PHYLOGEOGRAPHY AND MATING SYSTEM OF *SPIRAEA VIRGINIANA* BRITTON: A MULTI-SCALE EXPLORATION OF THE BIOLOGY OF A THREATENED SPECIES

A Thesis
by
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FOREWORD

This thesis is formatted to facilitate submission for journal publication. Citations, tables, figures, and headings are formatted in the style of *Plant Species Biology*.

ABSTRACT

PHYLOGEOGRAPHY AND MATING SYSTEM OF *SPIRAEA VIRGINIANA* BRITTON: A MULTI-SCALE EXPLORATION OF THE BIOLOGY OF A THREATENED SPECIES

(August 2010)

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This thesis explores the genetic structure and reproductive biology of *Spiraea virginiana*, a threatened shrub endemic to 2nd and 3rd order drainages in the southern Appalachians and Cumberland Plateau. Because little is known about the species, characterization of its mating system, potential for sexual reproduction, and evolutionary relationships within the species could lead to improved conservation management plans. A dataset created with eight Inter Simple Sequence Repeats (ISSRs) was analyzed, and phylogenetic analysis based on maximum parsimony revealed similarity within drainages, as well as monophyly of the species. To assess maternal relationships within the species, three regions of non-coding chloroplast DNA were sequenced, and a Bayesian phylogenetic analysis was conducted. In addition, a haplotype network was constructed, and the haplotype network and major clades identified in the Bayesian analysis were mapped. Both analyses showed little structure within drainages and potential relatedness across elevational gradients, although drainages were not sampled heavily. Pollination treatments were conducted in

a common garden setting and in three wild populations along the New River in Ashe County, NC. Results from these treatments tentatively suggest the species has a mixed mating system, and can reproduce sexually, contrary to prior observations. However, seed size, seed weight, and fruit set were variable among populations. With the knowledge provided by both studies, I conclude that sexual reproduction in the species may be more common than previously thought, and that, based on the patterns revealed by the phylogeography, seed may be wind-dispersed. Heavier sampling within drainages and population genetic studies should be conducted to reveal biological processes on smaller scales to confirm these hypotheses.

DEDICATION

For my grandmother, Lena Pate, who never stopped learning, never took herself too seriously, and taught me that you have to work hard for the things you really care about.

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Chapter One: Introduction

Phylogeography

The climate within the past three million years has fluctuated greatly, resulting in multiple ice age ages. Glaciers have expanded and contracted, and biotic assemblages have adjusted their distributions depending on the climate. During the late glacial interval of the Pleistocene (Riccardi 2009), the boreal and temperate forests expanded into more southern regions of North America (Delcourt & Delcourt 1984). The geographical extent to which this flora reached has been debated for many decades.

According to Braun (1950), very few species within the highest elevations of the Cumberland Mountains in the southeastern United States would be considered northern, and no spruce-fir forest remains in this region. Many coastal plain species have been found in bog-like stream headwaters on the Cumberland Plateau (Braun 1950). In light of this information, Braun suggested that Pleistocene flora did not migrate as far south as the Cumberland Plateau (Braun 1950), deciduous forests remained on the Appalachian Plateaus in Ohio and Kentucky, and Southern Appalachian endemics persisted in their current locations throughout glaciation (Braun 1951).

Deevey (1949), however, came to an alternate conclusion. Citing evidence from plant chromosome studies (Anderson 1936) which show that a tetraploid species of *Iris* was formed when Pleistocene glaciation forced two northern species (*setosa* var. *interior* from

central Alaska and *virginica* var. *shrevi* from northern Appalachia) southward, Deevey suggested both species would have had to migrate very far south in order to hybridize. He also cited a study of the distributions of *Plethodon* salamanders within eastern North America (Hairston and Pope 1948), which can be more easily explained in light of vicariance by multiple glacial ages. He suggested that the diversification caused by many cycles of glacial advance and retreat would create the environments likely to harbor the number of endemics found in the southeastern United States (Deevey 1949).

Since the mid-20th century, multiple paleoecological studies have emerged that address this issue. According to the fossil pollen data, when the climate supported growth of glaciers into Wisconsin and Pennsylvania, many Appalachian plants migrated southward towards warmer climates near the Gulf of Mexico and Atlantic Ocean, outside of their current range of the Ohio River watershed (Delcourt & Delcourt 1991). Palynological evidence of boreal-like forest has been found as far south as the Shenandoah Valley in Virginia, and macrofossils of boreal species such as jack pine (*Pinus banksiana)* have been found as far south as Louisiana (Critchfield & Little 1966), indicating the expansion of tundra throughout the northern portion of the continent (Delcourt 1979). While some of these species were unable to survive the extreme climate change (Stewart 2009; Hewitt 2000), mountain climates provided multiple refugia during the last glacial retreat (Gonzales & Hamrick 2005; Watts 1970; Soltis *et al*. 2006). A diverse flora and a large number of endemic species within the southeastern United States is likely due to the divergence of genomes within these refugia during the process of glacial metamorphosis (Hewitt 2000).

Within the early Holocene (12,500-8500 years BP) following the Last Glacial Maximum, the climate warmed, glaciers receded, and the region west of the Appalachians

responded quickly to the change in temperatures, while the region east of the Appalachians responded more slowly (Delcourt & Delcourt 1984). Species began a slow northern progression, radiating from the southeast. Adams (1902) identified three potential paths of dispersal to repopulate previously glaciated northern areas: the Mississippi Valley and its tributary streams (especially the Tennessee and Cumberland Rivers), the Coastal Plain, and the southern Appalachians and their neighboring plateaus.

Newly available molecular techniques have since provided a means to test previous hypotheses of biogeography and dispersal. Within the Appalachian region, intraspecific phylogeographies have been conducted on annelids (Wirchansky & Shain 2009), arachnids (Hedin & Thomas 2009), amphibians (Camp *et al*. 2009; Timpe *et al*. 2009; Tilley *et al*. 2008; Austin & Zamudio 2008), mammals (Arbogast *et al*. 2005; Sipe & Browne 2004), and plants (Godbout *et al*. 2005; Gonzales *et al*. 2008; Schlaepfer *et al*. 2008), among others, many in the interest of conservation. In their comparison of recent biogeographic studies, Soltis *et al*. (2006) found six major patterns in the breaks in genetic distributions across the southeastern United States and listed the Appalachian Mountains as one of these causes of vicariance. Several studies of angiosperms also identified a latitudinal contribution to genetic diversity (Dorken & Barrett 2004; Magni *et al*. 2005), but others reported no apparent genetic structure (Shaw & Small 2005; Morris *et al*. 2008). However, a large number of temperate species in North America show a genetic signal suggestive of rapid expansion north due to recent climatic change (Lessa *et al*. 2003).

The family Rosaceae provides an interesting group of species with which to address biogeographic questions (Potter *et al*. 2007a). Laurasian in distribution, most genera in Rosaceae are found primarily in temperate regions within North America, Europe, and

eastern Asia (Zomlefer 1994). Weakley (2008) lists 37 genera within Rosaceae as either native or naturalized in his Flora (which includes most of the southeastern U.S.). The family has been widely studied because of its large number of economically important species. Species within Rosaceae can be herbaceous or woody, and have perfect, actinomorphic flowers with a conspicuous cup-like hypanthium and many projecting stamens (Zomlefer 1994). Although poorly understood for decades because of the general lack of other morphological synapomorphies to unify Rosaceae, the monophyly of the family has been confirmed by recent phylogenetic work (Potter *et al*. 2007a). The evolution of this large group has been difficult to understand, and multiple genera within the family exhibit polyploidy, apomixis, and/or hybridization. Dickenson *et al.* (2007) found that the large number of species in the family was linked to the tendency of some genera to produce unreduced female gametes in polyploidy.

The subfamily Spiraeoideae within Rosaceae is distinguished by several morphological and chemical characteristics. Many species contain large quantities of sorbitol, which has been used as a sugar substitute in many products. Cyanogenic glycosides, which confer protection from herbivores, are also found throughout the group. Leaves are simple and alternate, stipules are typically present, and within the flowers, pistils number between one and five. Base chromosome number has been recorded as x = 8, 9, 15, or 17 (Potter *et al.* 2007b). Morgan *et al*. (1994) found Spiraeoideae non-monophyletic when using *rbc*L chloroplast sequence data, but the monophyly of the group was later affirmed using nuclear data (Potter *et al*. 2007a).

The tribe Spiraeeae within Spiraeoideae has been given multiple taxonomic treatments over the years. The tribe has been significantly reduced since described by de

Candolle (1825), and at one time included species now within *Physocarpus* (Cambess.) Raf., *Sorbaria* A. Braun, *Aruncus* Adans., and *Filipendula* Mill. in addition to *Spiraea* sensu stricto (Potter *et al*. 2007b). Currently it includes nine genera, the largest of which is the genus *Spiraea*. Rehder (1940) divided *Spiraea* into 3 sections based on inflorescence. These sections were not corroborated by a molecular study, but compound corymbs, found in section *Calospira*, have been suggested by that study to be the ancestral inflorescence type (Potter *et al*. 2007b). Monophyly of the group was well-supported based on internal transcribed spacer (ITS) sequences, as was monophyly of the genera (Potter *et al.* 2007b). A lack of stipules and unitegmic ovules has been suggested as a synapomorphy for the tribe (Evans & Dickinson 1999), and the origin of the group was determined to be in western North America (Potter *et al*. 2007b).

Spiraea L. is widely distributed throughout North America and temperate parts of Asia and Europe. Species within the genus are used horticulturally and are medicinally important in Asia because of the alkaloids they produce (Zhang *et al*. 2006). Ploidy levels vary widely from diploid (Sun *et al*. 1997) to octoploid (Oginuma *et al*. 1999). Within the eastern United States, species are diploid, triploid, tetraploid or hexaploid (see Table 1).

Three members of section *Calospira* occur in the southeastern United States: *S. corymbosa* Rafinesque, *S. virginiana* Britton, and *S. japonica* L. f., which is not native but has naturalized in the area. *Spiraea japonica* is easily distinguishable, but differentiating between *S. corymbosa* and *S. virginiana* has caused taxonomists considerable difficulty (Ogle 1991a). The two species overlap in many characters, although stamen length, size at maturity, leaf serration, and corymb size seem to separate them (Ogle 1991a). They also prefer very different habitats – while *S. virginiana* is found in riparian corridors, *S.*

corymbosa is found on dry outcrops and rocky woods (Ogle 1991a). In a brief examination of both species' distributions based on the Flora of the Southeast (Weakley 2008), they do not appear to occur within the same counties. Sax (1936) noted that *S. corymbosa* was completely pollen sterile but that the species was known to hybridize, so it must produce viable eggs. However, he did not list the number of accessions he examined before making this determination.

Table 1 Chromosome numbers of some *Spiraea* species in eastern North America.

Taxa	Chromosome Number	Researcher
Spiraea alba	4n	Baldwin 1951
Spiraea latifolia/ var. *septentrianalis*	4n, 6n	Baldwin 1951; Sax 1936
Spiraea tomentosa, forma *albiflora*	4n	Baldwin 1951
Spiraea japonica	2n, 4n	Baldwin 1951; Sax, 1936
Spiraea virginiana	4n	Glencoe 1961
Spiraea corymbosa	3n, 4n	Baldwin, 1951; Sax 1936

Understanding species boundaries is essential to the foundation of biological understanding and the description of biodiversity (Cracraft 2002). However, no species concept has been found to be agreeable to all biologists, so the debate over the definition of a species continues to thrive. The definition seems to vary by the motivation of the researcher. Mayden (1997) lists 21 species concepts in his review of the problem, many of which are similar in that they define a species as a related group of organisms that share evolutionary processes and are separate from other groups of organisms that lack those shared processes (Hey 2001). The most utilized of these, arguably, is the Biological Species Concept (BSC)

proposed by Mayr (1942; 1996). This concept defines a species as a group of reproducing, or potentially reproducing, organisms which are reproductively isolated from other organisms. The BSC is unwieldy in plant systematics because reproductive barriers often are absent between plant species, even when they remain separate in character. Plants can tolerate the polyploidy that can result from hybridization and even produce viable hybrids. Cracraft (1983) suggests that, in practice, the choice of species concepts is necessarily between the BSC and the Phylogenetic Species Concept (PSC), which defines a species as the smallest recognizable monophyletic group united by a shared character. The utility of the PSC is questionable, however. DNA sequencing can identify differences between individuals and, by definition, could place each individual into a separate species, regardless of the similarities that may unify them (Lowe *et al*. 2004). Bock (2004) suggests there is no species concept that can apply to all organisms because of the conflicts encountered when placing evolutionarily dynamic organisms in static categories. However, the Evolutionary Species Concept proposed by Simpson (1961), which describes a species as a lineage evolving separately from others and having its own evolutionary role and tendencies, unifies these various concepts, although it is non-operational (Mayden 1999).

Spiraea virginiana, occupies a very specific niche. Listed as Threatened by the US Fish and Wildlife Service (USFWS) since 1990 (Blanchard 1990), this riparian shrub occurs within the Ohio River watershed on high-quality tributaries of the Cumberland, Tennessee, and Ohio Rivers. It is also considered endangered by the states of North Carolina, Ohio, Tennessee, and Virginia. Of all the tributaries in the lower Mississippi drainage, the Ohio River receives the most input and most evenly distributed amount of precipitation throughout the year (Thomas 1928). Flooding was somewhat common in these waters, at least

historically, and occurred primarily in December – March during snow melt (Thomas 1928). These rivers are typical of the large upland rivers described by Bornette *et al*. (2008), which deposit material along edges or point-bars and deposit silt and clay sediments in larger floods before those waters recede to the floodplain. However, all three rivers' flood regimes have changed since the early 1900s due to impoundments (Newell & Rice 1978; Beckett & Miller 1982; Hedgecock *et al*. 2007) leading to a reduction in flood frequency and intensity. The areas where *S. virginiana* occurs seem to be upstream of dams (more extensive mapping would elucidate this further), so this may explain the patchy distribution of the species. Because this species was only described in 1878 (by Vasey), it is possible that the species was somewhat more widespread in the past, but that damming may have reduced the disturbances to which it was adapted.

The species has white to yellow/green flowers in terminal compound corymbs ranging from 5-22 cm wide. Flowering occurs from late May to late July (Britton & Brown 1913, Ogle 1991a). Stamens are twice the length of the sepals and protrude well beyond the rim of the floral cup (Ogle 1991a; Pate, pers. obs.) In addition to Flower Long-Horn Beetles (Cerambycidae), Flower Beetles (Phalacridae), and Soldier Beetles (Cantharidae) (Ogle 1991b), flowers are also visited by members of Halictidae, Apidae, and Vespidae (Pate, pers. obs.). *Spiraea virginiana* seedlings have never been reported in the wild, although when populations from different drainages were placed together in a common garden at Virginia Highlands Community College (VHCC), the plants produced seed (Ogle 1991b). Ogle hypothesized that the low numbers of seed produced in wild populations may be due to a lack of genotypic diversity within locales (Ogle 1991b). Because of potential isolation within drainages, populations may be limited by low genetic diversity.

According to Ogle (1991b), reproduction in wild populations depends on scouring floodwaters transporting broken rhizomes downstream, suggesting the species may be highly clonal. Gene flow may be very limited in species where ramets are connected by short internodes and populations grow in close proximity to one another (Eckert 2000; Charpentier 2001). A previous study showed variance in leaf morphology within the species, correlating with the drainage in which the plants are found. Random Amplified Polymorphic DNA (RAPD) analysis of the genomes showed greater diversity in the Cumberland and Tennessee River drainages than in the Ohio River drainage (Anders & Murrell 2001), but this study had limited resolution because of the nature of the markers and did not include the headwaters of the Ohio in the New River of Ashe and Watauga Counties in North Carolina. Even though the plant is restricted to riverine systems, other species within *Spiraea* are wind-dispersed (Zasada & Stickney 2009), so if seeds are viable, gene flow across drainages may be possible in several areas, such as the Northern Peaks area of northeast North Carolina, Lookout Mountain near Chattanooga, and the Cumberland-Tennessee divide in Tennessee.

Understanding the genetic variation among populations is critical in order to practice effective and efficient conservation plans for rare plants (Xiao *et al*. 2004). Molecular markers can be particularly useful in determining the amount of genetic diversity within species (Escaravage *et al*. 1998). The Murrell lab has conducted two previous molecular studies of *S. virginiana*, the first using RAPDs and the second using Inter Simple Sequence Repeats (ISSR) (Anders & Murrell 2001; Williams 2003). These studies have been informative, but because of the lack of reproducibility of RAPDs and the need to test the conclusions of these previous studies, more research was required. This study examines the phylogeography of the species in order to provide answers concerning microevolution in the

southern Appalachians, the genetic structure of the species, and the life history details necessary to manage this threatened species.

Mating system

Information about the mating system of species is important to phylogeographic studies because of the influence the breeding system has on gene flow. Selfing species will have lower gene flow between and within populations, allowing genetic drift or selection to cause differentiation among populations, whereas outcrossing tends to have the opposite effect and leads to more similarities among populations (Glémin *et al*. 2006). Limited seed and pollen dispersal is also responsible for a low level of gene flow and high levels of interpopulation variation (Wallace 2002). Since the reproductive biology determines the amount and distribution of diversity within the system, the initial step in designing a rare-plant management plan is to identify the species' breeding system (Demauro 1993). Species within Rosaceae are typically outcrossing, and many studies have found self-incompatibility within these systems (Bell & Hough 1986; Brown 1910; Lewis & Modlibowska 1942).

Self-incompatibility (SI) is the failure of a fertile hermaphroditic seed plant to produce zygotes after self-pollination (de Nettancourt 1977) due to identical genotype expressed in the pollen and female sporophyte tissue (Mulcahy & Mulcahy 1986). Two plants can be cross-incompatible if they have the same self-incompatibility genotype, as well (Demauro 1993). Self-incompatible pollination systems evidently evolved through selection to generate new combinations of genes, thereby preventing possible inbreeding depression which may occur in self-pollinating plants (Niklas 1997) Self-incompatibility has been

found in many families (e.g. Onagraceae, Brassicaceae), and one study estimated that 60% of flowering plants have some form of SI (Hiscock *et al.* 1999).

Two major types of SI systems exist in homomorphic plants – gametophytic and sporophytic – and these systems control the pollen-pistil interactions and the resulting genetic consequences for each plant (Pandey 1960; Arasu 1968; de Nettancourt 1977; Heslop-Harrison 1978; Charlesworth 1985). Gametophytic and sporophytic SI systems differ in the molecular mechanism that determines incompatibility, although both occur at one locus (the S-locus, or the S-genes complex, per Castric & Vekemans 2004) in most systems. Gametophytic systems rely on the haploid genotype of the pollen grain, the gametophyte, to reject the pollination (Newbigin *et al*. 1993). In this system, if a pollen grain and the stigma on which it is deposited express a common S-allele, then a pollen tube will germinate, but growth is suppressed before it reaches the ovary. If two plants share only one S-allele, half of the pollen grains will be able to fertilize ovules, as heterozygotes produce two types of pollen (Castric & Vekemans 2004). Sporophytic systems depend on the sporophytic tissue on the outside of the pollen grain to identify the presence of another genotype. If a pollen grain produced by a plant with an identical genotype lands on the stigma of an angiosperm with this type of system, then the pollen grain typically will not germinate, so no pollen tube is formed. In sporophytic systems only one pollen type is produced, and compatibility is determined by dominance or co-dominance (Castric & Vekemans 2004). Either of these systems is hypothesized to be beneficial in large, continuous populations, as outcrossing increases genetic diversity and the potential for populations to adapt to their environments.

Individuals in large SI populations containing multiple S-locus alleles have many mate options and easily produce seed. As human activities continue to fragment the

landscape, however, large continuous populations are becoming less common. Smaller, more isolated populations have fewer buffers against environmental stochasticity and genetic drift, and SI populations may be even more at risk because of a decrease in mate availability. Expression of SI may reduce reproduction if a substantial amount of the pollen received is incompatible and may even prevent reproduction in small groups of structured populations (Levin *et al*. 2009, Byers & Meagher 1992; Goodell *et al.* 1997). A study of multiple angiosperm species with a range of life histories surveyed factors contributing to angiosperm pollen limitation and showed SI populations were significantly more pollen-limited than self-compatible populations (Larson & Barrett 2000). In populations that have been through a bottleneck and have little genetic diversity, it has been previously suggested that this strategy can threaten populations to the point of extinction (Demauro 1993). If multiple genotypes do not occur near each other, and one allele is fixed at the S-locus, then the population could no longer reproduce. Once sexual reproduction is not possible, the rarity of the species would inevitably increase, unless vegetative reproduction is part of the species' life history, and even then, the population does not have long-term viability.

The most famous example of this is Demauro's (1993) study of the lakeside daisy, *Hymenoxys herbacea* (syn. *acaulis* var. *glabra*). The species is endemic to the Great Lakes area and is federally threatened. A pollination study revealed strong evidence of sporophytic SI, and closer examination revealed that a site in Illinois was fixed for one S-allele, rendering it effectively extinct. Computer simulations suggested that population sizes of less than 16 would go extinct because of their inability to maintain the number of S-alleles to allow reproduction, and this explained the rarity of the subspecies.

The positive relationship between population density and growth is known as the Allee effect (Allee 1931). An Allee effect occurs when a decrease in population density leads to reduced reproduction, and may result in extinction if the population numbers fewer than the threshold (Allee 1931). In the case of SI systems, this effect occurs as a result of reduced numbers of S-alleles when population density decreases.

Because populations of *S. virginiana* are clonal and may be isolated within drainages, an SI mating system could severely limit potential sexual reproduction. Based on observations by Ogle and studies completed by the Murrell lab, there are four possible scenarios for the species regarding its mating system and the migration of the species. Regarding the species distribution, I expect that if the species is reproducing by genets, populations in different drainages will be genetically distinct because dispersal would only be possible downstream. Because of the evidence from previous studies concerning ancestry of the species, I hypothesize that southern populations will have more ancestral characteristics because of migration during glaciation. In regards to its mating system, I hypothesize that *Spiraea virginiana* has a self-incompatible mating system, suggesting low mate availability may be limiting sexual reproduction.

Objectives

My study has three primary objectives:

- Use three regions of non-coding chloroplast DNA to distinguish among populations and infer evolutionary relationships within *S. virginiana*
- Determine if *S. virginiana* is self-incompatible
- Further determine if wild seeds are viable and if pollination can occur between wild populations.

Chapter Two: Methods

Phylogeography

Four different investigators extracted the DNA used in this study using two different methods (see Table 2). Estep (2002) extracted DNA from 16 of my samples of *S. virginiana* collected across the range by Ogle (1991b) and cultivated at Virginia Highlands Community College (VHCC) in Abingdon, VA. Ogle had previously deposited corresponding voucher specimens from his study at the Arnold Arboretum at Harvard University, MA (see Appendix D). Estill and Williams did not collect vouchers. I collected three samples of *Spiraea corymbosa* from fresh leaf material in the summer of 2009 from Alexander County, NC, and deposited voucher material at the herbarium at Appalachian State University, Boone, NC. I included an additional sample of frozen *Spiraea japonica* leaf material collected by Estill (1996) as an outgroup. Estep (2002) and I extracted DNA from fresh leaf tissue using the Qiagen DNeasy Plant Mini Protocol using the manufacturer's protocol (69104 QIAGEN, Inc.). I modified this protocol slightly by adding 1 teaspoon of sterile sand to leaf tissue before grinding with liquid nitrogen and I used a chilled (-20° C) mortar and pestle. Estill (1996) and Williams (2003) extracted DNA from three samples of frozen leaf tissue using the CTAB micro-extraction protocol developed by Torsten Eriksson (1994, pers. comm.) and described in Williams (2003). I evaluated all extractions for DNA concentrations and quality using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.)

