Genetic and Horticultural Characterization of *Hydrangea quercifolia* Bartr. (Oakleaf Hydrangea) Throughout its Natural Range of Occurrence

A THESIS

SUBMITTED TO THE FACULTY OF

THE UNIVERSITY OF MINNESOTA

BY

Andrew Sherwood

IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

ADVISED BY

Dr. Stan C. Hokanson and Dr. Matthew D. Clark

June 2020

Acknowledgements

I am extremely grateful for my advisors, Dr. Stan Hokanson and Dr. Matthew Clark for their support, advice and encouragement throughout my work on this thesis. Without them I would not have been able to accomplish this research. I am especially thankful to Stan for giving me the opportunity to study such a wonderful species in such detail. I appreciate how unique the opportunity is to study a species I am so passionate about.

I would like to thank all the staff members at the Horticulture Research Center for their help on growing all the hydrangeas I could get my hands on. I am especially thankful to Steve McNamara who accompanied me multiple times into snake territory to collect hydrangeas and for lending his expertise on woody plant cold hardiness.

I am grateful to Dr. Lisa Alexander for managing the hydrangeas growing in Tennessee. Without her keeping those hydrangeas growing I would have missed out on some very important data.

I would like to acknowledge the various teachers that I have had along the way who have inspired my interest in plants and in science. Among these, I am especially grateful to Dr. David Zlesak for inspiring me to passionately study a group of plants that I love.

I am also grateful to all the members of the Clark and Luby labs who have raised important questions and provided valuable insight during our weekly lab meetings.

Finally, I am thankful to my friends and family who have supported me along this journey.

Abstract

Oakleaf hydrangea (*Hydrangea quercifolia* Bartr.) is an understory shrub native to the southeastern United States. The species occupies a small native range, and little is known about its genetic diversity, needs for conservation or range of phenotypic variation for horticultural traits. This study had two primary objectives. 1) To characterize the structure of the genetic diversity throughout the species range and begin to understand what factors contribute to this structure. 2) To characterize variation for horticulturally important traits.

Samples were collected from 188 plants in 73 locations throughout the species range and were genotyped using genotyping by sequencing. A Structure analysis identified 6 genetic clusters which are geographically structured. Although these clusters are weakly differentiated, each has unique alleles. An environmental association analysis determined that environmental variables explain 11.3% of genetic diversity while population structure explains 13.5%. Further, 231 putative adaptive alleles were identified, the majority of which are correlated with precipitation related variables, indicating that precipitation has an impact on genetic diversity in *H. quercifolia.* Many historically documented populations were found to be either extirpated or at risk of extirpation. The genetic clusters on the southern extent of the species range are relatively small and contain putative adaptive alleles at relatively high allele frequencies. This highlights the importance of preserving representative germplasm from throughout the species range.

Seed was collected from 55 populations throughout the species range for the horticultural characterization. Seed germination percentage was characterized in a greenhouse and growth chamber. Plant architecture was characterized as plant height, number of nodes, internode length, number of branches and plant width. Plant architecture was measured in potted and field grown plants in two locations. Tolerance to leaf spot (*Xanthomonas campestris* L.) was characterized in wild collected seedlings and cultivars by measuring disease severity under natural exposure to inoculum. Cold hardiness was characterized in two winters with a controlled freezing experiment. The first winter, seedlings were tested in January only and the second winter seedlings and cultivars were tested monthly throughout winter. Significant variation among wild populations and cultivars was found for all traits measured in all environments. Mean population seed germination percent ranged from 11% to 93% (mean=61% in greenhouse, 74% in growth chamber). Plant architecture varied by environment, with plants growing larger in Tennessee than in Minnesota. Plant height was correlated with collection site latitude (r=-0.66) with populations from the northeastern extent of the species range being the most compact and populations from Florida being the largest. Leaf spot severity varied significantly among populations and cultivars and was also correlated with latitude in the wild seedlings (r=0.70). Two populations in Florida were

identified as sources of resistance to leaf spot while ‘Flemygea’ and ‘Alice’ were identified as having moderate tolerance to leaf spot. Cold hardiness varied among populations and cultivars and among months of the winter. Overall maximum cold hardiness was observed in February (mean LT_{50}=-33.7°C), and several populations maintained an extreme level of cold hardiness into late winter. Midwinter cold hardiness also varied by latitude (r=-0.65). These results indicate that certain wild oakleaf hydrangea populations will be useful for introgressing novel variation into breeding programs.

Table of Contents

List of Tables

List of Figures

Chapter 1:
Literature Review

Introduction

Hydrangeas have been important plants throughout history, with recorded mentions as far back as ancient China (Mallet et al. 1992). Hydrangeas have had cultural impacts such as being used for medicine and ornamental horticulture. Various species have traditionally been used medicinally throughout east Asia (Long and Li 2003; Abe and Ohtani 2013; Singh 2016) and in southeast North America (Banks 1953; Cozzo 2004) for a variety of purposes including treating abdominal pain, colds, fevers and arthritis.

In addition to being popular florist crops, plants in the genus *Hydrangea* are flowering shrubs that have been increasing in popularity in recent years as versatile and attractive landscape plants. This increase can at least in part be attributed to the introduction of remontant *H. macrophylla* Thunb. cultivars, notably, the best seller 'Bailmer' (Endless Summer ® The Original) in 2004. Since then, other hydrangea species have also experienced an increase in popularity. Although *H. macrophylla* has been well studied, other *Hydrangea* species have not received as much attention; *H. quercifolia* Bartr. (oakleaf hydrangea) is particularly understudied for being such a unique species.

Although it has not been utilized to the same extent as *H. macrophylla*, oakleaf hydrangea displays great potential to make a major contribution to any landscape. The attention currently being received by the genus due to the discovery and improvement of reblooming *H. macrophylla* can be capitalized on as gardeners begin to look for diversity in hydrangeas. *H. quercifolia* is distinguished among hydrangeas by its lobed leaves, attractive exfoliating bark and potential for intense fall colors (Fig. 1.1). This combination of traits allows oakleaf hydrangea to add four-season interest to the landscape, unlike any other hydrangea species.

History and Taxonomy of Hydrangea

Hydrangeas were cultivated for many years in Asia, with several clonal varieties (such as 'Rosea' and 'Sir Joseph Banks') having been selected prior to being exported to Europe and renamed (Church 2001). *Hydrangea arborescens* L. was exported to Europe from North America in 1736 by John Bartram, making it the first *Hydrangea* species to be cultivated on that continent, this occurring before the genus was even named. The name *Hydrangea* was first used by Johann F. Gronovius to describe *H. arborescens* in *Flora Virginica* (Gronovius 1739) and was later used by Linnaeus for the same species in *Species Plantarum* (Linnaeus 1753). However, previous to the establishment of the *Hydrangea* genus, the first plants which are now known as *Hydrangea* were described by Engelbert Kaempfer as *Sambucus* (Church 2001). In 1784, Carl Peter Thunburg described what are now known as *H. macrophylla* ssp. *macrophylla* and *H. macrophylla* ssp. s*errata* as *Viburnum macrophyllum* and *V. serratum,* respectively, in his *Flora Japonica*; Sir Joseph Banks then introduced these species into Europe for the first time in 1789 (McClintock 1957). Ruiz and Pavón first described the South American *Hydrangeas* in 1789, placing them in the genus *Cornidia* (Ruiz and Pavón 1789). *Hydrangea quercifolia* was first described by William Bartram in 1791; this is discussed below in further detail. Later in 1859, Philipp Franz von Siebold described *H. paniculata* and several other Asian *Hydrangea* species for the first time (Mallet et al. 1992).

A monograph for the genus was written by McClintock (1957), which divided *Hydrangea* into 23 species. Although this study was based entirely on herbarium voucher specimens, it has been the authority on *Hydrangea* taxonomy until recently, as evidence has been building for an updated classification. The plants in this genus are woody shrubs, lianas or small trees, and either semi-evergreen or deciduous. The inflorescences of *Hydrangea* species consist of many inconspicuous perfect florets surrounded by few to many conspicuous florets; the conspicuous florets typically contain four large petaloid sepals and have reduced fertility. *Hydrangea* flowers are mostly corymbs, with two notable exceptions; *H. quercifolia* and *H. paniculata* Sieb., which are the only *Hydrangea* species to have inflorescences in the form of panicles.

Although *Hydrangea* was placed in the Saxifragaceae historically, newer genetic evidence (Morgan and Soltis 1993) supports Hydrangeaceae as its own family in the

order Cornales. The Hydrangeaceae family consists of about 270 species within about 17 genera (de Smet et al. 2015). The family is divided into two subfamilies, Jamesioideae and Hydrangeoideae (Hufford et al. 2001). Subfamily Hydrangeoideae contains two tribes, Philadelpheae and Hydrangeeae (Soltis et al. 1995). Hydrangeeae contains nine genera and is further broken into two clades (or subtribes), named Hydrangea I and Hydrangea II (Samain et al. 2010). Other genera in Hydrangeeae are *Deinanthe, Cardiandra, Schizophragma, Pileostegia, Decumaria, Platycrater* (Hydrangea I), and *Dichroa,* and *Broussaisia* (Hydrangea II).

A recent phylogenetic analysis based on chloroplast markers (de Smet et al. 2015) has considerably altered the structure of the genus *Hydrangea* by including all species in the tribe Hydrangeeae into the genus *Hydrangea* and reworking the old generic names into sections (Fig. 1.2). This taxonomic treatment is consistent with several previous studies and is well supported by molecular and morphological data (Soltis et al. 1995; Hufford et al. 2001; Samain et al. 2010). However, this new classification leaves *H. quercifolia* without a section, and places *H. arborescens* L., the type species, in its own section.

Natural Distributions of Hydrangea species

Hydrangea are distributed across the tropical to temperate regions of the world, primarily localizing in Asia and the Pacific islands near the Asian mainland (considered to be the center of origin; Cerbah et al. 2001) as well as North and South America. In North America at least three species are known to exist, *H. arborescens, H. barbara* Y. De Smet & Granados and *H. quercifolia* according to the most recent revision (de Smet et al. 2015). This treatment of the genus places *Decumaria barbara* L. (climbing hydrangea) into *Hydrangea* section *Decumaria* as *H. barbara. Hydrangea arborescens* includes three subspecies which have been argued to be separate species (Pilatowski 1982), based on a study of trichome morphology and crossing experiments which indicated a cross-incompatibility between *H. arborescens* spp. *radiata*, spp. *cinerera* and spp. *arborescens.* However, the crossing experiments also suggested that all three taxa are self-compatible which is contrary to more recent studies (Reed 2000, 2004). Additionally, de Smet et al. (2015) found *H. arborescens* to form one genetically distinct (albeit morphologically diverse) species. Further research is necessary in order to

accurately resolve the taxonomy of the North American *Hydrangea* species. *H. arborescens* occurs from New York in the north to the Florida panhandle in the south, and west to Arkansas. *H. barbara* occurs from Louisiana in the west, north to Tennessee and northeast along the Atlantic coast to New York and into the peninsula of Florida in the southeast. *H. quercifolia* overlaps slightly with the range of *H. arborescens* and more substantially with *H. barbara*. Two and rarely all three of these species are found to co-occur within the region where the native ranges overlap (personal observation).

Hydrangea section *Cornidia* is located almost exclusively in Mexico, Central America and along the western coast of South America. The only exception within this section is *H. intergrifolia* Sieb. which is found in Taiwan and the Philippines. This section contains at least 12 known species, including the relatively recently discovered *H. albostellata* Samain, Najarro & E. Martinez (Samain et al. 2014). These are root-climbing lianas that occur in the primary rainforest and are unique by being functionally dioecious and having evergreen leaves. Several of these species are at risk of extinction due to habitat loss (Samain et al. 2014) and further exploration is required to determine if more previously undescribed taxa exist. Interestingly, many of the species which occur in the tropical regions of South America are confined to the high altitudes of the Andes Mountains where the climate is more temperate (McClintock 1957).

In Asia, *Hydrangea* are distributed from the Himalayan mountains in the west to Japan in the east and south to the Indonesian islands. Some of the Asian taxa are confined to the islands on the eastern edge of the continent (i.e. *H. sikokiana* Maxim., *H. hirta* Sieb. and *H. involucrata* Sieb. are mostly found in Japan). However, most of the Asian hydrangeas are also distributed across the continental mainland (McClintock 1957).

Several *Hydrangea* fossils have been discovered, including in Alaska (Hollick 1925), Idaho (Niklas and Brown 1981), and Oregon (Manchester 1994). Although the evidence is limited to sepal fossils for the Idaho and Alaska specimens, a more complete inflorescence represents the specimen from Oregon. Based on the available evidence it seems as though the specimens may represent extinct species and suggests that the historic range of hydrangea in North America was much larger.

History and Distribution of Oakleaf Hydrangea

Oakleaf hydrangea was first encountered by William Bartram during his 1773-1777 exploration mission throughout the southeastern United States and he published the first description of the species in his monograph (Bartram 1791). The first specimen he described was located along the "brook called Sweet Water" (which is possibly the same as what is now known as Sweetwater Creek west of Macon, GA) in south-central Georgia (Bartram 1791). The species was introduced to Europe in 1803 but details of the export remain elusive (Lawson-Hall and Rothera 1995).

According to the USDA Plants Database (USDA, NRCS 2020) and maps from McClintock (1957), the natural range of occurrence for the species covers six states; from Tennessee south along the western edge of Georgia into the panhandle of Florida and across to the western edge of Mississippi south to the border of Louisiana and Mississippi, only crossing the Mississippi river as disjunct populations in certain locations in Louisiana. No fine-scale map exists for the species, although locations of herbarium specimen collections inform of where certain populations have existed historically (Fig. 1.3).

Hydrangea quercifolia requires well drained soils and is typically found in the understory of undisturbed deciduous forest stands on steep riparian hillsides (personal observation). The species is currently found in relatively disjunct populations except for in the northern half of Alabama, where it grows as a nearly continuous population (personal observation). *H. quercifolia* generally occurs on steep slopes and outcroppings or bluffs, and occasionally on cliffs. *H. quercifolia* has been observed growing directly out of rock with little to no apparent soil present, indicating that rich soil is not a requirement for plants to grow and thrive. Although *H. quercifolia* often tends to occur in calcareous soil, this does not appear to be a requirement nor a limiting factor to its natural distribution as populations have also been observed growing in soils with various mineral compositions as well as rich organic soils on the banks of rivers. In these cases, the absence of stagnant or slowly moving water does seem to be a requirement, as *H. quercifolia* has only been observed along intermittent (seasonal) streams or quickly moving water and never in accordance with slowly moving rivers, standing water or saturated soil.

Hydrangea quercifolia appears to form clonal clumps in the wild which are likely propagated by layering of lower branches and rhizomes, and the ramets may or may not be still attached to the parent plant. A similar phenomenon has been observed for *H. arborescens* (Pilatowski 1982) although this was not studied in detail. Clonal spreading has also been shown to be occur in *H. paniculata* in Japan (Kanno and Seiwa 2004), which is similar in growth habit to *H. quercifolia* in that they both are able to form small trees or large slowly spreading shrubs. Kanno and Seiwa (2004) found that the relative distribution of clonal and seedling recruitment was largely due to light intensity, which were increased in forest gaps and decreased as gap succession proceeded. *H. paniculata* requires high light intensity to flower in comparison to *H. quercifolia*, which is more tolerant of heavy shade (Dirr 2004). However, *H. quercifolia* seeds are about half the size of *H. paniculata* (Hufford 1995) which decreases the ability to germinate in thick leaf litter (Kostel-Hughes et al. 2005). This suggests that the dynamics of clonal growth in *H. quercifolia* may also be affected by forest gap dynamics, although the response may differ from that observed in *H. paniculata.* This response could be manifested in *H. quercifolia* as an overrepresentation of clonal propagation in heavily shaded forests and higher seed representation on ridges with less competition for light. These responses could be hypothesized to be due to decreased flowering and therefore decreased seed production in heavy shade, as well as longer shoot growth under high competition growth conditions (unpublished data) which could contribute to increased propagation by layering. However, little is known about seed dispersal in oakleaf hydrangea and knowledge of this phenomenon would contribute to an understanding of propagation in the wild.

Morphology

The phenotypic diversity in *H. quercifolia* is less than that of other *Hydrangea* species. In her 1957 monograph of the genus, McClintock notes that oakleaf hydrangea is "remarkably uniform throughout its range" and a review of the available cultivars confirms this phenomenon (discussed in further detail below). There is little variation in the growth habit other than overall size; dwarf forms are available, but all display a

broadly mounded habit. Variation exists in floral characteristics to an extent; mostly in size of panicle and sepals, but also in coloration and flowering time.

Hydrangea quercifolia has opposite leaves with 5 to 7 lobes and pinnate venation, resulting in a superficial resemblance to oak (*Quercus*) leaves. The margins are coarsely serrate, and both leaf surfaces are pubescent with the abaxial surface more so than the adaxial surface. Apical buds and young leaves often have very high trichome densities and are therefore highly pubescent. Interestingly, *H. quercifolia* is the only *Hydrangea* species to have branched trichomes with tannin deposits (Stern 1978). Fall leaf color is variable according to genotype and environmental conditions such as shade and ranges from pale yellow to burgundy red.

The stems are densely pubescent and coarse, developing exfoliating bark with age. The growth rings of *H. quercifolia*, like that of several other *Hydrangea* species, are semi-porous; a distinguishing feature of *H. quercifolia* wood is the presence of axial xylem parenchyma (Stern 1978). Year-old and older stems have abundant root initials or latent buds, which likely aid the plant in self-propagation by layering.

In the wild, mature plants occasionally grow to greater than 5 m in height. Oakleaf hydrangea is often at least as wide as it is tall, and older plants have a tendency to develop a spreading habit as the lower branches naturally propagate by layering. Wild plants tend to produce few to several clumps in close proximity to one another, spreading both by stem layering and by rhizomatic suckering (personal observation). In the landscape, many cultivars can be 2 to 3 m in height with dwarf cultivars reaching only 0.5 to 1 m at maturity.

Oakleaf hydrangea blooms from May through June in its native range, flowering on the previous season's wood. Inflorescences are panicles with fully fertile perfect florets in the center and are partly covered by semi-sterile florets with conspicuous petaloid sepals around the exterior (Fig. 1.4B). Each fertile floret contains two stigmas and up to 10 stamens. Conspicuous florets indeed do possess all reproductive parts, albeit in reduced size. While the petals are inconspicuous, there are 4 orbicular conspicuous sepals which are typically white in color, fading to various shades of pink or brown as the inflorescence senesces. In a study of floral morphology in the tribe Hydrangeeae, Jacobs (2010) found *H. quercifolia* to have the highest number of conspicuous florets per

inflorescence out of 36 taxa examined, with up to 50 per inflorescence. This is twice as many as the species with the next highest number, *H. paniculata,* having up to 25 conspicuous florets per inflorescence. In that study, *H. quercifolia* was found to be the only species to consistently produce more than 20 conspicuous florets per inflorescence. These data show the promising potential for *H. quercifolia* to serve as a striking landscape plant with large conspicuous inflorescences.

Hydrangea quercifolia fruit are dry dehiscent capsules, which fade from green during the growing season to brown at maturity when the seeds dehisce. Once pollinated, the conspicuous florets invert and the fruit begin to ripen (Fig. 1.4, compare orientation of florets in panels B and C). The stigmas dry and form horn-shaped appendages on the top of the capsule with an aperture between them to allow seed dispersal. Each fruit contains several seeds, which are relatively small at only 0.6 mm in length, with longitudinal striations (Hufford 1995). Seed color ranges from light tan to dark brown, being ellipsoidal to ovate in shape (Fig. 1.5).

Biotic and Abiotic Considerations

Oakleaf hydrangea has the unique ability to flower in dense shade as well as tolerate full sun. The capacity to flower even in deep shade sets it apart from most other flowering shrubs (Dirr 2004). Nevertheless, blooming improves with sunlight as does flower color on genotypes which become pink with age. Similarly, fall foliage color is also enhanced on sun grown plants. However, if planted in prolonged direct sunlight in hot climates, the leaves have a tendency to burn. Oakleaf hydrangea are more sun tolerant in landscapes located in cooler climates, although flowering may be less impressive under such conditions (Lawson-Hall and Rothera 1995). In the wild, *H. quercifolia* tends to occur in shady forest understories and is rarely found in daylong, full sun situations.

Like most hydrangeas, *H. quercifolia* requires moist but well drained soil and will wilt in the heat of the day when moisture is limiting; even when adequately watered, plants may still wilt when under extremely high temperatures unless protected from afternoon sun. Oakleaf hydrangea is susceptible to several root rot diseases which are promoted by wet soil (discussed below), so care must be taken not to irrigate too

frequently. This reflects the finding that wild *H. quercifolia* most frequently occurs in well drained substrates such as cliff faces, steep ravines and rocky outcrops (Chapter 2).

Limited information is available regarding cold hardiness in *Hydrangea quercifolia*. Dirr et al. (1993) used controlled freezing tests on 'Alice' and 'Alison' to determine cold hardiness. They found the maximum hardiness was achieved in December with both accessions surviving -27°C (which corresponds to USDA Cold Hardiness Zone 5a). 'Alice' maintained cold hardiness longer into spring than 'Alison' did, suggesting that it may resist deacclimation longer, and therefore tolerate late freezes better. Additionally, this may indicate that genotypic variability for cold hardiness and deacclimation exists within the species.

H. quercifolia is susceptible to several vascular pathogens. *Phytophthora nicotiana* Breda de Haan, *Pythium* spp., and *Rhizoctonia solani* Kühn are fungal pathogens that infect the vasculature of oakleaf hydrangea through the roots and cause wilt disease. While no genetic resistance is currently known to exist against the various root rot pathogens, cultural practices can keep disease pressure down. Avoiding frequent irrigation and using a freely draining medium in containers can reduce the severity of infections (Baysal-Gurel et al. 2016). *Armillaria mellea* Kumm. and *A. tabescens* Emel are also vascular wilt fungi that can infect oakleaf hydrangea and are especially problematic with in-ground plantings. One possible source of *Armillaria* is wood mulch infected with the fungus, and therefore care should be used when mulching *H. quercifolia* with wood to determine the source is known to be free of pathogens.

There are an abundance of foliar pathogens that have been reported to infect hydrangeas, including *Cercospora hydrangea* Atk., *Corynespora cassiicola* Wei, *Phoma exigua* Sacc., *Glomerella* spp., *Myrothecium roridum* Sacc., *Alternaria alternate* Keissl., *Xanthomonas campestris* L. and *Botrytis cinereal* Pers. (Hagan et al. 2004; Mmbaga et al. 2012, 2015) Among these, *Ce. hydrangeae* and *Co. cassiicola* are the most frequently occurring on infected *H. macrophylla* leaves (Mmbaga et al. 2012). The fungus *Ce. hydrangeae* and the bacteria *X. campestris* have been identified on oakleaf hydrangea with foliar leafspot symptoms (Hagan and Mullen 2001; Mmbaga and Oliver 2007). Leafspots caused by *X. campestris* on oakleaf hydrangea are typically brown, angular lesions that are contained by the leaf veins (Fig. 1.6). Because dead leaves are a source of

inoculum, removing fallen leaves and avoiding overhead irrigation are effective control strategies (Baysal-Gurel et al. 2016). Further, Mmbaga and Oliver (2007) have shown a kaolin powder based natural pesticide to be as effective in protecting *H. quercifolia* from *X. campestris* infection as the broad-spectrum fungicides that were also tested. Powdery mildew (*Golovinomyces orontii* syn. *Erysiphe polygoni*) is a common foliar disease on *H. macrophylla* but *H. quercifolia* seems to be relatively more resistant (Baysal-Gurel et al. 2016); this difference could be hypothesized to be due to the pubescent leaf surfaces of *H. quercifolia* in comparison to the glabrous leaves of *H. macrophylla*.

Oakleaf hydrangea does not have any particular insect pest issues; however, generalist nursery and landscape pest insects will feed on *H. quercifolia* when given the opportunity. Anecdotally, Japanese beetle has been the most abundant insect feeding on *H. quercifolia* in our studies at the University of Minnesota. Mmbaga and Oliver (2007) demonstrated that kaolin powder is as effective at controlling Japanese beetle (*Popillia japonica* Newman) feeding on *H. quercifolia* as a broad-spectrum insecticide. This would be an option to use in nursery or greenhouse settings when additional pest defense is needed, however, kaolin powder may not be suitable for landscape use considering the unsightly appearance of the leaves after application.

