Ecology and horticultural potential of *Dirca palustris*

by

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ABSTRACT

Despite emerging interest in the use of native plants for horticulture, phenotypic traits and physiological tolerances of many indigenous species have not been formally evaluated. Because natural genetic resources represent both the historical and modern foundations for horticultural improvements, the status of such resources should receive greater consideration in the discipline. My first objective in the work presented in this thesis was to assess the horticultural potential and evolutionary diversity of five populations of *D. palustris* that span its range from Florida to North Dakota. The other populations assessed in this project were located in Wisconsin, Illinois, and Alabama. Plants in the populations in Florida and North Dakota were phenotypically distinct, with the former unique in its white-pubescent bud scales and the latter having more inflorescences, more flowers per inflorescence, and greater annual stem elongation than plants in the other populations. We found evidence that limited solar resources and tradeoffs in allocation of resources to floral and vegetative development may account for some of the phenotypic differences we observed. Nonetheless, the populations were genotypically distinct; genotyping by using inter-simple sequence repeats (ISSR) markers yielded 63% polymorphism and showed a range in population-level polymorphism from 20% for North Dakota to 36% for Alabama. The number of loci from each population varied from 230 for North Dakota to 264 for Alabama and Florida, whereas the number of population-specific loci varied clinally from none in North Dakota to 14 in Florida. Given the unique phenotypes of the populations in North Dakota and Florida and the distinct genotypes of the population in Florida, I conclude that these endangered peripheral populations represent valuable priorities for conservation and interesting targets

for horticultural evaluation. My second objective was to evaluate the responses of seedlings from Florida, North Dakota, and Maine to root-zone pH in soilless media, as the species has been reported both to favor alkaline or acidic soils. Although the provenances from which seeds were collected represented diverse soil pH, seedlings of *D. palustris* were nonetheless sensitive to root-zone pH and preferred acidic media. Seedlings from the three provenances differed in some ways in response to root-zone pH, but the overall effects of provenance on development were more pronounced. These results demonstrate both that horticultural gains may be made by selection of genotypes for increased shoot or root growth, and that horticultural production using acidic media offers the best root-zone environment for culture of *D. palustris*.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Thesis Organization

Three manuscripts are presented in this thesis. Chapter 2 presents a scientific note titled *Color of pubescence on bud scales conflicts with keys for identifying species of* Dirca *(Thymelaeaceae)*, which was published in the journal *Rhodora*. This manuscript illustrates a disparity between scientific keys for identifying species of *Dirca* and our own observations of a population in the accepted range for *Dirca palustris* L. Chapter 3 contains a manuscript titled *Phenotypic and genotypic diversity of eastern leatherwood in five populations that span its geographic distribution*. This manuscript is intended for submission to *The American Midland Naturalist* and it has been formatted for that journal. Chapter 4 contains a manuscript titled *Variation in development and response to root-zone pH among seedlings of* Dirca palustris *(Thymelaeaceae) from three provenances*, and it follows the format of *HortScience,* the journal in which it is in press. Overall conclusions emerging from these manuscripts are presented in chapter 5. An appendix, which follows the conclusions, presents observations and data that were collected but not incorporated into a manuscript.

Introduction

The invasion of indigenous ecosystems by non-indigenous plant species is an ecological problem of largely horticultural origin (Reichard and White, 2001; Perrings et al., 2005; Pimentel et al., 2005). Efforts to control invasive exotics in managed and natural environments cost the United States an estimated tens of billions of dollars annually (Pimentel et al., 2005). Nonnative, invasive species are second only to habitat loss as an

active threat to diversity of native plant species in the United States (Wilcove et al., 1998). Because of the risks inherent to the horticultural use of non-native species, indigenous species merit greater attention for horticulture. A perusal of books, magazines, and state and local organizations of the United States (e.g., Harper-Lore and Wilson, 2000; California Native Plant Society, 2009; State of Missouri, 2009) suggests there is abundant interest in the use of native species. Many nurseries specialize largely or exclusively in native plant species. The question arises whether there is a misplaced emphasis in horticulture on the evaluation and introduction of nonnative species at the expense of research on native species, many of which remain unused in horticulture. It seems state and local governments, the horticulture industry, and the gardening public are more receptive to and enthusiastic about the cultivation of native ornamental plants than at any time in the past (Woosaree, 2000; Helfand et al., 2006; Hooper et al., 2008; State of Missouri, 2009).