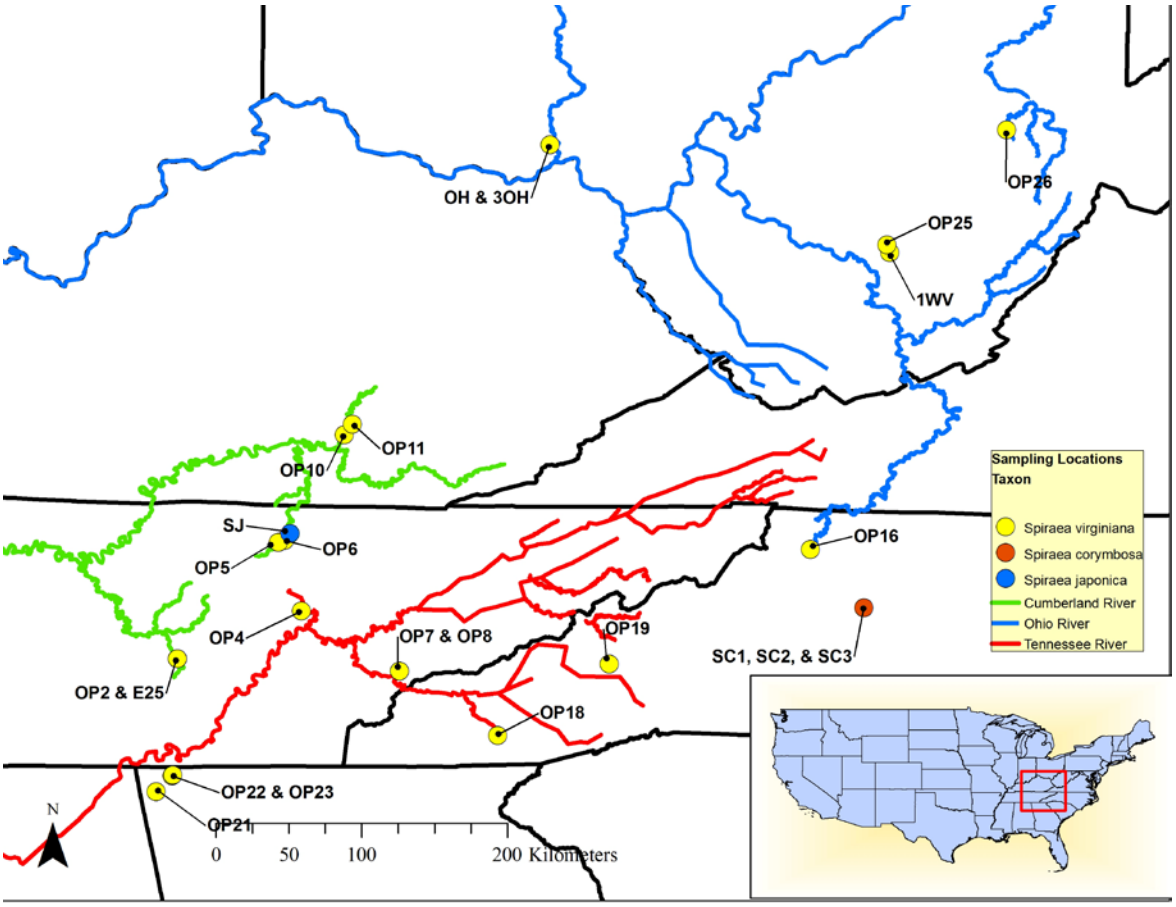

Fig. 1. Origin of *S. virginiana* populations sampled for sequencing. Base layer is World Physical Map, ESRI 2009.

In order to determine the level of statistical confidence I could place in the Williams (2003) analysis, I acquired the original dataset from Williams's (2003) study, and repeated her phylogenetic analysis using PAUP (Swofford 2003) with an additional bootstrap analysis. Because the initial dataset revealed identical banding patterns in samples collected from the same localities and this impeded the efficiency of the phylogenetic analysis, I reduced the dataset to 28 taxa from different locales. I formatted the data in MacClade (Maddison & Maddison 2005) and then conducted a full heuristic search with additions automatically added and all characters weighted equally to generate the best tree based on maximum parsimony in PAUP, using the tree bisection-reconnection (TBR) swapping algorithm. I

bootstrapped the trees, applying 500 replicates and utilizing the "keepall" option, which retains groupings occurring at lower frequencies as long as they are compatible with groupings occurring at higher frequencies (>50%) (see Appendix A for code from executable file). I used the 15 most parsimonious trees to construct strict and 50% majority consensus trees from the heuristic search. I used trees from the bootstrap analysis to construct a bootstrap consensus tree.

Of the extractions by Estep and Williams, I chose 22 that were representative of the range of *S. virginiana* (Fig. 1). For outgroup comparisons I used three samples of *S. corymbosa* and one sample of *S. japonica*. I screened ten sets of universal primers (Ebert & Peakall, 2009b) in non-coding chloroplast regions for potential utility. These primers were recently developed to facilitate phylogenetic research in orchids (Ebert *et al*. 2009), but because of their novelty, their phylogenetic utility is still being explored (although they have been tested on a large number of species). I PCR-amplified three regions within the large single-copy (LSC) region (*atp*Fex1-*atp*H, *rpo*C2-*rps*2, and *rps*16ex1-*rps*16ex2 (Fig. 2; Table 3)) using a GeneAmp PCR System 9700 thermocycler (PE Biosystems, Foster City, CA) and the "touchdown" PCR method (Don *et al*. 1991), resulting in single bands. Reactions began with a three-minute 94°C denaturation cycle, followed by 44 cycles of 30 s at 94°C, 30 s at a variable annealing temperature (see Table 3), and 45 s at 72°C. The annealing temperature began at the highest temperature in the range and decreased by 1°C every second cycle until reaching the lowest temperature in the range, where the remaining 36 amplification cycles occurred. Each 25 ul reaction consisted of 12.5 ul of GoTaq Green Master Mix (Promega), 9.5 ul of nuclease-free water, 1ul of each 10 umol primer, and 1 ul of template DNA.

Amplification products were electrophoresed in a 1% agarose/TBE gel containing ethidium bromide. Gels were visualized on a KODAK Gel Logic 100 Imaging System (Eastman Kodak Company, New Haven, CT, USA).

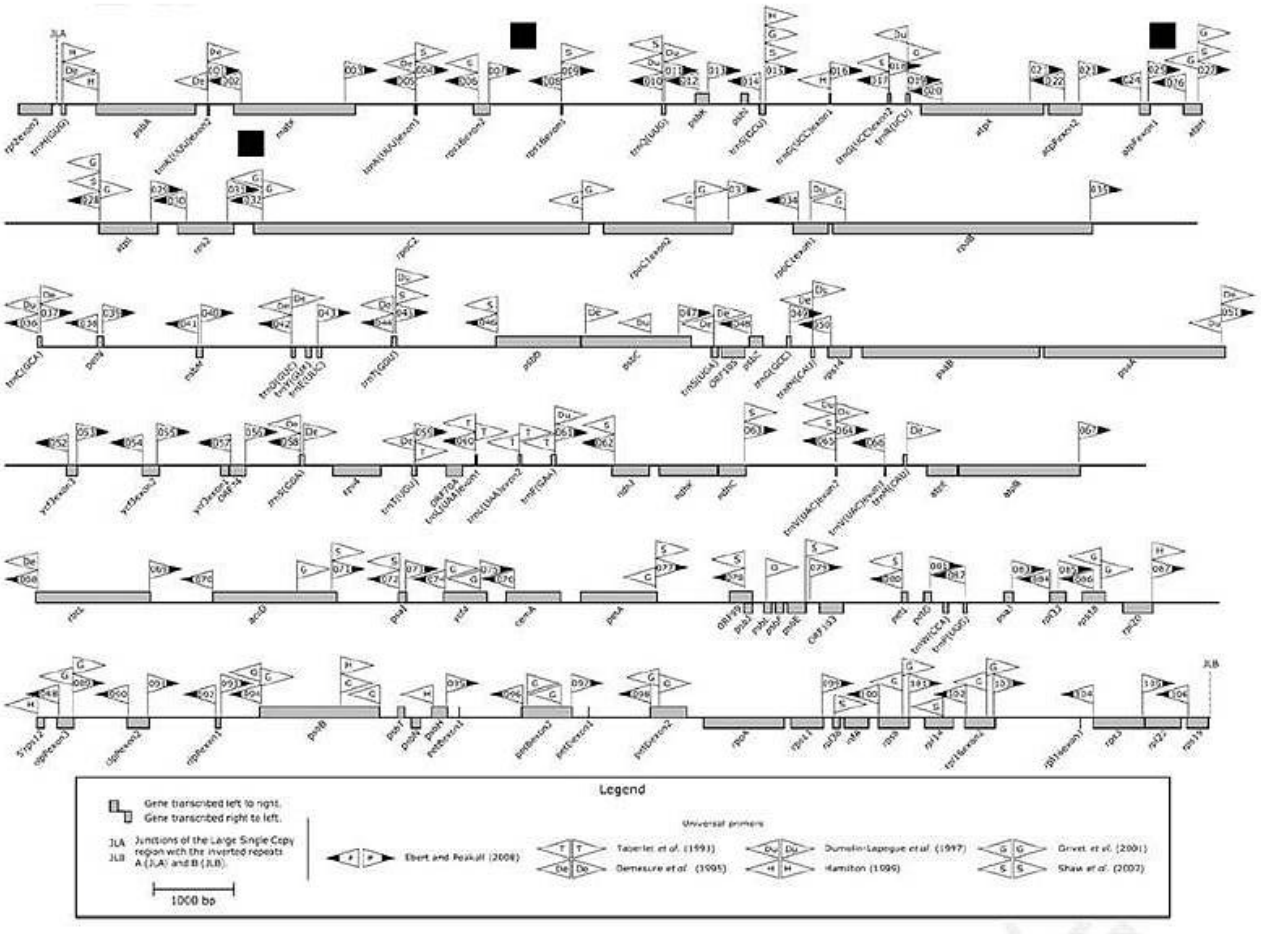

Fig. 2. Map of low single-copy (LSC) region of the chloroplast showing primer annealing locations. Black rectangles appear above regions sequenced for this study. Map modified from Ebert & Peakall (2009a) by permission.

I purified amplification products using a Qiagen Qiaquick PCR Purification Kit (QIAGEN, Inc.) following the manufacturer's protocol and including all optional steps for higher concentrations. I then sent cleaned products to Nevada Genomics or normalized them to 20 ng/ul and sent them to Retrogen, Inc. for direct sequencing. Nevada Genomics uses the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 for all sequencing reactions, and reactions are run on the ABI 3730 DNA Analyzer. Retrogen, Inc. uses capillary ABI 3730 sequencers.

I checked ambiguous bases in the chromatogram viewer provided by the sequencing facility and edited them in BioEdit v. 7.0.5 (Hall 1999). After aligning sequences in ClustalX v. 2.0 (Thompson *et al*. 1997) using the default settings, in a few cases, I inserted one or a few indels in order to retain the conserved sequence. After using MrAIC to designate the best evolutionary model for each region (Nylander 2004), I partitioned the data into three regions. I conducted a Bayesian phylogenetic analysis in Mr. Bayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) with Markov Chain Monte Carlo (MCMC) methods. I designated the *Spiraea japonica* sample SJ as an outgroup, and ran three million generations. I calculated burnin using the equation *(ngen/samplefreq) x 0.25* to eliminate the first 25% of the total trees from consideration in the construction of the consensus tree. I considered nodes with posterior probabilities equal to or greater than 0.90 to be well-supported (See Appendix B for code used to perform analyses.) All sequences will be deposited in GenBank at the conclusion of this study.

I constructed a haplotype network using the software TCS v.1.21 (Clement *et al*. 2000) to infer relationships among populations. TCS uses statistical parsimony to delineate gene genealogies and create networks when divergence is low. This method has been used to

delimit geographical structure within species or genera sections in previous studies (Gonzales *et al*. 2008, Shaw & Small 2005), and is modeled more closely after coalescent theory than traditional methods (Clement *et al*. 2000). Haplotypes and clades from the phylogeny were mapped used ArcGIS v.9.3 (ESRI) and species occurrence data were provided by state Natural Heritage Programs. Eight-digit watershed boundary and 30-second altitudinal shapefiles are available through USDA/NRCS and WorldClim, respectively.

Table 2 Origin of DNA utilized for chloroplast phylogeny.

Sample Name	Taxa	Collection Origin	Drainage	Investigator
OP2	*Spiraea virginiana*	Van Buren Co., TN	Cane Creek Falls	Estep
OP4	*Spiraea virginiana*	Roane Co, TN	Clifty Creek	Estep
OP5	*Spiraea virginiana*	Morgan Co., TN	Clear Fork River	Estep
OP6	*Spiraea virginiana*	Scott Co., TN	White Oak Creek	Estep
OP7	*Spiraea virginiana*	Blount Co, TN	Abrams Creek	Estep
OP8	*Spiraea virginiana*	Blount Co, TN	Abrams Creek	Estep
OP10	*Spiraea virginiana*	Pulaski Co., KY	Rockcastle River	Estep
OP11	*Spiraea virginiana*	Laurel Co., KY	Sinking Creek	Estep
OP16	*Spiraea virginiana*	Ashe Co., NC	S. Fork New River	Estep
OP18	*Spiraea virginiana*	Macon Co., NC	Little TN River	Estep
OP19	*Spiraea virginiana*	Buncombe Co., NC	Hominy Creek	Estep
OP21	*Spiraea virginiana*	Dade Co., GA	Bear Creek	Estep
OP22	*Spiraea virginiana*	Walker Co., GA	Lula Falls	Estep
OP23	*Spiraea virginiana*	Walker Co., GA	Upper Rock Creek	Estep
OP25	*Spiraea virginiana*	Nicholas Co., WV	Gauley River	Estep
OP26	*Spiraea virginiana*	Upshur Co., WV	Buckhannon River	Estep
#1fw27*/ 1-WV	*Spiraea virginiana*	Fayette Co., WV	Meadow River	Williams
#3fw27/ 3-OH	*Spiraea virginiana*	Scioto County, OH	Scioto Brush Creek	Williams
E25	*Spiraea virginiana*	Van Buren Co., TN	Upper Cane Creek	Estill
OH	*Spiraea virginiana*	Scioto Co., OH	Scioto Brush Creek	Pate
Sc-1	*Spiraea corymbosa*	Alexander Co., NC	N/A	Pate
Sc-2	*Spiraea corymbosa*	Alexander Co., NC	N/A	Pate
Sc-3	*Spiraea corymbosa*	Alexander Co., NC	N/A	Pate
SJ	*Spiraea japonica*	Fayette Co., TN	N/A	Estill

Table 3 Primers utilized for chloroplast sequencing and their annealing temperature ranges.

Primer Name	Location	Primer sequence 5' - 3'	Annealing Temperature Range (°C) of Touchdown PCR
ANU_cp007-L	*rps16*ex2	CTTCGAGATCGAACATCAAT	48-45
ANU_cp008-R	*rps16*ex1	AAAACGATGTGGTAGAAAGC	
ANU_cp025-L	*atpF*ex1	TCGGTATTAAGCCCGAAACT	50-46
ANU_cp026-R	*atpH*	GCTTTTATTTGCGAACCCTTT	
ANU_cp031-L	*rps2*	CCATGACCAAAATGAACTCC	48-45
ANU_cp032-R	*rpoC2*	GCGTCGGAAATGAGAGATATT	

Mating system

I conducted the mating system study over two years (2008 and 2009) in two locations. The first season I began a pilot study on five sets of stems collected by Douglas Ogle from across the species' range and transplanted in a common garden at VHCC in Abingdon, VA. I began monitoring for bud burst in early May, and bagged five inflorescences of maturing buds on five plants with bridal veil mesh. In the third week of May, shortly after buds opened, I applied one of the following treatments to each inflorescence: no hand-pollination (i.e. bagged) to test for autogamy, a hand-selfed pollination to test for self-compatibility, a pollination by an inflorescence from another set of stems within the common garden (VHCC), and an open-pollinated control. I re-bagged inflorescences following treatments. Table 4 demonstrates the cross-pollination design. Because of the large number of flowers per inflorescence (800-900 in some), I conducted pollinations by rubbing one corymb against the other, covering all adaxial surfaces of the pollen-receiving inflorescence. Note that this method can move self pollen in the process of transfering pollen from other inflorescences.

Table 4 Experimental design of cross-pollinations performed at VHCC.

Origin	Drainage	Pollinated With	Drainage
Nicholas Co., WV *	Gauley River	Dade Co., GA	Lower Bear Creek
Dade Co., GA *	Lower Bear Creek	Blount Co., TN	Little River
Wise Co., VA	Guest River	Morgan Co, TN	Clear Fork River
Blount Co, TN	Little River	Wise Co, VA	Guest River
Morgan Co., TN	Clear Fork River	Wise Co, VA	Guest River

* indicates plants used to measure seed length

At the end of the first season, I collected the inflorescences and counted and measured the length of ten potentially viable seeds from each treatment in two plants using a Leica Zoom 2000 and Leica ATC 2000 microscope, respectively (Leica Microsystems). Because of the minute size of the seeds (typically ≤ 3 mm), the numbers of flowers per inflorescence, and the difficulty of opening each follicle to look for potentially viable seeds, total seed production was approximated by calculating fruit set. I found when follicles were swollen and extended beyond the sepals, typically at least one seed per flower was larger and less chaff-like, whereas less swollen follicles had smaller, shriveled seeds (Scheffe & Pate, pers. obs.) (see Fig. 3; Fig. 4). I used this observation as an indicator of potentially viable seeds, and counted the number of flowers per inflorescence with these swollen follicles.

Following the analysis, I separated corymbs into three sections, and placed each section in a plastic bag with sphagnum, vermiculite, or soil collected from the site of a wild population and autoclaved. I then placed all treatments in a refrigerator at 3° C and left them to stratify for 90 days. I removed the stratified seeds from cold storage in mid-July, put them in pots containing the media with which they were stratified, and placed them in a controlled greenhouse setting at Appalachian State University. I placed one sample of each treatment under a grow light (Sun System Tek Light, Sunlight Systems, Inc) on a 16/8 hour light cycle, and moved the remaining samples to the conservatory, where light conditions more closely

resembled seasonal light (14.5/9.5 hour light). Temperature during this time fluctuated from 18-25° C. Germinated seeds were counted and left to grow in the greenhouse.

In the summer of 2009, I performed a field study on three populations of *S. virginiana* identified as EO16, EO17, and EO19 by the North Carolina Natural Heritage Program (Table 5). All populations occur along the South Fork of the New River in Ashe County, NC, but are located at least 1.5 km apart from each other. Even though *S. virginiana* is clonal (Ogle 1991b), the extent of clonality is not known. In this study, I considered groups of plants isolated from each other by more than one km to be separate populations. Elevations at all three sites range from 852 - 884 meters above sea level. This area receives an average of 1220 mm of rainfall per year and the average yearly temperature is 11.7° C (Chamber of Commerce, 2005).

Table 5 Characteristics of wild populations sampled in pollination study.

Population	Estimated Population Size	Population Description	Elevation (m)
EO16	350-450 stems	Many flowering stems	848
EO17	350-400 stems	Many flowering stems	852
EO19	1000-1500 stems	Largely vegetative	884

I monitored populations for mature flower buds beginning in late May of 2009. Beginning the first week of June 2009, I bagged five inflorescences of maturing buds on five to ten plants per population with bridal veil mesh (NCPCP Permit ID #100). Shortly after bud opening (the third week of June), these inflorescences received the same treatments given to those at the common garden, except that I included an additional treatment of pollination from an inflorescence collected from within the population to test for low mate availability in the case of self-incompatibility (Table 6). Each treatment was applied to each

plant at least once. I also performed the outcrossing pollination with an inflorescence from a Clear Fork, TN specimen cultivated at VHCC to prevent a false negative in case genotypes I sampled are identical.

Table 6 Pollination treatments applied to wild populations of *S. virginiana*

Breeding system test	Inflorescence bagged	Pollen source
Spontaneous autogamy	Yes	No pollination
Induced geitonogamy	Yes	Pollination from a different inflorescence on the same plant
Induced xenogamy	Yes	Pollination from an inflorescence on a different plant within the population
Artificial xenogamy	Yes	Pollination from an inflorescence on a different plant (from Clear Fork River drainage, TN)
Control	No	Open pollination

To prevent possible seed establishment from the artificial crosses, I did not remove the bags following the treatments, and left the treated inflorescences to mature until the last week of October. After collecting all bagged inflorescences, I began analyzing the products of the pollinations. Retaining the protocol from the previous summer, I counted the number of follicles swollen and extended beyond the sepals, as well as the total number of flowers on each inflorescence. I also weighed 20 seeds from each treatment from two populations using a Sartorius CP2P (Sartorius AG, Inc.). Although the scale is accurate to the microgram, it was necessary to weigh ten seeds at once in order to achieve an accurate reading because of the low weight of individual seeds. The large amount of variability in individual seed weight should be taken into consideration when interpreting the results of the analysis.

After calculating the percent of potential seed set in Microsoft Excel, I tested for significant differences in the percent of fruit set to number of flowers per inflorescence

among treatments and among populations using analysis of variance (ANOVA: general linear model) in SAS software, v. 9.1 for Windows (SAS Institute Inc, 2006). I applied a Bonferroni correction before performing multiple comparisons using the least squares means in order to conservatively evaluate the effects of the treatments within and among populations. I considered values of $p \leq 0.05$ to indicate significant differences. I also analyzed seed size and seed weight using the same methods.