Genetics and Breeding

Ploidy and Genome Size

Genome size and ploidy have been well studied in *Hydrangea*. Although there is some speculation that the ancestral base chromosome number is n=9 (Cerbah et al. 2001), the current base chromosome number in *Hydrangea* is n=18, with most species (including *H. quercifolia*) having 2n=2x=36 chromosomes (Van Laere et al. 2008). Exceptions to this include *H. aspera* D. Don and *H. involucrata* which have 2n=34 and 2n=30, respectively (Mortreau et al. 2010). Another exception to this is *H. platyarguta* Y. De Smet & Granados, which has 2n=2x=24 chromosomes (Qiu et al. 2009). Cerbah et al. (2001) found that the North and South American hydrangeas had the smallest genome sizes with *H. quercifolia* being the smallest of all species investigated (1.95 pg 2C DNA; 1.9 Gb). Zonneveld (2004) reported similar findings, noting *H. quercifolia* with the

smallest genome at 2.17 pg 2C DNA (2.1 Gb). However, *H. seemannii* L. Riley has been reported to have a similar genome size at 2.09 pg (2.1 Gb; Cerbah et al. 2001).

Although most *Hydrangea* are diploid, higher ploidy levels are not uncommon in the genus. Triploidy has been determined to arise from unreduced gamete production in *H. macrophylla* and triploid plants were noted to have an increased stem thickness and decreased number of flowers (Jones et al. 2007; Alexander 2017). Interestingly, no tetraploid *H. macrophylla* have been documented; however, *H. febrifuga* Y. De Smet & Granados (syn. *Dichroa febrifuga* Lour.), which is the one of the most closely related taxa to *H. macrophylla*, has been determined to exhibit diploid, tetraploid and hexaploid cytotypes (Rinehart et al. 2010). *H. paniculata* has also been documented in the diploid, triploid, tetraploid, pentaploid and hexaploid states with tetraploidy being the most common (Cerbah et al. 2001; Funamoto and Ogawa 2002; Zonneveld 2004; Beck and Ranney 2014). Oakleaf hydrangea has thus far only been documented in the diploid state, however, morphological traits could potentially be enhanced by induced polyploidy. Polyploidy can be induced using antimitotic chemicals or by the strategic use of unreduced gametes, although no *H. quercifolia* genotypes have been reported to produce 2n gametes.

Pollination Biology

Gametophytic self-incompatibility seems to be widespread throughout *Hydrangea* (Reed 2000, 2004, 2005; Mortreau et al. 2003). Reed (2000, 2004) reported the capacity of self-pollen to germinate on *H. quercifolia* stigmas, but not grow long enough to reach the ovaries except in a very low percentage of cases. Additionally, it was shown that cross-pollen typically outcompetes self-pollen when present. The stigmas are receptive to pollen the day after anthesis until five days post-anthesis (Reed 2004). The pollen tube grows to the bottom of the style within 48 hours after pollination and has fertilized the ovule within 72 hours of pollination. It has been shown with *H. arborescens* and *H. macrophylla* that pollen is able to be stored at -20°C for at least three months with only a marginal decrease in viability (Kudo and Niimi 1999). However, viability of stored pollen has yet to be confirmed empirically in *H. quercifolia*.

Breeding for Disease Tolerance/Resistance

Breeding for root rot resistance should be a priority in *H. quercifolia* considering the disease is often lethal. No resistance genes have been reported to date, but by screening diverse wild germplasm the possibility exists to find tolerant material. Opportunities for high throughput screening for resistance to *Phytophthora* has been demonstrated in crops such as *Nicotiana, Solanum,* and *Lycopersicon* using culture filtrates incorporated into a tissue culture medium (Behnke 1979; van den Bulk 1991). Although promising, more information about the host-pathogen interaction is needed in order to implement such procedures in *H. quercifolia.* Because *Phytophthora* is a vascular disease and may not use a chemical toxin as a pathogenic mechanism, screening with culture filtrates may not be a viable option. Instead, it may be necessary to screen seedlings by inoculating with the pathogen itself to identify variation in tolerance. Selecting for tolerance to foliar pathogens is also highly desirable as foliar pathogens have an ability to induce an unsightly appearance to plants in production as well as in the landscape.

Breeding for Compactness

Wild plants of the species are on average about 2 m tall and 1.8 m wide (with mature plants often being much larger; unpublished data) making breeding for smaller forms desirable in order to allow *H. quercifolia* to be grown in smaller garden settings. Considerable progress has been made in breeding more compact *H. quercifolia* cultivars. By using 'Sike's Dwarf' and 'Pee Wee' as parents in breeding populations, The USDA-ARS Floral and Nursery Crops Laboratory has introduced three cultivars that are considerably smaller than the species (Reed 2010; Reed and Alexander 2015). Using these cultivars as parents in crosses should yield progeny with smaller stature. Mature plant size is often cited as a quantitative trait in other crops (Miller and Hammond 1990), although in certain cases there may be a single major effect gene which induces compactness (Li et al. 2011).

Breeding for Cold Hardiness

Cold hardiness is the major limiting factor determining where oakleaf hydrangea is able to be cultivated, and therefore is a breeding priority in order to expand the

cultivated range. Current hardiness estimates indicate USDA Zone 5 may be the extent of cold hardiness for the species (Dirr et al. 1993; Halcomb and Reed 2012), although screening wild germplasm from the northern extent of the latitudinal cline may uncover variation in cold tolerance (Hurme et al. 1997; Friedman et al. 2008; Pagter et al. 2010).

Because winters are variable and unpredictable, controlled freezing experiments offer the most reliable information on cold tolerance at a given point during the winter (Hokanson and McNamara 2013). These controlled freezing experiments can be used to identify populations or individuals that are more cold hardy or deacclimate later than average (McNamara and Hokanson 2010). Identifying populations with increased cold tolerance or later deacclimation can provide an advantageous starting point for improvement. Although midwinter hardiness was not significantly different, the variation in deacclimation between 'Alice' and 'Alison' in controlled freezing tests (Dirr et al. 1993) indicates that genetic variation for winter survival traits may exist in *H. quercifolia.* A similar pattern was detected between *H. macrophylla* and *H. paniculata* where the latter, in addition to being far hardier in midwinter, retained its ability to withstand cold temperatures longer into an experimental warming period (Pagter et al. 2008a, 2008b, 2011a, 2011b). However, *H. paniculata* deacclimated faster than *H. macrophylla*, suggesting that midwinter hardiness and rate of deacclimation may be different breeding objectives.

Breeding for Floral Characteristics

Variation exists in *H. quercifolia* for floral characteristics such as flower color, flowering time, flower size, and double flowers (Dirr 2004). Because the white sepals become pink or brown as the inflorescence ages, the flower color variation consists of whether they turn pink, the timing of pinking and the shade of pink. To date, there are no known genotypes which have flowers that open pink. Inheritance of flower color in *Hydrangea* is not well studied, but it seems to be a quantitative trait considering the variability based on environment (light intensity likely plays a role) and the seemingly infinite number of intermediate phenotypes. Variation in flowering time is available in cultivars such as 'Late Hand', which blooms about a month later than typical (Dirr 2004), and 'Queen of Hearts', which blooms about 7-10 days later than other cultivars (Reed and Alexander 2015). In the wild, individual plants have been observed flowering later in

the season than the surrounding plants as well as flowering multiple times in one season (personal observation), however further research will be required to disentangle the environmental effects from the genetic components. Considerable variation also exists for flower size, with panicles ranging from 7 cm long in some wild plants (unpublished data) up to approximately 30 cm long in many cultivars. In *H. macrophylla*, double flowers are a recessively inherited trait that may be controlled by a single major gene (Suyama et al. 2015; Waki et al. 2018). However, double flowered *H. quercifolia* genotypes produce multiple whorls of sepals in place of sexual organs and therefore produce little to no seed or pollen. The fertility of any pollen or seed that is produced by double flowers in *H. quercifolia* has not been studied. Additionally, the double flowered *H. quercifolia* cultivars tend to produce a considerably higher number of sepals (up to 20 per floret) compared to most of the double flowered *H. macrophylla* cultivars which produce around 8 sepals per floret (Dirr 2004).

Germplasm Resources

There are at least 48 named cultivars of oakleaf hydrangea that have been introduced (Table 1.1), many of which were selected directly out of the wild or from chance seedlings. Several have unique characteristics that are available for use as breeding material.

The USDA-ARS Floral and Nursery Crops Laboratory has been breeding *H. quercifolia* since 1996, selecting for compactness. Two of the cultivar introductions, 'Munchkin' and 'Ruby Slippers' are more compact than the species, reaching only about 1 m in height (Reed 2010). As the name implies, 'Ruby Slippers' has sepals that age to deep pink, nearly red and 'Munchkin' has a tighter growth habit with lighter pink sepals. 'Ruby Slippers' is an F_2 selection from a cross between 'Pee Wee' and 'Flemygea' (Snow Queen), while 'Munchkin' is an F_2 selection from open-pollinated 'Sike's Dwarf'. More recently 'Queen of Hearts' was introduced which is not as compact, growing to approximately 2 m tall (Reed and Alexander 2015). Blooming about 7 to 10 days later than most with slightly larger panicles than the previous introductions, 'Queen of Hearts' is from the same F_2 population as 'Ruby Slippers'.

'Pee Wee' is a relatively compact cultivar reaching 1.5 m tall but has fairly small panicles up to 12 cm long and with sepals that do not age to pink, but brown. A yellow

leaved sport of ‘Pee Wee’ was identified and is available as ‘Little Honey’. ‘Sike’s Dwarf’ is another compact variety growing 1.5 m tall and wide, with flowers larger than those of ‘Pee Wee’ and sepals that age to pink.

There are at least four double flowered cultivars available on the market. ‘Brido’ (Snowflake) has dense panicles to 30 cm in length with double flowers that weigh down the branches and tend to make them droop. Discovered in the wild in Alabama in 1969, ‘Brido’ is a full-size cultivar reaching 3 m tall at maturity. ‘Shannon’ is also double flowered, with up to 20 sepals per floret and less dense panicles that are held more upright than ‘Brido’ (Dirr 2004). ‘Shannon’ is also a relatively compact cultivar reaching around 1.5 m tall. Other possible sources of double flowers are ‘Cloud Nine’ and ‘Horvaria’, both of which are comparable to ‘Brido’ (van Gelderen and van Gelderen 2004).

A commonly grown full size cultivar, ‘Flemygea’ grows to 2 m tall, with larger sepals than the species on 18 cm long upright panicles that age to light pink. Three cultivars, ‘Roanoke’, ‘Harmony’, and ‘Vaughn’s Lillie’ have extremely dense mophead-like rounded inflorescences that cause the branches to sag under the weight; all three are full size cultivars. ‘Alice’ is one of the largest cultivars available, reaching 4.5 m tall and wide, with large 30 cm panicles and sepals that age to pink. ‘Late Hand’ has large deeply lobed leaves that have the appearance of a hand shape; as mentioned previously, this cultivar is also reported to bloom up to a month later than is typical for the species (Dirr 2004; van Gelderen and van Gelderen 2004).

Wild collected germplasm is currently available as seed from the USDA National Plant Germplasm System in limited quantities. The accessions represent only a small portion of the species’ endemic range (Table 1.2). There are 8 accessions available (5 from Alabama and 3 from Mississippi) as of April 2020. This likely does not fully represent the existing genetic diversity for the species.

Molecular Resources for Breeding

Molecular markers can be used to facilitate the selection process by screening germplasm for markers that are linked to a gene of interest. Many types of molecular markers exist and have been used for phylogenetic analyses (De Smet et al. 2015), hybrid verification, linkage mapping (Waki et al. 2018), identification of unknown species

(Joung et al. 2010), genetic diversity estimates (Rinehart et al. 2006; Reed and Rinehart 2007, 2009; Ito et al. 2013; Choi et al. 2017) and predicting success of interspecific hybridizations in *Hydrangea* (Granados Mendoza et al. 2013). Many types of markers have been used for these studies including simple sequence repeats (SSR, or microsatellites), single nucleotide polymorphisms (SNP), randomly amplified fragment polymorphisms, *rbcL* sequence, plastid sequence markers (chloroplast and mitochondrial DNA), and internal transcribed spacer sequences.

Molecular markers can be used to develop marker-trait associations which can then be used to guide crossing or to select seedlings (Collard and Mackill 2008; Beyene et al. 2016). Genetic markers have yet to be used for selection in *H. quercifolia*, although success in other species has been realized and the first genetic map has recently been developed in *H. macrophylla* (Waki et al. 2018). Selecting for traits which are not able to be reliably phenotyped until maturity with markers will increase breeding efficiency by allowing the breeder to know what characteristics to expect early in the breeding cycle, thereby potentially reducing the breeding cycle by several years. SSR markers linked to double flower and inflorescence type genes in *H. macrophylla* (Waki et al. 2018) are a prime example.

Microsatellites have been developed for *Hydrangea* that are informative at both the inter- and intraspecific level (Rinehart et al. 2006; Reed and Rinehart 2007, 2009, Choi et al. 2017; Hempel et al. 2018; Waki et al. 2018). Microsatellites are sequences of several nucleotides that repeat several times consecutively (Gupta et al. 1996). Thirty-nine SSRs have been developed for *H. macrophylla* (Reed and Rinehart 2007), 14 of which were informative for determining species relationships within the genus including *H. quercifolia* (Rinehart et al. 2006). Microsatellites have also been utilized in *H. luteovenosa* Koidz. to estimate the genetic diversity and conservation status of a critically endangered population in South Korea (Ito et al. 2013; Choi et al. 2017). Recently, a genetic map of *H. macrophylla* with 147 SSR loci was developed (Waki et al. 2018); it is unknown if these markers would be useful in *H. quercifolia*.

SNPs are highly abundant mutations throughout genomes (Semagn et al. 2006). SNPs are either single base-pair substitutions or insertion/deletion polymorphisms and are therefore considered to be biallelic markers. Genotyping by sequencing (GBS) is a

technique which harnesses high-throughput sequencing technology to simultaneously identify and genotype thousands of SNPs by reducing genomic complexity using restriction enzymes prior to sequencing (Elshire et al. 2011). GBS has recently been used in *H. macrophylla* (Tränkner et al. 2019; Wu and Alexander 2019).

Genetic Variation in Hydrangea

Genetic variation is an important metric for plant breeding and conservation alike. For plant breeders, genetic variation is the raw material to be utilized in order to make gains from selection. In conservation, genetic variability is likewise extremely important. A species that possesses higher genetic variability has an increased ability to adapt to changing environmental conditions and selective pressures.

Natural variation has been increasingly utilized for breeding and genetic studies (Xue et al. 2008; Alonso-Blanco et al. 2009; Weigel 2012). A major benefit of using natural variation in studying plant genetics or plant improvement is that natural intraspecific variation can exceed (or greatly differ from) that found in commonly cultivated lines. This can be applied to hydrangea breeding by selectively incorporating wild germplasm into a breeding program in addition to cultivars.

Although genetic variation between species of *Hydrangea* and within *H. macrophylla, H. paniculata* and *H. febrifuga* (Rinehart et al. 2006; Reed and Rinehart 2007, 2009; Rinehart et al. 2010) has been determined, no comprehensive studies have previously been undertaken for *H. quercifolia.* Additionally, none of these studies examined the full range of variation in the species, but rather focused on the available germplasm in the form of cultivars and limited numbers of wild accessions.

A recent high-resolution study of population structure of a disjunct and geographically isolated island *H. luteovenosa* population found extremely low genetic diversity in the isolated population compared to nearby mainland populations (Choi et al. 2017). Using five SSR markers, it was determined that only two distinct genotypes exist on Jeju Island, South Korea, one of which dominates the population (251 out of 285 samples). In contrast, high diversity was found among three populations on the mainland of Japan. The fact that one of the two genotypes is found in 88% of the island population, indicates that the population has an extremely limited ability to adapt to changing

conditions, and as such is at a higher risk of extirpation and is more susceptible to inbreeding than the Japanese populations.

Population genetic structure and phylogeography of *H. platyarguta* (syn. *Platycrater arguta*) has also been studied across the extent of its natural range in eastern China and southern Japan (Qiu et al. 2009). Using chloroplast DNA sequences, this study found two genetically and geographically distinct groups which correspond to var. *sinensis* and var. *arguta.* Overall, it was shown that *H. platyarguta* has high genetic variation.

Conservation

Although *H. quercifolia* is abundant in the center of its range in central and northern Alabama, there is nevertheless a conservation concern. Many of the existing populations on the extremities of the range are small and therefore are susceptible to genetic drift and inbreeding. This could also be an early indication of range contraction and local extirpation. Additionally, the extent of gene flow between populations as either seed or pollen is unknown, which has a direct influence on the extent of genetic drift in small populations. Knowledge of these factors will be useful in assessing the need for conservation action as well as directing any action that is deemed necessary.

A survey of 421 herbarium specimens of *H. quercifolia* indicate that the median collection year was 1979 with only 16% having been collected since 2000. Searching populations throughout the native range indicates that greater than 23% (16 of 69 searched) of the populations that were previously documented no longer exist (personal observations). Many of these extirpated populations are located on the edges of the species range and therefore put an emphasis on protecting the populations in these areas. These locations have either undergone land-use change (housing developments, clear cutting, roadside vegetation control, etc.) or encountered habitat degradation due to invasive species, indicating that land preservation could be an effective strategy in protecting the remaining populations.

Propagation

Hydrangea quercifolia is fairly easy to propagate by seed and vegetative cuttings. Seeds are extremely small (0.6 mm long; Hufford 1995) but require no stratification treatment in order to achieve germination. Surface sown seeds typically germinate in 7-14 days with or without a light covering so long as seeds are not allowed to dry out or become buried under germination substrate (Halcomb and Reed 2012). Oakleaf hydrangea roots readily from softwood cuttings, although IBA may increase rate of rooting (Halcomb and Reed 2012).

H. quercifolia can be propagated via tissue culture. When introducing explants into culture, microbial contamination is a widespread problem in *Hydrangea* (unpublished data; Kitamura et al. 2008). However, the use of dormant buds as explant material has been used successfully in *H. quercifolia* (Sebastian and Heuser 1987; Ledbetter and Preece 2004). Both type and concentration of cytokinin were shown to have significant effects on number and length of shoots induced in tissue culture, with 5 µM thiadiazuron (TDZ) inducing the highest number of shoots but also the smallest shoot size; therefore, intermediate concentrations (0.1 – 0.5 µM TDZ) are recommended to produce a moderate number of adequately elongated shoots (Ledbetter and Preece 2004). Additionally, inclusion of gibberellic acid in the medium can enhance shoot elongation when higher cytokinin concentrations are used (Sebastian and Heuser 1987). It was also determined that micropropagated shoots have a high rooting percentage, on average 93%. Sebastian and Heuser (1987) demonstrated that shoots derived from callus tissue rooted more consistently than shoots derived directly from dormant buds; this difference was attributed to increased juvenility in the callus derived shoots. Conversely, Kästner et al. (2017) reported that tissue cultures of *H. quercifolia* declined in vigor after many repeated subcultures and that it was necessary to reestablish explants periodically. Cochran et al. (2014) found significant (but inconsistently) improved branching and symmetry in container produced *H. quercifolia* ‘Alice’ which were propagated in tissue culture compared to cutting propagated.

Future Prospects

Hydrangea quercifolia is a particularly understudied and underutilized species with unique horticultural potential. Because it is unrivaled among hydrangeas for its year-round aesthetic value and intriguing foliar characteristics, it can be described as "a shrub with a difference" (Lawson-Hall and Rothera 1995). *Hydrangea quercifolia* has immense potential waiting to be tapped. The amount of improvement seen in *H. macrophylla, H. paniculata* and *H. arborescens* over the previous century serve as an example of the possibilities the genus has to offer. To date, progress has been made in selecting for compactness and floral traits; however, there are other traits which would benefit from improvement.

Resistance to biotic and abiotic stressors are still a priority and have not yet received enough attention. Cold tolerance and deacclimation timing are limiting factors in cold climates. This is currently being examined in the species across its latitudinal range which will reveal the extent of cold hardiness that is available to select upon. Resistance to wilt and foliar pathogens still needs to be identified in order for *H. quercifolia* to be robust enough to survive nursery production reliably and still be attractive in the landscape with minimal pesticide applications. Tolerance to heat and direct sunlight are important traits to identify for southern growers.

Another trait that has yet to receive any appreciable amount of attention is floral scent. The floral scent in wild oakleaf hydrangea plants is sweet and although not strong it is generally stronger than that of other *Hydrangea* species (personal observation). Because *Hydrangea* are typically not considered to be fragrant flowers, a variety with a stronger fragrance would be quite significant. It is unknown what compounds make up the scent, nor what floral organs produce them. This trait is important as a breeding target and likely plays a role in pollination in the wild.

Tables

Table 3.1 Cultivars of oakleaf hydrangea with notes and origin. Trade names of cultivars are in parentheses.

Variety	Plant Height (m)	Panicle Length (cm)	Notes	Origin
Alice[1,2]	4	30	One of tallest cultivars; sepals turn pink	Selected by Dirr on the University of Georgia Athens campus
Alison[1,2]	3	25	-	Selected by Dirr on the University of Georgia Athens campus
Amethyst[1]	2	15	Sepals turn bright pink; nearly red	Selected by Dirr
Applause[1,2]	-	-	-	Cultivated for a long time without a cultivar name
Back Porch[1]	-	-	Early flowers; sepals turn pink	Introduced by Louisiana Nurseries
Burgundy[2]	2	-	Burgundy fall color; sepals turn pink	Bred by Rein and Mark Bulk, Netherlands 1995
Camelot[1,2]	-	-	deep red fall color; sepals turn light pink	Bred by Mary Nell McDaniel, Urbana, IL, (introduced by Louisiana Nurseries, prior to 1995)
Cloud Nine[1,2]	-	-	Double flowers; pointed sepals	Introduced by Louisiana Nurseries, prior to 1997
Dayspring (Day Spring) [1]	-	-	-	Introduced by Flowerwood Nursery

Table 1.1 Continued

Variety	Plant Height (m)	Panicle Length (cm)	Notes	Origin
Ellen Huff[1]	-	-	Heat tolerant	From Gulf Coast
Emerald Lake[1,2]	2	-	Weak branches; possibly same as 'Wade Mahlke'	Introduced by Louisiana Nurseries, prior to 1997
Brenhill (Gatsby Gal)	-	-	-	Bred in Cleveland, AL, introduced by Spring Meadow Nurseries
Brother Edward (Gatsby Moon)	-	-	Dense panicle, similar to Harmony	Bred in Cleveland, AL, introduced by Spring Meadow Nurseries
JoAnn (Gatsby Pink)	-	-	Sepals turn pink	Bred in Kingsville, MO, introduced by Spring Meadow Nurseries
Doughill (Gatsby Star)	-	-	Double flowers	Bred in Cleveland, AL, introduced by Spring Meadow Nurseries
Gloster (Gloster Form) [1,2]	-	-	Five sepals per floret; does well in the south	Introduced by Louisiana Nurseries, prior to 1997
Harmony[1,2]	3	30.5	Dense panicles weigh branches down; sepals turn light pink	Selected by Th. A. McDaniel in Harmony Baptist Church Cemetery, Attalla, AL; prior to 1985
Quercifolia (Horvaria)[1]	-	-	Double flowers, similar to ‘Brido’	Hofstede Nursery

Table 1.1 Continued

Variety	Plant Height (m)	Panicle Length (cm)	Notes	Origin
PIIHQ-I (Jetstream)[1]	-	-	Dark green foliage; sepals turn pink	Bred by Plant Introductions, Inc.
Joe McDaniel[1,2]	-	-	Large sepals	Wild collection by J.C. McDaniel. Raised by Louisiana Nurseries prior to 1997
John Wayne[1,2]	2	-	-	Selected from FL; Introduced by Louisiana Nurseries prior to 1997
Late Hand[1,2]	-	-	One month late blooming; leaves have deep sinuses	Introduced by Louisiana Nurseries, prior to 1997
Little Honey[1]	1.5	12	Sepals do not turn pink	Yellow leaf sport of Pee Wee selected by Peter Catt in the UK
Luverne Pink[1,2]	-	-	Sepals turn pink	Introduced by Louisiana Nurseries prior to 1997
Lynn Lowrey[1]	2	20	Possibly same clone as 'Angola Prison'	Selected by Tom Dodd III in Louisiana
Marshall[2]	-	-	Relatively small flowers; sepals turn pink	-
Montmorenci Rose[1]	-	-	Sepals turn pink quickly	Selected out of a South Carolina garden by Paul Crosby. Introduced by Nurseries Caroliniana
Munchkin[3]	0.9	17	Compact growth habit; sepals turn light pink	USDA-ARS Floral and Nursery Crops Laboratory

Table 1.1 Continued

Variety	Plant Height (m)	Panicle Length (cm)	Notes	Origin
Patio Pink[1,2]	-	-	Early flowers; large leaves; sepals turn pink	Introduced by Louisiana Nurseries prior to 1997
Pee Wee[1,2]	1.5	12	Sepals do not turn pink	Introduced by Louisiana Nurseries
Picnic Hill[1,2]	-	-	Short internodes; sepals turn light pink	Introduced by Louisiana Nurseries prior to 1997
Queen of Hearts[4]	1.9	30	7-10 days late blooming; sepals turn light pink	USDA-ARS Floral and Nursery Crops Laboratory
Roanoke[1,2]	3	-	Flopping branches	Introduced by Louisiana Nurseries, prior to 1993
Ruby Slippers[3]	1	25	Sepals turn deep pink, nearly red	USDA-ARS Floral and Nursery Crops Laboratory
Semmes Beauty (Semmes Select) [1]	-	-	Heat tolerant	-
Shannon[1]	1.5	-	Double flower, less dense than 'Brido'; more upright panicles	Theodore Klein, Crestwood, KY
Sike's Dwarf[1,2]	1.5	-	Flowers July-August; sepals turn light pink; larger than 'Pee Wee'	Introduced by Louisiana Nurseries, prior to 1990

Table 1.1 Continued

Variety	Plant Height (m)	Panicle Length (cm)	Notes	Origin
Snow Giant[1,2]	3	-	Double flower; possibly same clone as 'Brido'	Japanese origin
Flemygea (Snow Queen)[1,2]	2.5	18	Upright panicles; flowers June-September	B. Flemer, Princeton Nurseries, prior to 1980
Snowdrift[2]	-	-	Flowers are similar to those of 'Harmony'; thin branches	Bred in the United States before 2000
Brido (Snowflake) [1,2]	3	30	Double flower, flowers slightly later	Edgar Aldridge, 1960
Summit[1]	1.5	18	-	Theodore Klein, Crestwood, KY
Tennessee (Tennessee Clone) [1,2]	2	25	Four or five wavy sepals; flowers July-August; wrinkled leaves; wider than high; sepals greenish in color	From seed collected in Tennessee by Jelena de Belder of Arboretum Kalmthout, Belgium 1974
Turkey Hill[1]	-	-	Similar to 'Harmony'	Hayes Jackson, Anniston AL
Vaugh's Lillie[1]	-	-	Dense rounded mophead	Vaughn Billingsley, Georgia

Table 1.1 Continued

Variety	Plant Height (m)	Panicle Length (cm)	Notes	Origin
Wade Mahlke (Wade Malke) [1,2]	-	25	Possibly same clone as 'Emerald Lake'	Louisiana Nurseries prior to 1997

[1] Dirr (2004)

[2] van Gelderen and van Gelderen (2004)

[3] Reed (2010)

[4] Reed and Alexander (2015)

Table 1.4 Wild *Hydrangea quercifolia* Accessions Available through USDA-GRIN. As of 27 April 2020.