Despite growing interest in the use of native plants, many have not been formally evaluated for phenotypic traits or physiological tolerances valuable to horticulture (O'Brien, 1996). Furthermore, evaluations of species of interest for horticulture should address the potential for variation in important traits among plants from disparate provenances. It long has been understood that noteworthy variation in adaptive traits can be found within the ranges of many species, especially those with far-reaching geographic distributions (Turesson, 1923; Donselman and Flint, 1982). Genetic divergence can lead to intraspecific differences not only in aesthetic qualities, but in tolerances to stresses such as drought, high soil pH, low temperature, soil salts, and so on. For these reasons, the assessment of natural genetic resources with horticultural applications in mind should be of interest to the discipline.

Horticultural themes also may be found in the disciplines of conservation and restoration ecology. With increasing efforts to reintroduce native species and restore ecosystems (Young, 2000), the discipline of horticulture may have the opportunity to contribute information on the propagation, production, and establishment of native species in restored landscapes (Dreesen and Harrington, 1998; Woosaree, 2000; Harrington et al., 2001; Dreesen et al., 2002; Franco et al., 2006). Moreover, successful restoration strategies also require specific knowledge of the physiological tolerances of each species, as well as of patterns of genetic and phenotypic diversity (Dreesen et al., 2002; O'Brien et al., 2007). Regarding conservation, it is evident the flora of North America is threatened by a host of ecological problems, including habitat fragmentation, invasion by non-native species, chemical pollution, and overexploitation (Wilcove et al., 1998). Scientists are also concerned that global climate change may negatively impact local and global plant populations, lead to irreversible genetic changes to indigenous gene pools, and result in widespread extinctions (Hewitt, 2000; Schwartz et al., 2006; Skelly et al., 2007).

As natural genetic resources represent both the historical and modern foundations for horticultural improvements, the status of these resources should receive greater consideration in the discipline (Morico et al., 1998; Forsline et al., 2003; ten Kate and Laird, 2003). In this context, a case can be made for robust interdisciplinary efforts between horticulture and ecology to preserve, identify, and utilize genetic resources. Consideration of the diversity of largely overlooked native species as potential ornamentals could offer greater ecological benefits than the development of nonnative species for horticulture.

Horticulture can serve a role as a strong interdisciplinary science that contributes knowledge pertinent to a wide range of social, agricultural, and environmental challenges.

For instance, assessing the horticultural potential, morphological and physiological variation, and patterns of genetic diversity of indigenous species may yield valuable information not only to horticulture, but also to the biological disciplines of ecology, plant genetics, agronomy, plant physiology, and plant taxonomy. The first portion of this review focuses on the rationale for, and knowledge gained from, studying variation in phenotypic characters, physiological tolerances, and genetic diversity of indigenous plants. In the second half of the review, I will discuss specifically the ecology and horticultural status of *Dirca palustris* L., and avenues of research that could contribute to a more integrated horticultural and ecological understanding of the species.

