCC_xy <- read.genalex("EPCC_8_174_15_Routput_103017xy.csv", ploidy = 1, geo
= TRUE, region = FALSE)
EPCC_xy
EPCC_xy@other$xy

Dgen <- dist(EPCC_xy$tab)
Dgen
Dgeo <- dist(other(EPCC_xy)$xy)
Dgeo
ibd <- mantel.randtest(Dgen, Dgeo)
ibd

##### Mantel test#####
mantel.randtest(m1 = Dgen, m2 = Dgeo, nrepet = 100000)
plot(ibd)
plot(Dgeo, Dgen, xlab = "Geographic Distance", ylab = "Genetic Distance", pch = 20)

abline (lm(Dgen ~ Dgeo), col = "red", lty = 6, lwd = 2)
text(15.15, 0.55, paste("p < 0.01"), cex = 1)

pop(EPCC)
EPCC$pop

```

VITA

Christopher R Wyman was born in Stamford, CT, USA on September 1, 1987. He attended and graduated from Tallwood High School, Virginia Beach, VA, USA in 2005. Christopher served a two year full time mission for the Church of Jesus Christ of Latter-day Saints from 2007-2009. After completion of his missionary service, Chris earned his B.S. in Biology with a Biotechnology emphasis in 2014 from Brigham Young University-Idaho, Rexburg, ID, USA. He then joined the Department of Entomology and Plant Pathology under the mentorship of Dr. Robert N Trigiano to further pursue his professional and educational goals.