ID	Plant Name	Date Collected	State	Coordinates or Location
NA 74915	RJLGA2003-049	1 November 2003	AL	N33.38361 W86.84194
NA 74925	MCCAL2004-009	7 November 2004	AL	N34.31472 W87.51222
NA 80223	MS-2011-033	21 September 2011	MS	N33.29412 W90.13285
NA 80229	MS-2011-039	21 September 2011	MS	N 33.91675 W89.26345
NA 80232	MS-2011-042	22 September 2011	MS	N34.22403 W89.36282
NA 78055	LRC #7	19 September 2008	AL	Little River Canyon National Preserve
NA 78023	AL #32	17 September 2008	AL	Kathy Stiles Freeland Preserve Habitat
NA 77995	AL #4	-	AL	Bankhead National Forest

Figures

Figure 1.1 Photographs showing the unique aspects of oakleaf hydrangea. Lobed leaves, showy panicles, intense fall foliage color and exfoliating bark.

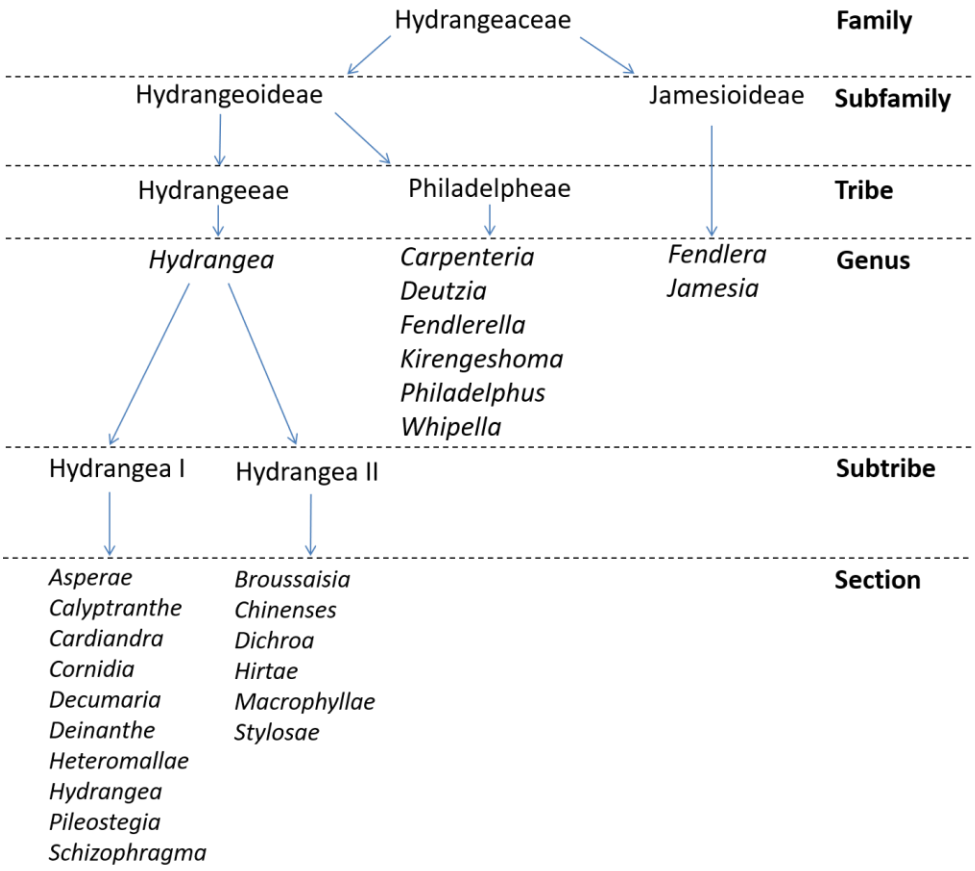

Figure 1.2 Diagram of Hydrangeaceae taxonomy. The taxonomy between family and tribe is based on Hufford et al. 2001 and the taxonomy within Hydrangeeae is based on De Smet et al. 2015.

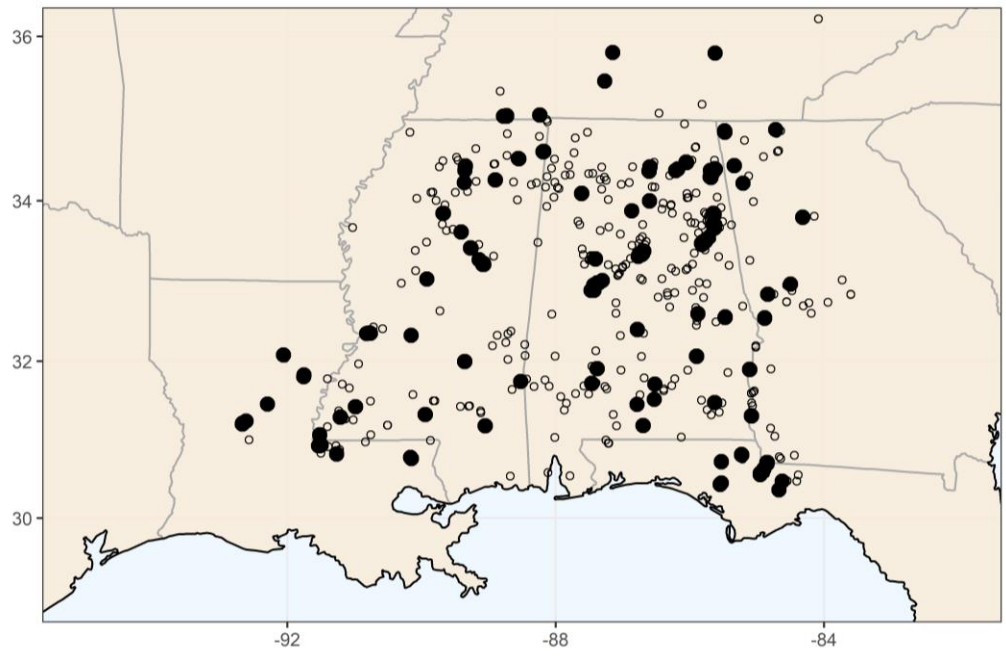

Figure 1.3 Map of historic herbarium specimens and recent samplings. Small open circles indicate approximate location of herbarium specimens collected between 1882 and 2017. Larger solid points indicate location of oakleaf hydrangea confirmed by the author in 2017 and 2018. Note many of the herbarium specimen collection sites were also searched but hydrangea could not be relocated as discussed in the Conservation section.

Figure 1.4 Stages of floral development. A) Young flower buds, B) full flower, C) senescing flower with developing fruit, D) senesced flower with dehiscent fruit.

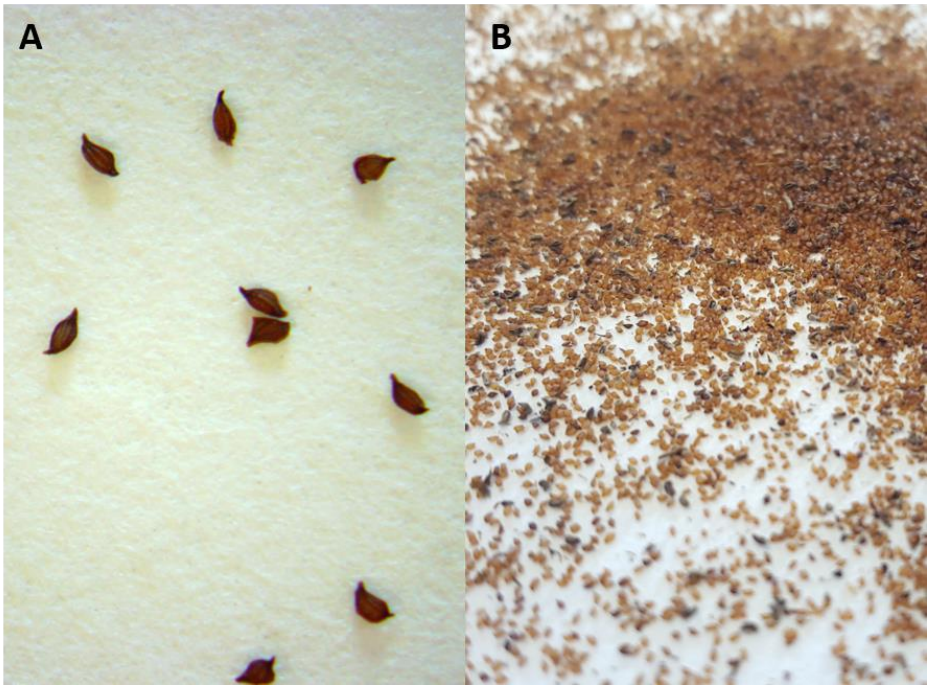

Figure 1.5 Oakleaf hydrangea seeds. A) Close up of individual seeds (12x magnification). B) Non-magnified close up photograph of seed.

Figure 1.6 Photograph showing range of bacterial leafspot severity in *Hydrangea quercifolia*.

Chapter 2:

A SNP-based Characterization of Genetic Diversity of *Hydrangea quercifolia* Throughout its Native Range of Occurrence

Introduction

Genetic diversity is important for species to adapt to changing environments. Having an understanding of how the diversity of a species is structured can help to guide conservation efforts by identifying populations with unique or especially at-risk diversity (Allendorf et al. 2010). Generalizations about the structure of genetic diversity have been made based on the life history of a species. For example, woody plants are expected to have higher diversity within than between populations, whereas insect pollination is expected to increase the differentiation between populations (Hamrick et al. 1992). Similar patterns based on life history have been found in response to processes such as habitat fragmentation (Vranckx et al. 2012). However, species-specific patterns have been identified which highlight the need to assess each species individually (Hahn et al. 2017).

Genetic diversity has been studied in several insect pollinated woody plants with the goal of addressing conservation concerns. Several examples can be taken from *Rhododendron,* where many species have been studied that are native to Asia (Zhao et al. 2012; Li et al. 2015; Wu et al. 2015) and North America (Chappell et al. 2008). These studies determined that many of the *Rhododendron* species are endangered and are in need of conservation. Another genus in which genetic diversity analysis uncovered a conservation need is *Forsythia,* where it was found that populations of *F. ovata* Vahl (Chung et al. 2013) and *F. suspensa* Thunb. (Fu et al. 2016) have critically low genetic diversity and are in need of management intervention.

Hydrangea quercifolia Bartr. is a native understory shrub or small tree found in a six state region in the southeastern United States (Fig. 2.1). Since its first scientific description by William Bartram in 1791, it has largely been considered as an ornamental plant but has been overlooked in comparison to some more commercially exploited

species (Mallet et al. 1992). *H. quercifolia* is an insect pollinated, obligate outcrossing species (Reed 2000, 2004) and grows in the shady understory of hardwood forests. It is most often found in well-drained soil on steep slopes such as bluffs, cliffs and riverbanks (Fig. 2.2). *H. quercifolia* has been observed growing directly out of rocks, suggesting that rich soil is not a requirement for growth. Like other *Hydrangea* spp. (Pilatowski 1982; Kanno and Seiwa 2002), it can naturally propagate by branch layering and occasionally forms dense clonal stands.

Herbarium records indicate that *H. quercifolia* was once common in the southeast region; however, our preliminary exploration suggested that many of the historic populations either no longer exist or are of questionable viability. Many of these extirpated populations have been impacted by land use change (clear cutting, housing developments, etc.) or habitat degradation (e.g. invasive species). Additionally, many of the remaining populations are very small, often having less than 10 individuals and in some cases less than five. This brings into question the need for conservation action for *H. quercifolia*, which requires data on genetic diversity to assess.

The objectives of this chapter were to 1) characterize the structure of the genetic diversity of *H. quercifolia*, 2) determine the impact that geographic and climatic factors have on this genetic structure and 3) relate these findings to identify conservation priorities for the species.

Methods and Materials

Identifying Populations

The range of occurrence for *H. quercifolia* is reported by McClintock (1957) and the USDA Plants Database (USDA, NRCS 2020) to be within the states of Alabama, Florida, Georgia, Louisiana, Mississippi and Tennessee. Herbarium databases from these states were searched for voucher specimens of *H. quercifolia* and locations from which they originated were estimated as accurately as possible (Table 2.1). Collection sites of herbarium specimens and public lands in the immediately surrounding areas were searched.

Leaf Collections

Samples were collected from 188 individuals from 73 collection sites (populations) in May 2017 and between April and July 2018 (Fig. 2.1, Table 2.2). Three to five young expanding leaves per plant were collected from one to seven individuals per population (mean=2.6). Sampled plants were generally at least 3 m apart to avoid resampling an individual clone. Leaves were placed into coin envelopes with silica gel immediately during collection and lyophilized upon return to the laboratory. Dried leaf samples were then frozen at -20°C until DNA extraction. Geographic coordinates (latitude and longitude) were recorded for each sampled plant with EpiCollect5 (v3.0.3) which has a 10 m accuracy along with notes on the habitat in which the plant was growing.

DNA Extraction

DNA was extracted using the DNeasy 96 plant kit (Qiagen, Hilden, Germany). The manufacturer's instructions were modified to include 2.5% beta-mercaptoethanol in the lysis solution, and elution incubations were extended to 10 minutes with elution buffer starting at 80°C. Quality and quantity of extracted DNA was assessed using agarose gel electrophoresis and Nanodrop spectrophotometry (ThermoFisher Scientific, Wilmington, DE) on a subset of samples and PicoGreen fluorimetry (Invitrogen, Carlsbad, CA) on all samples.

Genotyping

DNA samples were genotyped using a genotyping by sequencing (Elshire et al. 2011) protocol at the University of Minnesota Genomics Center. Samples were first digested with the restriction enzyme BamHI, barcoded, then sequenced on an Illumina NextSeq 550 (Illumina, San Diego, CA) with 150 bp single end reads in a single multiplexed pool. Approximately 300 million sequence reads were generated.

SNP variants were called using the Stacks de novo pipeline (v2.3b) (Catchen et al. 2011). Parameters for SNP calling were determined by first running the pipeline on a subset of eight random samples with varying parameter values to identify the parameter

set which maximized the number of polymorphic loci identified. The final parameters used on the full data set were M=2, n=4, and m=2.

Loci with biallelic SNPs were filtered to include those with a read depth between 15 and 100, a minor allele frequency ≥0.05, mean genotyping quality ≥39, allele balance between 0.4 and 0.6 for heterozygotes, and allele balance ≥0.9 for homozygotes using *vcftools* (v0.1.13) and custom Python scripts. Many of the polymorphic loci (150 bp in length) contained multiple SNPs. Therefore, instead of each SNP being analyzed independently, each locus was considered a multiallelic haplotype marker made up of multiple SNPs. Having a higher allele number per locus allowed for a higher information content per marker.

Genetic Diversity Analyses

To identify the number of genetic clusters in *H. quercifolia,* Structure (v2.3.4) (Pritchard et al. 2000) was used with 50,000 MCMC replications after a 5,000 replication burnin period. Number of clusters (K) between K=1 and K=10 were tested, with 10 Structure runs for each value of K. Structure runs were combined using Structure Harvester (v0.6.94) and the most likely number of genetic clusters was determined using the delta-K method (Evanno et al. 2005).

Principal Components Analysis (PCA) on allele frequencies at all loci, implemented in the R (R Core Team 2018) package *ade4* (v1.7-15) (Thioulouse and Dray 2007), was used as an additional method of detecting and visualizing population structure (Jombart et al. 2009). Because PCA does not handle missing data, missing genotype calls were replaced with the mean allele frequency for the allele and therefore this analysis slightly underestimates the true amount of genetic diversity.

A Procrustes analysis was used to test the congruency between PCA and geographic location (Wang et al. 2010), implemented in the R package *MCMCpack* (v1.4-6) (Martin et al. 2011). A Procrustes analysis minimizes the squared differences between two distance matrices by rescaling and stretching one matrix to fit the other without distorting it. In this case, the matrix of PCA values was fitted to the matrix of latitude and longitude coordinates and differences between them were visualized by

connecting the two points (Procrustes transformed PC values and geographic location) with a line.

Pairwise F_{st} was used to quantify the genetic differentiation among the detected genetic clusters. Isolation by distance was tested using a Mantel test on Nei's genetic distance (Nei 1972) and geographic distance using the R package *ade4* (v1.7-15). Genetic diversity within each cluster was also quantified as expected heterozygosity and average number of alleles per locus, calculated in the R package *poppr* (v2.8.5) (Kamvar et al. 2014).

An environmental association analysis was used to determine the effects of environmental factors on population structure. This was implemented with a Redundancy Analysis (RDA) in the R package *vegan* (v2.5-6) (Dixon 2003). An RDA is a multidimensional extension of linear regression, in this case a PCA ordination of allele frequencies is constrained by environmental variables (Forester et al. 2018). Environmental variables used were from the BioClim database (Fick and Hijmans 2017) for the location of each sample. To reduce collinearity among the variables, a PCA was used on the environmental variables and the variables which explained >50% of the variation on the first two PCs were included in the RDA model (Rellstab et al. 2015). Including only these variables captures a large amount of environmental variation without over-fitting the model. Population structure was controlled for by conditioning the RDA with the individual Q-matrix from Structure. Loci which were outliers (± 4 standard deviations from the mean) on RDA1 or RDA2 were considered to be candidate loci which are putatively under selection by environmental variables. Correlations were then tested between each candidate locus and the environmental variables.

Results

H. quercifolia populations were found in all six states for which it had been previously reported to occur. Population sizes varied widely, with a few only having a single individual while some had hundreds of plants throughout large forests. Many of the populations were very fragmented with little to no connection to other sites with suitable habitat. An exception to this occurred in northern Alabama, where *H. quercifolia* grows as nearly continuous populations. As an understory species, *H. quercifolia* tends to

grow on steep slopes such as ravines, bluffs and riverbanks. Many populations in the southern portion of the species range were growing in calcareous soils, however a wide range of soil types in *H. quercifolia* populations were observed.

Of the locations where *H. quercifolia* had previously been documented, 23% (16 of 69 searched) were found to no longer have populations when searched for sampling. These sites had either undergone land use change (housing developments, clear-cutting, roadside vegetation control, etc.) or habitat degradation due to logging or invasive species (primarily *Ligustrum* spp.). Observations during sampling indicate that *H. quercifolia* does not tolerate high competition. Many of these extirpated populations were located on the edges of the species range.

After all filtering steps, 6,006 polymorphic loci remained with between two and 25 alleles (haplotypes) per locus (mean=3.49). The samples had 22x sequencing coverage on average. The loci had a mean of 1.9 SNPs per locus which contributed to the high allelic diversity relative to most studies using genotyping by sequencing.

The Structure analysis indicated that the most likely number of genetic clusters in *H. quercifolia* is K=6. These genetic clusters are geographically structured with substantial admixture in certain parts of the species range (Fig. 2.3A). The numbers (1-6) assigned to each genetic cluster are arbitrary and are merely a convenient way to unambiguously refer to each cluster. A large cluster exists throughout Mississippi, eastern Louisiana and western Tennessee (cluster 5). The small, disjunct populations in Louisiana west of the Mississippi River belong to a distinct genetic cluster (cluster 2). In the eastern half of the species range, the genetic clusters are structured latitudinally with one cluster existing in northern Alabama, northern Georgia and eastern Tennessee (cluster 4). The populations in southeastern Alabama and southern Georgia belong to one genetic cluster (cluster 1). The populations in south-central Alabama (cluster 3) and the populations in the panhandle of Florida (cluster 6) are each in their own genetic cluster.

PCA provided results congruent with the Structure analysis (Fig 2.4A). PC1 spreads out cluster 5 and separates cluster 3 from the others (Fig. 2.3B, 2.4A). PC2 separates the remaining clusters in the eastern half of the species range latitudinally, with northern populations having higher PC2 values (Fig. 2.3C, 2.4A). PC2 also separates

cluster 6 from cluster 1. Cluster 4 is in the center of the PCA biplot, with varying degrees of overlap from the adjacent genetic clusters, indicating substantial admixture.

Procrustes analysis shows that geography indeed corresponds to genetic diversity (Fig. 2.3D). Cluster 3 is most impacted by the Procrustes transformation, with the populations occurring in south-central Alabama, but the transformed PCA values moving to the top of the plot. Cluster 5 experiences substantial shrinkage during the Procrustes transformation, with the geographic extent of the cluster being the largest, but the range of PCA values not being correspondingly large. After the Procrustes transformation, cluster 2 maintained its relationship to cluster 5 being at the extremity of both the geographic range and PCA values. Cluster 6 is the least affected by the Procrustes transformation, staying very close to the geographical location of the populations. As expected by the correlation between PC2 and latitude in the eastern half of the species range, clusters 1 and 4 have similar shifts to the west but to a slightly larger degree.

Pairwise F_{st} values among the clusters is shown in Table 2.3; overall, F_{st} values are quite low (mean=0.027). Cluster 6 had the highest pairwise F_{st} values with the other genetic clusters which indicates its genetic uniqueness. The two highest pairwise F_{st} values were between cluster 6 and cluster 4 (0.056) and between cluster 6 and cluster 5 (0.052).

The results of the Mantel test do not support isolation by distance in *H. quercifolia* (p=0.998). Genetic diversity statistics are shown in Table 2.4. Mean expected heterozygosity at the species level was 0.359, and observed values are comparable within each genetic cluster with the exception of cluster 2 (0.294). Mean number of alleles per locus within each cluster was substantially lower than at the species level with the lowest being cluster 2 (1.47). However, when using rarefaction to account for differing numbers of individuals in each cluster, all values decrease to a similar level (Table 2.4) due to each cluster effectively being a subsample of the entire species.

The environmental association analysis indicates that environmental factors have a significant impact on genetic structure in *H. quercifolia* (Fig. 2.4B). RDA indicates that, when conditioned by population structure, all 19 BioClim variables explain 11.25% of the variation with population structure explaining 13.5% (p=0.001). Even when controlling for both geographic locations and population structure, environmental

variation still explains 10.9% of the diversity observed. Performing PCA on the environmental variables revealed that nine of the variables account for greater than 50% of the variation on each of the first two PCs (Table 2.5). When performing RDA on these nine variables, they are able to explain 5.9% of the genetic variation (p=0.001).

Outlier analysis found 232 loci that were significant outliers on RDA1 (143 loci) and RDA2 (89 loci). One of these loci was equally correlated with two environmental factors and therefore was removed from further analyses due to lack of interpretability. The number of loci which are most correlated with each environmental variable is presented in Table 2.5. Figure 2.5B-D show examples of three outlying loci and the environmental variable with which they are most highly correlated. All nine environmental variables had at least one outlying locus which was most correlated with it. The variable with the highest number of outlying loci correlated is precipitation in the driest quarter (bio17) with 61 loci. Most of the outlying loci (71.4%) were most correlated with a precipitation related variable. Of the 66 temperature associated loci, 47 were most correlated with a variable related to annual variation in temperature (annual temperature range and temperature seasonality), rather than either high or low temperature. The environmental variable with the highest correlation coefficient with an outlying locus is minimum temperature in the coldest month (bio6), which is most correlated with Hq_locus29211 (r=0.49; Table 2.5, Fig. 2.5D).