Literature Review

Intraspecific variation

It long has been known that species cannot be regarded as static taxonomic units (Turesson, 1929; Langlet, 1971). New species may emerge from existing species by divergence through either adaptive or largely random processes. Species universally have some degree of differentiation among individuals and populations within their ranges (Linhart and Grant, 1996). Such variation creates ample opportunity for human benefits. Adaptation of plants to local environments can promote variation of interest to horticulturists engaged in selection for aesthetically superior genotypes (O'Brien, 1996). Furthermore, natural selection may yield physiological adaptations that enhance tolerance of stressors, including extremes in irradiance, temperature, moisture, edaphic factors, and insect and disease pressures (Chapin et al., 1993; Linhart and Grant, 1996; O'Brien, 1996). Rapid horticultural gains may be made by careful observation and selection for provenance

differences, whereas similar gains through selective breeding could take many generations. In species that do not reach sexual maturity for a number of years, the time involved in selective breeding for traits can be considerable.

On the global scale, plants show diverse adaptations to different ecosystems. On more regional scales, variation within hundreds of species has been documented since the advent of genecology and focused domestication efforts. Genecology, a term coined by Turesson (1923), is the study of the genetic composition and distribution of a species as a function of ecological factors acting on many individual genotypes. The term ecotype was proposed early on by Turesson to describe discrete subunits within a species. Following this, subcategories of climatic, edaphic, and biotic ecotypes were proposed to acknowledge the variety of factors that might promote localized divergence (Langlet, 1971). The potential complexities of describing discrete subunits of a species soon became obvious, with climatic, edaphic, and biotic factors interacting to create potentially overlapping or multidimensional ecotypic classifications. Huxley (1938) coined the term cline, described as a graded spatial variation of any morphological or physiological trait within a species or complex of species. Clinal variation may be found along gradients in temperature, elevation, soil moisture, edaphic conditions, available pollinators, community structure, and so on (Gregor and Watson, 1961). Given the subjectivities of dividing species into discrete ecotypes, the clinal approach may offer a generally more realistic framework for studying the genecology of a species (Gregor and Watson, 1961).

Evaluating plants from populations at the periphery of a species range may be advantageous if tolerance to an environmental extreme is of interest. Range margins represent both a set of environmental conditions at which further advancement of the species

is inhibited, and conditions under which strong selection for useful environmental tolerances might be occurring. For instance, marginal populations may acquire novel adaptations to a variety of temperature, moisture, soil, insect, and disease pressures (Lesica and Allendorf, 1995; O'Brien, 1996). Even if populations possessing tolerances of interest are not aesthetically superior to other members of the species, their resistance genes may be of interest in horticultural breeding programs (O'Brien, 1996). Marginal populations, however, need not be geographically marginal to be of interest. Additional sites to consider within the range of a species are ecologically marginal sites characterized by challenging edaphic conditions such as serpentine (Rajakaruna, 2004), alkaline (Snaydon, 1970), droughty (Zhang et al., 2005), or flood-prone (Fenster, 1997) soils.

Because gene flow from non-adapted populations can restrict local adaptation (Garcia-Ramos and Kirkpatrick, 1997), disjunct populations may be more likely than populations with gene flow among them to exhibit significant morphological or physiological variation. By virtue of their physical and reproductive isolation, disjunct populations may experience significant local adaptations to novel environments (Lesica and Allendorf, 1995; Garcia-Ramos and Kirkpatrick, 1996). Such differentiation arising from natural selection may have intrinsic value in horticulture and agriculture.

Phenotypic traits

Differences in phenotypic traits among natural populations have been documented in various species, although genotypic and environmental influences on phenotypic expression may be difficult to separate (Dorken and Barret, 2004; Brock and Weinig, 2007). Regardless, decisions regarding taxonomic classification historically have relied on measurements