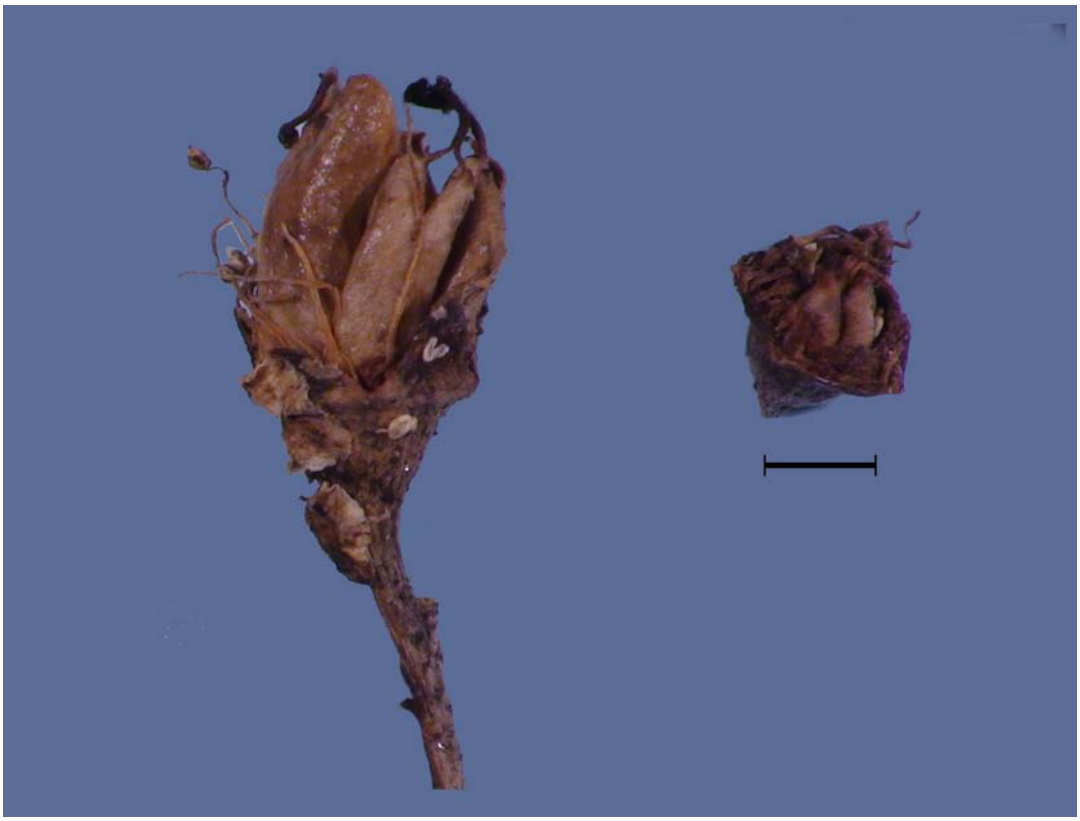

Fig. 3. Comparison of receptacle with swollen follicles indicating potential seed set (left) and a receptacle without swollen follicles (right). The black bar represents 1 mm.

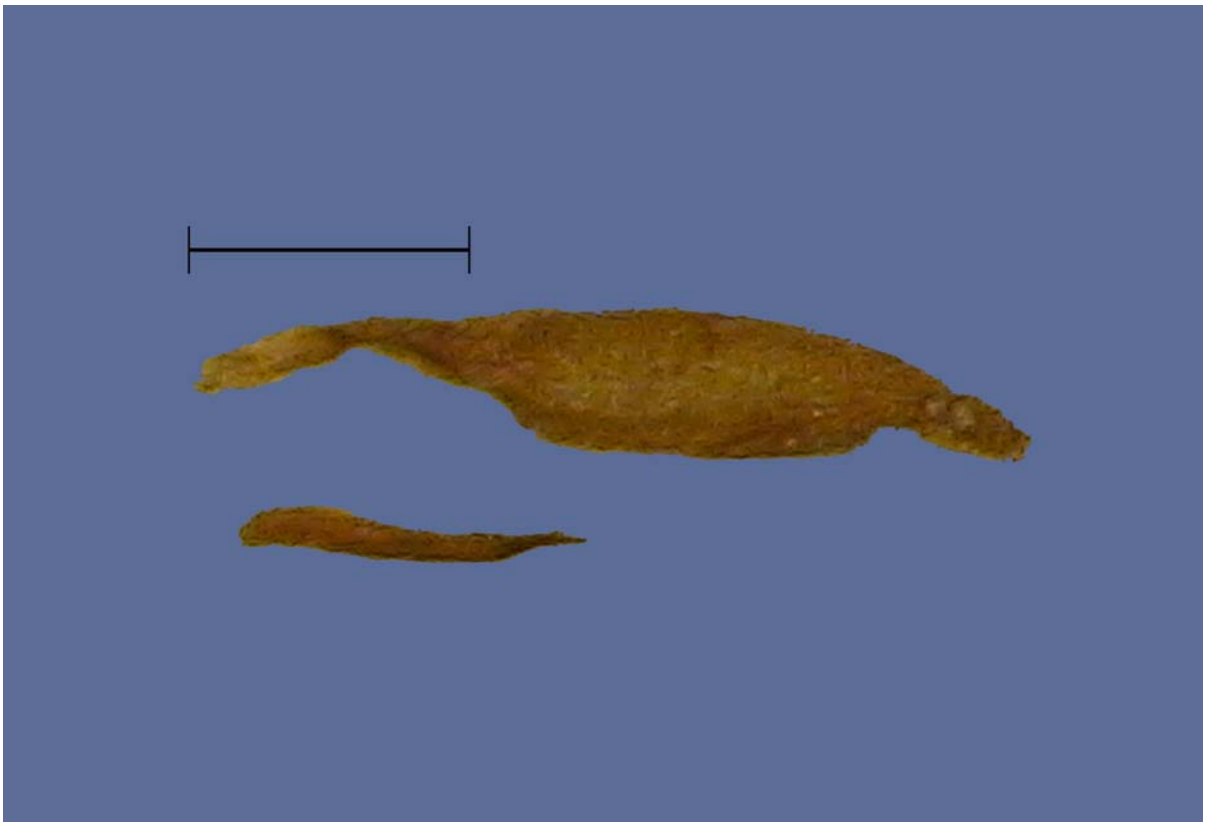

Fig. 4. Comparison of large seed from swollen follicles to smaller, chaff-like seed. The black bar represents 1 mm.

Chapter Three: Results

Phylogeography

The heuristic analysis of the ISSR data in PAUP found 40 of the 43 characters were parsimony-informative, and the analysis resulted in a total of 15 most parsimonious trees (Fig. 5). The strict and 50% majority consensus trees found *S. virginiana* to be monophyletic (Fig. 6; Fig. 7). In addition, all three trees placed the Clear Fork River populations basal to the rest of the clade. I saved bootstrapped trees to a file and calculated a consensus tree (Fig. 8). The bootstrapped consensus tree also showed the species to be monophyletic with a bootstrap value of 100. However, little else was resolved within the tree. Bootstrap values were somewhat higher at the tips, often showing relatedness within drainages or similar locales, but internal nodes had little to no support.

Sequencing of the *rps*ex2-*rps*ex1 region resulted in an 875-character long region and the *atp*Fex1-*atp*H region was found to be 534 characters long. The *rps*2-*rpo*C2 intron had 414 characters. Alignments revealed few polymorphisms specific to *S. virginiana*.

The best model of evolution selected for two of the datasets (*rps*ex2-*rps*ex1 and *atp*Fex1-*atp*H) by MrAIC (Nylander 2004) (based on the Aikake Information Criteria) was F81-I. The General Time Reversal (GTR+IG) model was identified as the minimum evolution model for the *rps*2-*rpo*C2 intron. The dataset partitioned based on these criteria

and analyzed by three million Markov Chain Monte Carlo (MCMC) generations resulted in a phylogenetic tree consisting primarily of two clades which contained all but one sample of *S. virginiana* and all three *S. corymbosa* samples (Fig. 9; Fig. 10). Almost half of the nodes had weak support (posterior probability < 0.90), although the nodes placing two of the *S. corymbosa* samples with a sample from Scioto Brush, OH had strong support. The node relating samples of *S. virginiana* from Lula Falls, GA and Sinking Creek, KY to another sample of *S. corymbosa* also had strong support. Additionally, this clade was contained within a clade of eastern populations and Cumberland Plateau populations which was well-supported.

To explore the impact of the Ohio populations and *S. corymbosa* on the tree topology, I excluded these samples from the matrix. I realigned the data and performed the Bayesian analysis again using the same methodology. The resulting tree had similar topology (Fig. 11) but stronger support at internal nodes (Fig. 12). When I mapped the clades with strong support within drainages, clades were not specific to drainages (Fig. 13). However, when I mapped these clades along an elevational gradient, the pattern appeared to fit (Fig. 14).

The haplotype network analysis segregated samples into 11 haplotypes using the longest intron, *rps*16ex1-*rps*16ex2, which had the highest probability of parsimony (>98%) (Fig. 15). Although I conducted limited sampling within short reaches of the same drainages, only two samples sharing a drainage had the same haplotype (Fig. 16, see Appendix C for key to haplotypes).

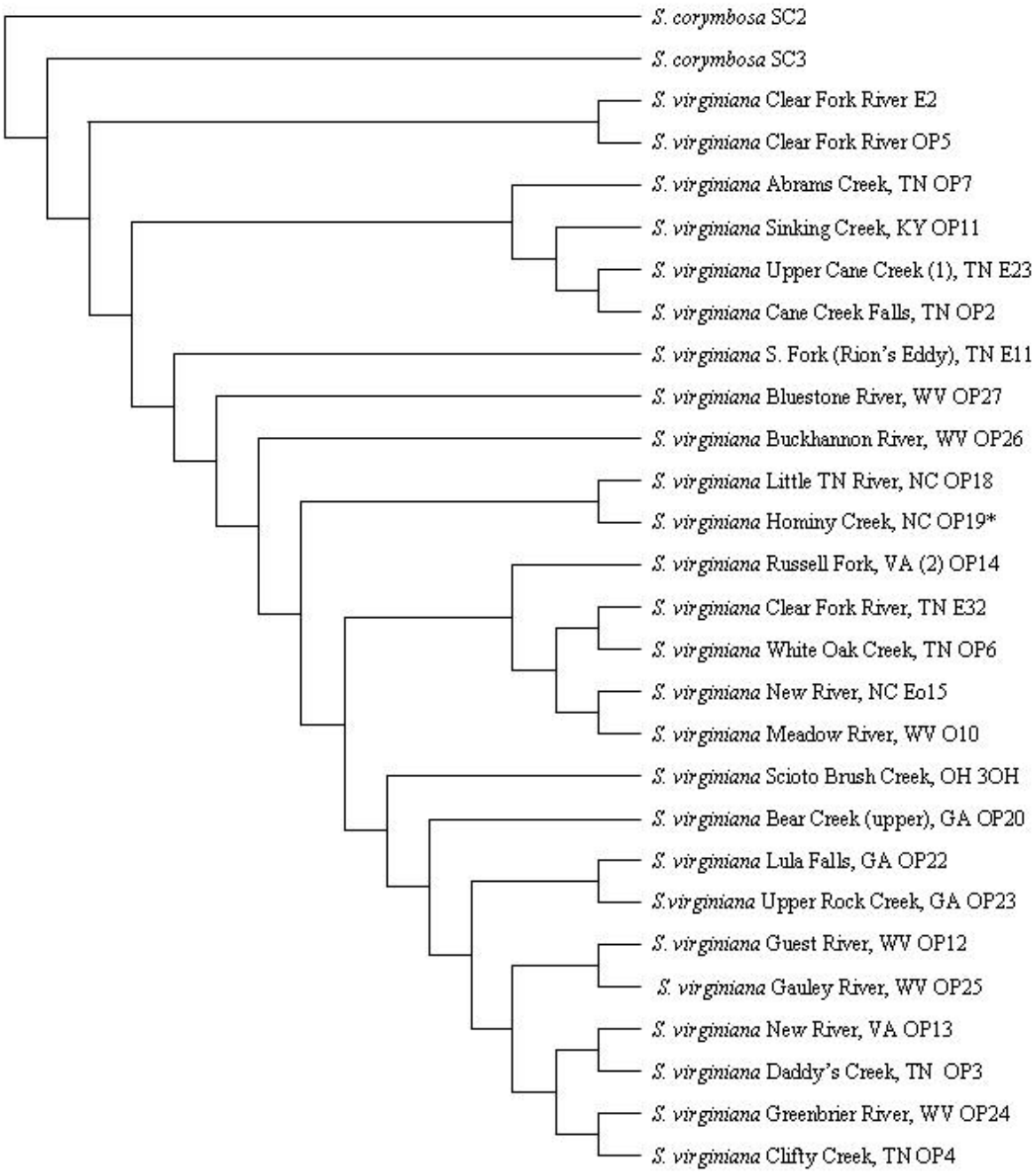

Fig. 5. One of 15 best trees, filtered from 1029. Tree length = 144, Consistency Index (CI) = 0.2986, Retention Index (RI) = 0.5476

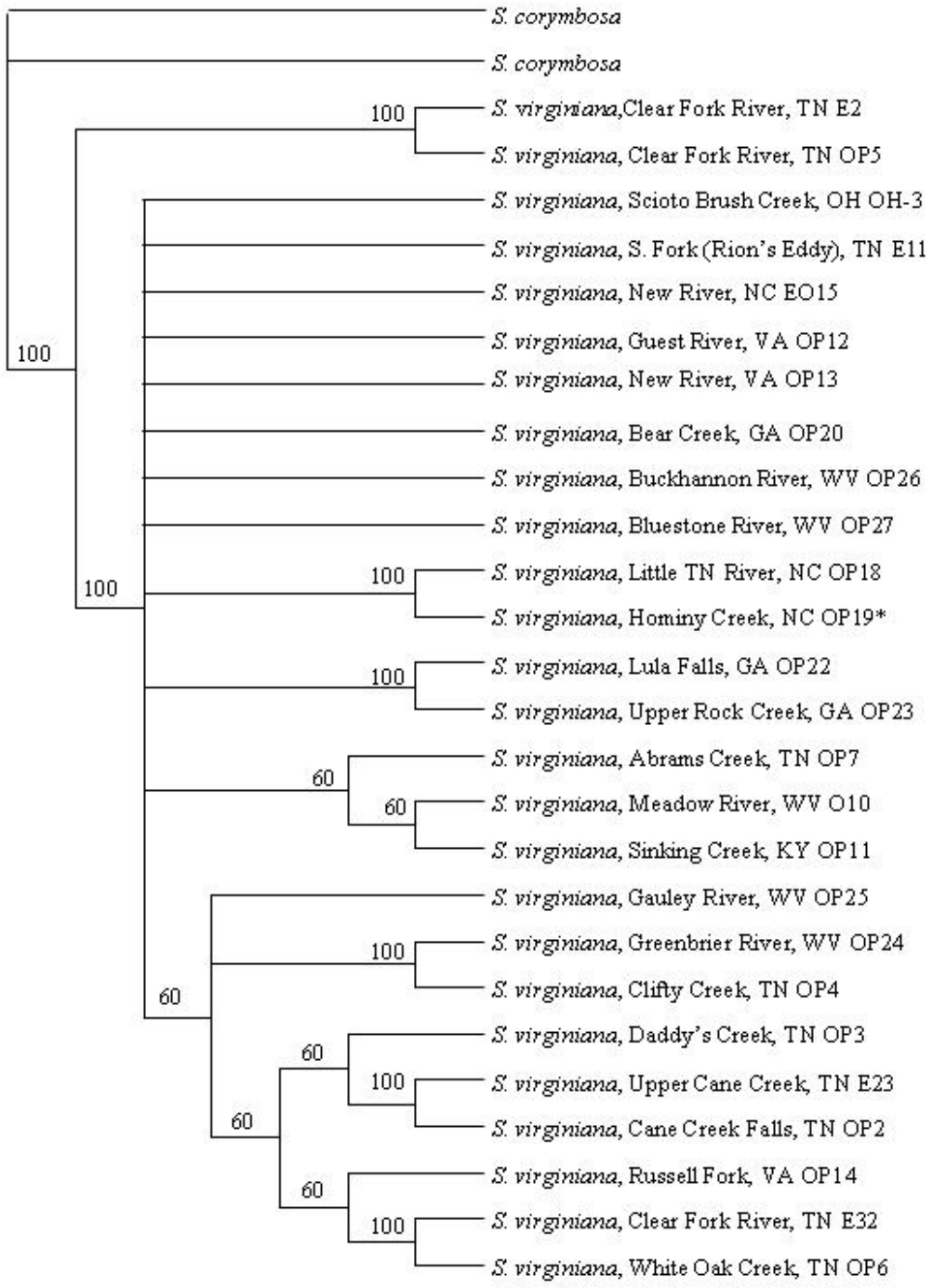

Fig. 6. Majority Consensus Tree based on ISSR heuristic search. Tree length= 144 Consistency index (CI) = 0.2986 Retention index (RI) = 0.5471 Homoplasy index = 0.7014
* indicates a population no longer extant

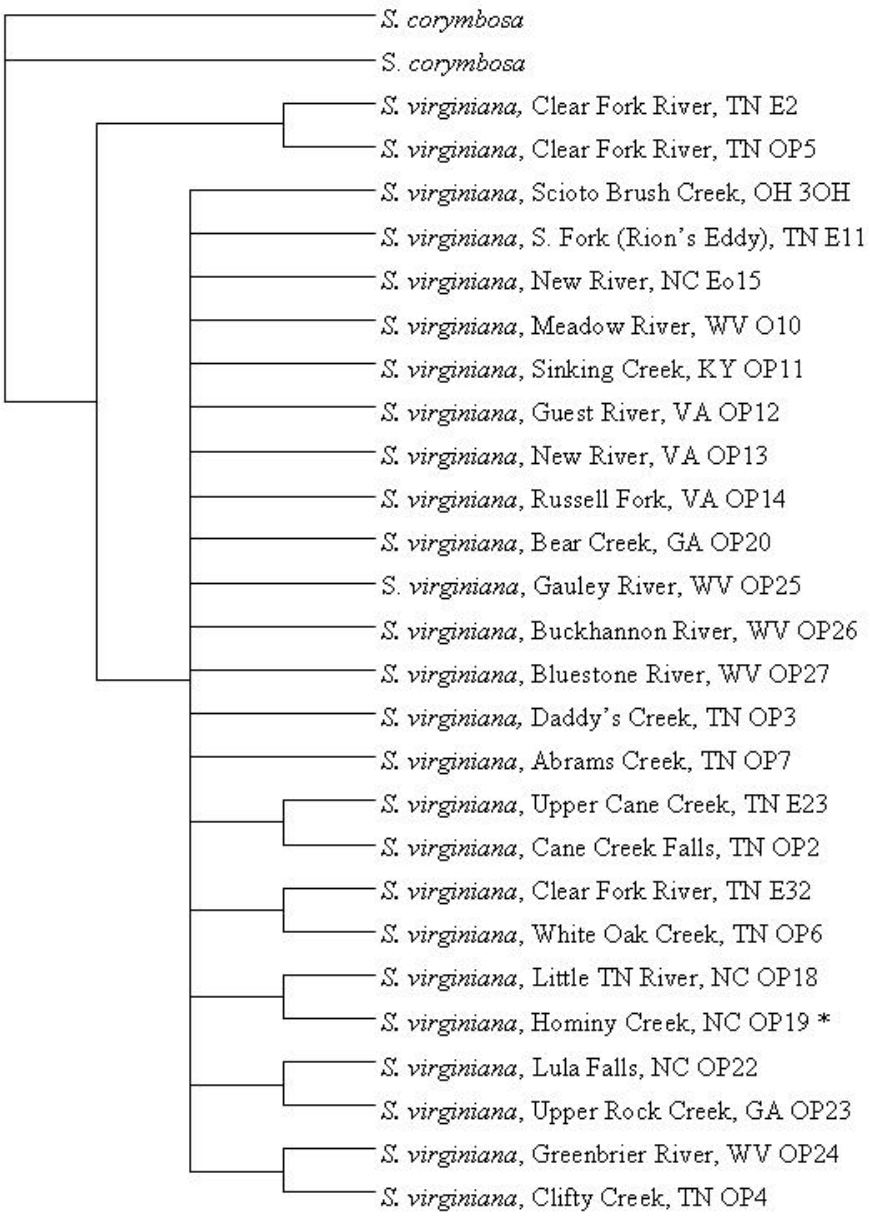

Fig. 7. Strict Consensus of 15 Best Trees based on ISSR heuristic search. * indicates a population no longer extant

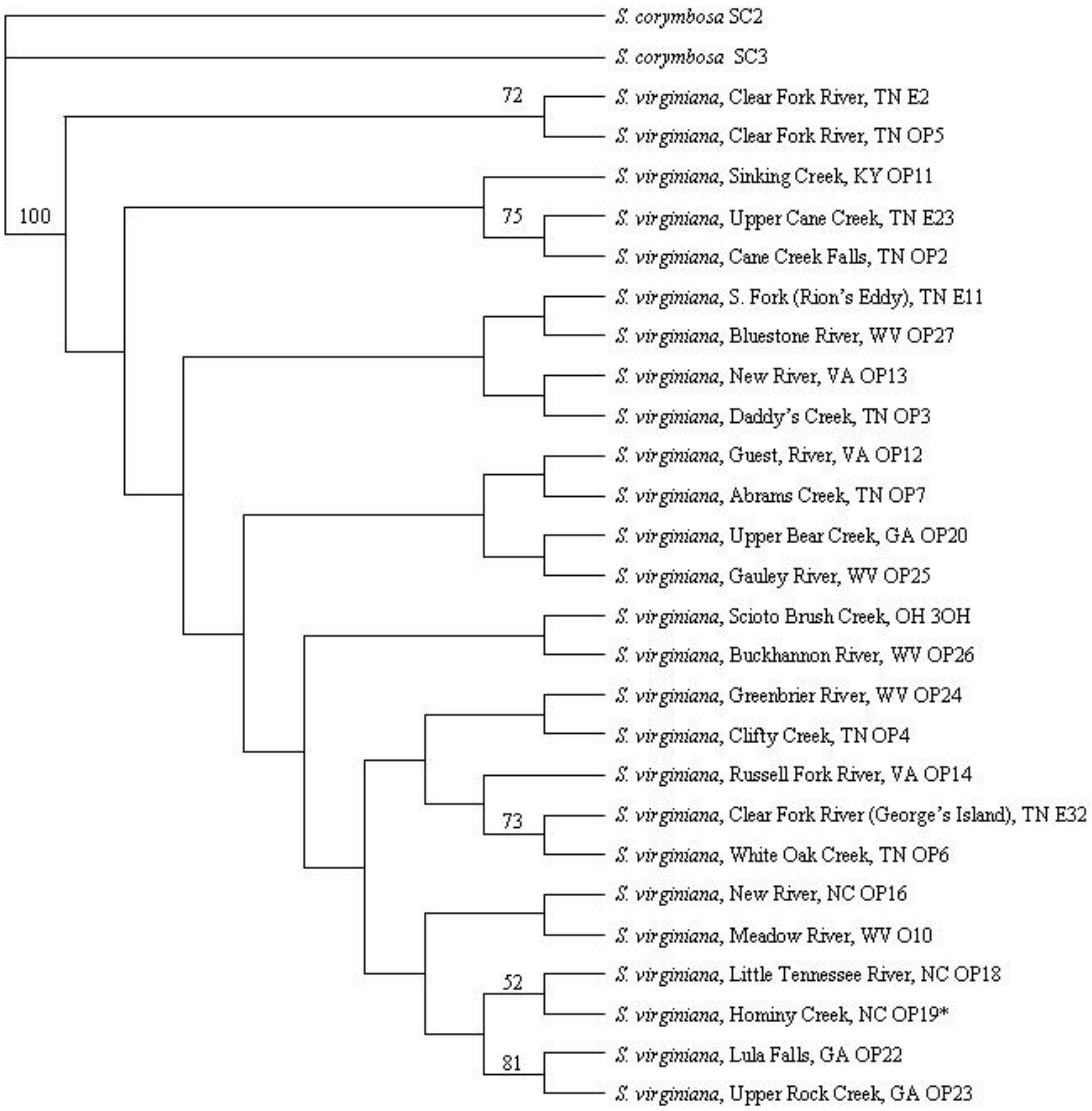

Fig. 8. Bootstrap 50% Majority Tree of 1314 trees from ISSR analysis. * indicates a population no longer extant