Discussion

The sampling strategy utilized largely reflected the disjunct nature of the *H. quercifolia* populations. However, in northern Alabama, sapling density was higher due to the high density and large size of the *H. quercifolia* populations. The finding that many previously documented *H. quercifolia* populations no longer exist suggest that there is indeed a need to develop conservation management plans for the remaining populations. Additionally, with the extirpated and smallest populations mainly being on the extremities of the species range, the edge populations are of highest priority when considering conservation action.

Although the genetic clusters are weakly differentiated, there is support from multiple analyses for the genetic diversity of *H. quercifolia* to be geographically

structured as six genetic clusters. The low F_{st} among clusters as well as the admixture detected with Structure and PCA indicate that there is substantial gene flow among the clusters. However, the presence of structuring in the genetic diversity indicates that effective gene flow is higher among populations within a cluster than among populations in different genetic clusters. Low genetic differentiation with significant geographic structure has previously been observed in other insect pollinated species including several *Rhododendron* species (Zhao et al. 2012; Li et al. 2015) and *Prunus sibirica* L. (Wang et al. 2014), and also in wind pollinated species including *Abies cilicia* Carr. (Awad et al. 2014). In general, populations of woody plants are expected to have lower levels of differentiation due to their relatively long generation time (Hamrick et al. 1992).

Additionally, the lack of significant isolation by distance further supports the hypothesis of long-distance geneflow. Even within cluster 5, which covers the largest geographic area, support for isolation by distance is not significant (p=0.151). This suggests that long distance migration plays a role in limiting genetic differentiation among the populations and clusters. Seeds of *H. quercifolia* are very small (3.2 mg on average; unpublished data) and have small appendages which may aid in dispersal. Water dispersal of seeds is a possibility for long distance gene flow among riverine populations; however, this could not account for all dispersal considering not all populations occur along rivers. A study of wind dispersal distances found that *H. paniculata* Sieb. seed travels typically one to two m from the plant, although on rare occasions can travel at least 100 m (Egawa 2017). In high wind situations such as storms or hurricanes, which are common in the southeast region of the United States, long distance seed dispersal can increase (Lugo 2000). The obligate outcrossing nature of *H. quercifolia* (Reed 2000, 2004) and pollination by generalist pollinators (personal observation) could additionally contribute to long distance dispersal. Although pollinator movement within *Cornus florida* L. populations has been observed to vary by forest cover (Dyer et al. 2012), insect mediated long distance pollen dispersal has been observed among fragmented habitats in other woody species (Jha and Dick 2010; Lander et al. 2010; Ismail et al. 2012). Therefore, it is likely that both long distance seed and pollen dispersal contribute to the low genetic differentiation among populations and clusters in *H. quercifolia.*

PCA within each genetic cluster shows that there is almost no substructure within the clusters (data not shown). Although the samples from the same populations tend to have similar PC values, there is no obvious detectable pattern. There are certain populations, however, which seem to be genetically unique within their cluster. For example, in cluster 6 two of the populations are genetically distinct and are separate from the other populations. A similar pattern was found in *Magnolia officinalis* Rehder & Wilson, where there was little structure within the genetic clusters (Yu et al. 2011). In *Fraxinus mandshurica* var. *japonica* Maxim., substructure was identified in the Honshu Island cluster but not the Hokkaido Island cluster (Hu et al. 2010). In contrast, *Salix purpurea* L. was found to have substantial substructure within the large genetic clusters detected both in its native range in Europe and in naturalized populations in North America (Gouker et al. 2019). These two examples with substructure are mostly explained by the relatively large geographic distances that the clusters occupy in those species. The general lack of substructure in *H. quercifolia* is also consistent with long distance gene flow among populations.

The Procrustes analysis shows that although clusters 3 and 6 are geographically very close to each other, they are genetically quite different. This is further supported by the relatively high F_{st} between these two clusters as well as the almost complete lack of admixture between them. There is likely not a physical geographic barrier to gene flow in that region, and the differentiation is likely not due to a phenological reproductive barrier as individuals in both clusters were observed to be flowering during the same week of sample collection. Instead, the PCA on environmental variables (Fig. 2.4A) suggests that cluster 6 has a considerably different climate compared to the rest of the species range which impacts the genetic variation in that cluster (Fig. 2.4B). Therefore, the most likely explanation is selection due to precipitation and temperature variables.

Cluster 2 has the lowest measures of genetic diversity as measured by expected heterozygosity and mean alleles per locus. This is largely explained by the small population sizes as well as the small number of populations in the genetic cluster (Table 2.4). Small populations are expected to have lower diversity due to the lower number of total alleles present in the population (Ellegren and Galtier 2016). The small sample sizes for cluster 2 reflect actual differences in the wild rather than lower sampling density. The

total number of plants per population in this cluster was five or less for all three populations. This low diversity on the extremity of the species range indicates a low potential for those populations to adapt to changing conditions and a high potential for genetic drift (Ellstrand and Elam 1993). Therefore, the populations in western Louisiana (cluster 2) are at risk of local extirpation. Although cluster 2 is not strongly differentiated from the nearby cluster 5 (F_{st}=0.026), the minimal admixture between the two clusters could indicate that the Mississippi River is a barrier to gene flow. Fragmentation of populations has been shown to have a negative effect on genetic diversity of woody species, especially those that are insect pollinated such as *H. quercifolia* (Vranckx et al. 2012).

Overall, the number of alleles per locus in any given genetic cluster is considerably lower than the species as a whole. Additionally, when using rarefaction to account for uneven sample sizes, both expected heterozygosity and number of alleles per locus decrease substantially in all genetic clusters. This is due to each genetic cluster being a nonrandom subset of the species and therefore having only a subset of all alleles at a given locus. This indicates that germplasm preservation needs to focus on maintaining as large a number of populations as is possible from all genetic clusters in order to preserve the greatest amount of diversity possible. Because each genetic cluster has unique diversity, losing populations in any genetic cluster will lead to the loss of genetic diversity in the species. Furthermore, each genetic cluster can be considered as a unit with unique conservation needs (van Zonneveld et al. 2014). The observation that land use change and invasive species may be driving population size reductions and local extirpation suggests that *in situ* conservation management needs to be prioritized to protect the existing populations.

The substantial amount of genetic diversity which can be explained by precipitation and temperature variables indicates that environmental factors can have nearly as large of an effect on genetic diversity as neutral population structure. Considering the effect of past climate on genetic diversity in *H. quercifolia,* one could expect that the species will also be impacted by future climate change. Such an effect on genetic diversity has been found in *P. taeda* L. (Eckert et al. 2010), which also grows in the southeastern United States, indicating that multiple woody plant species in the region

will be impacted by climate change. Figure 2.4B shows that when population structure is accounted for, the genetic clusters do not differ substantially in the genetic variation which is explainable by climatic factors. Cluster 6 is a clear exception to this and the diversity in that cluster accounts for most of the variation on RDA1, but existing in two groups with opposite RDA1 values. The six samples with high RDA1 values are from the two populations mentioned above which are genetically distinct within that cluster. There are two environmental variables which are contributing to the separation of cluster 6 in the RDA, precipitation in the wettest quarter (bio16) and precipitation in the coldest quarter (bio19). Therefore, the high precipitation in Florida is selecting for unique genetics in cluster 6. Figure 2.5A indicates that while most of the genetic clusters have substantial overlap in their overall environment, cluster 6 is the only one without any overlap and therefore represents a completely unique ecotype for the species. The adaptation to the unique climatic conditions in Florida makes cluster 6 a conservation priority. Future research could use this information to develop a habitat suitability model for *H. quercifolia* to further guide conservation action.

The overrepresentation of precipitation related variables among the correlations with outlying loci indicates that precipitation has a stronger impact on genetic diversity in *H. quercifolia* than temperature. This is not surprising given the fact that *H. quercifolia* is never found in dry locations, but always in moist forest understories with high drainage (e.g. often found on steep slopes along riverbanks). Additionally, recommendations for growing *H. quercifolia* include providing adequate moisture (Dirr 2004) as the species is considered to be generally intolerant of hot, dry conditions. Precipitation has been found to have a significant impact on genetic diversity in other species such as *Cotinus coggygria* Scop. (Miao et al. 2017), *Larix decidua* Mill., *Pinus mugo* Turra (Mosca et al. 2012), *Arabis alpina* L. (Manel et al. 2010) and *P. taeda* (Eckert et al. 2010). Of these examples, *P. taeda* is notable, as mentioned earlier, for growing in the same region as *H. quercifolia* although with a wider longitudinal distribution. When considering the portion of the *P. taeda* native range in which *H. quercifolia* cooccurs, high spring precipitation with fall aridity in Florida is also found to be a large factor contributing to the distribution of genetic diversity.

The finding that most temperature associated loci are correlated to variation in annual temperature rather than either high or low temperature alone suggests that both temperature extremes (both high and low temperatures within a year) are shaping genetic diversity in *H. quercifolia*. Plants in a location with a high range in temperature need to withstand both extreme heat and cold, which is a more complex selection pressure than either high or low temperature alone. Temperature variables have also been found to significantly impact genetic diversity in other woody species including *Abies alba* Mill. (Mosca et al. 2012) and *Alnus glutinosa* L. (De Kort et al. 2014). Effects of temperature on diversity in *H. quercifolia* are expected due to the general heat intolerance of the species. Even with adequate soil moisture *H. quercifolia* wilts under extreme heat conditions (personal observation). Consistent with the effect of temperature on genetic diversity, tolerance to low temperatures in winter has been found to vary throughout the native range of *H. quercifolia* with cluster 6 having the least tolerance to low winter temperatures (Chapter 3). Future research could further the understanding of the effects of climatic variables on *H. quercifolia* by studying the effects of heat and drought on growth.

Conclusions

These analyses indicate that *H. quercifolia* exists in 6 weakly differentiated genetic clusters which are geographically structured. The genetic clusters on the southern edge of the species range are most genetically unique and contain putatively adaptive alleles. Based on the observations of extirpated populations primarily on the edges of the species range, it appears as though the range for *H. quercifolia* is shrinking. Therefore, the populations on the edges of the species range are indeed of conservation concern, especially those in Louisiana west of the Mississippi River and those in Florida. Environmental association analysis suggests that climatic variables significantly affect genetic diversity with precipitation being the primary environmental factor affecting genetic variation in *H. quercifolia.* Furthermore, the analysis identified candidate loci for environmental adaptation for both precipitation and temperature variables.

Tables

Table 2.1 Herbaria in which *Hydrangea quercifolia* specimens were surveyed for population identification.

State	Herbarium
AL	Alabama Plant Atlas, Alabama Herbarium Consortium and The University of West Alabama
FL	Atlas of Florida Plants, Institute of Systematic Botany Robert K. Godfrey Herbarium, Florida State University
GA	The Atlas of Georgia Plants, University of Georgia Herbarium and Valdosta State University Herbarium
LA	Shirley C. Tucker Herbarium, Louisiana State University
MS	Thomas M. Pullen Herbarium, University of Mississippi

Table 5.2 Locations and number of samples of *Hydrangea quercifolia* populations.

State	Latitude (°N)	Longitude (°W)	Site Name	Number of DNA Samples
AL	34.47173	86.0501	Buck's Pocket State Park	3
AL	34.4167	86.5965	Hugh's Spring	5
AL	34.37129	86.2123	Hwy 227	2
AL	34.36452	86.6073	Crawford Chute	2
AL	34.28716	85.6843	Little River Canyon	5
AL	34.09298	87.6116	Natural Bridge	2
AL	33.99769	86.6013	Swann Covered Bridge	2
AL	33.87496	86.8651	Rickwood Caverns State Park	2
AL	33.83529	85.6372	Dugger Mtn.	2
AL	33.66558	85.6325	Skyway Mtn.	5
AL	33.46074	85.8182	Cheaha Mtn.	5
AL	33.35335	86.7045	Oak Mountain State Park	2
AL	33.28056	87.4068	Rocky Branch Public Use Area	2
AL	32.88895	87.4279	Ocmulgee District	7
AL	32.5959	85.8803	Coon Creek	2
AL	32.54893	85.4778	Chewacla State Park	3
AL	32.39916	86.7821	Bob Woodruff Park	2
AL	32.06199	85.8993	High Ridge	2
AL	31.7197	87.4618	Haines Island Park	2
AL	31.71095	86.522	Pigeon Creek Tributary	2
AL	31.51681	86.5307	Hwy 23	2
AL	31.47532	85.6284	Dale County Lake	2
AL	31.45441	86.7863	Sepulga River at Travis Bridge	1
AL	31.18501	86.6953	Conecuh River	4
FL	30.8114	85.2295	Florida Caverns State Park	4
FL	30.72475	85.5305	Falling Waters State Park	2
FL	30.69707	84.8491	Angus Gholson Nature Park	2
FL	30.62958	84.896	I-10 Rest Stop	2
FL	30.56973	84.9402	Torreya State Park	2
FL	30.4764	84.6235	Bear Creek Educational Forest	1
FL	30.43172	85.5484	Econfina Creek Water Management Area	2
FL	30.36302	84.671	Rock Bluff	2
GA	34.87422	84.7192	Mill Creek	2
GA	34.84345	85.4789	Cloudland Canyon State Park	2
GA	34.43213	85.3365	James H. Floyd State Park	3
GA	34.21739	85.2075	Blacks Bluff Preserve	2
GA	33.79689	84.3175	Lullwater Preserve	2
GA	32.96549	84.4978	Camp Thunder	2
GA	32.8409	84.8392	Franklin D. Roosevelt State Park	3
GA	32.54643	84.8849	Flat Rock Park	1
GA	31.89453	85.1059	River Bluff	2

Table 2.2 Continued

State	Latitude (°N)	Longitude (°W)	Site Name	Number of DNA Samples
GA	31.30734	85.0817	Coheelee Creek	2
GA	30.71394	84.8515	Jim Woodruff Cliff	6
LA	31.80523	91.7576	Sicily Island	4
LA	31.45902	92.2993	Camp Beauregard	2
LA	31.21508	92.6711	Kisatchie National Forest	3
LA	30.92754	91.5287	Tunica Hills	7
LA	30.8235	91.2657	Marry Ann Brown Nature Preserve	2
LA	30.77954	90.1629	Bogue Chitto State Park	2
MS	34.60074	88.1853	Tishomingo State Park	3
MS	34.51675	88.547	Hwy 366	3
MS	34.37356	89.3521	Puskus Lake	3
MS	34.25801	88.9006	Trace State Park	2
MS	34.2237	89.3634	Tula Cemetery	2
MS	33.84466	89.6729	Carver's Point State Park	3
MS	33.61316	89.4099	The Old Cove	3
MS	33.41539	89.264	Jeff Busby Park	4
MS	33.22734	89.0906	Tombigbee National Forest	2
MS	33.02973	89.9198	Holmes County State Park	1
MS	32.35323	90.7748	I-20 at Vicksburg	3
MS	32.3259	90.1561	LeFleur’s Bluff State Park	2
MS	31.99717	89.3564	Cat's Den Preserve	2
MS	31.74519	88.523	Old US 84	2
MS	31.42324	90.9846	Clear Springs	2
MS	31.32607	89.9449	Red Bluff	2
MS	31.29311	91.2103	Homochitto National Forest	2
MS	31.18147	89.0506	DeSoto National Forest	3
MS	31.06661	91.5179	Clark Creek	2
TN	35.809	87.1477	Dutch Potts Hollow	2
TN	35.80094	85.623	Rock Island State Park	2
TN	35.46251	87.2685	Stillhouse Hollow Falls	2
TN	35.0513	88.2367	Pickwick Landing State Park	2
TN	35.03504	88.7289	Big Hill Pond State Park	2

Table 2.3 Pairwise F_{st} between genetic clusters of *Hydrangea quercifolia*. Genetic clusters were determined using Structure on 188 samples from 73 populations throughout the species native range.

	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**
Cluster 2	0.015				
Cluster 3	0.014	0.020			
Cluster 4	0.019	0.027	0.025		
Cluster 5	0.020	0.026	0.025	0.039	
Cluster 6	0.022	0.032	0.033	0.056	0.052

Table 2.4 Sample sizes, expected heterozygosity (H_e) and mean alleles per locus (A) for each genetic cluster and *Hydrangea quercifolia* as a whole.

Cluster	Individuals (N)	Populations (N)	H_e	Rarefied[1] H_e	A	Rarefied[1] A
1	40	14	0.352	0.289	2.67	1.48
2	9	3	0.294	0.294	1.47	1.47
3	19	8	0.343	0.295	2.11	1.48
4	38	14	0.357	0.297	2.62	1.46
5	59	25	0.358	0.301	2.95	1.50
6	23	9	0.343	0.288	2.24	1.47
Total	188	73	0.358	0.296	3.49	1.48

[1] Expected heterozygosity and alleles per locus were also calculated using rarefaction to account for unequal sample sizes.

Table 2.5 Environmental variables included in the Redundancy Analysis (RDA) of the environmental association analysis of *Hydrangea quercifolia*.

Environmental Variable	BioClim Code	PC axis[1]	Variation (%)[2]	Number of Associated Loci[3]	Highest Correlation with Outlying Locus (r)
Precipitation of Driest Quarter	Bio17	PC2	18.4	61	0.47
Precipitation of Warmest Quarter	Bio18	PC1	8.0	40	0.44
Precipitation Seasonality	Bio15	PC2	14.9	33	0.39
Precipitation of Coldest Quarter	Bio19	PC2	16.8	31	0.44
Temperature Annual Range	Bio7	PC1	8.2	31	0.42
Temperature Seasonality	Bio4	PC1	9.4	16	0.40
Annual Mean Temperature	Bio1	PC1	9.8	15	0.48
Minimum Temperature of Coldest Month	Bio6	PC1	9.8	3	0.49
Mean Temperature of Coldest Quarter	Bio11	PC1	10.0	1	0.47

[1] PC axis that the variable contributed the most amount of variation to.

[2] Percent of the variation on the PC axis that is contributed by the variable.

[3] Number of RDA outliers that were most highly correlated with the variable.

Figures

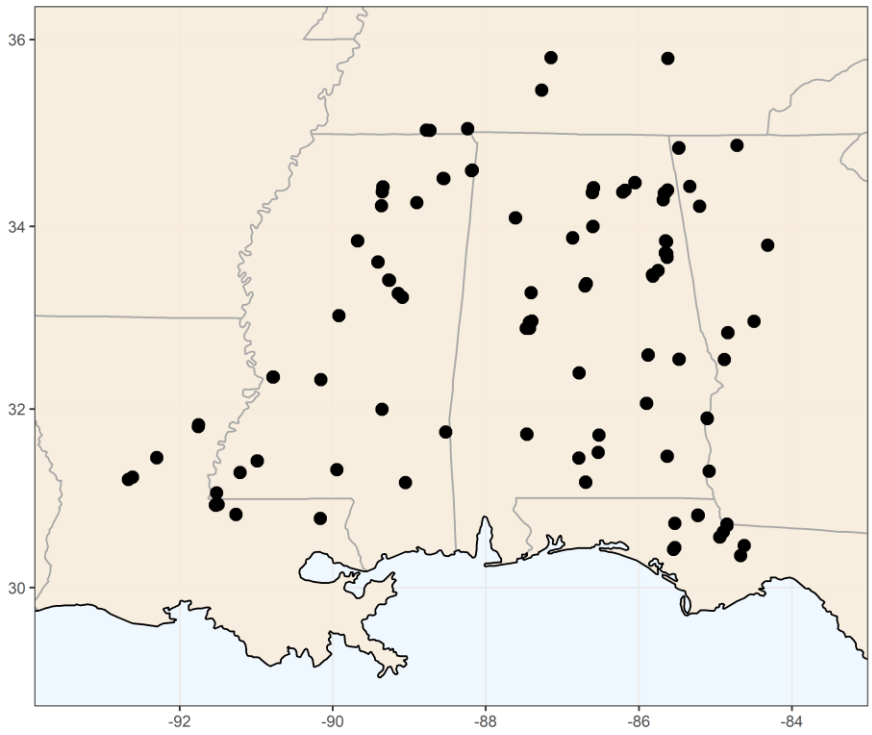

Figure 2.1 Map of *Hydrangea quercifolia* DNA sample collection locations. Each black point represents one of the 188 samples collected from 73 locations in the six-state native range of *H. quercifolia.*

Figure 2.2 Photographs of *Hydrangea quercifolia* in its natural habitat. Left photograph shows representative riverbank population. Center photograph shows flowering *H. quercifolia* growing out of bedrock. Right photograph shows small population growing out of near-vertical bluff.

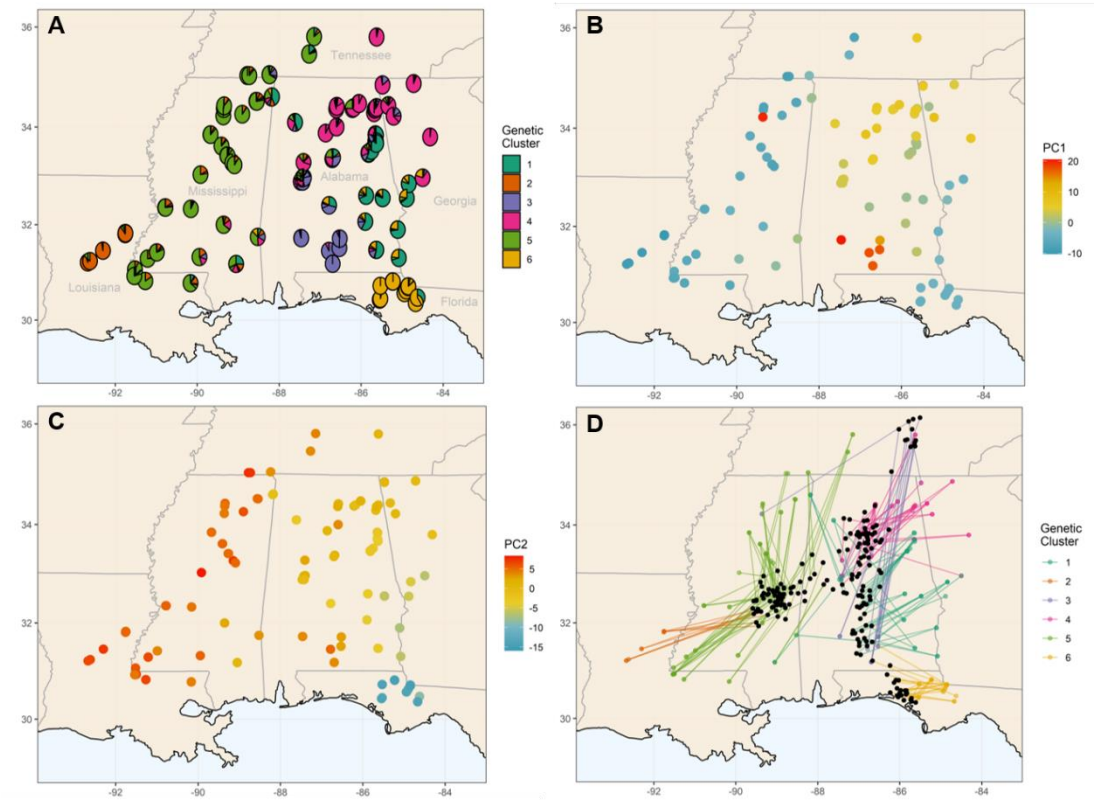

Figure 2.3 Geographic structure of genetic diversity in *Hydrangea quercifolia.* A) Proportion of each pie chart on the map indicates assignment probability to each respective genetic cluster. B) Map of PC1 values. Here color represents PC1 value rather than genetic cluster. C) Map of PC2 values. Color represents PC2 value. D) Map showing Procrustes transformed PCA values as black points and sampling locations as points colored by their respective genetic cluster. Points representing the same sample are connected with a line.

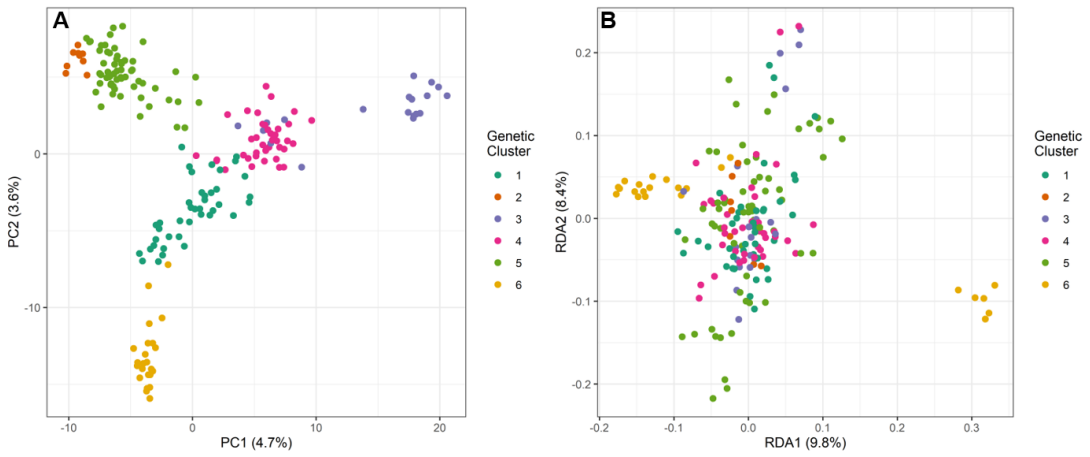

Figure 2.4 Unconstrained and constrained ordination biplots of allele frequency. A) Principal Components Analysis (unconstrained ordination) with point color representing genetic cluster with highest assignment probability. B) Redundancy Analysis (constrained ordination) constrained by environmental factors and conditioned by population structure. This displays the genetic variation which is unaccounted for by population structure and can be explained by environmental variation.