collected from indigenous specimens, and there is good evidence that phenotypic differences can act as a partial surrogate for genetic differences (Waitt and Levin, 1998). In this spirit, Jian-xun et al. (2005) reported variation in morphology of *Picea asperata* Mast., information which could be used as a starting point for selection and breeding of superior genotypes. Zeneli (2005) evaluated indigenous populations of *Juglans regia* L. and found potentially exploitable differences in phenology and nut characteristics. In situ and ex situ evaluations of sweet cherry (*Prunus avium* L.), however, showed interactions between genotype and environment for the expression of several phenotypic traits (Hjarlmarsson and Ortiz, 2000). The expression of floral traits, in contrast, is expected to be less plastic than that of vegetative or fruit-related traits, because strong stabilizing selection and genetic control presumably fix floral morphology for optimization of pollen transfer (Cresswell, 1998; Lendvai and Levin, 2003; Herrera, 2005; Pérez-Barrales et al., 2007). Galen and Newport (1988) and Boyd (2002) reported divergence in flower size of indigenous species that corresponded to morphology of available pollinators. Herrera (2005) found variation in flower size among natural populations of *Rosmarinus officinalis* L. among habitats of different elevations with different likely pollinators. Galen (1999) discusses several other evolutionary mechanisms that might account for intraspecific divergence of flower size.

Identifying and selecting for genetically controlled differences in phenotypes within species often are accomplished by using provenance trials. Propagules from multiple provenances are grown in a common environment to limit environmentally induced variations among phenotypes from each provenance. The phenotype of an individual plant depends on the interaction between its genotype and the environment in which it develops. Provenance trials have the unique capacity to resolve whether differentiation within a species

is the result of plastic responses to heterogeneous environments or to genetic differentiation (O'Brien et al., 2007).

Provenance trials have demonstrated intraspecific variation in vegetative traits of horticultural interest among many woody taxa. For instance, Gustavsson (2001) found that average height and width of plants of *Vaccinium vitis-idaea* L. differed among provenances when grown in a common environment, and suggested that horticultural selection for growth habit may be possible. Cornelius and Mesén (1997) reported differences among plants from various provenances of *Vochysia guatemalensis* Donn. Sm. in height and stem diameter at breast height. Smithberg and Weiser (1968) found growth habit varied from nearly decumbent to upright, and mean annual stem growth varied more than tenfold, among cuttings of *Cornus sericea* L. collected from diverse provenances. Donselman and Flint (1982) found evidence for morphological adaptations to xeric environments among genotypes of *Cercis canadensis* L. originating from provenances low in available moisture.

Variation among provenances in growth of young seedlings has been studied with the assumption that such variation reflects performance of genotypes over the long-term. There is evidence that this assumption often holds true (Schuler, 1994; Mattsson, 1996; Harmer, 2000). Schrader and Graves (2000) identified differences among provenances of *Alnus maritima* in seedling leaf morphology, density of foliation, rate of dry weight accumulation, and caliper of stems; a cultivar was subsequently selected after further confirmation of these provenance differences (Graves and Schrader, 2004). Ginwal et al. (2005) discuss the implications for forestry of variation in survival, stem elongation, root collar diameter, leaves per plant, and biomass among seedlings from diverse provenances of *Jatropha curcas* L.

Intraspecific divergence of floral traits also may be illustrated using provenance trials. Gustavsson (2001) reported clinal variation in color of *Vaccinium vitis-idaea* corollas from white to pinkish, as well as a negative correlation between latitude of origin and both abundance of flowers and duration of anthesis. Jonas and Geber (1999) presented evidence for a negative correlation between petal size and elevation of provenance, and for a positive correlation between petal size and latitude of provenance of *Clarkia unguiculata* Lindl. Rates of flower production in 15-year-old *Eucalyptus marginata* Donn ex Smith varied with latitude of Australian provenances (O'Brien et al., 2007). Worley and Barrett (2001) demonstrated that both flower size and flower number per plant differed significantly between populations grown from seed in a greenhouse environment.