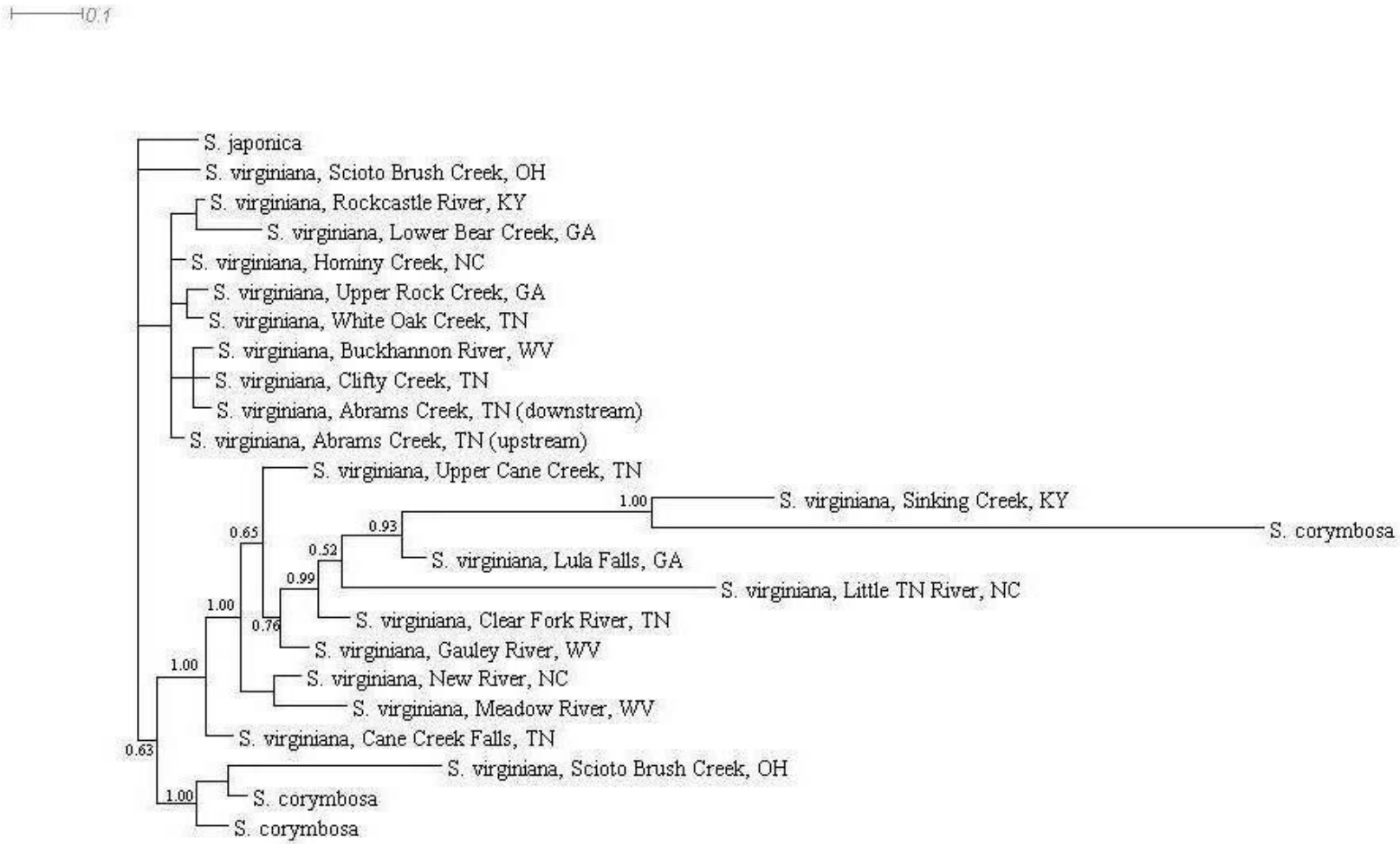

Fig. 9. Tree inferred from Bayesian analysis of all chloroplast sequences showing branch lengths. * indicates population no longer extant

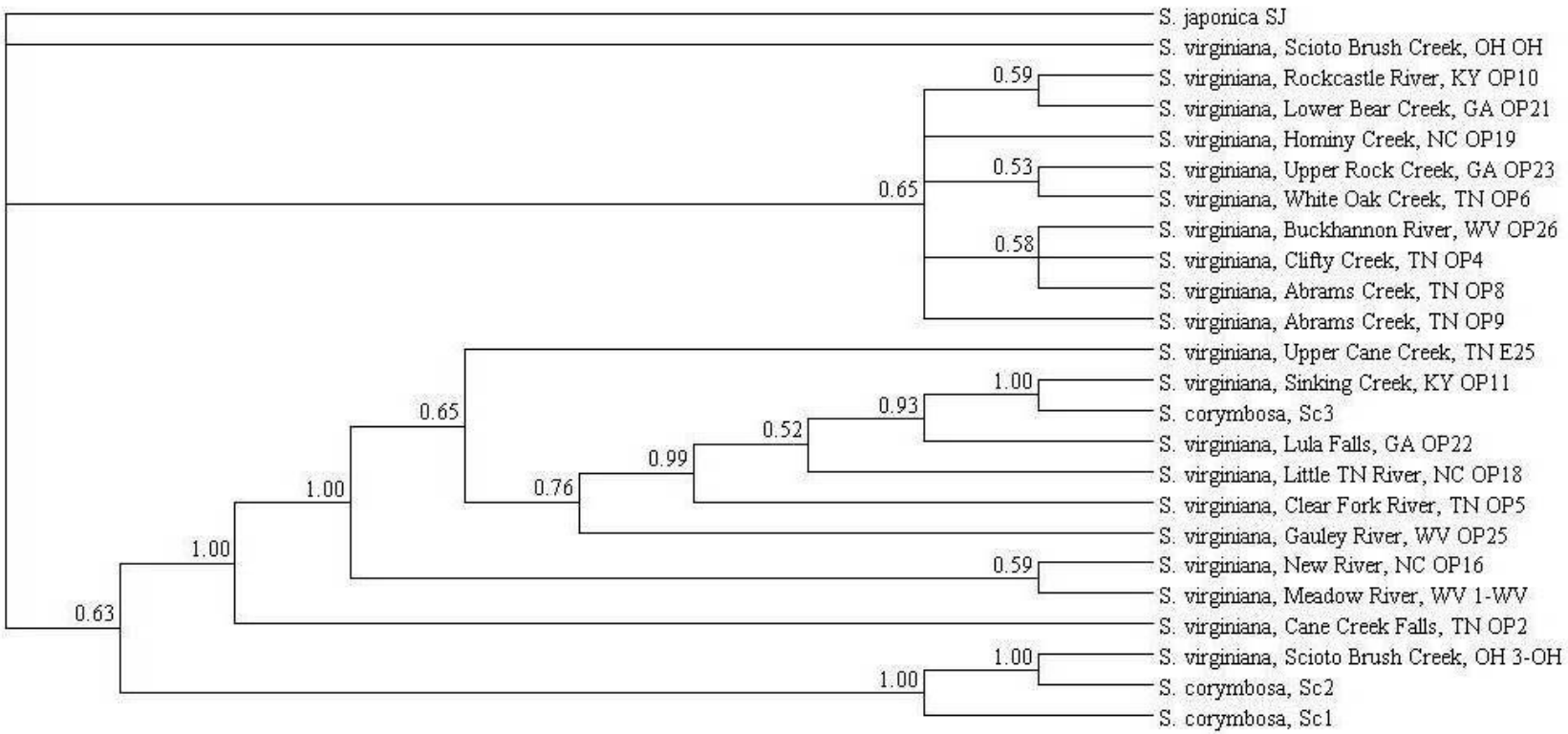

Fig. 10. Tree inferred from Bayesian analysis of chloroplast sequences of all three species (without branch lengths).

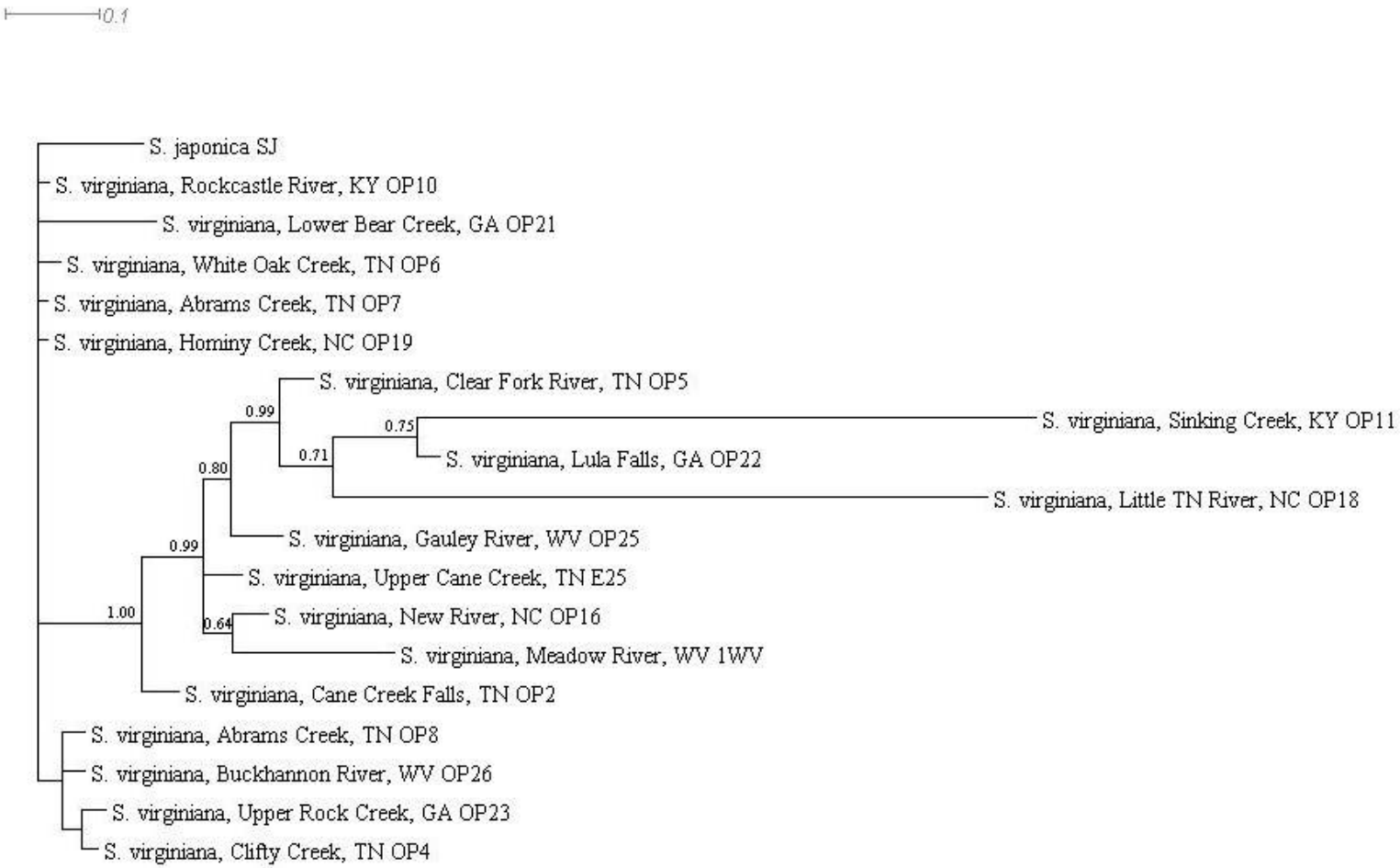

Fig. 11. Tree inferred from Bayesian analysis of chloroplast regions after the removal of *S. corymbosa*

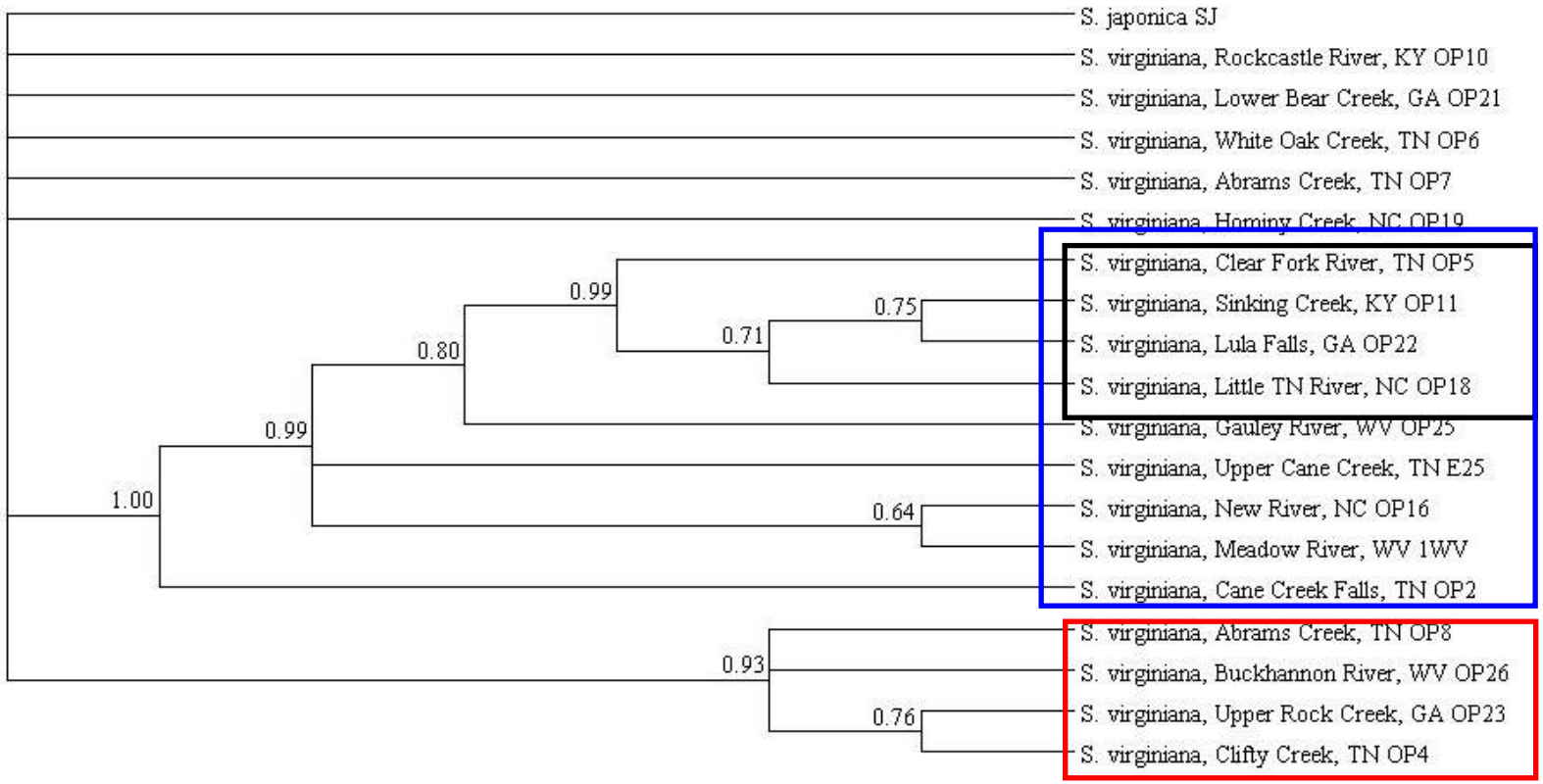

Fig. 12. Tree inferred from chloroplast Bayesian analysis after the removal of *S. corymbosa* (shown without branch lengths). Numbers are posterior probabilities. The blue box represents Clade 1, the black box represents the Internal Clade, and the red box represents Clade 2.

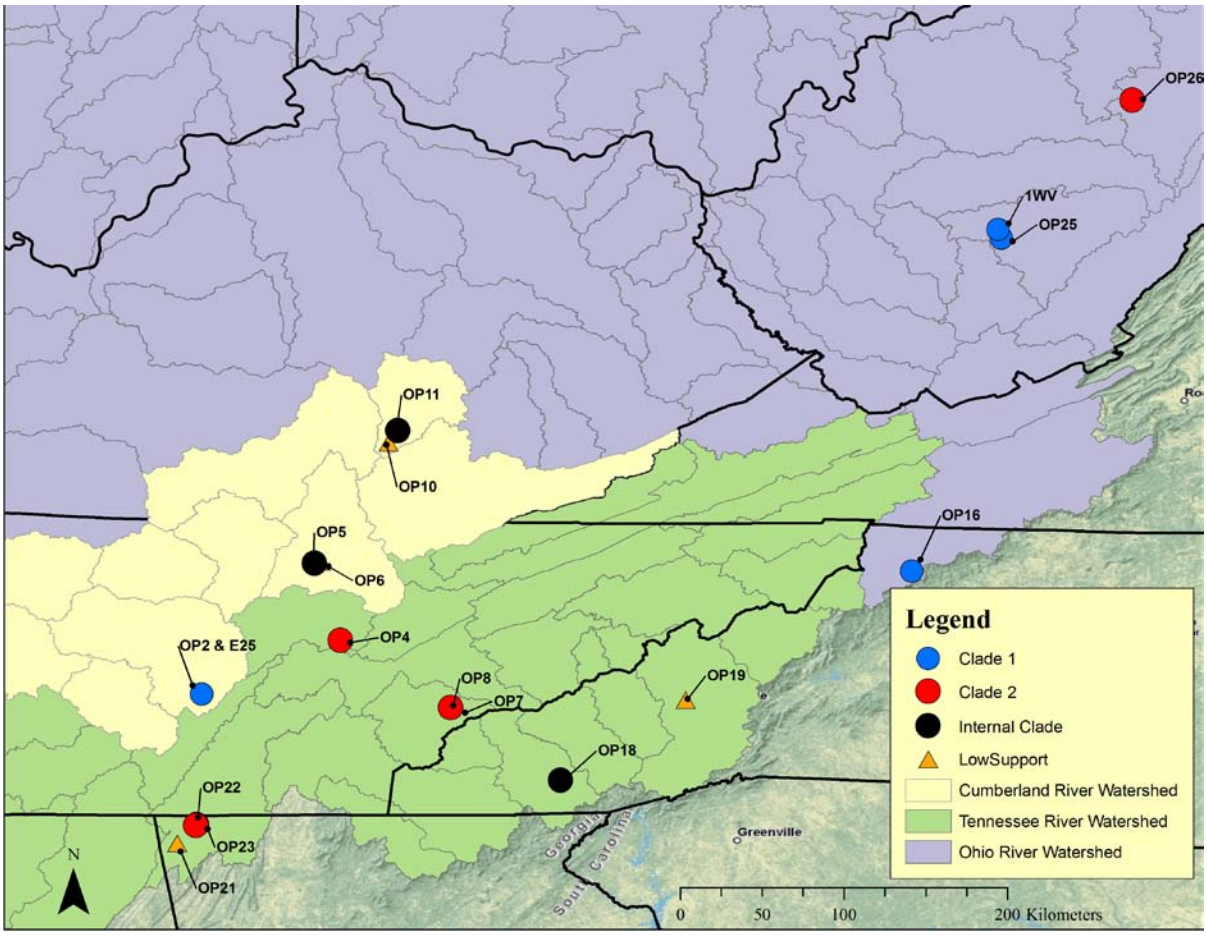

Fig. 13. Chloroplast haplotypes within major watersheds.

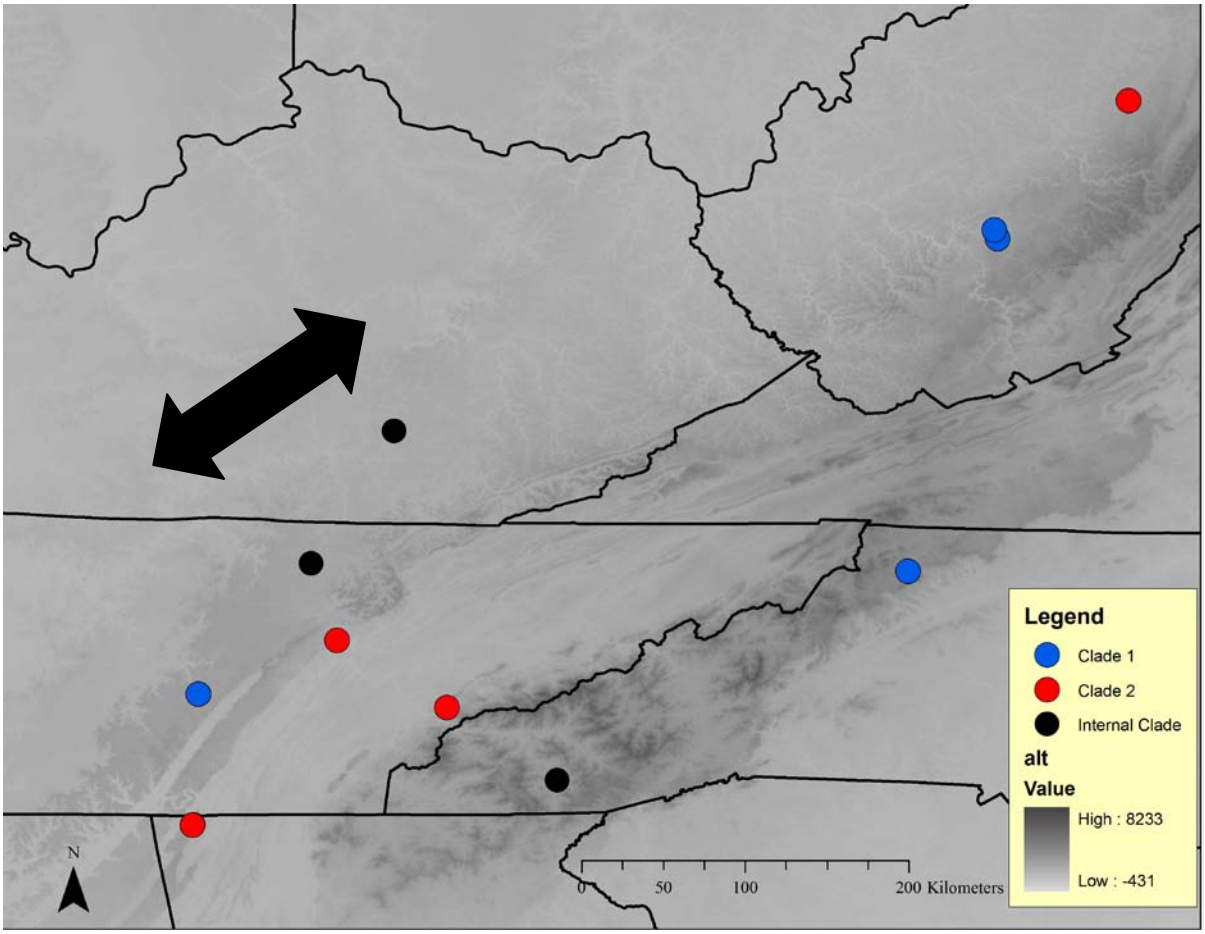

Fig. 14. Chloroplast haplotypes along elevational gradient. Arrow represents prevailing wind direction. 30-second altitudinal data available through WorldClim.