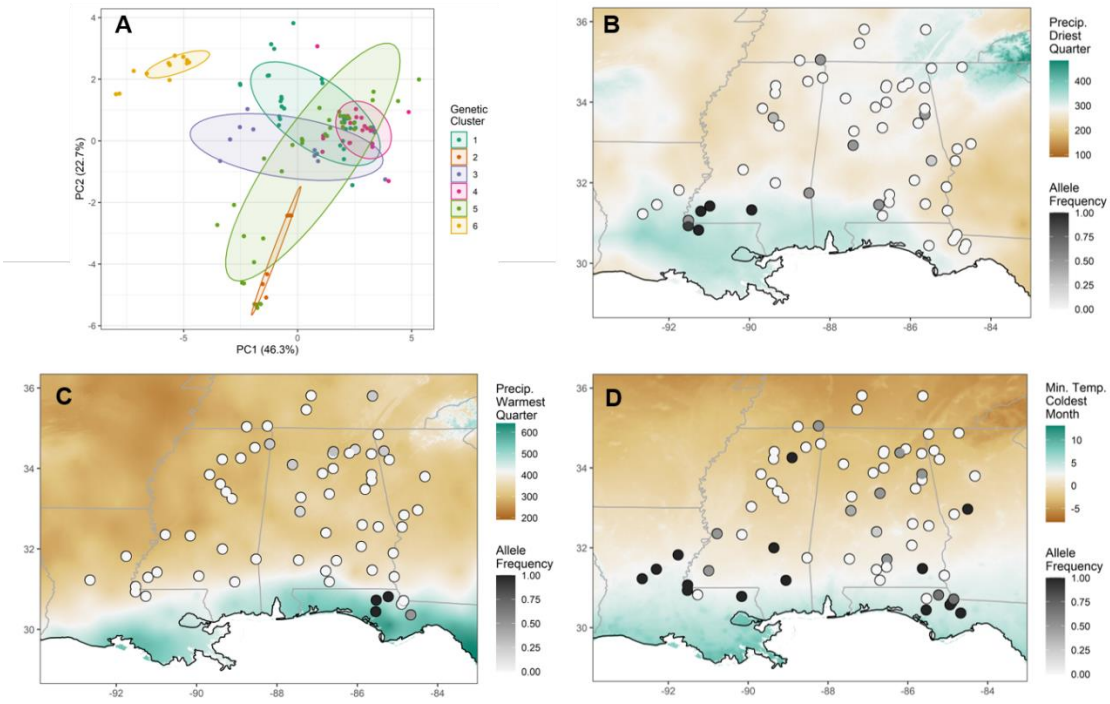

Figure 2.5 Environmental variation within the natural range of *H. quercifolia* and allele frequencies of putative environment associated loci. A) PCA of 19 environmental variables from the BioClim database with 80% ellipses surrounding the genetic clusters. B) Map of precipitation in the driest quarter (bio17) and allele frequency of Hq_locus526520 (mean allele frequency=0.144). C) Map of precipitation in the warmest quarter (bio18) and allele frequency of Hq_locus34460 (mean allele frequency=0.118). D) Map of minimum temperature in the coldest month (bio6) and allele frequency of Hq_locus29211 (mean allele frequency=0.337).

Chapter 3:

Horticultural Characterization of Wild Collected *Hydrangea quercifolia* Seedlings

Introduction

Hydrangea L. is a popular genus of flowering plants which has high ornamental value and is often grown as a landscape plant. There are several species of *Hydrangea* which are grown for their horticultural value, with *H. macrophylla* Thunb. being the most popular (Dirr 2004). Despite the recent interest in developing new hydrangea cultivars, *H. quercifolia* Bartr. (oakleaf hydrangea) remains an underutilized species with very little information available.

Plant breeders rarely begin a new breeding program with a comprehensive evaluation of the species, instead beginning with whatever germplasm (typically cultivars) is most readily available. However, a systematic evaluation of wild germplasm prior to breeding would allow one to determine the full extent of variation for traits of interest and therefore allow for selection of the best germplasm available in the species. Breeding in *H. quercifolia* is relatively recent with only a few cultivars that have known pedigrees available (Reed 2010; Reed and Alexander 2015). Additionally, the species' native range is relatively small in the southeastern United States and can be sampled thoroughly (Fig. 3.1). Therefore, there is an opportunity to undergo such a characterization of oakleaf hydrangea to further inform future breeding efforts for the species.

Oakleaf hydrangea is a multi-stem shrub which can spread by branch layering. Traits relating to plant architecture are important for oakleaf hydrangea because at maturity plants can reach greater than 3 m tall. Despite an increasing demand for compact genotypes of ornamental plants and there are very few available for H. quercifolia (Dirr 2004). Additionally, the few that are available have been derived from the same two cultivars, Pee Wee and Sike's Dwarf (Reed 2010; Reed and Alexander 2015). Therefore, novel sources of compactness would contribute to generating new compact varieties while maintaining a wide genetic base.

Plant architecture of shrubs is a multi-component trait which can be quantified in various ways (Crespel et al. 2012, 2013). Important components of plant architecture include the total size of the plant, measured as height and width. Number of branches and internode length impact how compact a plant is visually, as even a relatively tall plant that is highly branched with small internodes can appear compact.

Although hydrangea seed is generally easy to germinate and does not need a stratification treatment (Halcomb and Reed 2012), germination rates are important to growers and breeders. For growers who grow oakleaf hydrangea from seed, being able to predict the germination rate allows for better resource use when scheduling a crop. Likewise, for breeders, knowing the germination rate helps to reach target progeny numbers efficiently.

Oakleaf hydrangea is susceptible to a bacterial leaf spot disease caused by *Xanthomonas campestris* L. (Hagen and Mullen 2001; Mmbaga and Oliver 2007). Leaf spot causes unsightly lesions on the leaf, which greatly diminishes the ornamental quality of the plants. Leaf spot severity is especially high under production conditions which utilize overhead irrigation, thereby decreasing the value of the product (Hagen and Mullen 2001). There is currently no known source of leaf spot tolerance or resistance in oakleaf hydrangea, and therefore identifying variation in tolerance to leaf spot would introduce the possibility of breeding for it. By screening diverse germplasm, tolerance to *X. campestris* has been found in other species (Maas et al. 2000; Naqvi et al. 2012; Hayes et al. 2014) and therefore is a promising strategy in oakleaf hydrangea.

Cold hardiness is a complex trait which limits the northern extent where oakleaf hydrangea can be grown in landscapes. Published estimates for cold hardiness in oakleaf hydrangea are USDA Hardiness Zone 5a (-28.9 to -26.1°C; Halcomb and Reed 2012). However, this estimate is supported by testing only two cultivars (Dirr et al. 1993) and therefore it is unknown how much if any variation exists for cold hardiness in the species. In wild populations of woody plants, cold hardiness has been found to vary by latitude (Hurme et al. 1997; Aldrete et al 2008; Pagter et al. 2010) and therefore it is hypothesized that northern *H. quercifolia* populations can be a source of increased cold hardiness. In woody plants cold hardiness level varies throughout a winter while the plant undergoes a process of acclimation in the fall during which hardiness increases, and deacclimation in

the spring during which hardiness decreases (Arora and Rowland 2011). Plants are expected to be at their most hardy point in midwinter, which can serve as simple estimate of winter hardiness. However unseasonably late cold periods after the plant has begun to deacclimate, and lose hardiness, can have just as large of an effect on winter damage. Additionally, midwinter hardiness and deacclimation timing are different traits in *Hydrangea* (Pagter et al. 2011a) and therefore both need to be tested in order to best understand a plant's ability to survive winter. Although Dirr et al. (1993) found 'Alice' and 'Alison' to have the same cold hardiness level in midwinter, the two cultivars did deacclimate at different times which suggests that variation exists for deacclimation timing in *H. quercifolia*.

Methods and Materials

Plant Material

Seeds were collected from 530 maternal plants from 55 wild *H. quercifolia* populations from October-December in 2017 and 2018. For the purposes of this study, a population was defined as a semi-continuous group of hydrangeas at a sampling location. Population identification strategy was more thoroughly described in Chapter 2. Populations were greater than 2 km apart. Number of seed samples per population ranged from one to 29. Each seed sample consisted of one open-pollinated inflorescence from one maternal plant (mean = 24,771 seeds per sample). Because *H. quercifolia* is self-incompatible (Reed 2000, 2004), each sample is considered to be a maternal half-sibling family. Latitude and longitude were recorded for each maternal plant with EpiCollect5 (v3.0.3) which has 10 m accuracy. Each inflorescence was placed into a plastic bag for transport to the laboratory. Seed was extracted from each inflorescence and frozen at -20°C until sowing. The seed were sown in spring of 2018 and 2019, with each cohort consisting of the seed from the populations which were sampled the previous fall (Fig. 3.1). In 2018, seed from 17 populations were grown which represent the latitudinal range of the species (older cohort) from Tennessee, Mississippi, and Florida. In 2019, seed from 38 populations were grown which cover a broader geographical representation of the species (younger cohort) extending to Louisiana, Georgia, and Alabama.

After germination (described below), seedlings were transplanted into 7.62 cm square pots with a soilless potting medium (Sungrow Horticulture, Agawam, MA) and were grown in the greenhouse until May or June. Greenhouse temperatures were maintained between 25 and 30°C with natural daylength. Seedlings were transplanted into 9.21 cm square pots with peat-pine bark (1:1) potting mix and transferred to an outdoor container nursery with overhead irrigation.

In spring 2019, plants in the older cohort were cut back to three nodes above the soil to induce branching in their second year of growth. They were then randomly assigned to three groups and either transplanted into 7.57 L pots or planted in the field at the Horticulture Research Center in Chanhassen, Minnesota (44.859, -93.634) or in the field at the Otis L. Floyd Nursery Research Center in McMinnville, Tennessee (35.709, -85.744).

A selection of cultivars (Table 3.1) were obtained from commercial nurseries in 2019 and grown under the same conditions as the wild collected seedlings in 7.57 L pots. These plants were used for leaf spot tolerance assessment and cold hardiness testing as described below.

Percent Seed Germination

In 2018 seed was germinated under two conditions, in a greenhouse in Minnesota and in a growth chamber in Tennessee. Seed from each maternal parent was surface sown onto soilless germination mix (Sungrow Horticulture, Agawam, MA). In the greenhouse, flats were grown on heat mats (Hydrofarm Horticulture, Petaluma, CA) and covered with clear plastic domes to maintain high relative humidity. Greenhouse temperatures were maintained between 25 and 30°C with natural daylength. In 2019, seeds were only germinated in the greenhouse in Minnesota under the same conditions described for 2018. In both years, number of seed sown per half-sib family and population varied based on amount of seed available. After the first seedling germinated, germination was recorded every other day until no new seedlings germinated for one week. Percent germination was determined for each sample by dividing the number of seedlings germinated by the number of seeds sown and multiplying by 100. Population means were

assessed with Analysis of Variance (ANOVA) and Bonferroni corrected for multiple comparisons.

Plant Architecture

In 2018 a minimum of 100 random seedlings from each population were measured after terminal buds had been set. Plants were measured from the top of the soil to the top of the apical bud on the tallest shoot. Nodes were counted on the tallest shoot of 100 random seedlings from each population. Internode length was estimated by dividing the total height (of the same individuals) by the number of nodes.

In 2019 seedlings were measured using the same methods. The older cohort of seedlings were measured in 7.57 L pots and in both field locations to assess genotype by environment interactions. In addition to the traits measured in 2018, number of primary branches was also measured in 2019 in the older cohort. Due to the lack of secondary branches, branch counts were unambiguous. Additionally, plant width was measured in the field by averaging the width in the widest dimension and the width perpendicular to the widest dimension.

Population and environment means were assessed with ANOVA and Bonferroni corrected for multiple comparisons.

Leaf Spot Tolerance

One leaf per plant was sampled from 100 random seedlings (or three leaves from each of 10 plants per cultivar) growing in 7.57 L pots from each population of the older cohort of seedlings in September 2019. Plants were exposed to ambient, naturally occurring *X. campestris* inoculum under container nursery conditions with overhead irrigation. The fourth fully expanded leaf was chosen for sampling based on the observation that the leaf spot symptoms were less severe on the top of the canopy, whereas further down in the canopy leaves were severely senesced or otherwise deteriorated. Leaves were then transported to the laboratory for imaging against a white background with uniform, cool white fluorescent lighting. Images were taken in the jpeg file format with a Samsung SM-G950U1 camera (Samsung Electronics Co., Seoul, South Korea) with 4032 x 3024 pixel resolution and a 4.25 mm focal length.

Images were analyzed using Food Color Inspector software (v4.0; http://www.cofilab.com/portfolio/food-color-inspector/) to determine the percent leaf area affected by lesions and secondary symptoms. This software allows the user to define each of the categories in a training set, then assigns all pixels in the image to one of the categories based on the color using a Bayesian algorithm. One leaf per population was used as the initial training set for the population, which was updated iteratively as needed for each leaf to ensure accurate classifications. Pixels were assigned to four categories: background, healthy leaf, necrotic tissue, and discoloration (Fig. 3.2). Discoloration was either chlorosis or other discoloration surrounding or immediately adjacent to necrotic tissue and typically represented the leading edge of the lesion. Percent leaf area affected by leaf spot (necrotic tissue and discoloration) was calculated for each leaf by dividing the number of pixels classified as each of the diseased categories by the number of non-background pixels, then multiplying by 100. Population and cultivar means were assessed with ANOVA and Bonferroni corrected for multiple comparisons.

Several representative infected leaves were submitted to the University of Minnesota Plant Disease Clinic to verify the identity of the pathogen. The pathogen was determined to be *Xanthomonas campestris*, consistent with the expectation. No additional pathogens were detected.

Cold Hardiness

The older seedling cohort was tested for cold hardiness in January 2019 with a controlled freezing test. These methods were based on those used previously for controlled hardiness screening (McNamara et al. 2002; McNamara and Hokanson 2010). The seedlings were overwintered in a minimally heated structure which exposed the plants to ambient temperatures to approximate natural acclimation conditions (Fig. 3.3A). The structure was heated only to maintain a minimum temperature of -9.5°C to avoid cold damage prior to the experiment. The seedlings were sampled on four dates over the course of two weeks (January 6, 10, 13, 17) in a completely randomized experimental design. On each sample date, stems from seedlings of each population were taken to the laboratory and cut into 3.5 cm sections and then randomly assigned to one of 6 temperature treatments, plus a non-frozen control (Fig. 3.3B). Stem sections from each

population at each temperature treatment were placed into bags with moist paper towels (3 replicate bags per temperature per sample date; Fig. 3.3C) and the bags were placed in a ScienTemp freezer (ScienTemp Corporation, Adrian, MI) controlled by a Watlow series 942 temperature controller (Watlow, St. Louis, MO; Fig. 3.3D). Each bag contained stem sections from all populations. A thermocouple was inserted into the pith of at least one stem section per temperature treatment to monitor stem temperature throughout the experiment. Temperatures were slowly decreased to the first test temperature (-10°C) overnight to allow stem temperatures to equilibrate. Temperatures were then decreased at a rate of 3°C per hour and three replicate bags for each treatment were removed from the freezer at -5°C increments to -35°C. Stem sections were slowly thawed at 3°C overnight and then were incubated at room temperature for one week to allow damage symptoms to appear. Stem sections were evaluated for cold damage by slicing the stem longitudinally with a scalpel and rating stems as live or dead by observing the damage under a dissecting microscope (12x magnification; Fig. 3.4). Cold hardiness was determined as LT_{50}, calculated for each population using a binomial logit model to interpolate the temperature where 50% of the stems are expected to die (Soujala and Lindén 1997). The binomial logit model was implemented in the R packages MASS (v7.3-50) and stats (v3.5.1).

The experiment was repeated in winter 2019-2020 with a subset of populations from both the younger and older seedling cohorts as well as a selection of cultivars. In this experiment, plants were tested once per month from October to April in order to identify timing of fall acclimation and spring deacclimation using the methods described above. The tested temperatures were varied each month to exceed the expected range of LT_{50} values (Table 3.2). In each month, the sample from a population or cultivar was taken as one random stem from multiple individual plants and therefore each plant was resampled every month.

Results

Seed Germination

Significant variation among populations in seed germination percentage was observed in both 2018 ($p<0.001$, $F=4.67$) and 2019 ($p=0.005$, $F=20.2$). In the greenhouse,

the overall mean percent germination was 61.6% and 60.5% for 2018 and 2019 respectively, with the difference between years being insignificant (two-sample t-test p=0.76). In 2018, significant differences were detected between greenhouse and growth chamber germinated seed (p=0.002, F=15.2; Fig. 3.5F) with 61.6% and 73.5% respectively. In 2018, population 14 had the highest percent germination (92.7%), while population 2 had the lowest percent germination (21.2%). In 2019, population 9 had the highest percent germination (90.0%), while population 35 had the lowest percent germination (11.0%). Population mean seed germination percentage is given in Table 3.3. Almost no variation was observed for number of days to first germination with all populations having their first germination between nine and 11 days after sowing.

Plant Architecture

Significant differences were found among populations in both years and in all environments tested for all plant architecture traits ($p<0.001$; Table 3.3, Fig. 3.5A-E). In 2018 population mean plant height ranged from 37.5 cm (population 14) to 76.3 cm (population 2); mean node number ranged from 7.4 (population 14) to 10.4 (population 20); mean internode length ranged from 3.6 cm (population 14) to 7.5 cm (population 5). Figure 3.6 shows representative variation observed in the seedlings in 2018.

In 2019, the older cohort of seedlings that were grown in pots had mean population heights ranging from 33.7 cm (population 22) to 76.6 cm (population 3); mean node numbers ranging from 9.6 (population 6) to 11.5 (population 13); mean internode lengths ranging from 3.4 cm (population 22) to 7.0 cm (population 3); and mean number of branches ranging from 3.2 (population 11) to 4.6 (population 21).

In the older seedling cohort that was grown in the field in Minnesota, mean population height ranged from 28.6 cm (population 14) to 44.3 cm (population 3); mean number of nodes ranged from 8.1 (population 6) to 9.9 (population 20); mean internode length ranged from 3.4 cm (population 22) to 5.4 cm (population 3); mean number of branches ranged from 3.5 (population 3) to 4.8 (population 14); and mean width ranged from 12.0 cm (population 22) to 18.4 cm (population 5).

In the older seedling cohort that was field grown in Tennessee, the mean population heights ranged from 59.3 cm (population 12) to 101.0 cm (population 17);

mean number of nodes ranged from 7.2 (population 13) to 21.1 (population 23); mean internode length ranged from 2.1 cm (population 3) to 11.3 cm (population 13); mean number of branches ranged from 2.8 (population 13) to 8.0 (population 23); and mean plant width ranged from 5.9 cm (population 13) to 63.4 cm (population 4).

The younger cohort of seedlings was only grown in containers in 2019 and mean population heights ranged from 18.1 cm (population 64) to 60.1 cm (population 43). Mean number of nodes ranged from 4.9 (population 70) to 10.7 (population 43). Mean internode length ranged from 2.8 cm (population 64) to 5.7 cm (population 43).

Plant architecture varied by environment and year (Fig. 3.5A-E) with the plants generally being the largest when grown in Tennessee ($p<0.001$, $F=755.2$). Within the Minnesota grown plants, the seedlings grew the tallest in their first year and were shortest when grown in the field. However, when averaged across growing environments, plant height was inversely correlated with collection site latitude. The association was slightly stronger in the older cohort ($r=-0.68$; $p=0.003$) than in the younger cohort ($r=-0.64$; $p<0.001$).

Leaf Spot Tolerance

Total percent leaf area affected by leaf spot ranged from 3.5% in population 2 to 26.5% in population 14 and significant differences were detected among populations ($p<0.001$, $F=17.6$; Table 3.3, Fig. 3.7A). Among the cultivars, total percent leaf area affected ranged from 8.8% in Flemygea to 24.2% in Queen of Hearts (Table 3.1, Fig. 3.7B). On average, cultivars had higher leaf area affected than the wild seedlings did (two-sample t-test $p=0.006$), although there was more variation among the wild seedling populations.

Percent leaf area affected by necrotic tissue and discoloration were correlated ($r=0.44$, $p<0.001$) and leaves typically had greater area affected by necrosis than discoloration. However, there were two exceptions to this with ‘Queen of Hearts’ having greater leaf area affected by discoloration and ‘Alice’ having nearly equal leaf area affected by discoloration and necrosis. Total percent leaf area affected was positively correlated with latitude ($r=0.70$; $p=0.002$), with the populations originating in Florida having the lowest disease severity.

Cold Hardiness

In January 2019, significant differences were found among populations for estimated LT_{50}. The LT_{50} values for the populations ranged from -27.1°C (population 4) to -33.2°C (population 19). 'Ruby Slippers' had an estimated LT_{50} of -35°C. Among the wild populations, LT_{50} was inversely correlated with latitude ($r=-0.71$, $p=0.003$) with the northern populations generally being more cold hardy than the southern populations.

In the winter of 2019-2020, significant differences were detected among cultivars and wild populations in each month (Tables 3.1 and 3.2). Additionally, significant differences were detected among months of the winter ($p<0.001$, $F=74.4$) with maximum cold hardiness being achieved in December, January or February depending on the population (Fig. 3.8). Overall mean LT_{50} was at the lowest (most cold hardy) in February with an LT_{50} of -33.7°C. More variation was found among populations and cultivars in early and late winter than in midwinter, with the highest amount of variation during deacclimation in March and April (Fig. 3.8B). With the broader sampling of wild populations, the inverse correlation between January LT_{50} and latitude remained significant, but the association was weaker ($r=-0.58$; $p=0.006$).

Population 4 was consistently the least hardy population with the exception of October. No population was consistently the most hardy throughout winter, however there was considerable variation in deacclimation timing with two populations (populations 9 and 58) maintaining extreme cold hardiness into March and several surviving the lowest temperature tested (-25°C) in April (populations 10, 37 and 47). Sike's Dwarf was the most cold hardy cultivar throughout the middle of winter, but had the highest rate of deacclimation between March and April. 'Sike's Dwarf' no longer tolerated freezing in April after a 28.7°C increase in LT_{50}. In contrast, 'Ruby Slippers' had moderate cold hardiness in midwinter, but maintained a high level of cold hardiness into March and April. Flemygea was consistently among the least cold hardy cultivars throughout winter.

Discussion

Considerable variation was observed in seed germination percentages, however most of the population means were within the range of what has been observed in

Hydrangea macrophylla (4.8% - 68.5%) and *H. paniculata* (37.3% - 65.4%) (Greer and Rinehart 2009). The higher germination percentage in the growth chamber is likely due to more uniform environmental conditions, e.g. optimized temperature and humidity. There was no detectable geographic pattern in percent seed germination, although studies in *Campanula americana* L. (Zettlemoyer et al. 2017) and *Acer saccharum* Marsh. (McCarragher et al. 2011) indicate that germination temperature could confound the effects of latitude on seed germination. Therefore, wild populations from different latitudes may have different optimal germination temperatures which may, at least in part, explain the observed differences among populations.

Although plant architecture was significantly different in each of the growing environments tested, the largest differences were between Minnesota and Tennessee (Fig. 3.5A-E). This difference could potentially be explained by the longer growing season in Tennessee compared to Minnesota. The differences in growth within Minnesota (2018 in pots, 2019 in pots and 2019 in the field) could be due to the closer spacing of the potted plants in their first year of growth, which induced a competitive response and shoot elongation. Even within 2019 in Minnesota, the plants had different growth responses to container or field growth with the plants being generally more compact and higher branched in the field. This is also likely explained by the lower plant density and therefore lower competition in the field. These results indicate that to select on plant architecture traits in a breeding program, selections should be grown in multiple environments (or the environment most relevant to the breeding objectives) to ensure selections will have the desired phenotype in the target environment.