Physiological tolerances

Variation among provenances in physiological responses also is an important aspect to consider in the selection of genotypes for horticulture. Many species exhibit variation in physiological attributes among genotypes (Robakowski et al., 2005; Martin et al., 2007). Rajakaruna (2004) reviewed the role of edaphic selection in reproductive isolation, divergence, and speciation of plants. Conditions imposing strong selection, including serpentine soils, limestone outcrops, mine tailings (rich in heavy metals), and dolomite soils can promote immediate reproductive isolation or evolved isolation for improved fitness (Rajakaruna, 2004). Research further demonstrates the capacity for plants to undergo rapid genetic adaptation to edaphic stress, including copper-nickel pollution (Eränen, 2008), high zinc content (Al-Hiyaly et al., 1993), or high pH and soluble salt content (Dawson et al., 2007). The evolutionary mechanisms responsible for increased fitness on stressful soils are

often unidentified, with tolerance, true resistance, and evolved plasticity representing competing explanations (Eränen, 2008). Regardless, the role of edaphic variation in plant speciation and the evolution of horticulturally useful adaptations should be considered. For instance, provenance trials have been used commonly to identify genotypes with noteworthy edaphic tolerances (Anderson and Ladiges, 1978; Al-Hiyaly et al., 1993; Zhang et al., 2005; Eränen, 2008).

Despite the value of genotypes adapted to novel environments, evidence suggests that strong environmental selection may lead to decreased genetic variation within adapted populations (Aitken and Libby, 1994). Decreased genetic variation coupled with a high degree of adaptation to particular environmental conditions may diminish the ability of such indigenous populations to respond to climate change through migration or evolution. If, however, plasticity is the mechanism for the success of populations on diverse soils, genetic variation is not expected to be diminished within populations on extreme sites. Provenance trials become useful to a) determine the extent of differentiation among provenances that differ in edaphic conditions, b) provide a scientific foundation for focused preservation of true edaphic ecotypes that may be rare, endemic, or lacking genetic variability, and c) identify provenances of greatest promise in horticultural and ecological applications.

Genetic structure of indigenous species

Although provenances with novel genotypes offer value in horticultural selection programs, several possibilities regarding the genetic nature of such populations are worth mentioning. Such populations may be at risk for extinction because of low genetic variation (Yakimowski and Eckert, 2007). For example, northernmost populations of *Sorbus*

torminalis L. reproduce at low rates compared with more centrally located populations due to pollen limitation and inefficient self-pollination, with some evidence for inbreeding depression as an additional factor in reproductive failure (Rasmussen and Kollmann, 2004). In contrast, Yakimowski and Eckert (2007) found no apparent decrease in sexual reproduction, seed viability, or genetic diversity at northernmost populations of *Vaccinium stamineum* L., and suggested that these provenances might be valuable in shifting its range in response to anthropogenic climate change. Intense bottlenecking selection to overcome a particular climatic or edaphic stress may be responsible for diminished allelic richness within divergent populations (Parsons, 1991). Populations low in genetic diversity can be especially sensitive to environmental change (Guo et al., 2005). Knowledge of genetic diversity within and among populations can inform efforts to select genotypes for human benefit, preserve the integrity of indigenous populations, and assess potential influences of global climate change on the persistence of indigenous genetic resources.

Genetic markers such as inter-simple sequence repeats (ISSR) are tools useful for the study of genetic structure and diversity within species. Inter-simple sequence repeats (ISSR) markers may be particularly valuable in population-level genetic studies because they exhibit higher levels of polymorphism than other markers but maintain excellent reproducibility of banding patterns (Esselman et al., 1999). ISSR markers have proven cost-effective compared with random amplified polymorphic DNA (RAPD) and restriction fragment length poplymorphism (RFLP) markers (Yang et al., 1996).

Description and ecology of eastern leatherwood

Dirca palustris L. (eastern leatherwood, Thymelaeaceae) is a deciduous understory shrub of patchy distribution throughout eastern North America. Its range extends from New Brunswick west to North Dakota, and south to Oklahoma and Florida (Steyermark, 1963; Gleason, 1968). In its broad range, *D. palustris* generally occurs infrequently but can be locally abundant (Nevling, 1962; Godfrey, 1988; Ward and Horn, 1998). It is found in rich, moist woods, on stream banks and forested bluffs, and often favors north- or east- facing slopes (Steyermark, 1963; Gleason, 1968). *D. palustris* is one of three species of leatherwood; the others are *Dirca occidentalis* Gray and *Dirca mexicana* Nesom & Mayfield.