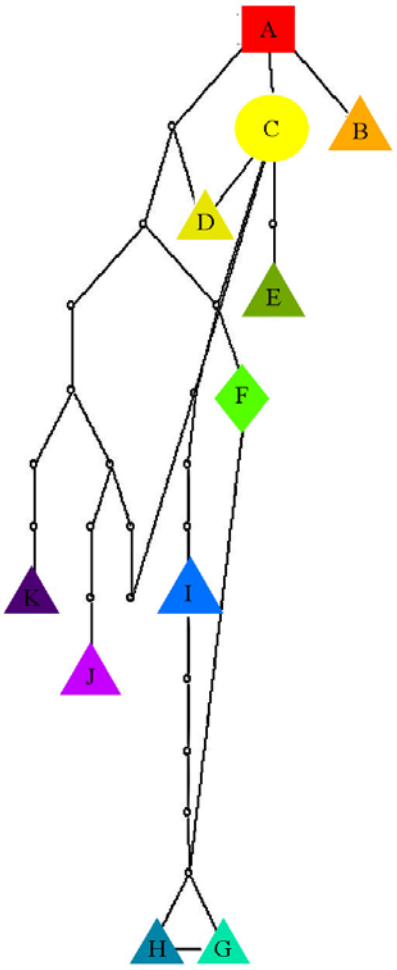

Fig. 15. Haplotype network based on the *rps*16ex1-*rps*16ex2 chloroplast region

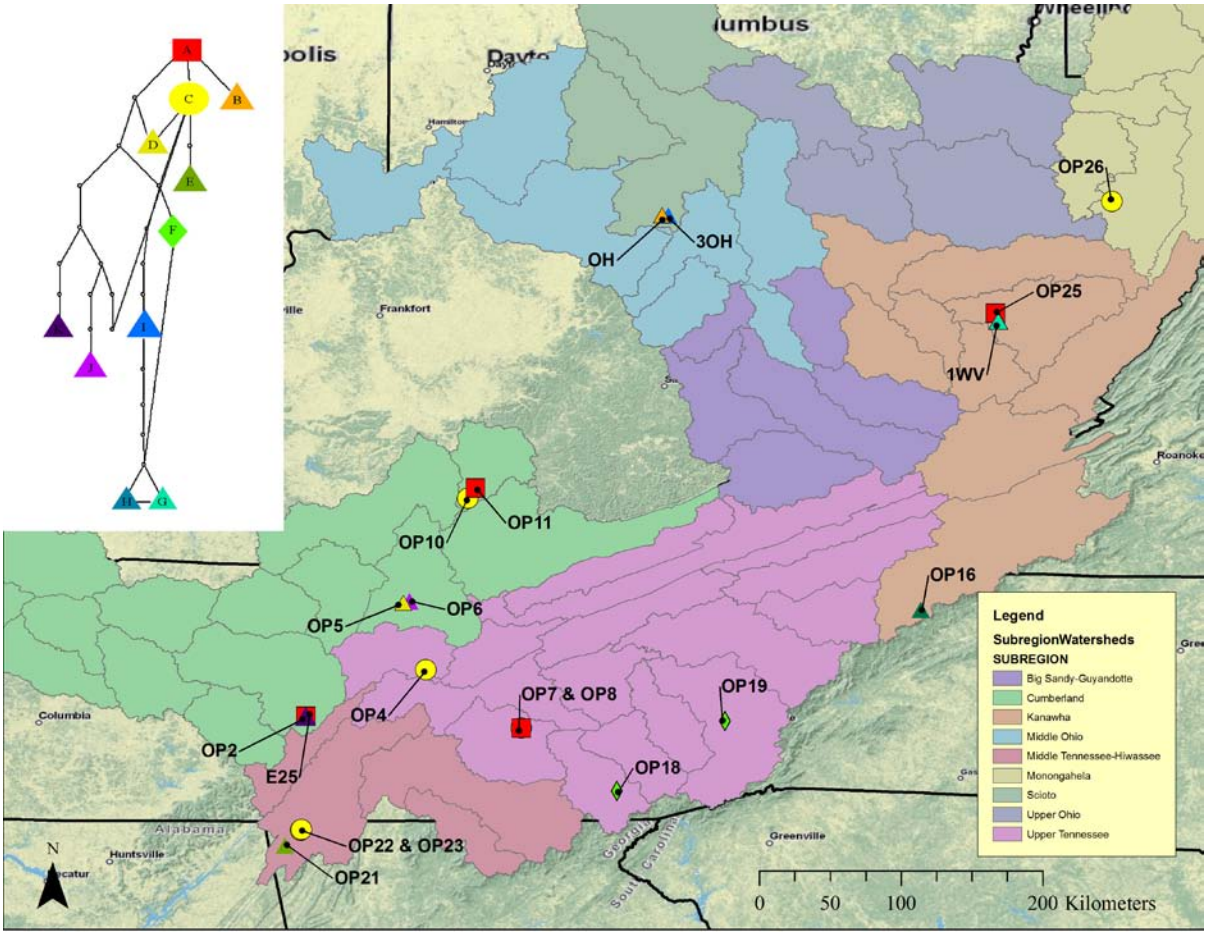

Fig. 16. Chloroplast haplotypes shown within subregion watersheds.

Mating system

Year One Experiments

Seeds were produced in all treatments within the common garden (# of crosses = 20, total seeds produced= 252), although flowers were not abundant enough to perform every treatment on every plant. Seed length was significantly different between the autogamy treatment and the open pollination (n= 150, p= 0.0199); average seed length in the open pollination was more than double the average of the autogamy treatments (792 μm vs. 1641 μm). The remaining treatments were not different from each other, although the two plants responded differently to the treatments. The plant originating from Nicholas County, WV produced larger seeds in the spontaneous autogamy treatment than any of the other treatments ($p \leq 0.0053$). The plant originating from Dade County, GA produced the largest seeds in the open pollination, and these seeds were significantly larger than the autogamy treatment (p= 0.0100), but not significantly larger than those produced in the other treatments (Fig. 17).

Few seeds germinated from seeds produced at the common garden. No seedlings germinated in the sphagnum media and two germinated in the vermiculite. Ten seedlings germinated in the autoclaved soil. Of these twelve seedlings, seven were products of the artificial xenogamy treatment, four were from the geitonogamy treatment, and one was produced by spontaneous autogamy. No seeds germinated from the open pollination, and all seedlings produced were products of treatments performed on Blount or Morgan County, TN plants. The percentages of seeds germinated in each treatment were not significantly different. Eighteen percent of seeds produced in autogamy germinated, seven percent produced in geitonogamy germinated, and six percent germinated from xenogamy.

Year Two Experiments

Across wild populations the open-pollinated treatment set significantly more fruits than the other four treatments (47.9 %, $p< 0.0001$, # of pollinations = 96, # of fruits produced = 7479). Production of fruits from the bagged, hand-selfed, and two outcrossed (within the population and outside the population) treatments was not significantly different from each other (24.5 %, 22.6%, 15.5%, and 29.7%, respectively). However, fruit production varied by population. In one of the populations (EO16), none of the treatments were significantly different from each other, and inflorescences produced approximately the same number of fruits as a result of each treatment. In the second population (EO17), fruit set in the open pollination treatment was significantly higher than both the spontaneous autogamy ($p= 0.0074$) and the induced xenogamy ($p= 0.0057$). However, there were no differences among the rest of the treatments within the population. The third population (EO19) produced significantly more fruits in the open pollination than the induced xenogamy ($p= 0.0032$). The origin of the pollen in the outcrosses, as compared between induced xenogamy and artificial xenogamy, nearly affected fruit set in this population but ultimately was not significant ($p= 0.0557$). Compared to the other populations, EO19 produced significantly more fruit by all treatments except by induced xenogamy. When the autogamy and induced geitonogamy treatments were combined as selfed treatments and compared to the two combined xenogamy treatments, the means were not significantly different (Fig. 18). Outcrossed seed weighed significantly more, on average, than seed from autogamy and open pollination treatments (Fig.19; Table 7). In addition, the seeds produced by the artificial xenogamy treatment also weighed significantly more than the seeds produced by induced geitonogamy ($p< 0.0020$ in all cases). However, this was due to only one of the populations (EO19), which produced

heavier seeds in both xenogamy treatments than in the induced geitonogamy ($p \leq 0.0300$ in all cases) and heavier seeds by artificial xenogamy than by autogamy ($p = 0.0465$). The treatments had no effect in the other population (EO17).

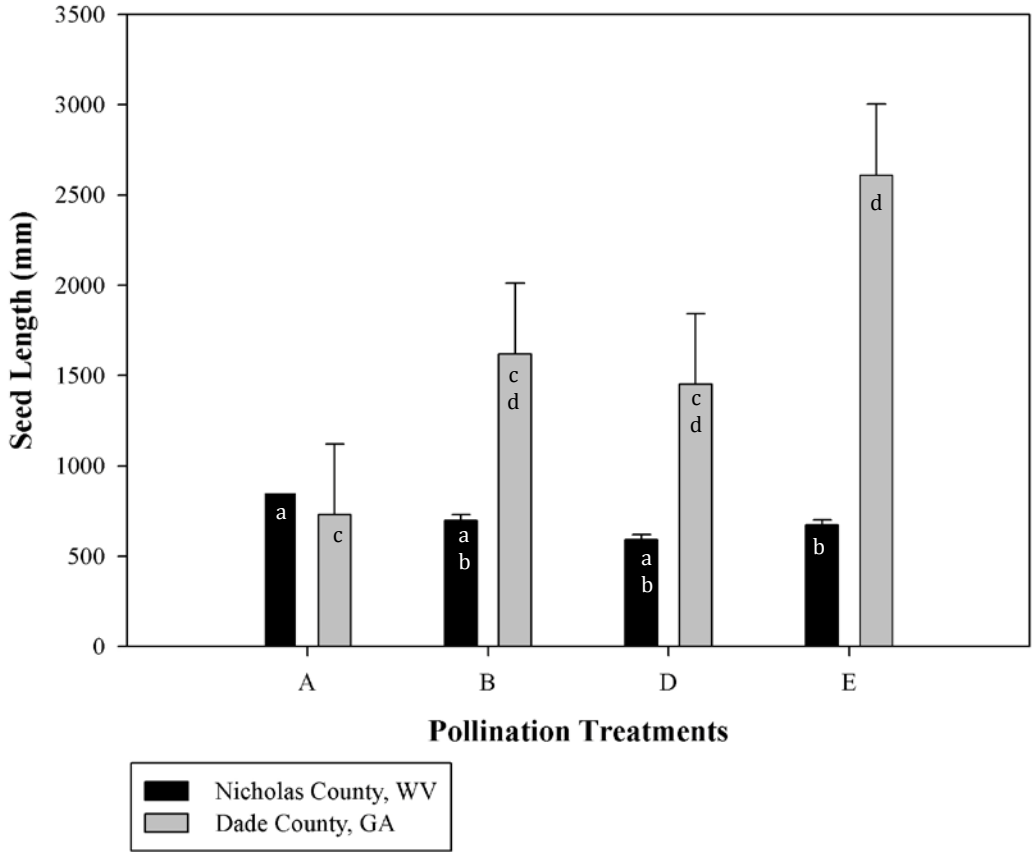

Fig. 17. Seed size in pollination treatments at Virginia Highlands Community College. Treatments which were significantly different have different letters (alpha=0.05). Bars equal one standard error. Treatments were as follows: A = bagged, B = hand-selfed, D = outcrossed, and E = open pollinated.

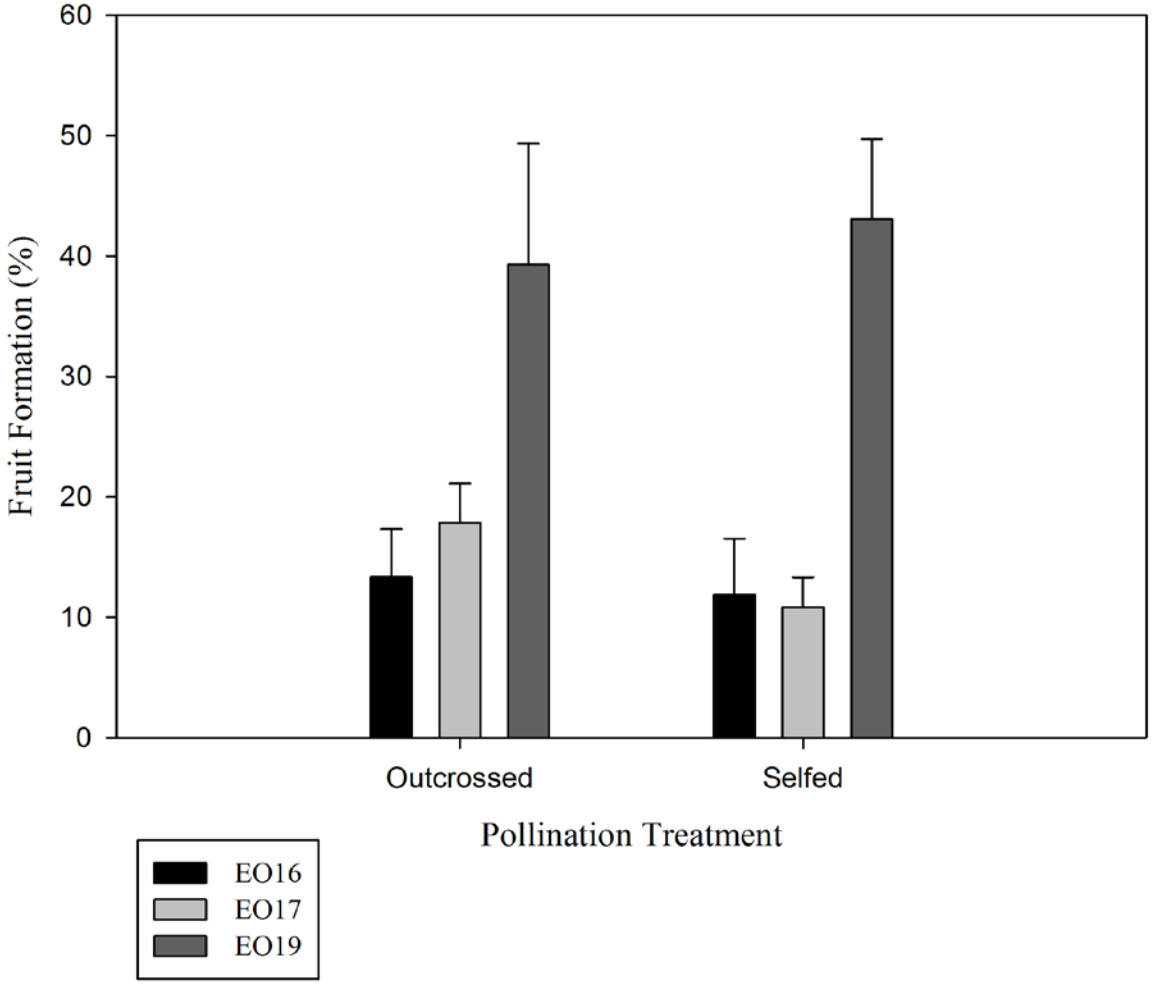

Fig. 18. Fruit set in three populations of *S. virginiana* as a result of pollination treatments. Outcrossed treatments include induced and artificial xenogamy. Selfed treatments include autogamy and induced geitonogamy. Bars represent one standard error.

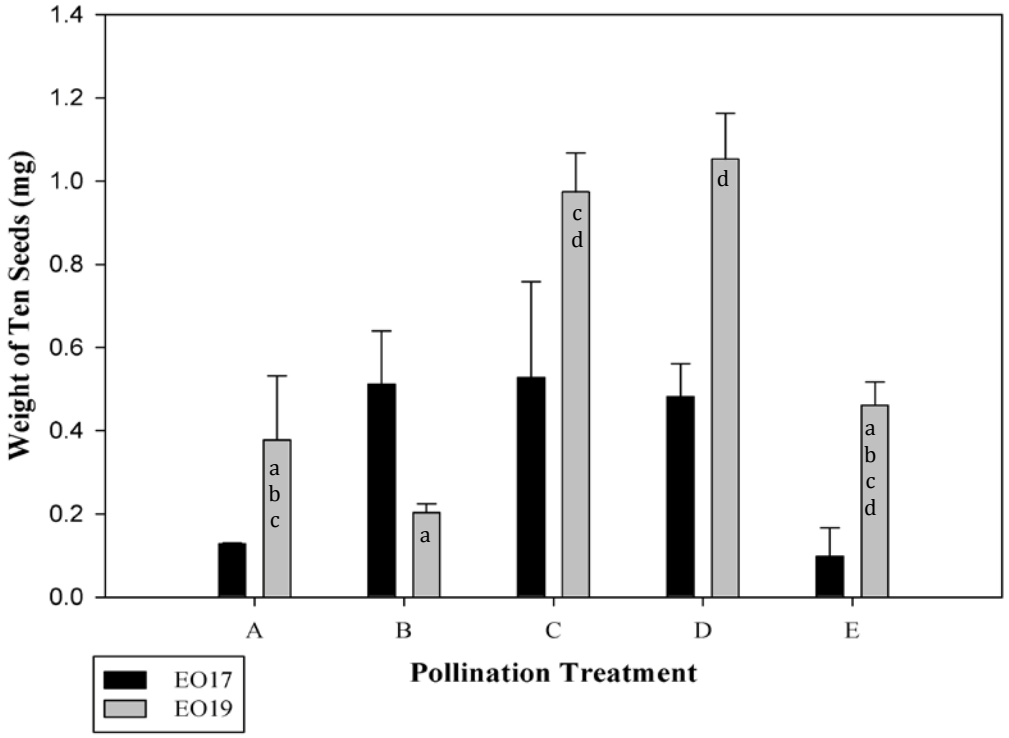

Fig. 19. Weight of ten seeds in two populations resulting from pollination treatments. Treatments which were significantly different have different letters (alpha=0.05). Treatments were as follows: A = bagged, B = hand-selfed, C = outcrossed within population D = outcrossed with Clear Fork inflorescence, and E = open pollinated. Treatments in population EO17 were not significantly different. Seeds from EO16 were not used in this analysis.

Table 7 Multiple comparisons of treatment effect on seed weight. Results that are significantly different from each other (where alpha ≤ 0.05) are shown in bold.

Population	Treatment (A)	Treatment (B)	Difference in Mean (A-B)	Significance
EO17	Autogamy	Induced Geitonogamy	-0.383	0.8561
		Induced Xenogamy	-0.3995	0.7622
		Artificial Xenogamy	-0.3535	1.0000
		Open Pollination	0.0295	1.0000
	Induced Geitonogamy	Induced Xenogamy	-0.0165	1.0000
		Artificial Xenogamy	0.0295	1.0000
		Open Pollination	0.4125	0.6960
	Induced Xenogamy	Artificial Xenogamy	0.046	1.0000
		Open Pollination	0.429	0.6208
	Artificial Xenogamy	Open Pollination	0.383	0.8561
EO19	Autogamy	Induced Geitonogamy	0.1745	1.0000
		Induced Xenogamy	-0.5965	0.0778
		Artificial Xenogamy	-0.675	**0.0465**
		Open Pollination	-0.0835	1.0000
	Induced Geitonogamy	Induced Xenogamy	-0.771	**0.0262**
		Artificial Xenogamy	-0.675	**0.0170**
		Open Pollination	-0.0835	1.0000
	Induced Xenogamy	Artificial Xenogamy		1.0000
		Open Pollination	-0.0785	0.1414
	Artificial Xenogamy	Open Pollination	0.5915	0.0805

Chapter Four: Discussion

Mating system

Based upon the results from the mating system analysis, I tentatively conclude that *S. virginiana* is self-compatible, and appears to have a mixed mating system since both self-fertilization and outcrossing occur at varying degrees within the species (Goodwillie *et al*. 2005). This is in conflict with my expectations based on Ogle's observations that plants produced seed in the common garden after exposure to pollen from other locales (Ogle 1991a). However, before this determination can be made with confidence, emasculation of flowers should be performed and apomixis should be ruled out, germination rates should be calculated, and flowers should be examined for arrested pollen tubes. I did not emasculate flowers in any of the treatments, so I can not rule out the possibility that some agamospermy exists within the species. Facultative agamospermy is known to occur within Rosaceae (Baturin 2009; Talent 2009), although not within Spiraeeae. Germination rates from wild populations may differ from the common garden; germination should be considered before I can conclude this study. Fruits were set in the bagged, unpollinated treatments, so I conclude that insects are not required to move pollen.

In the common garden study, seed size varied by plant. This prevents me from drawing strong conclusions from this experiment. However, the heavier weight of seeds in outcrossed treatments may be a result of the treatment and may indicate fitness in outcrossed progeny. None of the seeds measured for length germinated, making it also impossible to determine from this experiment if seed size is related to seed viability. The seeds from

Blount and Morgan County, TN were the only seeds that germinated from the common garden, so this should be taken into account in future experiments. It may be interesting to compare haplotypes from these areas with those from areas where seeds did not germinate to determine potentially reliable seed banks. If the haplotype can predict the viability of seeds, information concerning haplotypes may prove to be a valuable conservation tool.

Because outcrossed seed weighed more than autogamous seed in the common garden, *S. virginiana* may reproduce more effectively when other pollen sources are introduced. Seed mass has been correlated with progeny fitness in previous studies (Paz & Martinez-Ramos 2003; Du & Huang 2008), and the advantages of outcrossing are well known (Schemske 1983; Williams 1975). It may also indicate levels of inbreeding depression within populations, since the introduction of a different pollen genotype may mask the effects of deleterious recessive alleles that are fixed in a population (Charlesworth & Charlesworth 1987). The results of an ongoing germination study with larger sample size from the wild populations may shed light more light on this possibility.

Based on what we know about *S. virginiana*, perhaps it should not be surprising that the species is self-compatible. Although diploid Rosaceae typically are self-incompatible, it is thought that some polyploids are likely to have at least 50% or more self-compatible pollen (de Nettancourt 2001). Several empirical studies of tetraploid species have found a relationship between a polyploid origin and self-compatibility (Dickinson *et al.* 2007; Husband & Schemske 1997; Cook & Soltis 2000; Masuyama & Watano 1990), although this relationship may only hold for certain groups (Mable 2004). Three species within Rosaceae have shown a breakdown in gametophytic self-incompatibility accompanied by polyploidy

(Miller & Venable 2000). The *Spiraea virginiana* ploidy level and self-compatible mating system may be another example of this relationship within Rosaceae.

Outcomes of selfing are well-studied, and models by Lloyd (1979; 1992) and Schoen (1996) suggest three possibilities. Self-pollination may result in an increase in seed production resulting from a lack of pollinators or potential mates. Alternatively, it may lead to a reduction in paternity to other plants due to the pollen that is spent on the self-pollination (pollen discounting) or reduced availability of outcrossed pollen to the maternal plant, because the ovules have already been fertilized (seed discounting). The outcome, and whether it positively or negatively affects the population, is dependent on the timing and the mode of self-fertilization (Schoen & Lloyd 1992). Germination of seeds collected from native populations will give us a better idea of the success of seedlings in each treatment, considering this experiment more closely mimicked natural settings.