In other ornamental plants, components of plant architecture have been shown to have a genetic basis. For example, in rose there have been several studies to identify the components of plant architecture (Crespel et al. 2013, 2014) and genetic loci which underly these traits (Li-Marchetti et al. 2017). This study identified between three and seven quantitative trait loci (QTL) for the traits analyzed. Similar studies in *Lagerstroemia* have quantified genotype by environment interactions (Pounders et al. 2010) and QTL linked to compactness (Ye et al. 2016), where three QTL were also identified. In both of these species, like the current study in *H. quercifolia*, plant architecture was found to have a genetic component (variation among populations)

despite the significant effect of environmental factors. Furthermore, as expected for quantitative traits such as plant architecture, there are likely several loci controlling each of these traits.

Three populations (population 12, 13 and 14) were more consistent across environments than the others for the plant architecture traits. Interestingly, these were also among the most compact populations. Stability in various environments for compactness would be an important production trait in that the phenotype would be relatively predictable regardless of growing environment.

The correlation of plant height with latitude is congruent with the variation that has been documented among species worldwide (Moles et al 2009). This was attributed to varying environmental conditions which are correlated with latitude. In particular, Moles et al (2009) found precipitation in the wettest month to be the best predictor of plant height. This is also consistent with the finding that precipitation has a significant impact on genetic diversity in *H. quercifolia,* indicating the selective pressure it has on the species (Chapter 2). For *H. quercifolia* the variation in plant height is largely due to the fact that the plants from Florida were the tallest averaged across years and environments. With the younger seedling cohort not having populations from Florida, the trend was weaker, although still significant.

Potential novel sources of compactness were identified from at least two sources. Plants from northern latitudes (especially in the northeastern portion of the range) tended to be shorter with smaller internodes. Although the number of branches did not follow a clear geographical pattern, one family from population 22 had eight seedlings which were highly branched with very small internodes (mean number of branches in the putative mutant seedlings = 8.1). This likely represents a source of qualitative variation considering the discrete categories the seedlings in this family can be assigned to (Fig. 3.10). Both of these sources could be utilized for breeding novel compact oakleaf hydrangea varieties.

A more than seven-fold difference in leaf spot severity was detected among the wild plants and cultivars. Wild *H. quercifolia* populations in Florida, especially populations 2 and 3, can serve as sources of *Xanthomonas* tolerance for breeding (Fig. 3.11). Populations in Florida encounter substantially different environmental conditions

than the remainder of the species' native range, having higher precipitation and higher temperatures (Chapter 2). These conditions likely favor growth of the pathogen (Dixon et al. 2002) and therefore wild populations could have developed disease resistance. This might be expected to be a horizontal resistance due to the environmental conditions favoring any pathogen present as well as the lack of complete resistance detected in this study. Although no plants were found to be entirely resistant, the leaf with the lowest disease severity was from a population 3 seedling having 0.03% total leaf area affected. By using ambient, natural inoculum, it is possible that a leaf such as this may have encountered a lower inoculum level than others. However, at the population level it is likely that the detected differences represent true resistance considering that the populations were grown in the same nursery and therefore had equivalent disease pressure. Two cultivars can also be used as sources of moderate leaf spot tolerance. 'Alice' and 'Flemygea' are the only two tested cultivars with less than 10% leaf area affected. Additional testing with controlled inoculations could potentially identify individuals within each of the Florida populations with higher leaf spot tolerance than either 'Alice' or 'Flemygea'.

Resistance to leaf spot caused by *X. campestris* has been identified in other species. By screening diverse germplasm, leaf spot resistance has been identified in strawberry (Maas et al. 2000; Roach et al. 2016), sesame (Naqvi et al. 2012) and lettuce (Hayes et al. 2014). Resistance to *X. campestris* has been shown to have a very high heritability in mulberry (Banerjee et al. 2012), which suggests that selecting for leaf spot resistance should lead to efficient genetic gain.

It is interesting to note that all three compact cultivars included in this study (Sike's Dwarf, Pee Wee and Queen of Hearts) had the highest disease severity of the tested cultivars. This is similar to that which was observed in the wild seedlings with the most compact population (population 14) also having the highest disease severity. One possible explanation is unfavorable linkage between genetic loci controlling both traits. Another possible explanation is a pleiotropic effect of plant height on leaf spot severity by making the leaves more accessible to inoculum which spreads upward through a plant from fallen leaves. The increased canopy density on compact plants could further this effect by creating a microclimate around the plant with increased humidity and decreased

air flow as well as facilitating the disease spread among leaves. Although this is a potential confounding factor on the wild seedlings, the cultivars were relatively the same height throughout the growing season in which they were phenotyped and therefore it is unlikely that this accounts for the differences observed.

In January of both winters, cold hardiness varied as a latitudinal cline with the northern populations being more cold hardy. However, like height, the populations from Florida had a greater contribution to this pattern as they were consistently the least cold hardy. Indeed, despite being from similar latitudes as the populations from Louisiana and southern Mississippi, the Florida populations were substantially less cold hardy. A correlation between cold hardiness and latitude has been found in several other woody species such as *Pinus sylvestris* L. (Hurme et al. 1997), *P. greggii* Engelm. (Aldrete et al. 2008) and *Acer platanoides* L. (Pagter et al. 2010) and therefore appears to be a general phenomenon in woody plants. Of these examples, *P. greggii* is particularly interesting as it is the only species from North America and exhibits a similar phenomenon of withstanding temperatures colder than would be expected based on its native range of occurrence. The northernmost population tested by Aldrete et al. (2008) was from approximately 25°N (northern Mexico) and had an LT_{50} of -18°C. This follows the pattern observed in this study where the southernmost *H. quercifolia* population was from approximately 30°N and had an LT_{50} of -27°C. Minimum winter temperatures vary by latitude (r=-0.98; Fig. 2.5D) and contribute to the genetic diversity of *H. quercifolia* (Chapter 2), and therefore likely have a selective influence on cold hardiness.

The cultivar that exhibited the lowest LT_{50} was Sike's Dwarf in Februrary (-37.7°C). Among the wild seedlings, population 8 had the lowest LT_{50} in Februrary (mean -38.7°C), although population 46 was very similar (mean -38.3°C). These LT_{50} values were lower than expected for the species based on a previous study with 'Alison' and 'Alice' (Dirr et al. 1993). In that study, 'Alice' was found to be most hardy in December (LT_{50} = -27°C), while in this study 'Alice' was most cold hardy in January (LT_{50} = -35°C). The previous study took place in Georgia, which likely reduced the level of acclimation achieved (McNamara et al. 2002). McNamara et al. (2002) assessed cold hardiness in several species of woody plants acclimated in Georgia compared to Minnesota and found that the same genotype acclimated in Minnesota achieved a greater

degree of cold hardiness for all species tested. Although population LT_{50} estimates were different in January 2019 and January 2020, the two samples were not significantly different ($p=0.64$), indicating that under the controlled winter conditions tested in this experiment, between year variation was minimal. These annual differences would likely be larger under natural acclimation conditions in field grown plants (Pagter et al. 2011b). The average LT_{50} among all cultivars and populations was lowest in February (LT_{50} = -33.7°C) which is lower than previous estimates for the hardiest *H. macrophylla* cultivars (LT_{50} = -24°C; Adkins et al. 2003).

In the winter of 2019-2020, timing of acclimation and deacclimation varied among populations and cultivars. Greater variation was detected in deacclimation than in acclimation or midwinter hardiness. In late winter 2020, the populations and cultivars split into a group which deacclimates rapidly and a group which maintains cold hardiness through April (Fig. 3.9). Therefore, midwinter cold hardiness does not seem to be the primary limiting factor for winter survival, but rather deacclimation timing and cold hardiness in late winter. However, because oakleaf hydrangea flowers on second-year wood and hydrangea flower buds are less hardy than stems (Pagter and Williams 2011), survival of stems does not necessarily indicate that the plants will flower in the subsequent summer. Furthermore, although cold hardiness determined by laboratory-based freezer assays has previously been demonstrated to correlate well with field survival in *Weigela florida* Bunge cultivars ($r=0.80$; McNamara and Hokanson 2010), variable conditions in the field (e.g. air temperatures, soil moisture, plant health, etc.) can impact realized winter survival. Therefore, because there is not yet data for cold hardiness in the field for this species, multi-year field testing on these seedlings will be required before a definitive statement can be made regarding the true cold hardiness level.

Considering cold hardiness and bacterial leaf spot tolerance, for which both cultivars and wild germplasm were evaluated, variation in wild populations exceeded that which was observed in the cultivars. Although wild oakleaf hydrangea cannot be said to be generally superior to the cultivars in either trait, certain populations did have either lower leaf spot severity or lower LT_{50}. Therefore, selectively including wild oakleaf hydrangea germplasm in a breeding program has the potential to provide novel variation

which exceeds that which is available in the commercial germplasm. For example, crossing individuals from one of the Florida populations with individuals from the northeastern portion of the species range would be expected to yield compact progeny with cold hardiness and leaf spot tolerance. The seed collected in this study has been submitted to the USDA National Plant Germplasm System to be maintained and made available to breeders and researchers.

Tables

Table 3.1. Leaf spot severity and cold hardiness in *Hydrangea quercifolia* cultivars. Leaf spot values represent percent of leaf area affected by *Xanthomonas campestris* on plants exposed to natural inoculum. LT_{50} values represent the temperature where 50% of stem samples were killed during laboratory based controlled freezer testing.

		Monthly LT_{50} (°C)						
Cultivar	**Leaf spot (%)**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**
Alice	8.8	-8.3	-26.0	-30.6	-35.0	-31.2	-22.7	0.0[3]
Brido	14.3	-13.7	-29.9	-32.8	-33.2	-35.1	-27.4	-19.7
Brother Edward	-[1]	-	-	-	-31.9	-	-	-
Flemygea	8.8	-13.8	-26.1	-29.3	-30.5	-29.3	-18.0	0.0[3]
Harmony	14.0	-9.6	-	-	-32.9	-35.4	-17.1	0.0[3]
Munchkin	-	-13.6	-26.8	-	-32.0	-33.2	-26.7	0.0[3]
Pee Wee	20.3	-14.0	-30.0	-30.0	-31.6	-34.1	-15.0[1]	0.0[3]
Queen of Hearts	24.2	-12.2	-29.2	-34.0	-31.2	-37.1	-17.3	-2.0
Ruby Slippers	-	-13.7	-25.6	-31.0	-31.9	-33.6	-30.7	-25.0[2]
Sike's Dwarf	21.0	-15.0[2]	-28.8	-35.5	-36.9	-37.7	-28.7	0.0[3]

[1] Dashes (-) indicate the cultivar was not analyzed for leaf spot or for cold hardiness during that month.

[2] Less than 50% of stem samples were killed at coldest temperature tested. Temperature listed was the coldest temperature tested.

[3] Greater than 50% of stem samples were killed at warmest temperature tested. Temperature listed was the warmest temperature tested.

Table 3.2 Test temperatures for controlled freezing experiment in each month of winter 2019-2020. In addition to the non-frozen control treatments, stems were frozen to six temperature treatments ranging from the high to the low temperatures with the interval listed between treatments.

	Oct.	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**
High Temperature (°C)	0	-10	-20	-20	-20	-15	0
Low Temperature (°C)	-15	-30	-40	-40	-40	-35	-25
Interval (°C)	-3	-4	-4	-4	-4	-4	-5

Table 3.3 Population mean for traits measured in wild collected *Hydrangea quercifolia* seedlings. Latitude and longitude indicate location where the population was collected. Leaf spot values represent percent of leaf area affected by *Xanthomonas campestris* on plants exposed to natural inoculum. LT_{50} values represent the temperature where 50% of stem samples were killed during laboratory based controlled freezer testing.

Pop. ID	State	Lat. (°N)	Long. (°W)	Year Seed Sown	Seed Germ. (%)[1]	Leaf Spot (%)	Height (cm)[1]	Node Number[1]	Internode Length (cm)[1]	Branch Number[1]	Width (cm)[1]	LT_{50} (°C)[1]
2	FL	30.57	84.95	2018	21.9	3.5	60.4	10.2	5.4	3.9	16.4	-
3	FL	30.63	84.90	2018	82.2	3.7	56.4	10.7	5.8	4.1	31.4	-27.8
4	FL	30.70	84.85	2018	64.1	9.9	57.7	11.1	5.3	4.4	33.3	-26.9
5	FL	30.71	84.85	2018	62.3	9.5	59.1	10.6	5.8	4.0	26.3	-
6	FL	30.81	85.23	2018	87.3	10.3	49.0	10.0	4.7	4.8	31.2	-27.9
8	AL	31.91	87.38	2019	88.2	-[2]	32.5	6.8	4.7	-	-	-38.7
9	AL	33.34	86.72	2019	90.0	-	29.0	6.6	4.2	-	-	-35.0[3]
10	AL	34.09	87.61	2019	81.0	-	25.9	6.1	4.0	-	-	-36.4
11	TN	35.04	88.73	2018	74.2	10.6	53.0	10.8	4.6	3.9	23.7	-31.6
12	TN	35.05	88.24	2018	71.8	19.2	41.5	9.1	4.2	4.5	15.8	-31.1
13	TN	35.46	87.27	2018	89.0	13.3	37.8	8.9	4.3	3.6	12.6	-29.6
14	TN	35.80	85.62	2018	92.7	26.5	36.0	8.4	4.0	4.5	14.1	-34.1
16	MS	34.26	88.90	2018	60.7	14.8	48.8	10.9	4.4	4.4	25.5	-32.7
17	MS	34.60	88.18	2018	62.9	19.8	52.4	11.2	4.3	4.3	25.3	-30.5
18	MS	33.61	89.41	2018	57.8	16.5	52.2	10.2	4.8	3.8	17.9	-30.5
19	MS	33.42	89.26	2018	53.5	13.8	52.2	10.6	4.6	4.4	23.0	-33.6
20	MS	31.18	89.05	2018	73.6	12.2	50.7	11.9	4.7	5.0	31.1	-30.9
21	MS	31.75	88.52	2018	66.5	17.1	45.9	10.1	4.4	4.9	23.1	30.2
22	MS	32.00	89.36	2018	50.8	15.6	47.0	10.3	4.3	4.4	20.5	-29.1
23	MS	32.33	90.16	2018	59.6	13.5	49.3	11.6	4.3	4.7	25.6	30.7
24	LA	31.42	90.99	2019	28.0	-	30.0	7.2	4.1	-	-	-
27	LA	30.93	91.51	2019	71.0	-	39.9	7.6	5.2	-	-	-37.2
28	LA	31.81	91.76	2019	29.4	-	26.4	6.7	3.5	-	-	-
29	LA	31.22	92.67	2019	80.0	-	24.4	6.2	3.8	-	-	-36.3
31	LA	30.82	91.26	2019	40.0	-	38.6	8.2	4.8	-	-	-
35	AL	31.52	86.53	2019	11.0	-	31.0	7.4	3.9	-	-	-
37	AL	31.19	86.70	2019	85.0	-	39.6	7.9	4.9	-	-	-31.3
40	GA	31.90	85.11	2019	73.0	-	47.5	8.9	5.2	-	-	-31.6
41	AL	32.06	85.90	2019	37.0	-	28.9	6.6	4.2	-	-	-

Table 3.3 Continued

Pop. ID	State	Lat. (°N)	Long. (°W)	Year Seed Sown	Seed Germ. (%)[1]	Leaf Spot (%)	Height (cm)[1]	Node Number[1]	Internode Length (cm)[1]	Branch Number[1]	Width (cm)[1]	LT_{50} (°C)[1]
42	GA	31.31	85.08	2019	41.2	-	43.2	7.9	5.4	-	-	-
43	AL	31.48	85.63	2019	16.0	-	60.1	10.7	5.7	-	-	-
44	AL	32.96	87.37	2019	63.0	-	31.6	7.0	4.3	-	-	-34.6
45	AL	32.40	86.78	2019	45.0	-	35.3	7.7	4.4	-	-	-
46	AL	32.60	85.88	2019	73.5	-	27.4	7.1	3.7	-	-	-38.3
47	AL	32.55	85.48	2019	65.0	-	40.5	8.5	4.6	-	-	-35.8
49	GA	32.84	84.84	2019	47.0	-	22.0	5.6	3.8	-	-	-
50	GA	32.97	84.50	2019	58.0	-	32.1	7.4	4.2	-	-	-
51	GA	33.80	84.32	2019	47.0	-	20.7	5.5	3.6	-	-	-
52	AL	34.47	86.05	2019	68.0	-	22.7	5.7	3.8	-	-	-
54	AL	34.42	86.59	2019	42.0	-	20.0	5.9	3.1	-	-	-
56	AL	33.88	86.87	2019	66.0	-	17.5	5.3	3.1	-	-	-
57	AL	33.28	87.41	2019	83.0	-	31.9	6.7	4.6	-	-	-34.8
58	AL	34.35	85.67	2019	76.0	-	25.3	6.2	3.9	-	-	-36.0
60	GA	34.43	85.34	2019	78.0	-	34.7	7.1	4.8	-	-	-33.2
61	GA	34.87	84.72	2019	30.7	-	27.2	7.0	3.4	-	-	-
62	GA	34.85	85.48	2019	67.5	-	22.8	6.6	3.4	-	-	-
64	AL	33.49	85.80	2019	63.0	-	21.8	6.1	3.3	-	-	-
66	AL	33.84	85.65	2019	61.0	-	25.7	5.9	4.0	-	-	-
67	MS	31.33	89.94	2019	67.0	-	46.1	9.5	4.8	-	-	-33.1
68	MS	32.35	90.78	2019	47.0	-	41.9	7.9	5.2	-	-	-
70	MS	34.42	89.35	2019	12.0	-	24.2	5.5	3.8	-	-	-
71	MS	34.52	88.56	2019	77.0	-	24.1	6.0	3.8	-	-	-
72	MS	33.24	89.10	2019	79.0	-	28.7	7.1	4.0	-	-	-35.0
73	MS	33.84	89.67	2019	65.0	-	28.7	6.7	4.1	-	-	-
75	LA	32.08	92.06	2019	42.0	-	44.9	8.3	5.3	-	-	-

[1] Values for populations that were phenotyped in multiple environments or years represent means across all environments tested.

[2] Dashes (-) indicate the population was not analyzed for the trait.

[3] Less than 50% of stem samples were killed at coldest temperature tested. Temperature listed was the coldest temperature tested.

Figures

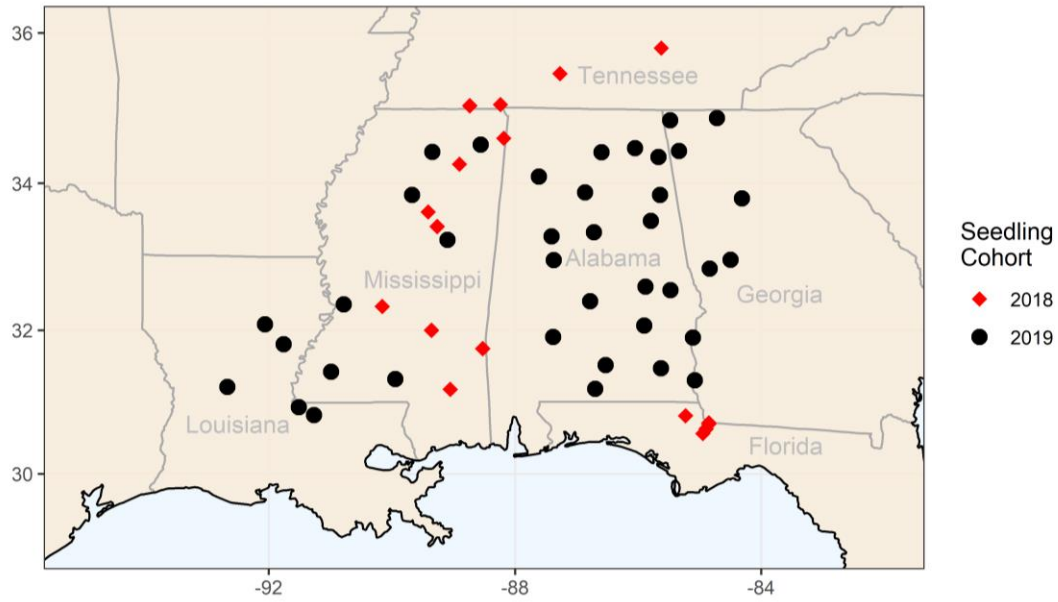

Figure 3.1 Map of *Hydrangea quercifolia* seedling cohorts and the locations of their collection. Red diamonds indicate locations of seed collected in 2017, which was germinated in 2018 (older cohort). Black circles indicate locations of seed collected in 2018, which was germinated in 2019 (younger cohort).

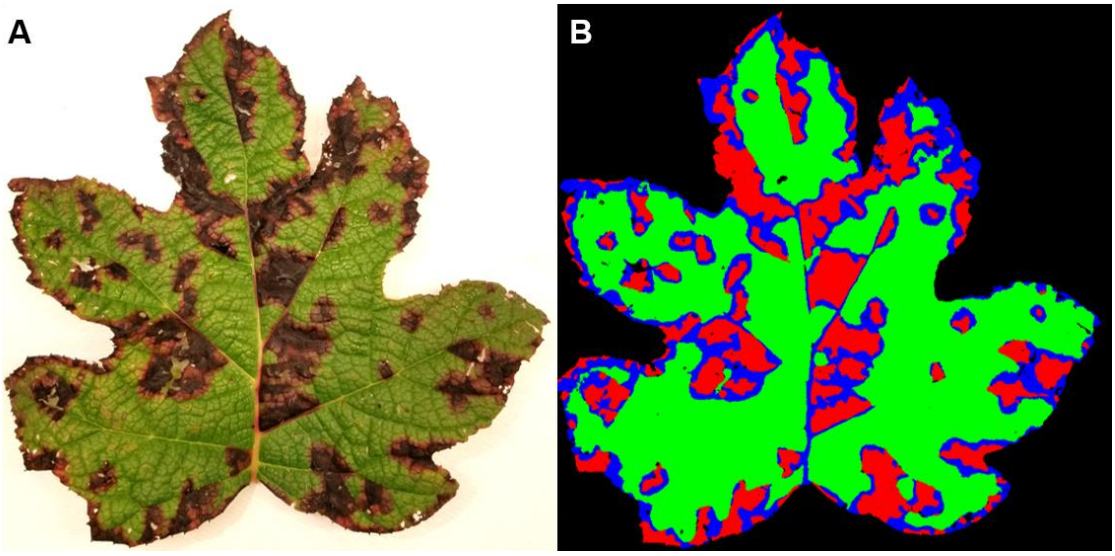

Figure 3.2 Example of leaf spot and image analysis. A) Leaf image with leaf spot. B) Image segmented into background pixels (black), healthy leaf (green), necrotic tissue (red) and discoloration (blue).

Figure 3.3 Photographs of sampling for cold hardiness. A) Overwintering structure protects plants from cold damage prior to sampling. B) Stems are cut into 3.5 cm sections and color-coded by population or cultivar. C) Stems from all populations or cultivars are placed into bags representing a temperature treatment with a thermocouple inserted into the pith of one representative stem. D) Bags are placed into programmable freezer and subsequently removed at the designated temperature.

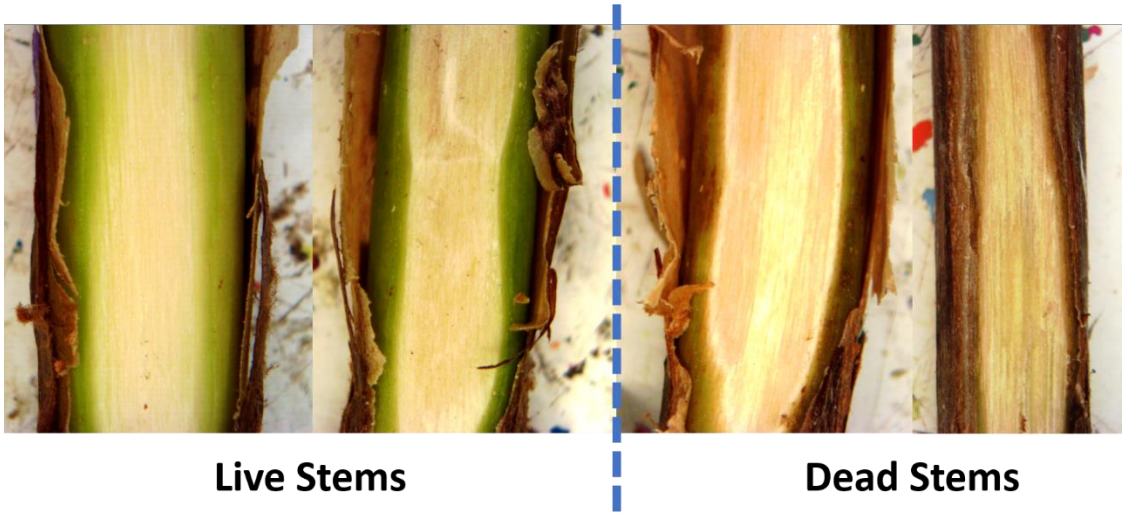

Figure 3.4 Photographs showing cold damage in *Hydrangea quercifolia* stems. Vascular browning is the primary symptom differentiating live and dead stems. All four photographs are taken at 12x magnification.