The three species of *Dirca* may be distinguished both by their geographic ranges and their morphological traits. Whereas *D. palustris* is found throughout eastern North America, *D. occidentalis* and *D. mexicana* are narrowly endemic species found near the San Francisco Bay in California, and in the mountains of Tamaulipas, Mexico, respectively (Nesom and Mayfield, 1995). Morphologically, *D. occidentalis* and *D. mexicana* share more traits in common than does *D. palustris* with either *D. occidentalis* or *D. mexicana* (Nesom and Mayfield, 1995). Brown pubescence on bud scales, glabrous young stems and leaves, pedicellate flowers, and unlobed calyx tubes are among the morphological traits that distinguish *D. palustris* from the other two (Nesom and Mayfield, 1995). A population of *Dirca* was discovered in Kansas, which is within the accepted range of *D. palustris*, that does not exhibit the characters listed above. Rather, it shares traits in common with *D. occidentalis* and *D. mexicana* (Floden and Mayfield, 2006). Although the taxonomic status of this population is unresolved (Floden and Mayfield, 2006), this discovery suggests that the accepted range of *D. palustris* may encompass greater variation in phenotypes than generally has been acknowledged.

Of the three species of *Dirca*, *D. palustris* has undergone the least genetic differentiation from ancestral characters (Schrader and Graves, 2004). This may be due in part to the large gene pool to which *D. palustris* has historically belonged. Its current distribution, which includes scattered and likely reproductively isolated populations, is in part the result of logging of old-growth forests (Schrader and Graves, 2004). This recent fragmentation of *D. palustris* has likely not contributed to significant regional differentiation of the species, despite the potential for reproductive isolation among populations. However, its historically large distribution in eastern North America suggests a potential for significant clinal variation, and the presence of populations historically isolated from others may have promoted noteworthy ecotypic adaptation.

Despite apparent security on the global scale, *D. palustris* is regarded as either imperiled or critically imperiled at latitudinal and longitudinal extremes of its distribution in North Dakota, Florida, and Nova Scotia (NatureServe, 2007). It is uncertain whether these statuses represent shrinking or especially vulnerable populations or simply limited occurrence near the edges of the species range. Regardless, timely ecological study and assessment of germplasm from the geographic range limits of *D. palustris* is warranted, as evidence suggests peripheral populations tend to be particularly vulnerable to climate change but important to future adaptation (Davis and Shaw, 2001; Hampe and Petit, 2005). If small, fragmented populations exist in these areas with little interpopulation gene flow, there is the potential for high levels of differentiation among them (Ellstrand and Elam, 1993).

Although the genetic structure of *D. palustris* has not been studied, Graves and Schrader (2008) used ISSR markers to elucidate patterns of genetic diversity in *D. occidentalis*. Populations of *D. occidentalis* were shown to be genetically distinct, despite

the localized endemism of the species near the San Francisco Bay. Graves and Schrader (2008) also identified low genetic diversity within populations of *D. occidentalis*, and suggested that low diversity within populations is a threat to persistence of the species.

Dirca palustris blooms earlier than most other species in its range and anthesis precedes or coincides with its leaf emergence in early spring (Gleason, 1968; Godfrey, 1988). Timing of anthesis varies climatically, with populations from the southern edge of the range flowering in February and populations from the northern edge flowering in late May (Graves et al., 2006). The inflorescence of *D. palustris* typically consists of small yellow flowers in groups of three and rarely as many as seven (Godfrey, 1988; Zasada et al., 2008). The flower consists of a yellowish calyx tube 5-6 mm long with notching at its summit, inconspicuous petals obscured by the tube, and conspicuous stamens protruding approximately 3 mm (Gleason, 1968; Godfrey, 1988).