The extent of clonality within *S. virginiana* is not known, although U.S. Fish and Wildlife has funded a grant to a group studying this issue (K. Smith, pers. comm.). It is likely that rates of geitonogamy are high throughout the species range because of the species' large inflorescences. The species produces many flowers within one inflorescence, and multiple inflorescences per branch in healthier populations. If populations are highly clonal and multiple stems within one area share identity, selfing is much more likely to occur and the species is more likely to incur reproductive costs (Lloyd 1992; Klinkhamer *et al*. 1994; Barrett & Harder 1996). In a study on another mass-flowering, self-compatible, clonal shrub, Eckert (2000) found this abundance of selfing to be disadvantageous to the species as abundant inbreeding depression was found. However, clonality is not typically associated with self-compatible species in other genera (Vallejo-Marín & O'Brien 2007).

Although self-compatibility combined with clonality could be extremely detrimental to the species because of the amount of subsequent inbreeding depression, there are benefits to self-compatibility as well. Self-compatible variants have a two-fold advantage over outcrossing variants because of the biparental genetic transmission that takes place in self-fertilization (Fisher 1941; Charlesworth & Charlesworth 1987). In a world where habitat fragmentation increasingly leads to small population size, the reproductive assurance of self-compatibility is a potential survival strategy when outcrossed pollen is not available. If agamospermy does not occur in the species and the germination rates of wild populations are similar to those from the common garden, the species has a mixed mating system. Many models predict the stability of this type of mating systems (Goodwillie *et al*. 2005), even though the retention of some outcrossing can maintain inbreeding depression.

In addition to the benefits of reproductive assurance, self-fertilization may allow species to purge deleterious alleles through expression of homozygosity, whereas this option is not available to outcrossing species with gametophytic SI that may still contend with inbreeding depression in small populations (Glemin *et al.* 2001). Evidence beginning as early as the mid-1800s suggests that organisms inbred for multiple generations show initial declines in fitness but can often recover (Crnokrak & Barrett 2002). However, it may be difficult to purge inbreeding depression if outcrossing maintains a large number of deleterious alleles because few selfed progeny survive to reproduce (Goodwillie *et al*. 2005).

Supplementation of seeds or plants between populations is the most common solution offered to alleviate inbreeding depression. However, many conservation practitioners hesitate to recommend genetic rescue, as it is termed, because it may lead to outbreeding depression (Willi *et al*. 2007). This reduction in fitness results from a fragmentation in co-

adapted gene complexes or a breakdown in local adaptation (Edmands 2007). Outbreeding depression has been studied for over two decades, but little consensus has been reached on the degree of its effects. Edmands (2007) reviewed inbreeding and outbreeding in multiple species of plants and animals, and found a number of species that exhibited a greater reduction in fitness because of outbreeding when compared to inbreeding. In addition, most of these studies, as well as others not included in her review, only measured fitness in the first generation of offspring, and many species are known to show symptoms of outbreeding depression only in the second generation or later when heterozygosity breaks down and homozygosity is expressed (Lynch 1991; Dudash & Fenster 2000).

Several factors may influence the success of crosses between populations. Two plants from populations that have diverged a great deal and have become locally adapted may be less likely to produce viable seed in a cross-pollination because a breakdown in co-adapted gene complexes may result in unfit progeny. One factor to consider may be how long the two populations have been isolated or how much genetic distance lies between them. In the self-incompatible species *Grevillea repens*, populations limited by mate availability had more reproductive success in crosses with increasing genetic distance, but in populations where mate availability was not problematic, increasing genetic distance resulted in a decrease in fruit and seed set (Holmes *et al*. 2008). However, in the clonal species *Ranunculus reptans*, Willi *et al*. (2007) found no significant effect of genetic distance on fitness of their crosses within and between inbred populations in two generations, although it is possible that polyploidy in this species may have buffered the decline of heterozygosity.

According to some studies, the benefits of genetic rescue may have more to do with the origin of the maternal plant. In their study of *Aster amellus*, Raabová *et al*. (2009) found

that the maternal population source significantly affected seed set and germination rates. Seed from between-region crosses, when geographic distances between populations was more than 70 km, had much higher seed set than within-population crosses. Plants from between-population crosses, when the geographic distance was only 10 km between populations, had lower seed set than within-population crosses or between-region crosses. Although this study only measured fitness in the first generation, this phenomenon seen through multiple generations would imply that in some cases, immigration from populations beyond those that are locally adapted may have a positive effect on depressed populations. Outbreeding depression is a serious risk, but evidence suggests the benefits of genetic rescue may compensate for its risks in small populations succumbing to inbreeding, a large number of fixed mutations, or limitation by a lack of cross-compatible mates (Willi *et al*. 2007). Allowing small amounts of immigration into these populations may allow the populations to recover some variation, and the increase in fitness, if only brief, may alleviate stress long enough to increase the census size to a number that may be able to better weather the environmental burden (Willi *et al*. 2007).

Phylogeny

Interpretation of my data is difficult because of the common problems of inconsistencies between markers and the many possible causes of polyphyly. However, I have established incongruence between *S. virginiana* and *S. corymbosa* in arbitrarily primed regions, which suggests phenotypic plasticity is unlikely as a possible explanation of their differences (Aagaard *et al*. 2009), and confirms the results of the comparative study of the two species performed by Ogle (1991a). Although *S. virginiana* was not found to be

monophyletic based on the chloroplast genome regions I examined, monophyly was supported in the randomly sampled regions. Several possibilities exist to explain the non-monophyly found in the chloroplast phylogeny of *S. corymbosa* and *S. virginiana.*

One explanation for the polyphyly and paraphyly observed in chloroplast regions could be a difference in rates between speciation and the evolution of the chloroplast regions. If the regions under study evolve more slowly than the rate at which speciation has occurred, the chloroplast may show haplotypes identical to the maternal species. Recent speciation may be associated with little differentiation between lineages due to the small amount of time during which mutations could have accumulated (Lowe *et al.* 2004). Chloroplast DNA evolves slowly because the chloroplast does not recombine, and differences are only incorporated through rare indels or other mutations. Many intraspecific studies have found incomplete lineage sorting within species (Willyard *et al*. 2009, Bänfer *et al*. 2006). Lineage sorting reduces the number of lineages through natural selection and chance (Lowe *et al*. 2004). When times differ between a speciation event and that required for an allele to coalesce (Lowe *et al*. 2004), this may create problems in a molecular analysis because we are observing the gene or molecular history, rather than the species' history. However, if the species is evolving at slower rates, but the DNA region is rapidly changing, two lineages may appear identical because of homoplasy (Funk & Omland 2003). This could lead to long branch attraction in the phylogenetic analysis (Bergsten 2005). The removal of *S. corymbosa* and the Ohio samples did little to change the tree topology, although the samples from the Little TN River, NC (OP-18) and Sinking Creek, KY (OP-11) appear to have changed rapidly. Additional sampling and/or removal of these samples may provide further resolution.

According to Funk and Omland (2003), relictual ancestral polymorphisms are expected to be seen in basal parts of the tree, whereas more recent introgression is typically expected to be closer to the tips. Reticulation, or introgression, which is common in plants, can complicate analyses because this re-introduces alleles that were once isolated, which complicates the ability to infer species' history from molecular history. *Spiraea corymbosa* appeared near branch tips in our chloroplast phylogeny. Hybridization is common in *Spiraea* (Kůdela 2007), and interspecific crosses have been conducted successfully in the genus (Iizuka *et al*. 2001). Information concerning chloroplast relationships within related species prove essential in inferring some intraspecific phylogeographies (Jakob & Blattner 2006), so more complete sampling of congeners which overlap in distribution with *S. virginiana* may be helpful in determining if this is the cause of polyphyly. It may also help to compare morphologies of other species that share the *S. virginiana* distribution pattern. However, if the exchange in genes occurred in the distant past, the chances of tracing these lineages become more unlikely (Funk & Omland 2003).

Geographic structure

Previous studies of *S. virginiana* have suggested that the primary mode of reproduction in the species is through vegetative propagules (i.e. stolon or rhizome fragments) (Ogle 1991b; Anders & Murrell 2001). However, only two samples within drainages shared haplotypes (the Lula Lake and Upper Rock Creek populations). In fact, there was little geographic structure in the distribution of the haplotypes, although the most common haplotypes (A and C) were located primarily along the Cumberland Plateau. Chloroplasts are passed down maternally, so geographical distribution would show both

vegetative propagation and seed dispersal. I would expect that vegetative dispersal would lead to identical haplotypes within drainages. The presence of two samples within haplotype F near different headwaters of the Little Tennessee (which are not connected by drainage) and the widespread dispersal of haplotypes A and C throughout the Cumberland Plateau and into West Virginia suggests seed dispersal by wind may be more likely than previously thought. Other species within *Spiraea* are wind-dispersed (Zasada & Stickney 2009), and the reproductive data collected in my study suggests that some seed may be viable. However, more thorough sampling within drainages would be required to answer this question.

Ancestry

In order to summarize my data and infer questions about ancestry, I can draw on coalescent theory. Coalescent theory allows the study of population-level processes through time without the assumption of Hardy-Weinberg equilibrium (Lowe *et al*. 2004), and is constructed around mathematical and statistical calculations of processes such as selection and gene flow on a small sample of alleles (Hudson 1990). Coalescent theory has four main predictions (Lowe *et al*. 2004): a) older alleles are expected to be more geographically widespread than younger alleles; b) rare alleles are expected to be younger; c) older alleles are more likely to be placed on internal nodes in phylogenetic trees; and d) rare alleles are more likely to be related to alleles from within populations than from other populations (Hudson 1990).

In all three data sets, I see evidence supporting the conclusions of Anders and Murrell (2001). In their RAPD study, they found the highest diversity of alleles in the south within the Cumberland Plateau, suggesting these populations are ancestral. My re-analysis of

Williams' (2003) ISSR study provided strong support for the Clear Fork River as a basal lineage – this river is on the Cumberland Plateau. Internal nodes in the chloroplast phylogeny are on the Cumberland Plateau. In addition, the haplotype network analysis collapsed the basal haplotype into the Pulaski County, KY sample (OP-10). Pulaski County, KY is also on the Cumberland Plateau. While it is true that many of my samples originate from the Cumberland Plateau, Anders and Murrell (2001) and Williams (2003) sampled a larger number of populations with similar results. I would suggest that a southern ancestry is likely.

Bibliography

Aagaard, S. M. D., Vogel, J. C. & Wikström, N. (2009) Resolving maternal relationships in the clubmoss genus *Diphasiastrum* (Lycopodiaceae). *Taxon* **58:** 835-848.

Adams, C. C. (1902) Southeastern United States as a center of geographical distribution of flora and fauna. *Biological Bulletin* **3:** 115-131.

Allee, W. C. (1931) *Animal Aggregations: a Study in General Sociology.* University of Chicago Press, Chicago.

Anders, C. M. & Murrell, Z. E. (2001) Morphological, molecular, and biogeographical variation within the imperiled Virginia Spiraea. *Castanea* **66:** 24-41.

Anderson, E. (1936) The species problem in *Iris*. *Annals of the Missouri Botanical Garden* **23:** 457-509.

Arasu, N. T. (1968) Self-incompatibility in angiosperms: A review. *Genetica* **39:** 1-24.

Arbogast, B. S., Browne, R. A., Weigl, P. D. & Kenagy, G. J. (2005) Conservation genetics of endangered flying squirrels (*Glaucomys*) from the Appalachian mountains of eastern North America. *Animal Conservation* **8:** 123-133.

Austin, J. D. & Zamudio, K. R. (2008) Incongruence in the pattern and timing of intra-specific diversification in bronze frogs and bullfrogs (Ranidae). *Molecular Phylogenetics and Evolution* **48:** 1041-1053.

Baldwin, J. T., Jr. (1951) Chromosomes of *Spiraea* and of certain other genera of Rosaceae. *Rhodora* **53:** 203-206.

Bänfer, G., Moog, U., Fiala, B., Mohamed, M., Weising, K. & Blattner, F. R. (2006) A chloroplast genealogy of myrmecophytic *Macaranga* species (Euphorbiaceae) in Southeast Asia reveals hybridization, vicariance and long-distance dispersals. *Molecular Ecology* **15:** 4409-4424.

Barrett, S. C. H. & Harder, L. D. (1996) Ecology and evolution of plant mating. *Trends in Ecology and Evolution* **11:** 73-79.

Baturin, S. O. (2009) Agamospermy in outbred crosses of *Fragaria vesca* L. (Rosaceae). *Russian Journal of Genetics* **45:** 322-326.

Beckett, D. C. & Miller, M. C. (1982) Macroinvertebrate colonization of multiplate samplers in the Ohio River - the effect of dams. *Canadian Journal of Fisheries and Aquatic Sciences* **39:** 1622-1627.

Bell, R. L. & Hough, L. F. (1986) Interspecific and intergeneric hybridization of *Pyrus*. *Horticultural Science* **21:** 62-64.

Bergsten, J. (2005) A review of long-branch attraction. *Cladistics* **21:** 163-193.

Blanchard, M. (1990) Endangered and threatened wildlife and plants; threatened status determined for *Spiraea virginiana* (Virginia Spiraea). *Federal Register* **55:** 24241-24247.

Bock, W. J. (2004) Species: the concept, category and taxon. *Journal of Zoological Systematics and Evolutionary Research* **42:** 178-190.

Braun, L. (1950) *Deciduous Forests of Eastern North America.* Hafner Publishing Company, Inc., New York and London.

Braun, L. (1951) Plant Distribution in Relation to the Glacial Boundary. *The Ohio Journal of Science* **51:** 139.

Britton, N. L. & Brown, A. (1913) *An Illustrated Flora of the United States, Canada, and the British Possessions.* Charles Scribner's Sons, New York.

Brown, H. B. (1910) The genus *Crataegus* with some theories of the origin of its species. *Bulletin of the Torrey Botanical Club* **37:** 251-260.

Byers, D. L. & Meagher, T. R. (1992) Mate availability in small populations of plant species with homomorphic sporophytic self-incompatibility. *Heredity* **68:** 353-359.

Camp, C. D., Peterman, W. E., Milanovich, J. R., Lamb, T., Maerz, J. C. & Wake, D. B. (2009) A new genus and species of lungless salamander (family Plethodontidae) from the Appalachian highlands of the south-eastern United States. *Journal of Zoology* **279:** 86-94.

Castric, V. & Vekemans, X. (2004) Plant self-incompatibility in natural populations: A critical assessment of recent theoretical and empirical advances. *Molecular Ecology* **13:** 2873-2889.

Chamber of Commerce, A. C. (2005) Ashe County, NC Relocation Guide, Version. Ashe County Chamber of Commerce, West Jefferson, NC. [cited 10 February 2010]. Available from URL: http://www.ashechamber.com/.

Charlesworth, D. (1985) Distribution of dioecy and self-incompatibility in angiosperms. In Greenwood, J. J. & Slatkin, M. (eds.) *Evolution - Essays in Honor of John Maynard Smith.* Harvard University Press, Cambridge.

Charlesworth, D. & Charlesworth, B. (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology & Systematics* **18:** 237-268.

Charpentier, A. (2001) Consequences of clonal growth for plant mating. *Evolutionary Ecology* **15:** 521-530.

Clement, M., Posada, D. & Crandall, K. A. (2000) TCS: A computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1659.

Cook, L. M. & Soltis, P. S. (2000) Mating systems of diploid and allotetraploid populations of *Tragopogon* (Asteraceae). II. Artificial populations. *Heredity* **84:** 410-415.

Cracraft, J. (2002) The seven great questions of systematic biology: An essential foundation for conservation the sustainable use of biodiversity. *Annals of the Missouri Botanical Garden* **89:** 127-144.

Critchfield, W. B. & Little, E.L., Jr. (1966) *Geographic Distribution of the Pines of the World*. Misc. Pub. 991. Washington, DC: U.S. Department of Agriculture, Forest Service.

Crnokrak, P. & Barrett, S. C. H. (2002) Perspective: Purging the genetic load: A review of the experimental evidence. *Evolution* **56:** 2347-2358.

de Candolle, A. P. (1825) *Prodromus systematis naturalis regni vegetabilis, sive, Enumeratio contracta ordinum generum specierumque plantarum huc usque congnitarium juxta methodi naturalis, normas digesta.* Treuttel et Würtz, Paris.

de Nettancourt, D. (1977) *Incompatibility in Angiosperms.* Springer-Verlag, Berlin.

de Nettancourt, D. (2001) *Incompatibility and Incongruity in Wild and Cultivated Plants.* Springer, Berlin.

Deevey, E. S. (1949) Biogeography of the Pleistocene. *Geological Society of America Bulletin* **60:** 1315-1416.

Delcourt, H. R. (1979) Late quaternary vegetation history of the Eastern Highland Rim and adjacent Cumberland Plateau of Tennessee. *Ecological Monographs* **49:** 255-280.

Delcourt, H. R. & Delcourt, P. A. (1991) *Quaternary Ecology: A Paleoecological Perspective.* Chapman & Hall, London.

Delcourt, P. A. & Delcourt, H. R. (1984) Late Quaternary paleoclimates and biotic responses in eastern North America and the western North Atlantic Ocean. *Palaeogeography, Palaeoclimatology, Palaeoecology* **48:** 263-284.

Demauro, M. M. (1993) Relationship of breeding system to rarity in the Lakeside Daisy (*Hymenoxys acaulis* var. *glabra*). *Conservation Biology* **7:** 542-550.

Dickinson, T. A., Lo, E. & Talent, N. (2007) Polyploidy, reproductive biology, and Rosaceae: understanding evolution and making classifications. *Plant Systematics & Evolution* **266:** 59-78.

Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K. & Mattick, J. S. (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research* **19:** 4008.

Dorken, M. E. & Barrett, S. C. H. (2004) Chloroplast haplotype variation among monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae) in eastern North America. *Molecular Ecology* **13:** 2699-2707.

Du, Y. J. & Huang, Z. L. (2008) Effects of seed mass and emergence time on seedling performance in *Castanopsis chinensis*. *Forest Ecology and Management* **255:** 2495-2501.

Dudash, M. & Fenster, C. (2000) Inbreeding and outbreeding depression in fragmented populations. In: Young, A. G. & Clarke, G. M. (eds.) *Genetics, demography and viability of fragmented populations.* Cambridge University Press, Cambridge, pp. 55-74.

Ebert, D., Hayes, C. & Peakall, R. (2009) Chloroplast simple sequence repeat markers for evolutionary studies in the sexually deceptive orchid genus *Chiloglottis*. *Molecular Ecology Resources* **9:** 784-789.

Ebert, D. & Peakall, R. (2009a) Chloroplast simple sequence repeats (cpSSRs): Technical resources and recommendations for expanding cpSSR discovery and applications to a wide array of plant species. *Molecular Ecology Resources* **9:** 673-690.

Ebert, D. & Peakall, R. (2009b) A new set of universal *de novo* sequencing primers for extensive coverage of noncoding chloroplast DNA: New opportunities for phylogenetic studies and cpSSR discovery. *Molecular Ecology Resources* **9:** 777-783.

Eckert, C. G. (2000) Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology* **81:** 532-542.

Edmands, S. (2007) Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* **16:** 463-475.

Escaravage, N., Questiau, S., Pornon, A., Doche, B. & Taberlet, P. (1998) Clonal diversity in a *Rhododendron ferrugineum* L. (Ericaceae) population inferred from AFLP markers. *Molecular Ecology* **7:** 975-982.

Estep, M. C. (2002) *Phylogeographic Analysis of Selected Populations of* Paruroctonus utahensis *(Williams, 1968) from the Desert Southwest*. Master's Thesis. Appalachian State University, Boone.

Estill, J. C. (1996) *Demographic and molecular genetic analysis of* Spiraea virginiana. Master's Thesis. Western Kentucky University, Bowling Green.

Evans, R. C. & Dickinson, T. A. (1999) Floral ontogeny and morphology in subfamily Spiraeoideae Endl. (Rosaceae). *International Journal of Plant Sciences* **160:** 981-1012.

Fisher, R. A. (1941) Average excess and average effect of a gene substitution. *Annals of Eugenics* **11:** 53-63.

Funk, D. J. & Omland, K. E. (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* **34:** 397-423.

Gillespie, E. L. (2005) *Phylogeography of* Carex eburnea *(Cyperaceae) and the systematics of the* Carex eburnea *complex*. Master's Thesis. Appalachian State University, Boone.

Glemin, S., Bataillon, T., Ronfort, J., Mignot, A. & Olivieri, I. (2001) Inbreeding depression in small populations of self-incompatible plants. *Genetics* **159:** 1217-1229.

Glémin, S., Bazin, E. & Charlesworth, D. (2006) Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proceedings: Biological Sciences* **273:** 3011-3019.

Glencoe, J. F., Jr. (1961) Spiraea virginiana *Britton: a rare southern Appalachian endemic*. Master's Thesis. West Virginia University, Morgantown.

Godbout, J., Jaramillo-Correa, J. P., Beaulieu, J. & Bousquet, J. (2005) A mitochondrial DNA minisatellite reveals the postglacial history of jack pine (*Pinus banksiana*), a broad-range North American conifer. *Molecular Ecology* **14:** 3497-3512.

Gonzales, E. & Hamrick, J. L. (2005) Distribution of genetic diversity among disjunct populations of the rare forest understory herb, *Trillium reliquum*. *Heredity* **95:** 306-314.

Gonzales, E., Hamrick, J. L. & Chang, S.-M. (2008) Identification of glacial refugia in south-eastern North America by phylogeographical analyses of a forest understorey plant, *Trillium cuneatum*. *Journal of Biogeography* **35:** 844-852.

Goodell, K., Elam, D. R., Nason, J. D. & Ellstrand, N. C. (1997) Gene Flow Among Small Populations of a Self-Incompatible Plant: An Interaction between Demography and Genetics. *American Journal of Botany* **84:** 1362-1371.

Goodwillie, C., Kalisz, S. & Eckert, C. G. (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, & Systematics* **36:** 47-79.

Hairston, N. G. & Pope, C. H. (1948) Geographic variation and speciation in Appalachian salamanders (*Plethodon jordani* group). *Evolution* **2:** 266-278.

Hall, T. (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95-98.

Hedgecock, T. S., Feaster, T. D., Alabama Department of Transportation & Geological Survey. (2007) *Magnitude and frequency of floods in Alabama, 2003 [electronic resource] / by T.S. Hedgecock and Toby D. Feaster ; prepared in cooperation with the Alabama Department of Transportation* U.S. Dept. of the Interior, U.S. Geological Survey, Reston, Va.

Hedin, M. & Thomas, S. M. (2009) Molecular systematics of eastern North American Phalangodidae (Arachnida: Opiliones: Laniatores), demonstrating convergent

morphological evolution in caves. *Molecular Phylogenetics and Evolution* **54:** 107-121.

Heslop-Harrison, J. (1978) Genetics and physiology of angiosperm incompatibility systems. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **202:** 73-92.

Hewitt, G. (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907-913.