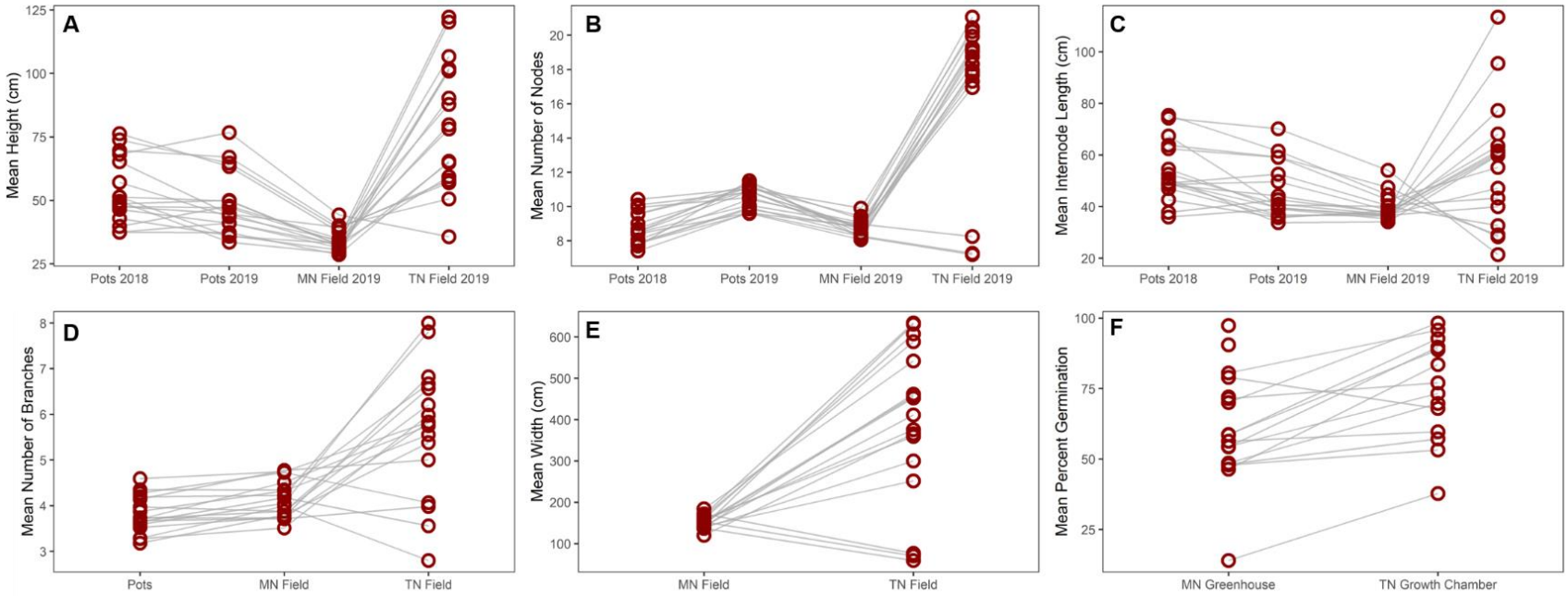

Figure 3.5 Interaction of population mean and environment in *Hydrangea quercifolia* plant architecture and seed germination. Each circle represents the mean of a wild seedling population in each environment and year combination. Circles representing the same population are connected with a line.

Figure 3.6 Photograph showing representative variation in seedling height and internode length in wild collected *Hydrangea quercifolia*.

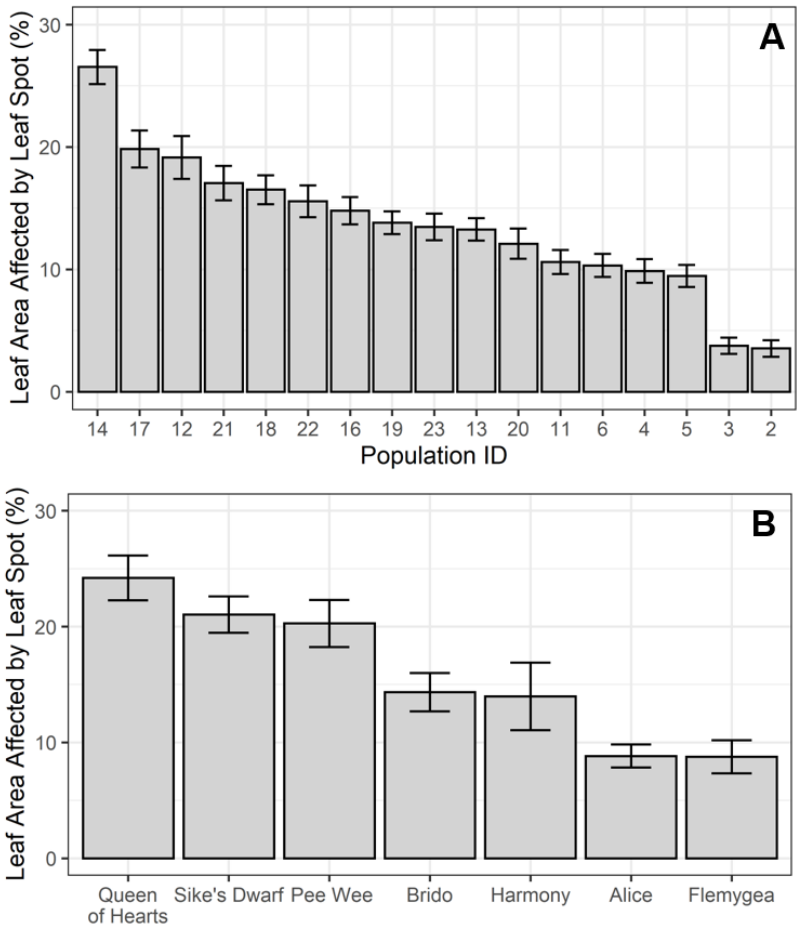

Figure 3.7 Bar plots indicating mean percent leaf area affected by leaf spot (*Xanthomonas campestris*) in *Hydrangea quercifolia* which were exposed to natural inoculum. A) Wild collected seedling populations and B) cultivars. Error bars indicate standard error of the mean.

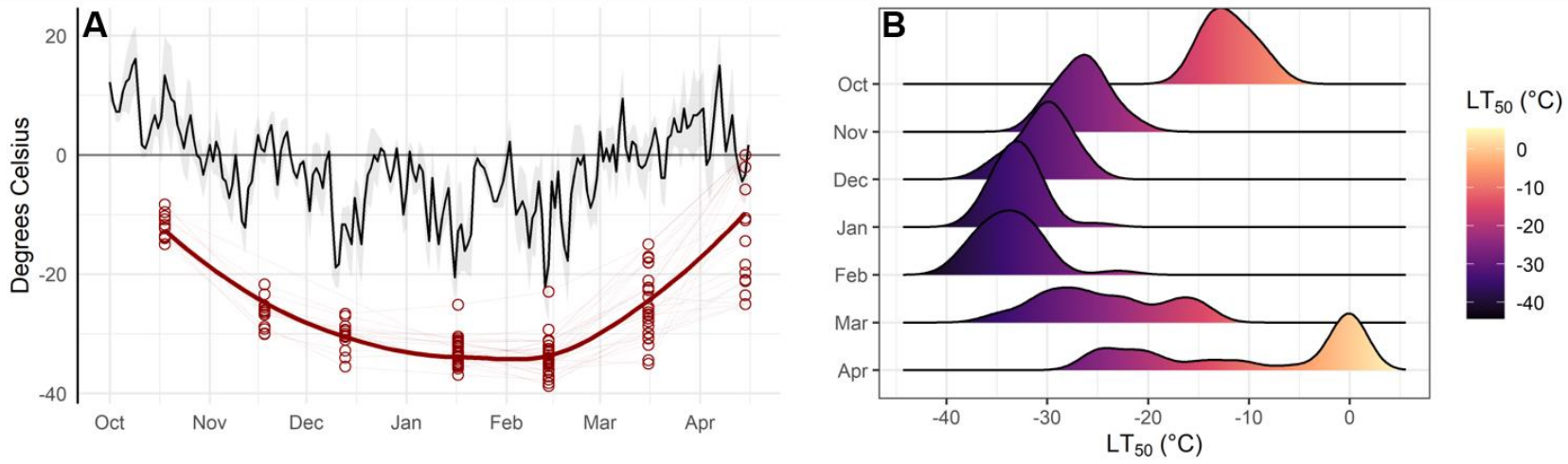

Figure 3.8 Cold hardiness in *Hydrangea quercifolia* measured with controlled freezing assay measured as the lowest temperature where 50% of the samples survive (LT_{50}). A) LT_{50} and outdoor temperature in winter 2019-2020. Black line indicates mean daily temperature in Chanhassen, MN while grey ribbon indicates daily high and low temperature. Circles represent LT_{50} for each population and cultivar tested each month. B) Ridgeline plot displays density distribution of estimated LT_{50} each month with color representing LT_{50}.

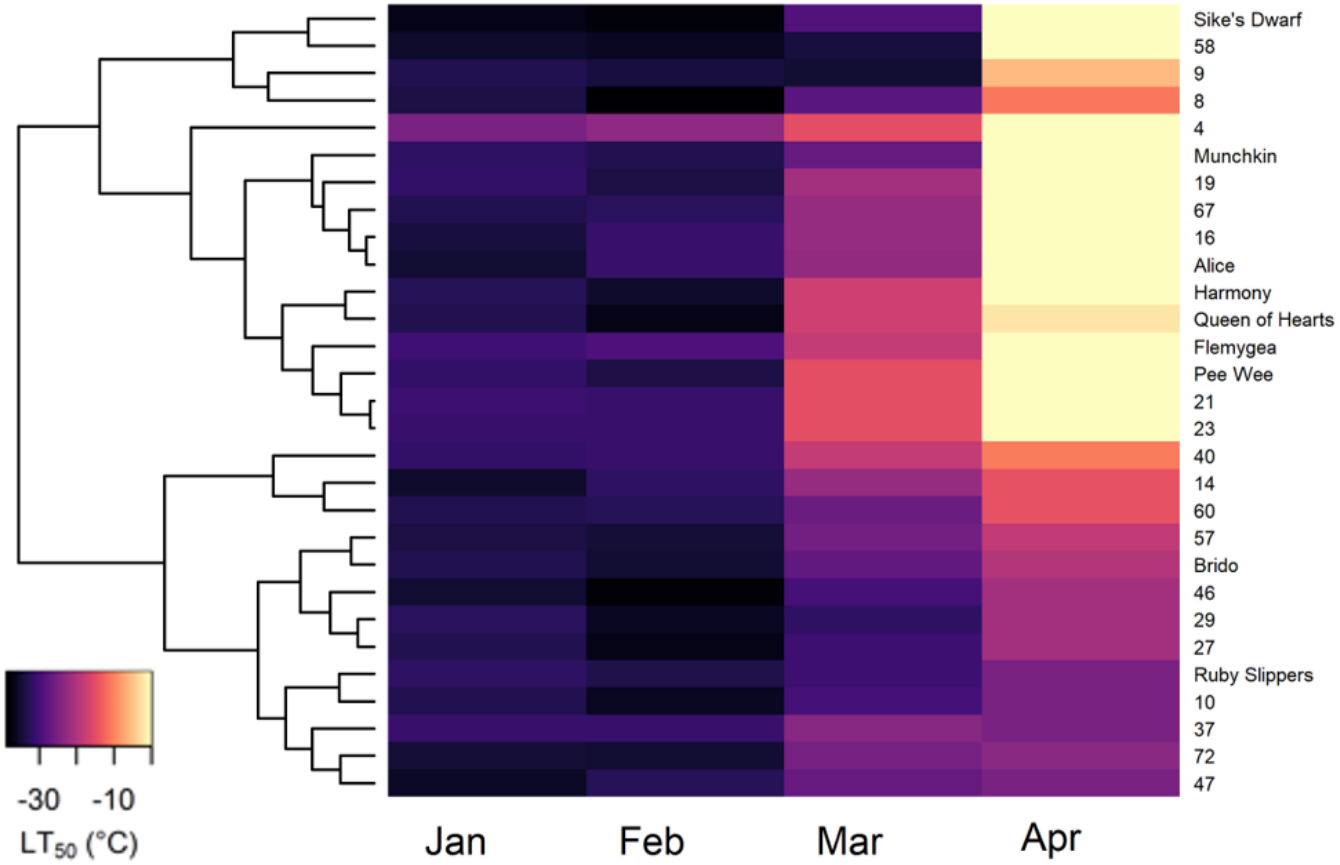

Figure 3.9 Heatmap of LT_{50} for each *Hydrangea quercifolia* population and cultivar during deacclimation. Dark colors represent lower LT_{50} and lighter colors represent higher LT_{50}.

Figure 3.10 Photos showing putative mutants with increased basal branching in wild collected *Hydrangea quercifolia* seedling populations. A) All eight putative mutants from population 22 in 2018. B) Comparison of one putative mutant next to a typical seedling from population 3.

Figure 3.11 Photograph of *Hydrangea quercifolia* seedlings with contrasting leaf spot severity. A representative seedling from population 14 (left) and a representative from population 5 (right).

Bibliography

Abe, R., & Ohtani, K. (2013). An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. J. Ethnopharmacol., 145:554–565.

Adkins, J. A., Dirr, M. A., & Lindstrom, O. M. (2003). Cold hardiness estimates for ten *Hydrangea* taxa. Acta Hort., 618:163–168.

Aldrete, A., Mexal, J. G., & Burr, K. E. (2008). Seedling cold hardiness, bud set, and bud break in nine provenances of *Pinus greggii* Engelm. For. Ecol. Manage., 255:3672–3676. doi:10.1016/j.foreco.2008.02.054

Alexander, L. (2017). Production of triploid *Hydrangea macrophylla* via unreduced gamete breeding. HortScience, 52:221–224. doi:10.21273/HORTSCI11358-16

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. Nat. Rev. Genet., 11:697–710. doi:10.1038/nrg2844

Alonso-Blanco, C., Aarts, M. G. M., Bentsink, L., Keurentjes, J. J. B., Reymond, M., Vreugdenhil, D., & Koornneef, M. (2009). What has natural variation taught us about plant development, physiology, and adaptation? Plant Cell, 21:1877–1896. doi.org/10.1105/tpc.109.068114

Arora, R., & Rowland, L. J. (2011). Physiological research on winter-hardiness: deacclimation resistance, reacclimation ability, photoprotection strategies, and a cold acclimation protocol design. HortScience, 46:1070–1078.

Awad, L., Fady, B., Khater, C., Roig, A., & Cheddadi, R. (2014). Genetic structure and diversity of the endangered fir tree of Lebanon (*Abies cilicica* Carr.): implications for conservation. PLoS ONE, 9:1–12. doi:10.1371/journal.pone.0090086

Banerjee, R., Das, N. K., Doss, S. G., Saha, A. K., Bajpai, A. K., & Bindroo, B. B. (2012). Narrow sense heritability estimates of bacterial leaf spot resistance in pseudo F_2 (F_1) population of mulberry (*Morus* spp.). Eur. J. Plant Pathol., 2, 537–544. doi:10.1007/s10658-011-9894-z

Banks, W. H., Jr. (1953). Ethnobotany of the Cherokee Indians. Master's Thesis, Univ. of Tennessee.

Bartram, W. (1791) Travels through North & South Carolina, Georgia, East & West Florida, the Cherokee Country, the extensive territories of the Muscogulges, or Creek Confederacy, and the country of the Chactaws; containing an account of the soil and natural productions of those regions, together with observations on the manners of the Indians. James & Johnson, Philadelphia.

Baysal-Gurel, F., Kabir, N., & Blalock, A. (2016). Foliar diseases of hydrangeas. Tennessee State Univ. Extension. 1–7.

Beck, W. T., & Ranney, T. G. (2014). Ploidy levels and interploid hybridization in panicle hydrangea (*Hydrangea paniculata*). SNA Research Conference, 59:181–187.

Beckman, T. G., & Pusey, P. L. (2001). Field testing peach rootstocks for resistance to armillaria root rot. HortScience, 36:101–103.

Behnke, M. (1979). Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. Theor. Appl. Genet., 55:69–71. doi:10.1007/BF00285192

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: building and genotyping loci de novo from short-read sequences. G3, 1:171–182. doi:10.1534/g3.111.000240

Cerbah, M., Mortreau, E., Brown, S., Siljak-Yakovlev, S., Bertrand, H., & C, L. (2001). Genome size and species relationships in the genus *Hydrangea*. Theor. Appl. Genet., 103:45–51.

Chappell, M., Robacker, C., & Jenkins, T. M. (2008). Genetic diversity of seven deciduous azalea species (*Rhododendron* spp. section *Pentanthera*) native to the eastern United States. J. Am. Soc. Hortic. Sci., 133:374–382.

Choi, H., Ito, T., Yokogawa, M., Kaneko, S., Suyama, Y., & Isagi, Y. (2017). Population and genetic status of a critically endangered species in Korea: *Hydrangea luteovenosa* (Hydrangeaceae), Korean J. Pl. Taxon. 47:1–5.

Chung, M. Y., Chung, J. M., López-Pujol, J., Park, S. J., & Chung, M. G. (2013). Genetic diversity in three species of *Forsythia* (Oleaceae) endemic to Korea: implications for population history, taxonomy, and conservation. Biochem. Syst. Ecol., 47:80–92. doi:10.1016/j.bse.2012.11.005

Church, G. (2001). Hydrangeas. Firefly Books, Buffalo, NY.

Cochran, D. R., Benitez-Ramirez, M., & Fulcher, A. (2014). Effect of branch-inducing treatments on growth of tissue culture and cutting-propagated *Hydrangea quercifolia* "Alice." J. Environ. Hortic., 32:182–188.

Cozzo, D. N. (2004). Ethnobotanical classification system and medicinal ethnobotany of the Eastern Band of the Cherokee Indians. Doctoral Thesis, Univ. of Georgia Athens.

Crespel, L., Le Bras, C., Relion, D., & Morel, P. (2014). Genotype × year interaction and broad-sense heritability of architectural characteristics in rose bush. Plant Breeding, 133:412–418. doi:10.1111/pbr.12157

Crespel, L., Morel, P., & Galopin, G. (2012). Architectural and genetic characterization of *Hydrangea aspera* subsp. *aspera* Kawakami group, *H. aspera* subsp. *sargentiana* and their hybrids. Euphytica, 184:289–299. doi:10.1007/s10681-011-0477-z

Crespel, L., Sigogne, M., Donés, N., Relion, D., & Morel, P. (2013). Identification of relevant morphological, topological and geometrical variables to characterize the architecture of rose bushes in relation to plant shape. Euphytica, 191:129–140. doi:10.1007/s10681-013-0902-6

De Kort, H., Vandepitte, K., Bruun, H. H., Closset-Kopp, D., Honnay, O., & Mergeay, J. (2014). Landscape genomics and a common garden trial reveal adaptive differentiation to temperature across Europe in the tree species *Alnus glutinosa*. Mol. Ecol., 23:4709–4721. doi:10.1111/mec.12813

De Smet, Y., Mendoza, C. G., Wanke, S., Goetghebeur, P., & Samain, M. S. (2015). Molecular phylogenetics and new (infra)generic classification to alleviate polyphyly in tribe Hydrangeeae (Cornales: Hydrangeaceae). Taxon, 64:741–753. doi:10.12705/644.6

Dirr, M. A. (2004). Hydrangeas for American gardens. Timber Press, Portland, OR.

Dirr, M. A., Lindstrom Jr., O. M., Lewandowski, R., & Vehr, M. J. (1993). Cold hardiness estimates of woody taxa from cultivated and wild collections. J. Environ. Hortic., 11:200–203.

Dixon, A. G. O., Ngeve, J. M., & Nukenine, E. N. (2002). Genotype × environment effects on severity of cassava bacterial blight disease caused by *Xanthomonas axonopodis* pv. *manihotis*. Eur. J. Plant Pathol., 108:763–770.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. J. Veg. Sci., 14:927–930.

Dyer, R. J., Chan, D. M., Gardiakos, V. A., & Meadows, C. A. (2012). Pollination graphs: quantifying pollen pool covariance networks and the influence of intervening landscape on genetic connectivity in the North American understory tree, *Cornus florida* L. Landscape Ecol., 27:239–251.

Eckert, A. J., Bower, A. D., González-Martínez, S. C., Wegrzyn, J. L., Coop, G., & Neale, D. B. (2010). Back to nature: ecological genomics of loblolly pine (*Pinus*

taeda, Pinaceae). Mol. Ecol., 19:3789–3805. doi:10.1111/j.1365-294X.2010.04698.x

Egawa, C. (2017). Wind dispersal of alien plant species into remnant natural vegetation from adjacent agricultural fields. Glob. Ecol. Conserv., 11:33–41.

Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity, Nat. Gen. Rev., 17:422–433. doi:10.1038/nrg.2016.58

Ellstrand, N. C., & Elam, D. R. (1993). Population genetic consequences of small population size: implications for plant conservation. Annu. Rev. Ecol. Syst., 24:217–242. doi:10.1146/annurev.es.24.110193.001245

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE, 6:1–10. doi:10.1371/journal.pone.0019379

Engelmann, F. (2011). Use of biotechnologies for the conservation of plant biodiversity. In Vitro Cell. Dev. Biol. - Plant, 47:5–16. doi:10.1007/s11627-010-9327-2

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol., 14:2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Fick, S.E. and R.J. Hijmans, 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. Int. J. Climatol., 37:4302-4315.

Forester, B. R., Lasky, J. R., Wagner, H. H., & Urban, D. L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype – environment associations. Molec. Ecol., 27:2215–2233. doi:10.1111/mec.14584

Friedman, J. M., Roelle, J. E., Gaskin, J. F., Pepper, A. E., & Manhart, J. R. (2008). Latitudinal variation in cold hardiness in introduced *Tamarix* and native *Populus*. Evol. Appl., 598–607. doi:10.1111/j.1752-4571.2008.00044.x

Fu, Z.-Z., Lei, Y.-K., Peng, D.-D., & Li, Y. (2016). Population genetics of the widespread shrub *Forsythia suspensa* (Oleaceae) in warm-temperate China using microsatellite loci: implication for conservation. Plant. Syst. Evol., 302:1–9. doi:10.1007/s00606-015-1241-y

Funamoto, T., & Ogawa, M. (2002). A cytogeographical study in *Hydrangea paniculata* Sieb. (Saxifragaceae s. l.) in Japan. Chromosome Science, 6:73–82.

Funamoto, T., & Tanaka, R. (1988). Karyomorphological studies in some taxa of *Hydrangea* from Japan. Kromosomo, 2:1583–1594.

Gouker, F. E., Difazio, S. P., Bubner, B., Zander, M., & Smart, L. B. (2019). Genetic diversity and population structure of native, naturalized, and cultivated *Salix purpurea*. Tree Genetics and Genomes, 15:1–14.

Granados Mendoza, C., Wanke, S., Goetghebeur, P., & Samain, M. S. (2013). Facilitating wide hybridization in *Hydrangea* s. l. cultivars: A phylogenetic and marker-assisted breeding approach. Mol. Breed., 32:233–239. doi:10.1007/s11032-012-9822-8

Greer, S. P., & Rinehart, T. A. (2009). In vitro germination and dormancy responses of *Hydrangea macrophylla* and *Hydrangea paniculata* seeds to ethyl methane sulfonate and cold treatment. HortScience, 44:764–769.

Gronovius, J. F. (1739). Flora Virginica. Reprinted (1946) by Arnold Arboretum, Boston, MA.

Gupta, P. K., Balyan, H. S., Sharma, P. C., & Ramesh, B. (1996). Microsatellites in plants: A new class of molecular markers. Curr. Sci., 70:45-54.

Hagan, A. K., & Mullen, J. M. (2001). Diseases of Hydrangea. Alabama Cooperative Extension System, Alabama A&M And Auburn Univ., 1–8.

Hagan, A. K., Olive, J. W., Stephenson, J., & Rivas-Davila, M. E. (2004). Impact of application rate and interval on the control of powdery mildew and *Cercospora* leaf spot on bigleaf hydrangea with azoxystrobin. J. Environ. Hortic., 22:58–62.

Hahn, C. Z., Michalski, S. G., Fischer, M., & Durka, W. (2017). Genetic diversity and differentiation follow secondary succession in a multi-species study on woody plants from subtropical China. J. Plant Ecol., 10:213–221. doi:0.1093/jpe/rtw054

Halcomb, M., & Reed, S. (2012). Hydrangea production. Univ. of Tennessee Extension Publication.

Hamrick, J. L., Godt, M. J. W., & Sherman-Broyles, S. L. (1992). Factors influencing levels of genetic diversity in woody plant species. New Forests, 6:95–124. doi:10.1007/978-94-011-2815-5

Hayes, R. J., Trent, M. A., Mou, B., Simko, I., Gebben, S. J., & Bull, C. T. (2014). Baby leaf lettuce germplasm enhancement: developing diverse populations with resistance to bacterial leaf spot caused by *Xanthomonas campestris* pv. *vitians*. HortScience, 49:18–24.