The sexual success of *D. palustris* in wild populations is largely unknown. Its flowering schedule may diminish the success of sexual reproduction and the genetic diversity of this insect-pollinated species (Williams, 2004), particularly at environmentally stressful or unpredictable limits of its distribution. Schulz et al. (2004) did not find evidence for low-temperature limits to fruit set within populations in Wisconsin. Although eastern leatherwood can persist and reproduce in understories especially low in insolation, Schulz et al. (2004) found a positive correlation between insolation and the production of inflorescences. Self-compatibility and/or apomixis in natural populations of *D. palustris* remain possible factors exerting influence on the rates of sexual and asexual success (Williams, 2004). Asexual spread by the production of rhizomes has been noted in the wild (Graves, 2004), though the rates of sexual and asexual reproduction are unknown. In

addition to uncertainty regarding rates of sexual recruitment, little about mechanisms for seed dispersal is known. While there is evidence for granivory by rodents (Ward and Horn, 1998; Rogers et al., 2008), evidence for important vectors capable of promoting long-distance dispersal is lacking. Ward and Horn (1998) suggested that problems with seed viability and/or dispersal might be restricting the local distribution of populations in South Carolina.

Soil moisture seems to be a consistent determinant of the distribution of *D. palustris*. Ward and Horn (1998) found that populations in South Carolina performed best on mesic or moist sites (e.g. along floodplains) and only infrequently occurred on more droughty sites. In the lake-states region, *D. palustris* demonstrates a nearly exclusive preference for mesic conditions (Stearns, 1951). It is unclear, however, whether frequent occurrence on mesic sites is related to a general physiological intolerance for less-mesic conditions or to difficulties in seed dispersal and seedling establishment in such environments (Ward and Horn, 1998). Small populations have been found on relatively dry sites in South Carolina, and Ward and Horn (1998) speculated that survival of seedlings may be limited under such conditions.

A perusal of literature also reveals discrepancy in the presumed tolerances of *D. palustris* to soil pH, another important edaphic factor. Del Tredici (1984) reported that *D. palustris* performs best in moist areas that have high limestone content, and Cooperrider (1962) found occurrence of the species in eastern Iowa to be limited to sites over limestone bedrock. Clark (1971) asserted that *D. palustris* occurs over circumneutral or basic soils in the southeastern United States. Nevling (1962) characterized *D. palustris* as a facultative calciphile, whereas Anderson (1933) claimed it grows in a range of environments but avoids

limestone. A survey of *D. palustris* in South Carolina demonstrated that it occurs on acidic soils (Ward and Horn, 1998), and Dirr (1997) asserted that the species prefers acidity.

Uncertainty regarding the phenotypic and genotypic diversity of *D. palustris,* coupled with the widespread but sporadic occurrence of the species, leads to interesting questions about its intraspecific diversity. The conflicting information about the edaphic tolerances of *D. palustris* also merits clarification. Finally, it is unclear whether peripheral populations of *D. palustris* should represent a priority for conservation.

Horticultural status of eastern leatherwood

Dirca palustris has received relatively little attention in the nursery and landscape industry despite its exceptionally early bloom time, unique and upright growth habit, yellow fall color, curiously flexible branches, and tolerance of full-shade conditions (Anderson, 1933; Esson, 1949; Del Tredici, 1984). These traits suggest that it may be a particularly unique shrub for shady landscapes. My observations at the University of Minnesota Landscape Arboretum (Chaska, MN) suggest the species also can be used effectively in formal, sheared hedges.