Hey, J. (2001) The mind of the species problem. *Trends in Ecology and Evolution* **16:** 326-329.

Hiscock, S. J. & Kües, U. (1999) Cellular and molecular mechanisms of sexual incompatibility in plants and fungi. *International Review of Cytology* **193**: 165-295.

Holmes, G. D., James, E. A. & Hoffmann, A. A. (2008) Limitations to reproductive output and genetic rescue in populations of the rare shrub *Grevillea repens* (Proteaceae). *Annals of Botany* **102:** 1031-1041.

Hudson, R. (1990) Gene genealogies and the coalescent process. In: Futuyma, D. & Antonovics, J. (eds.) *Oxford Surveys in Evolutionary Biology.* Oxford University Press, Oxford, pp. 1-44.

Huelsenbeck, J. P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754-755.

Husband, B. C. & Schemske, D. W. (1997) The effect of inbreeding in diploid and tetraploid populations of *Epilobium angustifolium* (Onagraceae): Implications for the genetic basis of inbreeding depression. *Evolution* **51:** 737-746.

Iizuka, M., Kudo, N., Kimura, Y. & Ogiwara, I. (2001) Interspecific hybrids between *Spiraea thunbergii* Sieb. ex Blume. and *S. japonica* L. fil. via ovule culture. *Journal of the Japanese Society for Horticultural Science* **70:** 767-773.

Jakob, S. S. & Blattner, F. R. (2006) A chloroplast genealogy of *Hordeum* (Poaceae): Long-term persisting haplotypes, incomplete lineage sorting, regional extinction, and the consequences for phylogenetic inference. *Molecular Biology and Evolution* **23:** 1602-1612.

Klinkhamer, P. G. L., De Jong, T. J. & Metz, J. A. J. (1994) Why plants can be too attractive: A discussion of measures to estimate male fitness. *Journal of Ecology* **82:** 191-194.

Kůdela, V. (2007) Inflorescence blast and flower bud abnormalities of *Spiraea* x *vanhouttei* and their causes. *Czech Journal of Genetics and Plant Breeding* **43:** 135-143.

Larson, B. M. H. & Barrett, S. C. H. (2000) A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* **69:** 503-520.

Lessa, E. P., Cook, J. A. & Patton, J. L. (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceedings of the National Academy of Sciences of the United States of America* **100:** 10331-10334.

Levin, D. A., Kelley, C. D. & Sarkar, S. (2009) Enhancement of Allee effects in plants due to self-incompatibility alleles. *Journal of Ecology* **97:** 518-527.

Lewis, D. & Modlibowska, I. (1942) Genetical studies in pears IV. Pollen-tube growth and incompatibility. *Journal of Genetics* **43:** 211-222.

Lloyd, D. G. (1979) Some reproductive factors affecting the selection of self-fertilization in plants. *American Naturalist* **113:** 67-80.

Lloyd, D. G. (1992) Self- and cross-fertilization in plants: II. The selection of self-fertilization. *International Journal of Plant Sciences* **153:** 370-380.

Lowe, A., Harris, S. & Ashton, P. (2004) *Ecological Genetics: Design, Analysis, and Application.* Blackwell Publishing, Malden.

Lynch, M. (1991) The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* **45:** 622-629.

Mable, B. K. (2004) Polyploidy and self-compatibility: Is there an association? *New Phytologist* **162:** 803-811.

Maddison, D. R. & Maddison, W. P. (2005) MacClade 4.08. Sinauer Associates, Sunderland.

Magni, C. R., Ducousso, A., Caron, H., Petit, R. J. & Kremer, A. (2005) Chloroplast DNA variation of *Quercus rubra* L. in North America and comparison with other Fagaceae. *Molecular Ecology* **14:** 513-524.

Masuyama, S. & Watano, Y. (1990) Trends for inbreeding in polyploid Pteridophytes. *Plant Species Biology* **5:** 13-18.

Mayden, R. L. (1997) A hierarchy of species concepts: The denouement in the saga of the species problem. In: Claridge, M. F., Dawah, A. H. & Wilson, M. R. (eds.) *Species: the Units of Biodiversity.* Chapman & Hall, London, pp. 381-424.

Mayden, R. L. (1999) Consilience and a hierarchy of species concepts: Advances toward closure on the species puzzle. *Journal of Nematology* **31:** 95-116.

Mayr, E. (1942) *Systematics and the Origin of Species.* Columbia University Press, New York.

Mayr, E. (1996) What is a species and what is not? *Philosophy of Science* **63:** 262-277.

Miller, J. S. & Venable, D. L. (2000) Polyploidy and the evolution of gender dimorphism in plants. *Science* **289:** 2335.

Morgan, D. R., Soltis, D. E. & Robertson, K. R. (1994) Systematic and evolutionary implications of *rbc*L sequence variation in Rosaceae. *American Journal of Botany* **81:** 890-903.

Morris, A. B., Ickert-Bond, S. M., Brunson, D. B., Soltis, D. E. & Soltis, P. S. (2008) Phylogeographical structure and temporal complexity in American sweetgum (*Liquidambar styraciflua*; Altingiaceae). *Molecular Ecology* **17:** 3889-3900.

Mulcahy, G. B. & Mulcahy, D. L. (1986) Pollen-Pistil Interactions. In: G.B. Mulcahy, D. L. M., and E. Ottaviano (ed.) *Biotechnology and Ecology of Pollen: Proceedings of the International Conference on the Biotechnology and Ecology of Pollen.* Springer-Verlag, pp. 173-178.

Newbigin, E., Anderson, M. A. & Clarke, A. E. (1993) Gametophytic self-incompatibility systems. *The Plant Cell* **5:** 1315-1324.

Newell, W. L. & Rice, D. D. (1978) Structural control of Cumberland River and its ancestral channels at Flat-Lick, Kentucky. *Journal of Research of the US Geological Survey* **6:** 359-367.

Niklas, K. (1997) *The Evolutionary Biology of Plants.* University of Chicago Press, Chicago.

Nylander, J. A. A. (2004) MrAIC.pl. Evolutionary Biology Centre, Uppsala University, Uppsala.

Oginuma, K., Chen, Z.-d., Kondo, K. & Hong, D.-y. (1999) Karyomorphology of three species of *Spiraea* (Rosaceae) from China with a new count of octoploid (2n=72). *Chromosome Science* **3:** 146.

Ogle, D. W. (1991a) *Spiraea virginiana* Britton: I. Delineation and Distribution. *Castanea* **56:** 287-296.

Ogle, D. W. (1991b) *Spiraea virginiana* Britton: II. Ecology and Species Biology. *Castanea* **56:** 297-303.

Pandey, K. K. (1960) Evolution of gametophytic and sporophytic systems of self-incompatibility in angiosperms. *Evolution* **14:** 98-115.

Paz, H. & Martinez-Ramos, M. (2003) Seed mass and seedling performance within eight species of *Psychotria* (Rubiaceae). *Ecology* **84:** 439-450.

Pittillo, J. D. (1994) Vegetation of three high elevation southern Appalachian bogs and implications of their vegetational history. *Water Air and Soil Pollution* **77:** 333-348.

Potter, D., Eriksson, T., Evans, R. C., Oh, S., Smedmark, J. E. E., Morgan, D. R., Kerr, M., Robertson, K. R., Arsenault, M., Dickinson, T. A. & Campbell, C. S. (2007a) Phylogeny and classification of Rosaceae. *Plant Systematics and Evolution* **266:** 5-43.

Potter, D., Still, S. M., Grebenc, T., Ballian, D., Božič, G., Franjiæ, J. & Kraigher, H. (2007b) Phylogenetic relationships in Tribe Spiraeeae (Rosaceae) inferred from nucleotide sequence data. *Plant Systematics and Evolution* **266:** 105-118.

Raabova, J., Munzbergova, Z. & Fischer, M. (2009) Consequences of near and far between-population crosses for offspring fitness in a rare herb. *Plant Biology* **11:** 829-836.

Rehder, A. (1940) *Manual of Cultivated Trees and Shrubs.* Dioscorides Press, Portland.

Riccardi, A. C. (2009) IUGS ratified ICS recommendation on redefinition of Pleistocene and formal definition of base of Quaternary. International Union of Geological Sciences.

Sax, K. (1936) Polyploidy and Geographic Distribution in *Spiraea*. *Journal of the Arnold Arboretum* **17:** 352-356.

Schemske, D. W. (1983) Breeding system and habitat effects on fitness components in three neotropical *Costus* (Zingiberaceae). *Evolution* **37:** 523-539.

Schlaepfer, D. R., Edwards, P. J., Widmer, A. & Billeter, R. (2008) Phylogeography of native ploidy levels and invasive tetraploids of *Solidago gigantea*. *Molecular Ecology* **17:** 5245-5256.

Schoen, D. J. & Lloyd, D. G. (1992) Self- and cross-fertilization in plants: III. Methods for studying modes and functional aspects of self-fertilization. *International Journal of Plant Sciences* **153:** 381-393.

Schoen, D. J., Morgan, M. T. & Bataillon, T. (1996) How does self-pollination evolve? Inferences from floral ecology and molecular genetic variation. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **351:** 1281-1290.

Shaw, J. & Small, R. L. (2005) Chloroplast DNA phylogeny and phylogeography of the North American plums (*Prunus* subgenus *Prunus* section *Prunocerasus*, Rosaceae). *American Journal of Botany* **92:** 2011-2030.

Simpson, G. G. (1961) *Principles of Animal Taxonomy.* Columbia University Press, New York.

Sipe, T. W. & Browne, R. A. (2004) Phylogeography of masked (*Sorex cinereus*) and smoky shrews (*Sorex fumeus*) in the southern Appalachians. *Journal of Mammalogy* **85:** 875-885.

Soltis, D. E., Morris, A. B., McLachlan, J. S., Manos, P. S. & Soltis, P. S. (2006) Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology* **15:** 4261-4293.

Stewart, J. R. (2009) The evolutionary consequence of the individualistic response to climate change. *Journal of Evolutionary Biology* **22:** 2363-2375.

Sun, B.-Y., Kim, T.-J. & Kim, C. H. (1997) A biosystematic study on polyploid populations of the genus *Spiraea* (Rosaceae) in Korea. *Journal of Plant Biology* **40:** 291-297.

Swofford, D. L. (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10 4.0b10 ed. Sinauer Associates, Sunderland.

Talent, N. (2009) Evolution of gametophytic apomixis in flowering plants: An alternative model from Maloid Rosaceae. *Theory in Biosciences* **128:** 121-138.

Thomas, L. F. (1928) Basic factors in flood frequency in the lower Mississippi River. *The Annals of the American Academy of Political and Social Science* **135:** 1-6.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25:** 4876–4882.

Tilley, S. G., Eriksen, R. L. & Katz, L. A. (2008) Systematics of dusky salamanders, *Desmognathus* (Caudata : Plethodontidae), in the mountain and Piedmont regions of Virginia and North Carolina, USA. *Zoological Journal of the Linnean Society* **152:** 115-130.

Timpe, E. K., Graham, S. P. & Bonett, R. M. (2009) Phylogeography of the brownback salamander reveals patterns of local endemism in Southern Appalachian springs. *Molecular Phylogenetics and Evolution* **52:** 368-376.

Vallejo-Marín, M. & O'Brien, H. E. (2007) Correlated evolution of self-incompatibility and clonal reproduction in *Solanum* (Solanaceae). *New Phytologist* **173:** 415-421.

Wallace, L. E. (2002) Examining the effects of fragmentation on genetic variation in *Platanthera leucophaea* (Orchidaceae): Inferences from allozyme and random amplified polymorphic DNA markers. *Plant Species Biology* **17:** 37-49.

Watts, W. A. (1970) The full glacial vegetation of northwestern Georgia. *Ecology* **51:** 17-33.

Weakley, A. S. (2008) Flora of the Carolinas, Virginia, Georgia, northern Florida, and surrounding areas. Working draft of 7 April 2008 version. [cited 10 February 2010]. Available from URL: http://www.herbarium.unc.edu/flora.htm.

Willi, Y., Van Kleunen, M., Dietrich, S. & Fischer, M. (2007) Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. *Proceedings of the Royal Society Biological Sciences Series B* **274:** 2357-2364.

Williams, F. (2003) *Examination of variation among populations of* Spiraea virginiana *Britton based upon multiple molecular methods*. Master's Thesis. Appalachian State University, Boone.

Williams, G. C. (1975) *Sex and evolution.* Princeton University Press, Princeton.

Willyard, A., Cronn, R. & Liston, A. (2009) Reticulate evolution and incomplete lineage sorting among the Ponderosa Pines. *Molecular Phylogenetics and Evolution* **52:** 498-511.

Wirchansky, B. A. & Shain, D. H. (2009) A new species of *Haemopis* (Annelida: Hirudinea): Evolution of North American terrestrial leeches. *Molecular Phylogenetics and Evolution* **54:** 226-234.

Xiao, L.-Q., Ge, X.-J., Gong, X., Hao, G. & Zheng, S.-X. (2004) ISSR Variation in the Endemic and Endangered Plant *Cycas guizhouensis* (Cycadaceae). *Annals of Botany* **94:** 133-138.

Zasada, J. C. & Stickney, P. F. (2009) *Spiraea* L. In: Bonner, F. T. & Karrfalt, R. P. (eds.) *The Woody Plant Seed Manual, Agric. Handbook No. 727.* U.S. Department of Agriculture, Forest Service, Washington, D.C., pp.1067-1070.

Zhang, Z., Fan, L., Yang, J., Hao, X. & Gu, Z. (2006) Alkaloid polymorhpism and ITS sequence variation in the *Spiraea japonica* complex (Rosaceae) in China: Traces of the biological effects of the Himalaya-Tibet plateau uplift. *American Journal of Botany* **93:** 762-769.

Zomlefer, W. B. (1994) *Guide to Flowering Plant Families.* The University of North Carolina Press, Chapel Hill & London.

APPENDIX A

Executable Code for Parsimony and Bootstrap Analysis in PAUP

```
Begin PAUP;
outgroup outgroup/only; set maxtrees=5000 increase=auto autoclose=no torder=right
tcompress=yes taxlabels=full storebrlens=yes storetreewts=yes outroot=paraphyl;
log file=reducedpresabsspiraea.log append=yes replace=yes;
set autoclose=yes;
hsearch start=stepwise addseq=random nreps=1000 savereps=yes randomize=addseq
rstatus=yes hold=1 swap=tbr multrees=yes;
savetrees file=reducedpresabsspiraea.all.tre brlen=yes;
filter best=yes permdel=yes;
savetrees file=reducedpresabsspiraea.best.tre brlen=yes;
log stop;
end;

begin paup;
outgroup outgroup/only;
log file=reducedpresabsboot.log append=yes replace=yes;
bootstrap nreps=500 keepall=yes treefile=bootreducedpresabs.tre format=nexus brlens=yes
search=heuristic/ addseq=random;
log stop;
end;
```

APPENDIX B

Executable Code for Bayesian Analysis of Partitioned DNA Sequences in MrBayes

```
BEGIN DATA;
        DIMENSIONS  NTAX=19 NCHAR=1788;
        FORMAT DATATYPE=mixed (DNA:1-860,DNA:861-1389,DNA:1390-1788)
INTERLEAVE=NO MISSING=? GAP=- ;
MATRIX
(Sequences in nexus format go here)
;
END;
begin mrbayes;
charset rps16 = 1-860;
charset atpH = 861-1389;
charset rps2 = 1390-1788;
partition favored = 3: rps16, atpH, rps2;
set partition = favored;
unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
prset applyto=(all) ratepr=variable;
lset applyto=(1) nst=1 rates=gamma;
lset applyto=(2) nst=1 rates=gamma;
lset applyto=(3) nst=6 rates=invgamma;
outgroup SJ;
mcmc ngen=3000000 samplefreq=100;
sump burnin=7500;
sumt burnin=7500;
END;
```

Notes: In partitioned datasets, the sequences from different regions are strung together, as if part of one long region. In a Bayesian or maximum likelihood analysis, you must determine the appropriate evolutionary model for each region first, then tell the model which part of the long sequence is the region to which you want the particular model assigned. In this example, the *rps*16 region is the part of the sequence that spans from the 1st nucleotide to the 860th. MrAiC determined the "nst" and "rates" values. The number of generations (ngen) should be determined by examining the literature, but 20,000 generations can give you a good idea of relationships. Burn-in values (sump burnin and sumt burnin) should be set so that convergence occurs before you begin sampling trees to create the consensus tree.

APPENDIX C

Haplotypes Identified by the Haplotype Network Analysis

Haplotype	Samples within haplotype
A	OP11, OP25, OP7, E25
B	OH
C	OP10, OP8, OP26, OP23, OP4, OP22
D	OP5
E	OP21
F	OP19, OP18
G	1-WV
H	OP16
I	3-OH, Sc-1, Sc-2, Sc-3
J	OP6
K	OP2

APPENDIX D

Voucher Accessions from Specimens Collected by Ogle and Pate

DNA sample	Arnold Arboretum Voucher No.
OP-26	AA 20-91
OP-25	AA 1093-88, 1165-89
OP-23	AA 1162-89
OP-22	AA 1161-89
OP-21	AA 1164-89
OP-19	AA 774-88
OP-18	AA 812-88
OP-16	AA 1189-88
OP-11	AA 23-91
OP-10	AA 25-91
OP-8	AA 1153-89
OP-7	AA 1152-89
OP-6	AA 1160-89
OP-5	AA 1157-89
OP-4	AA 1154-89
OP-2	AA 1155-89
Sc-3	BOON 20585

APPENDIX E

Molecular Sequences

Molecular Sequences for Primer 8R

Sequence	1	10	20	30	40	50
OP-8	TTT TTT TAT	ATT TTA TAT AGG AAT GAA AGTGCT CTT GGC TCGAC ATC ATT				
OP-26	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-10	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-21	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-6	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-23	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-5	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-11	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-25	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-7	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
E25	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
SJ	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-16	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
WV-1	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-4	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-19	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-18	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-22	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-2	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .

Sequence	60	70	80	90	100
OP-8	TGT TCT GTT CCA CTG GAACTC TCA TTT TTT	GAG TGT TGT GAT GTA ATA TG			
OP-26	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-10	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-21	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-6	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-23	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-5	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-11	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-25	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-7	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
E25	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
SJ	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-16	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
WV-1	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-4	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-19	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-18	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-22	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-2	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .

```
Sequence                110       120       130       140       150
OP-8        TACACG ATG GAGCTC GAG TAGAAA GTA TTG ATT CTC AAGGGC AAG AATCT
OP-26       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-11       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

Sequence                160       170       180       190       200
OP-8        AAG GTT AGTATC GATCAA TAA ATT GTA ACAACT TCG TAA GTA TAT TTT CG
OP-26       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-11       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
```

```
Sequence                210       220       230       240       250
OP-8      AGATAT AAA TCT AAA GTA TCC AAT TCG AGAAAATTG AAA AGT AAA ATT T
OP-26     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-11     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ        . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . .
OP-16     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

Sequence                260       270       280       290       300
OP-8      GTT  GAA ATT GGT AAA ACT CTT TCG ATCAAA TCAAAA GTA AAG TGTAGGAA
OP-26      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21      . . . . . . . . . . . . . . . . . . . . . . . . . . . . .G. . . . .
OP-6       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-11      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16      . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . .
WV-1       . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . .
OP-4       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
```

```
Sequence                310       320       330       340       350
OP-8      TCA ACCATT CGT ATG ATT CTT TGA TAG AAA TAA ATCCCC AAA AAT GGTAT
OP-26     . . . . . .
OP-10     . . . . . .
OP-21     . . . . . .
OP-6      . . . . . .
OP-23     . . . . . .
OP-5      . . . . . .
OP-11     . . . . . .
OP-25     . . . . . .
OP-7      . . . . . .
E25       . . . . . .
SJ        . . . . . T . . . . . .
OP-16     . . . . . .
WV-1      . . . . . .
OP-4      . . . . . .
OP-19     . . . . . .
OP-18     . . . . . .
OP-22     . . . . . .
OP-2      . . . . . .

Sequence          360       370       380       390       400
OP-8      GTTGCTGCCATTTTGAAACGATTTAAAGATCACCGAAGTAATGTCTAAAC
OP-26     . . . . . .
OP-10     . . . . . .
OP-21     . . . . . .
OP-6      . . . . . .
OP-23     . . . . . .
OP-5      . . . . . .
OP-11     . . . . . .
OP-25     . . . . . .
OP-7      . . . . . .
E25       . . . . . .
SJ        . . . . . .
OP-16     . . . . . .
WV-1      . . . . . .
OP-4      . . . . . .
OP-19     . . . . . .
OP-18     . . . . . .
OP-22     . . . . . .
OP-2      . . . . . .
```

Sequence	410	420	430	440	450
OP-8	CCA ATG ATT CAAGGC AAA GATAAAGAT CCT GGAACA AGT AAATAC CC TTT				
OP-26	. . . . .				
OP-10	. . . . .				
OP-21	. . . . .				
OP-6	. . . . .				
OP-23	. . . . .				
OP-5	. . . . .				
OP-11	. . . . .				
OP-25	. . . . .				
OP-7	. . . . .				
E25	. . . . .				
SJ	. . . . .				
OP-16	. . . . .				
WV-1	. . . . .				
OP-4	. . . . .				
OP-19	. . . . .				
OP-18	. . . . .				
OP-22	. . . . .				
OP-2	. . . . .				

Sequence	460	470	480	490	500
OP-8	TCAA TTG TCT CAA CAACTA GAT CAGAAT GAAGAA TAA AAGTAG ATT CTAA				
OP-26	. . . . .				
OP-10	. . . . .				
OP-21	. . . . .				
OP-6	. . . . .				
OP-23	. . . . .				
OP-5	. . . . .				
OP-11	. . . . .				
OP-25	. . . . .				
OP-7	. . . . .				
E25	. . . . .				
SJ	. . . . .				
OP-16	. . . . .				
WV-1	. . . . .				
OP-4	. . . . .				
OP-19	. . . . .				
OP-18	. . . . .				
OP-22	. . . . .				
OP-2	. . . . .				

Sequence	510	520	530	540	550
OP-8	AGG AGACAG ACA AAAAAGGGG TTAGAG ACC ACT CAATAA ATG AAATAC CT				
OP-26	. . . . .				
OP-10	. . . . .				
OP-21	. . . . .				
OP-6	. . . . .				
OP-23	. . . . .				
OP-5	. . . . .				
OP-11	. . . . .				
OP-25	. . . . .				
OP-7	. . . . .				
E25	. . . . .				
SJ	. . . . .				
OP-16	. . . . .				
WV-1	. . . . .				
OP-4	. . . . .				
OP-19	. . . . .				
OP-18	. . . . .				
OP-22	. . . . .				
OP-2	. . . . .				