Hempel, P., Hohe, A., & Tränkner, C. (2018). Molecular reconstruction of an old pedigree of diploid and triploid *Hydrangea macrophylla* genotypes. Front. Plant Sci., 9:1-13. doi:10.3389/fpls.2018.00429

Hokanson, S.C. and S. McNamara. (2013). Can't always get what we want! Finding and creating cold hardiness for screening at the University of Minnesota. Acta Hortic. 900:193-202.

Hollick, A. (1925). A new fossil species of *Hydrangea*. J. Torrey Bot. Soc., 52:21–22.

Hu, L.-J., Uchiyama, K., Saito, Y., & Ide, Y. (2010). Contrasting patterns of nuclear microsatellite genetic structure of *Fraxinus mandshurica* var. *japonica* between northern and southern populations in Japan. J. Biogeogr., 37:1131–1143.

Hufford, L. (1995). Seed morphology of Hydrangeaceae and its phylogenetic implications. Int. J. Plant Sci., 156:555–580.

Hufford, L., Moody, M. L., & Soltis, D. E. (2001). A phylogenetic analysis of Hydrangeaceae based on sequences of the plastid gene *matk* and their combination with *rbcL* and morphological data. Int. J. Plant Sci., 162:835–846.

Hurme, P., Repo, T., Savolainen, O., & Pääkkönen, T. (1997). Climatic adaptation of bud set and frost hardiness in Scots pine (*Pinus sylvestris*). Can. J. For. Res., 27:716–723. doi:10.1139/cjfr-27-5-716

Ismail, S. A., Ghazoul, J., Ravikanth, G., Shaanker, R. U., Kushalappa, C. G., & Kettle, C. J. (2012). Does long-distance pollen dispersal preclude inbreeding in tropical trees? Fragmentation genetics of *Dysoxylum malabaricum* in an agro-forest landscape. Mol. Ecol., 21:5484–5496. doi:10.1111/mec.12054

Ito, T., Kaneko, S., Yokogawa, M., Song, G., Choi, H., & Isagi, Y. (2013). Isolation and characterization of microsatellite markers for *Hydrangea luteovenosa* (Hydrangeaceae), an endangered species in Korea. Korean Journal of Plant Taxonomy, 43:30–33. doi:10.11110/kjpt.2013.43.1.30

Jacobs, S. J. (2010). Flag flower morphology and phylogeny of Hydrangeaceae tribe Hydrangeeae. Master's Thesis, Washington State Univ.

Jha, S., & Dick, C. W. (2010). Native bees mediate long-distance pollen dispersal in a shade coffee landscape mosaic. PNAS, 107:13760–13764.

Jombart, T., Pontier, D., & Dufour, A.-B. (2009). Genetic markers in the playground of multivariate analysis. Heredity, 102:330–341. doi:10.1038/hdy.2008.130

Joung, Y. H., Suh, J. K., Lee, N. S., Eum, S. M., Choi, I.-Y., & Roh, M. S. (2010). Identification of Hydrangeaceae accessions of wild origin from Jeju, Korea, using molecular markers. Plant Genetic Resources, 8:235–241. doi:10.1017/S1479262110000286

Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ, 1–14. doi:10.7717/peerj.281

Kanno, H., & Seiwa, K. (2004). Sexual vs. vegetative reproduction in relation to forest dynamics in the understory shrub, *Hydrangea paniculata* (Saxifragaceae). Plant Ecology, 170:43–53. doi:10.1023/B:VEGE.0000019027.88318.54

Kästner, U., Klocke, E., & Abel, S. (2017). Regeneration of protoplasts after somatic hybridization of *Hydrangea*. Plant Cell Tissue Organ Cult., 129:359–373. doi:10.1007/s11240-017-1183-x

Kitamura, Y., Hosokawa, M., Tanaka, C., & Yazawa, S. (2008). Identification and sterilization of epiphytic bacterial flora near hydrangea shoot apical meristems. J. Jap. Soc. Hort. Sci., 77:418–425. doi:10.2503/Jjshs1.77.418

Kostel-Hughes, F., Young, T. P., & Wehr, J. D. (2005). Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation to seed size. J. Torrey Bot. Soc., 132:50–61. doi:10.3159/10955674(2005)132[50:EOLLDO]2.0.CO;2

Kudo, N., & Niimi, Y. (1999). Production of Interspecific Hybrids between *Hydrangea macrophylla* f. *hortensia* (Lam.) Rehd. and *H. arborescens* L. J. Jap. Soc. Hort. Sci., 68:428–439. doi:10.1248/cpb.37.3229

Lander, T. A., Boshier, D. H., & Harris, S. A. (2010). Fragmented but not isolated: Contribution of single trees, small patches and long-distance pollen flow to genetic connectivity for *Gomortega keule*, an endangered Chilean tree. Biological Conservation, 143:2583–2590. doi:10.1016/j.biocon.2010.06.028

Lawson-Hall, T., & Rothera, B. (1995). Hydrangeas a gardeners' guide. Timber Press, Portland, OR.

Ledbetter, D. I., & Preece, J. E. (2004). Thidiazuron stimulates adventitious shoot production from *Hydrangea quercifolia* Bartr. leaf explants. Sci. Hortic., 101:121–126. doi:10.1016/j.scienta.2003.09.014

Li, M., Chen, S., Shi, S., Zhang, Z., Liao, W., Wu, W., Zhou, R., Fan, Q. (2015). High genetic diversity and weak population structure of *Rhododendron*

jinggangshanicum, a threatened endemic species in Mount Jinggangshan of China. Biochem. Syst. Ecol., 58:178–186. doi:10.1016/j.bse.2014.12.008

Li-Marchetti, C., Bras, C. Le, Chastellier, A., Relion, D., Morel, P., Sakr, S., Oyant Hibrand-Saint, L., Crespel, L. (2017). 3D phenotyping and QTL analysis of a complex character: rose bush architecture. Tree Genet. Genomes, 13. doi:10.1007/s11295-017-1194-0

Linnaeus, C. (1753). Species plantarum: exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. Stockholm: Impensis Laurentii Salvii.

Long, C., & Li, R. (2004). Ethnobotanical studies on medicinal plants used by the red-headed Yao People in Jinping, Yunnan Province, China. J. Ethnopharmacol., 90:389–395.

Lugo, A. E. (2000). Effects and outcomes of Caribbean hurricanes in a climate change scenario. The Science of the Total Environment, 262:243–251.

Maas, J. L., Gouin-Behe, C., Hartung, J. S., & Hokanson, S. C. (2000). Sources of resistance for two differentially pathogenic strains of *Xanthomonas fragariae* in *Fragaria* genotypes. HortScience, 35:128–131.

Mallet, C., Mallet, R., van Trier, H. (1992). Hydrangeas: species and cultivars. Center d'Art Floral, Varengeville, France.

Manchester, S. R. (1994). Fruits and seeds of the middle Eocene nut beds flora, Clarno Formation, Oregon. Palaeontographica Americana, 58:1-205.

Manel, S., Poncet, B. N., Legendre, P., Gugerli, F., & Holderegger, R. (2010). Common factors drive adaptive genetic variation at different spatial scales in *Arabis alpina*. Molecular Ecology, 19:3824–3835. doi:10.1111/j.1365-294X.2010.04716.x

Martin, A. D., Quinn, K. M., & Park, J. H. (2011). MCMCpack: markov chain monte carlo in R. J. Stat. Softw., 42:1–21.

McCarragher, S., Goldblum, D., & Rigg, L. (2011). Geographic variation of germination, growth, and mortality in sugar maple (*Acer saccharum*): Common garden and reciprocal dispersal experiments. Phys. Geogr., 32:1–21. doi:10.2747/0272-3646.32.1.1

McClintock, E. (1957). A monograph of the genus *Hydrangea*. Proc. Calif. Acad. Sci., XXIX:147–256. doi:10.1016/S0079-8169(08)61510-X

Mcnamara, S., & Hokanson, S. C. (2010). Cold hardiness of weigela (*Weigela florida* Bunge) cultivars. J. Environ. Hortic., 28:35–40.

Mcnamara, S., Pellett, H., Florkowska, M., & Lindstrom, O. (2002). Comparison of the cold hardiness of landscape tree and shrub cultivars growing at two disparate geographic locations. J. Environ. Hortic., 20:77–81.

Miao, C. Y., Li, Y., Yang, J., & Mao, R. L. (2017). Landscape genomics reveal that ecological character determines adaptation: a case study in smoke tree (*Cotinus coggygria* Scop.). BMC Evolutionary Biology, 17:1–11. doi:10.1186/s12862-017-1055-3

Miller, J. F., & Hammond, J. J. (1991). Inheritance of reduced height in sunflower. Euphytica, 53:131–136.

Mmbaga, M. T., & Oliver, J. B. (2007). Effect of biopesticides on foliar diseases and japanese beetle (*Popillia japonica*) adults in roses (Rosa spp.), oakleaf hydrangea (*Hydrangea quercifolia*), and crapemyrtle (*Lagerstroemia indica*). Arboric. Urban For., 33:210–219.

Mmbaga, M. T., Kim, M. S., MacKasmiel, L., & Li, Y. (2012). Evaluation of *Hydrangea macrophylla* for resistance to leaf-spot diseases. J. Phytopathol., 160:88–97. doi:10.1111/j.1439-0434.2011.01862.x

Mmbaga, M. T., Kim, M.-S., Mackasmiel, L., & Klopfenstein, N. B. (2015). Differentiation of *Corynespora cassiicola* and *Cercospora* sp. in leaf-spot diseases of *Hydrangea macrophylla* using a PCR-mediated method. Can. J. Plant Sci., 95:711–717. doi:10.4141/cjps-2014-354

Moles, A. T., Warton, D. I., Warman, L., Swenson, N. G., Laffan, S. W., Zanne, A. E., Pitman, A., Hemmings, F. A., Leishman, M. R. (2009). Global patterns in plant height. J. Ecol., 97:923–932. doi:10.1111/j.1365-2745.2009.01526.x

Morgan, D. R., & Soltis, D. E. (1993). Phylogenetic relationships among members of Saxifragaceae sensu lato based on *rbcL* sequence data. Ann. Mo. Bot. Gard., 80:631. https://doi.org/10.2307/2399851

Mortreau, E., Bertrand, H., Lambert, C., & Lallemand, J. (2003). Collection of *Hydrangea*: genetic resources characterization. Acta Hortic., 623:231–238. doi:10.17660/ActaHortic.2003.623.25

Mortreau, E., Siljak-Yakovlev, S., Cerbah, M., Brown, S. C., Bertrand, H., & Lambert, C. (2010). Cytogenetic characterization of *Hydrangea involucrata* Sieb. and *H. aspera* D. Don complex (Hydrangeaceae): Genetic, evolutional, and taxonomic

implications. Tree Genetics and Genomes, 6:137–148. doi:10.1007/s11295-009-0235-8

Mosca, E., Eckert, A. J., Di Pierro, E. A., Rocchini, D., La Porta, N., Belletti, P., & Neale, D. B. (2012). The geographical and environmental determinants of genetic diversity for four alpine conifers of the European Alps. Mol. Ecol., 21:5530–5545. doi:10.1111/mec.12043

Naqvi, S. F., Inam-ul-Haq, M., Tahir, M. I., & Mughal, S. M. (2012). Screening of sesame germplasm for resistance against the bacterial blight caused by *Xanthomonas campestris* pv. sesami. Pak. J. Agri. Sci., 49:131–134.

Nei, M. (1972). Genetic distance between populations. Am. Nat., 106:283–292. doi:10.1086/282187

Niklas, K. J., & Brown, R. Malcolm, J. (1981). Ultrastructural and paleobiochemical correlations among fossil leaf tissues from the St. Maries River (Clarkia) area, northern Idaho, USA. Am. J. Bot., 68:332–341.

Pagter, M., & Williams, M. (2011). Frost dehardening and rehardening of *Hydrangea macrophylla* stems and buds. HortScience, 46:1121–1126. doi:10.5344/ajev.2016.16078

Pagter, M., Hausman, J. F., & Arora, R. (2011a). Deacclimation kinetics and carbohydrate changes in stem tissues of *Hydrangea* in response to an experimental warm spell. Plant Sci., 180:140–148. doi:10.1016/j.plantsci.2010.07.009

Pagter, M., Jensen, C. R., Petersen, K. K., Liu, F., & Arora, R. (2008a). Changes in carbohydrates, ABA and bark proteins during seasonal cold acclimation and deacclimation in *Hydrangea* species differing in cold hardiness. Physiol. Plant., 134:473–485. doi:10.1111/j.1399-3054.2008.01154.x

Pagter, M., Kristoffersen, A., Brønnum, P., & Jensen, M. (2010). Phenotypic differences in development of cold hardiness in three latitudinal populations of *Acer platanoides* L. Scand. J. For. Res., 25:412–420. doi:10.1080/02827581.2010.512872

Pagter, M., Lefèvre, I., Arora, R., & Hausman, J. F. (2011b). Quantitative and qualitative changes in carbohydrates associated with spring deacclimation in contrasting *Hydrangea* species. Environ. Exp. Bot., 72:358–367. doi:10.1016/j.envexpbot.2011.02.019

Pagter, M., Liu, F., Jensen, C. R., & Petersen, K. K. (2008b). Effects of chilling temperatures and short photoperiod on PSII function, sugar concentrations and xylem sap ABA concentrations in two *Hydrangea* species. Plant Sci., 175:547–555. doi:10.1016/j.plantsci.2008.06.006

Pagter, M., Sergeant, K., Møller, S. M., Bertram, H. C., & Renaut, J. (2014). Changes in the proteome and water state in bark and xylem of *Hydrangea paniculata* during loss of freezing tolerance. Environ. Exp. Bot., 106:99–111. doi:10.1016/j.envexpbot.2013.11.009

Palsbøll, P. J., Bérubé, M., & Allendorf, F. W. (2007). Identification of management units using population genetic data. Trends Ecol. Evol., 22:11–16. doi:10.1016/j.tree.2006.09.003

Pilatowski, R. E. (1982). A taxonomic study of the *Hydrangea arborescens* complex. Castanea, 47: 84–98.

Pounders, C. T., Blythe, E. K., Fare, D. C., Knox, G. W., & Sibley, J. L. (2010). Crapemyrtle genotype x environment interactions, and trait stability for plant height, leaf-out, and flowering. HortScience, 45:198–207.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155:945–959. doi:10.1111/j.1471-8286.2007.01758.x

Qiu, Y.-X., Qi, X.-S., Jin, X.-F., Tao, X.-Y., Fu, C.-X., Naiki, A., & Comes, H. P. (2009). Population genetic structure, phylogeography, and demographic history of *Platycrater arguta* (Hydrangeaceae) endemic to East China and South Japan, inferred from chloroplast DNA sequence variation. Taxon, 58:1226–1241.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reed, S. M. (2000). Compatibility studies in *Hydrangea*. J. Environ. Hortic., 18:29–33.

Reed, S. M. (2003). Self-incompatibility in *Hydrangea paniculata* and *H. quercifolia*. SNA Research Conference, 48:485–487.

Reed, S. M. (2004). Self-incompatibility and time of stigma receptivity in two species of *Hydrangea*. HortScience, 39:312–315.

Reed, S. M. (2005). Pollination biology of *Hydrangea macrophylla*. HortScience, 40:335–338.

Reed, S. M. (2009). Advanced Generation Progeny from Hybrids of *Hydrangea macrophylla* and *Dichroa febrifuga*. SNA Research Conference, 54:358–360.

Reed, S. M. (2010). 'Ruby Slippers' and 'Munchkin' oakleaf hydrangeas. HortScience. 45:1908-1909.

Reed, S. M., & Alexander, L. W. (2015). 'Queen of Hearts' oakleaf hydrangea. HortScience. 50:310-311.

Reed, S. M., & Rinehart, T. A. (2007). Simple sequence repeat marker analysis of genetic relationships within *Hydrangea macrophylla*. J. Am. Soc. Hortic. Sci., 132:341–351.

Reed, S. M., & Rinehart, T. A. (2009). Simple sequence repeat marker analysis of genetic relationships within *Hydrangea paniculata*. HortScience, 44:27–31.

Resllstab, C., Gurgerli, F., Eckert, Andrew, J., Hancock, Angela, M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. Mol. Ecol, 24, 4348–4370. doi:10.1111/mec.13322

Rinehart, T. A., & Reed, S. M. (2010). Using SSR markers to correctly identify *Hydrangea* germplasm, assess parentage, and verify hybrids. Acta Hortic., 885:291–296.

Rinehart, T. A., Scheffler, B. E., & Reed, S. M. (2006). Genetic diversity estimates for the genus *Hydrangea* and development of a molecular key based on SSR. J. Am. Soc. Hort. Sci., 131:787–797.

Rinehart, T. A., Scheffler, B. E., & Reed, S. M. (2010). Ploidy variation and genetic diversity in *Dichroa*. HortScience, 45:208–213.

Roach, J. A., Verma, S., Peres, N. A., Jamieson, A. R., van de Weg, W. E., Bink, M. C. A. M., Bassil, N. V., Lee, S., Whitaker, V. M. (2016). FaRXf1: a locus conferring resistance to angular leaf spot caused by *Xanthomonas fragariae* in octoploid strawberry. Theor. Appl. Genet., 129:1191–1201. doi:10.1007/s00122-016-2695-1

Ruíz, H., & Pavon, J. (1789). Flora peruviana, et chilensis, sive, descriptione, et icones pantarum peruvianarum, et chilensium, secundum systema linneanum digestæ, cum characteribus plurium generum evulgatorum reformatis. Typis Gabrielis de Sancha.

Samain, M., Wanke, S., & Goetghebeur, P. (2010). Unraveling extensive paraphyly in the genus *Hydrangea* s. l. with implications for the systematics of tribe Hydrangeeae. Syst. Bot., 35:593–600.

Samain, M.-S., Najarro, F. H., Manuel, E., & Salas, M. (2014). First record of the critically endangered *Hydrangea steyermarkii* Standl. (Hydrangeaceae) in Mexico, and description of a new widespread *Hydrangea* species of Mesoamerica. Phytotaxa, 162:181–197.

Sebastian, T. K., & Heuser, C. W. (1987). In vitro propagation of *Hydrangea quercifolia* Bartr. Sci. Hortic., 31:303–309. doi:10.1016/0304-4238(87)90056-2

Semagn, K., Bjørnstad, Å., & Ndjiondjop, M. N. (2006). An overview of molecular marker methods for plants. Afr. J. Biotechnol., 5:2540–2568.

Singh, S. (2016). Ethnobotanical study of some climbers of Parsa district forest of Nepal. Journal of Medicinal Plants Studies, 4:6–10.

Soltis, D. E., Xiang, Q.-Y., & Hufford, L. (1995). Relationships and evolution of Hydrangeaceae based on *rbcL* sequence data. Am. J. Bot., 82:504–514.

Stern, W. L. (1978). Comparative anatomy and systematics of woody Saxifragaceae. *Hydrangea*. Bot. J. Linn. Soc., 76:83–113.

Suojala, T., & Lindén, L. (1997). Frost hardiness of *Philadelphus* and Hydrangea clones during ecodormancy. Acta Agric. Scand., 47:58–63. doi:10.1080/09064719709362440

Thioulouse, J., & Dray, S. (2007). Interactive multivariate data analysis in R with the ade4 and ade4TkGUI packages. J. Stat. Softw., 22:1–14.

Thunberg, C. P. (1784). Flora Japonica. Lipsiae: In Bibliopolio I. G. Mülleriano

Tränkner, C., Krüger, J., Wanke, S., Naumann, J., Wenke, T., & Engel, F. (2019). Rapid identification of inflorescence type markers by genotyping-by-sequencing of diploid and triploid F_1 plants of *Hydrangea macrophylla*. BMC Genet., 20:1–12. doi.org/10.1186/s12863-019-0764-6

USDA, NRCS. 2020. The PLANTS Database (http://plants.usda.gov, 19 April 2020). National Plant Data Team, Greensboro, NC 27401-4901 USA.

Van den Bulk, R. W. (1991). Application of cell and tissue culture and in vitro selection for disease resistance breeding - a review. Euphytica, 56:269–285. doi:10.1007/BF00042373

van Gelderen, C. J., & van Gelderen, D. M. (2004). Encyclopedia of hydrangeas. Timber Press, Portland, OR.

Van Laere, K., Van Huylenbroeck, J., & Van Bockstaele, E. (2008). Karyotype analysis and physical mapping of 45S rRNA genes in *Hydrangea* species by fluorescence in situ hybridization. Plant Breed., 127:301–307. doi:10.1111/j.1439-0523.2007.01456.x

Van Treuren, R., de Groot, E. C., & van Hintum, T. J. L. (2013). Preservation of seed viability during 25 years of storage under standard genebank conditions. Genet. Resour.Crop Evol., 60:1407–1421. doi:10.1007/s10722-012-9929-0

van Zonneveld, M., Dawson, I., Thomas, E., Scheldeman, X., van Etten, J., Loo, J., & Hormaza, J. I. (2014). Application of molecular markers in spatial analysis to optimize in situ conservation of plant genetic resources. Genomics of Plant Genetic Resources. p. 67–91. doi:10.1007/978-94-007-7572-5

Vranckx, G., Jacquemyn, H., Muys, B., & Honnay, O. (2012). Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. Conservation Biology, 26:228–237.

Waki, T., Kodama, M., Akutsu, M., Namai, K., Iigo, M., Kurokura, T., Yamamoto, T., Nashima, K., Nakayama, M., Yagi, M. (2018). Development of DNA markers linked to double-flower and hortensia traits in *Hydrangea macrophylla* (Thunb.) Ser. Hortic. J., 87:264–273. doi:10.2503/hortj.OKD-096

Wan, Q.-H., Wu, H., Fujihara, T., & Fang, S.-G. (2004). Which genetic marker for which conservation genetics issue? Electrophoresis, 25:2165–2176. doi:10.1002/elps.200305922

Wang, C., Szpiech, Z. A., Degnan, J. H., Jakobsson, M., Pemberton, T. J., Hardy, J. A., Singleton, A. B., Rosenberg, N. A. (2010). Comparing spatial maps of human population-genetic variation using procrustes analysis. Statistical Applications in Genetics and Molecular Biology, 9. doi:10.2202/1544-6115.1493

Wang, Z., Kang, M., Liu, H., Gao, J., Zhang, Z., Li, Y., & Wu, R. (2014). High-Level genetic diversity and complex population structure of Siberian apricot (*Prunus sibirica* L.) in China as revealed by nuclear SSR markers. PLoS ONE, 9:1–13. doi:10.1371/journal.pone.0087381

Weigel, D. (2012). Natural variation in Arabidopsis: From molecular genetics to ecological genomics. Plant Physiol., 158:2–22. doi.org/10.1104/pp.111.189845

Wilson, G., & Rannala, B. (2003). Bayesian inference of recent migration rates using multilocus genotypes. Genetics, 163:177–1191. doi:10.1073/pnas.081068098

Wu, F. Q., Shen, S. K., Zhang, X. J., Wang, Y. H., & Sun, W. B. (2015). Genetic diversity and population structure of an extremely endangered species: the world's largest *Rhododendron*. AoB PLANTS, 7:1–9. doi:10.1093/aobpla/plu082

Wu, X., & Alexander, L. W. (2019). Genetic diversity and population structure analysis of bigleaf hydrangea using genotyping-by-sequencing. J. Am. Soc. Hortic. Sci., 144:257–263. doi.org/10.21273/JASHS04683-19

Xue, W., Xing, Y., Weng, X., Zhao, Y., Tang, W., Wang, L., Zhou, H., Yu, S., Xu, C., Li, X., Zhang, Q. (2008). Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. Nat. Genet., 40:761–767. doi.org/10.1038/ng.143

Ye, Y., Cai, M., Ju, Y., Jiao, Y., Feng, L., Pan, H., & Cheng, T. (2016). Identification and validation of SNP markers linked to dwarf traits using SLAF-seq technology in *Lagerstroemia*. PLoS ONE, 1–18. doi:10.1371/journal.pone.0158970

Yu, H.-H., Yang, Z.-L., Sun, B., & Liu, R. (2011). Genetic diversity and relationship of endangered plant *Magnolia officinalis* (Magnoliaceae) assessed with ISSR polymorphisms. Biochem. Syst. Ecol., 39:71–78. doi:10.1016/j.bse.2010.12.003

Zettlemoyer, M. A., Prendeville, H. R., & Galloway, L. F. (2017). The effect of a latitudinal temperature gradient on germination patterns. Int. J. Plant Sci., 178:673–679. doi:10.1086/694185

Zhao, B., Yin, Z., Xu, M., & Wang, Q. (2012). AFLP analysis of genetic variation in wild populations of five *Rhododendron* species in Qinling Mountain in China. Biochem. Syst. Ecol., 45:198–205. doi:10.1016/j.bse.2012.07.033