Sequence	560	570	580	590	600
OP-8	AAA AGG TTT TTT TTT GAG TTA TTT TAG AAT TAT TCA ACT TGA GTT - ATGA				
OP-26	. . . . .				
OP-10	. . . . .				
OP-21	. . . . .				
OP-6	. . . . .				
OP-23	. . . . .				
OP-5	. . . . .				
OP-11	. . . . .				
OP-25	. . . . .				
OP-7	. . . . .				
E25	. . . . .				
SJ	. . . . .				
OP-16	. . . . .				
WV-1	. . . . .				
OP-4	. . . . .				
OP-19	. . . . .				
OP-18	. . . . .				
OP-22	. . . . .				
OP-2	. . . . . T				

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Sequence                610       620       630       640       650
OP-8      GGGTGC AAA TTT CAA ATACAA TTT TTG GTT  - - GGG AAA AAT AAG AAAAAA
OP-26     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-11     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2      . . . . . . . . . . . . . . . . . . . . . . . . . G . TG. .G. . . . . . . . . . . . . . .

Sequence                660       670       680       690       700
OP-8      A-G TAC TT -  AAATCA ATA ATC TAA TTA ATT TGA TGA TTT TAT GGA TAT AT
OP-26     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-11     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2      . A. . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . ? ? ?  ? ? ? ? ? ? ?  ? ?
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Sequence                710       720       730       740       750
OP-8        TTT ATA TTC GAA TTC TAT AT - - -- -- ATA GAC ATC ATC ATAA TTT CGAAT
OP-26       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5        . . . . . . . . . . . . . . . . . . . . CTATAT . . . . . . . . . . . .
OP-11       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . .
OP-18       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2        ?????????????????????????????????????????????????????????????????????

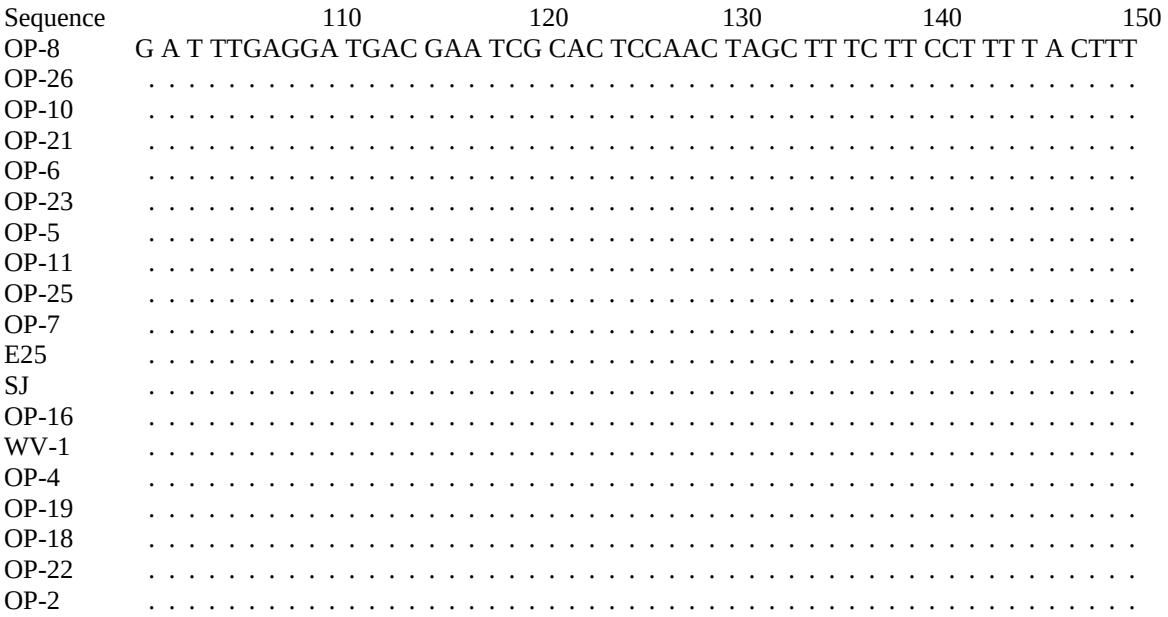
Sequence                760       770       780       790       800
OP-8        TTT CTC GAG CCG TAC GAGGAT AAAACT T – CTTA TAC GTT TCT AGG GGGGG
OP-26       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5        . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . N. . . . . .
OP-11       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25         . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . . . . .
SJ          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . ??????????
OP-2        ?????????????????????????????????????????????????????????????????????
```

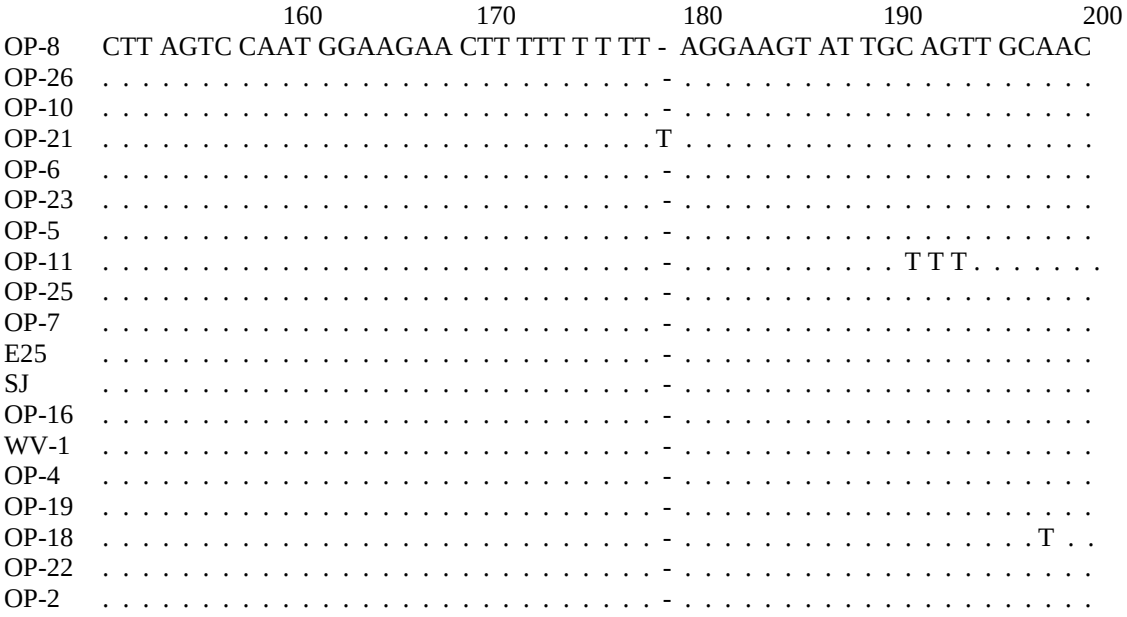
Sequence	810	820	830	840	850
OP-8	GGGG - - TAT NGT TCA TCT ATC CCA ATG AGC CAT TTA TCG AAT CGT TGCAATT				
OP-26	. . . . . T . . . . . ????????????????????				
OP-10	. . . . ??				
OP-21	. . . . NA. . . T				
OP-6	. . . . . - . . . T ??				
OP-23	. . . . . N. . . T .N				
OP-5	. . . . . N. . . T				
OP-11	. . . - . . . . . T				
OP-25	. . . - . . . . . T				
OP-7	. . . - . . . . . T . . . ??				
E25	. . . - . . . . . T . . . . . N. . .				
SJ	. . - - . . . . . T				
OP-16	. . - - . . . . . T				
WV-1	. . - - . . . . . T				
OP-4	. T??				
OP-19	. . - - . . . . ??				
OP-18	. . . T??				
OP-22	??				
OP-2	??				

Molecular Sequences for Primer 26R

Sequence	1	10	20	30	40	50
OP-8	? ? ? T T T T T TCT T GGAT T TACTGCT C T TGT T T T T TCGAAT TCTAT TAAGAT
OP-26	? ? ? ? . . . . .
OP-10	? ? ? ? ? ? ? ? ? ? ? ? . . . . .
OP-21	? ? ? ? ? ? ? ? ? ? ? ? . . . . .
OP-6	? ? T . . . . .
OP-23	? ? ? . . . . .
OP-5	TAT . . . . .
OP-11	TAT . . . . .
OP-25	TAT . . . . .
OP-7	? ? ? . . . . .
E25	TAT . . . . .
SJ	? ? ? ? ? ? ? ? ? ? ? ? . . . . .
OP-16	TAT . . . . .
WV-1	TAT . . . . .
OP-4	? ? T . . . . .
OP-19	? ? ? . . . . .
OP-18	TAT . . . . .
OP-22	TAT . . . . .
OP-2	TAT . . . . .

Sequence	60	70	80	90	100
OP-8	T TAAC TCCT ACAAT TAAA T T AC T T AGGGACAACAA T CCGC TGAAAGA TC T
OP-26	. . . . .
OP-10	. . . . .
OP-21	. . . . .
OP-6	. . . . .
OP-23	. . . . .
OP-5	. . . . .
OP-11	. . . . .
OP-25	. . . . .
OP-7	. . . . .
E25	. . . . .
SJ	. . . . .
OP-16	. . . . .
WV-1	. . . . .
OP-4	. . . . .
OP-19	. . . . .
OP-18	. . . . .
OP-22	. . . . .
OP-2	. . . . .


Sequence
110
120
130
140
150
OP-8 G A T TTGAGGA TGAC GAA TCG CAC TCCAAC TAGC TT TC TT CCT TT T A CTTT
OP-26
OP-10
OP-21
OP-6
OP-23
OP-5
OP-11
OP-25
OP-7
E25
SJ
OP-16
WV-1
OP-4
OP-19
OP-18
OP-22
OP-2


160
170
180
190
200
OP-8 CTT AGTC CAAT GGAAGAA CTT TTT T T TT - AGGAAGT AT TGC AGTT GCAAC
OP-26
OP-10
OP-21 T
OP-6
OP-23
OP-5
OP-11 T T T
OP-25
OP-7
E25
SJ
OP-16
WV-1
OP-4
OP-19
OP-18 T
OP-22
OP-2

```
Sequence                210       220       230       240       250
OP-8      A AA CGAGAT AT TT CCCA ACTG ACC TGACA TAAG ATCT GGT ACCT AAT AAG
OP-26     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-11     . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . .
OP-25     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18     . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . .
OP-22     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

Sequence          260       270       280       290       300
OP-8      TAT CAT TC TTAT TG GAAAT TT CCA ATTAAA AAAAAAAA AAAAA A TC CATT
OP-26     . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . . .
OP-10     . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . . .
OP-21     . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . T . .
OP-6      . . . . . . . . . . . . . . . . . . . . . . . . . . . . - . . . .
OP-23     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . .
OP-5      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-11     . . . . . . . . . . . . . . . . . . . . . . . . . . T – C . . . .
OP-25     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-7      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
SJ        . . . . . . . . . . . . . . . . . . . . . . . . . . . . - - . . .
OP-16     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . .
OP-19     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-22     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-2      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
```

```
Sequence                310       320       330       340       350
OP-8      T C TAAA T CAATAT ATA TA TT TGA CTC TA TT TAGT ATA TT CTA TG TTAGTA
OP-26     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5      . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . A . A . . . . . G . . . . . .
OP-11     . . A . . . . . T . T . C . T . G . . . . . . . . A . . . . . A A . G . A . . A . G . . . G . . .
OP-25     . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . A . . . . . . A . G . . . . . .
OP-7      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25       . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . A . A . . . . A . G . . . . . .
SJ        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16     . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . A . A . . . . A . G . . . . . .
WV-1      . . . . . . . . . . . . . . . . . T . . . . . . . . . T . . . . . A . A . . . . A . G . . . . . .
OP-4      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18     . . . . . . A . . . A . A . . . . T . . . . . G G . . T . . . . A A . . . . . . . . G . . A . . G .
OP-22     . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . A . A . . . . A . G . . . . . .
OP-2      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . A . . . . A . G . . . . . .


                        360       370       380       390       400
OP-8      T T TAAA AATA GGGAAAT T TA TAATAAAA AT AATA CAAAAA TAAT AGAAAT
OP-26     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-21     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-6      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-23     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-5      . . . . G . . . A . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . G . . . . C .
OP-11     G . . G G . . . A T . . . . . . . . . T . . . . . . . . . . . T . . . . . G G . A . G . G . G . C C
OP-25     . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . C .
OP-7      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
E25       . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . C .
SJ        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-16     . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . C .
WV-1      . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-4      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-18     . . . . G . . . A G . . . . . T . . . T . . . C . . . . . . . . A . A . . . . . . . . G . . . . G .
OP-22     . . . . G . . . A . . . . . . . . . . T . . . . . . . . . . . T . . . . . G . . A . G G G . . . C C
OP-2      . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
```

Sequence	410 420 430 440 450
OP-8	AATTA- - - - - - GT CTAT CTA TAAC TAT AAAAAAGAG GATAT CAT AT GAAA
OP-26	. . . . .------. . . . .
OP-10	. . . . .------. . . . .
OP-21	. . . . .------. . . . .
OP-6	. . . . .------. . . . .
OP-23	. . . . .------. . . . .
OP-5	. . . . .------. . . . .
OP-11	. . . . .------. . . . T . . . T . G . . G . . T . . . . A . . . C . . . . .
OP-25	. . . . .------. . . . . T . . . . A . . . .
OP-7	. . . . .------. . . . .
E25	. . . . .------. . . . . T . . . . A . . . .
SJ	. . . . .------. . . . .
OP-16	. . . . .------. . . . . T . . . .
WV-1	. . . . .------. . . . . T . . . .
OP-4	. . . . .------. . . . .
OP-19	. . . . .------. . . . .
OP-18	. . . . .TTATTA. . . . T . . . T . G . . . . . T . . . . T . . . . .
OP-22	. . . . .------. . . . . T . G . . . . A . . . .
OP-2	. . . . .------. . . . .

Sequence	460 470 480 490 500
OP-8	AATGTAA CCGAT T CTT TCC T TT CCT T- - -G GGTT AT TGG CCAT CCG CCGG
OP-26	. . . .---. . . . .
OP-10	. . . .---. . . . .
OP-21	. . . .---. . . . .
OP-6	. . . .---. . . . .
OP-23	. . . .---. . . . .
OP-5	. . G . . . G . . . .---. . . . .
OP-11	. . A . . . G . . . C . . . . . C . . . . . . T . . .---. . . G - . . . . T T G G C
OP-25	. . G . . . G . . . .---. . . . .
OP-7	. . . .---. . . . .
E25	. . G . . . .---. . . . .
SJ	. . . .---. . . . .
OP-16	. . G . . . .---. . . . .
WV-1	. . G . . . .---. . . . . C . . . .
OP-4	. . . .---. . . . .
OP-19	. . . .---. . . . .
OP-18	. . A . . . G . . . . C . . . .---. . . . . T T G G . . . .
OP-22	. . G . . . G . . . .---. . . . .
OP-2	. . G . . . .---. . . . .

Sequence	510	520	530
OP-8	GAGTT TCG G ?		
OP-26	. . . . . G ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-10	. . . . . G TTA? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-21	. . . . . G T? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-6	. . . . . G ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-23	. . . . . ? ?? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-5	. . . . . T . TTAAAAAAAAAAAAAAAAA?		
OP-11	C . .A G G GT . CAAAAAAAAAAAAAAAAAA		
OP-25	. . . . . G . . GTAAAAAAAAAAAAAAAAA?		
OP-7	. . . . . G ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
E25	. . . . . C ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
SJ	. . . . . G ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-16	. . . . . TAAAAAAAAAAAAAAAAAA?		
WV-1	. . . . . C ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-4	. . . . . G ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-19	. . . . . G ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		
OP-18	. . . . . G . T . T T T T T T TCCCCCCCCC? ? ? ?		
OP-22	. . . . . T . TTAAAAAAAAAAAAAAAAA		
OP-2	. . . . . C ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?		

Molecular Sequences for Primer 32R

Sequence	10	20	30	40	50
OP-8	? T AT TT GATT T TT GTAT TT AAAAGAA TT TA CATG ATA CAT CAGAACA CTC				
OP-26	?				
OP-10	? ? ?				
OP-21	? ?				
OP-6	? ?				
OP-23	?				
OP-5	? ? . . . . . -				
OP-11	? ? ? ? ? ? ? . . . . . -				
OP-25	? ? ? ? ? ? ? ? ? . . . . . -				
OP-7	?				
E25	? ? ? ? ? ? ? ?				
SJ	? ?				
OP-16	? ? ? ? ? ? . . . . . -				
WV-1	? ? ? ? ? ? ? ? ? ?				
OP-4	? ? ?				
OP-19	A				
OP-18	? ? ? ? . . . . . -				
OP-22	? ? ? ? ? . . . . . -				
OP-2	? ?				

Sequence	60	70	80	90	100
OP-8	ATTT TT AGGAT T TAAT GAT TC CTA GGAGTGAA TT CA TCC TT TCT AGGA TT				
OP-26	. . . . .				
OP-10	. . . . .				
OP-21	. . . . .				
OP-6	. . . . .				
OP-23	. . . . .				
OP-5	. . . . .				
OP-11	. . . . .				
OP-25	. . . . .				
OP-7	. . . . .				
E25	. . . . .				
SJ	. . . . .				
OP-16	. . . . .				
WV-1	. . . . .				
OP-4	. . . . .				
OP-19	. . . . .				
OP-18	. . . . .				
OP-22	. . . . .				
OP-2	. . . . .				

Sequence	110	120	130	140	150
OP-8	GGAT TTAA	TAAT TGGA TTCT TAAA AGCG AATT CAT CC TT TTA C GGT CATT			
OP-26	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-10	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-21	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-6	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-23	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-5	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-11	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-25	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-7	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
E25	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
SJ	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-16	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
WV-1	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-4	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-19	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-18	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-22	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-2	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .

Sequence	160	1 70	180	190	200
OP-8	TGA TTT GGT AAT ATT AATAAA GATAAT AAC GAATGG AAAGAAATG AACGG				
OP-26	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-10	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-21	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-6	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-23	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-5	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-11	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-25	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-7	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
E25	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
SJ	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-16	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
WV-1	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-4	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-19	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-18	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-22	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
OP-2	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .

Sequence	210	220	230	240	250
OP-8	TT TT GAT	CTGTAA CAA CGGCCAA TT TCC G	TTAAA AGAGAAGGT TCC ATGG		
OP-26	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-10	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-21	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-6	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-23	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-5	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-11	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-25	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-7	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
E25	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
SJ	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-16	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
WV-1	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-4	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-19	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-18	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-22	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-2	. . . . .	. . . . .	. . . . .	. . . . .	. . . .

Sequence	260	270	280	290	300
OP-8	GAA CAATTA	GTT AT T CAT	ATT TTC AGG GTA	CTT CAT CTC	TTT T T T TT CTT
OP-26	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-10	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-21	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-6	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-23	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-5	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-11	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-25	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-7	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
E25	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
SJ	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-16	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
WV-1	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-4	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-19	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-18	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-22	. . . . .	. . . . .	. . . . .	. . . . .	. . . .
OP-2	. . . . .	. . . . .	. . . . .	. . . . .	. . . .

```
Sequence                310       320       330       340       350
OP-8     TGA AAAAAA AAAAAAAAAAAAA AAA - -  AAAAAG TGGGGAAAA AAATG ACA
OP-26    . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . . . . . . . . . . G . . . . . . . .
OP-10    . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . . . . . .
OP-21    . . . . . . . . . . . . . . . . . . . . G . . . . . . TA . . . . . . G . . . . . . . . . . . . . . . .
OP-6     . . . . . . . . . . . . . . . . . . G . . . . . . T . -- . . . . . . G . . . . . . . G . . . . . . . .
OP-23    . . . . . . . . . . . . . . . . . . . . . . . . . T . -- . . . . . . . . . . . . . . . G . . . . . . . .
OP-5     . . . . . . . . . . . . . . . . . . . . . . . . . . A - . . . . . - . G . . . . . . . . . . . G . . . .
OP-11    . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . G . . . .
OP-25    . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . G . . . .
OP-7     . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . G . . . . . . . . .
E25      . . . . . . . . . . . . . . . . . . . . . . . . T . -- . . . . . . G . . . . . . . G . . . . . . . . .
SJ       . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . G . . . .
OP-16    . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . G . . . .
WV-1     . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . A . . . . . . . . . . . .
OP-4     . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . . . . . . . . . . . . . . . . . . .
OP-19    . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . . . . .
OP-18    . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . . . . .
OP-22    . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . . . . .
OP-2     . . . . . . . . . . . . . . . . . . . . . . . . . . -- . . . . . . G . . . . . . . . . . . . G . . . .

Sequence                360       370       380       390       400
OP-8     AAAAGGT ATTGGA ACA TCA ATT TGGAAAAAA TGA GGGAAG CGG GAGTTC
OP-26    . . . . . . . . . . . . . . . . . . . . T . . . . A . . . . . . . . . . . . . . . . . . . . . . . . .
OP-10    . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . G . . . . . . . . G . . . . . . . . .
OP-21    . . . . . . . . . . . A . . . . . . . T . . . . A . . . . . . G . . . . . . . . G . . . . . . . . .
OP-6     . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . G . . . . . . . . N . . . . . . . . .
OP-23    . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . G . . . . . . . . N . . . . . . . . .
OP-5     . . . . . . . . . G . A . . . . . . . T . . . . A . . . . . . G . . . . . . . . . . . . . . . . . .
OP-11    . . . . . . . . . G . A . . . . . . . T . . . . A . . . . . . . . . . . . . . . . . . . . . . . . .
OP-25    . . . . . . . . . . A . . . . . . . T . . . . A . . . . . . G . . . . . . . . . . . . . . . . . .
OP-7     . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . G . . . . . . . . . . . . . . . . . .
E25      . . . . . . . . . . . A . . . . . . . T . . . . A . . . . . . . . . . . . . . . . . . . . . . . . .
SJ       . . . . AA . . . . . A . . . . . . . T . . . . . . . . . . . G . . . . . . . . G . . . . . . . . .
OP-16    . . . . . . . . . G . A . . . . . . . T . . . . A . . . . . . . . . . . . . . . . . . . . . . . . .
WV-1     . . . . . . . . . . . A . . . . . . . T . . . . A . . . . . . . . . . . . . . . G . . A . . . . . .
OP-4     . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
OP-19    . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . G . . . . . . . . . . . . . . . . . .
OP-18    . . . . . . . . . . . A . . . . . . . T . . . . A . . . . . . . . . . . . . . . G . . . . . . . . .
OP-22    . . . . . . . . . . . A . . . . . . . T . . . . A . . . . . . G . . . . . . . . . . . . . . . . . .
OP-2     . . . . . . . . . . . A . . . . . . . T . . . . . . . . . . . G . . . . . . . . . . . . . . . . . .
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BIOGRAPHICAL SKETCH

Sarah Jo Pate was born in Johnson City, TN on September 27, 1978. After the age of five, she spent her time playing outside and reading everything she could, and wanted to be a dress designer, an obstetrician, or a paleontologist when she grew up. Little has changed in terms of her interests since that time. She loves all aspects of the natural sciences, and also enjoys drawing, painting, sewing, and knitting. She attended the University of Tennessee in Knoxville, TN to earn her Bachelor of Science degree, and graduated in 2001. The following year she accepted a position as an Americorps crew member with EarthCorps in Seattle, WA, a habitat restoration non-profit. Her experiences working there convinced her to pursue conservation work. In 2007 she accepted a teaching assistantship in the Department of Biology at Appalachian State University and began work towards her Master of Science degree in Biology. She completed her Master of Science degree in 2010, and plans to pursue endeavors in conservation research following graduation.