Although *D. palustris* has traits that merit horticultural attention, limitations to the production of the species for horticulture include the absence of protocols for asexual propagation (Dirr and Heuser, 1987), sensitivity to root-zone conditions (William Graves, personal communication), and the especially slow growth rate of the species (Steyermark, 1963). An absence of protocols for asexual propagation limits the potential for development of cultivars (Hendricks, 1985; Dirr and Heuser, 1987), but identification of superior provenances is nonetheless relevant to the selection of seed sources. Root-zone conditions that promote

growth of *D. palustris* should be identified if the species is to become more available in the horticulture and landscape industries. Furthermore, because of discrepancies in the reported tolerances of the species to root-zone pH, the possibility of provenance-specific responses merits investigation. The use of provenance trials also may help to identify seed sources that yield plants with greater annual stem elongation than has been generally observed for the species. The following manuscripts are the product of a dual approach to studying both the ecology and horticultural potential of *D. palustris.*

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CHAPTER 2. COLOR OF PUBESCENCE ON BUD SCALES CONFLICTS WITH KEYS FOR IDENTIFYING SPECIES OF *DIRCA* (THYMELAEACEAE)

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The genus *Dirca* (Thymelaeaceae) comprises three North American species of deciduous understory shrubs. *Dirca palustris* (eastern leatherwood) occurs in patchy populations in eastern North America. Nevling (1962) reported that *D. palustris* ranges from New Brunswick west to Minnesota and Oklahoma, and south to Florida. Guided by herbarium records, we recently confirmed the existence of a large population of *D. palustris* in northern North Dakota, and the persistence of the species in Nova Scotia, as reported by Gray (1873) and more recently by Soper and Heimburger (1994). Throughout its broad range, *D. palustris* generally occurs infrequently but can be locally abundant (Nevling 1962). It is found in populations of variable densities from few to hundreds of individuals per

hectare in rich mesic forests (Schulz et al. 2004), and the species often is associated with forested north- or east-facing stream banks and slopes (Kurz 1997; Nevling 1962). The two other species in the genus are *D. occidentalis*, endemic near the San Francisco Bay, and *D. mexicana*, narrowly endemic to the Sierra Madre Oriental in Tamaulipas, Mexico.

The three species are reported to be distinguishable by morphological features, including the color of pubescence on bud scales. Vogelmann (1953) presented a key to distinguish *Dirca palustris* and *D. occidentalis*, listing first the dark brown pubescence of the former and whitish pubescence of the latter. In a report of their discovery of *D. mexicana*, Nesom and Mayfield (1995) constructed a key differentiating all three species. Again, color of pubescence on bud scales was the first trait listed to separate the brown-pubescent *D. palustris* from the white-pubescent *D. occidentalis* and *D. mexicana*. A perusal of both historical (Gray 1868) and contemporary (Soper and Heimburger 1994; Swanson 1994) descriptions reveals the long-held contention that *D. palustris* has brown to dark brown pubescence on its bud scales throughout its range.

Here we contribute evidence that some populations within the established range of *Dirca palustris* exhibit bud scales with white pubescence, which is in direct conflict with the keys used to distinguish members of the genus. We collected seeds of *Dirca palustris* from Torreya State Park west of Tallahassee, Florida (30°34’632”N, 84°56’747”W), and germinated them in our greenhouse. The seedlings had white pubescence on bud scales instead of the expected brown. On 14 February 2008, we visited the population while it was in flower and confirmed the presence of white pubescence on bud scales (Figures 1-2). We estimated that there were several hundred plants within this population, and the pubescence on bud scales was white without exception among the many dozens of plants we inspected.

Voucher specimens from the population collected on 23 February 2008 have been deposited at the University of Florida Herbarium in Gainesville (*Sharma 2008-28*, FLAS), with duplicates to be distributed to the University of Georgia (*Sharma 2008-27*, GA) and Harvard University (*Sharma 2008-26*, GH).

Our observations are consistent with several existing herbarium records. A survey of herbarium specimens conducted at the University of South Florida Herbarium in Tampa (USF) showed white pubescence on bud scales of individuals from the southeastern extreme of the established distribution of *D. palustris*. Additionally, herbarium specimens of *D. palustris* at the Anniston Museum of Natural History in Anniston, Alabama, confirm white-pubescent bud scales on plants indigenous to that state (Daniel Spaulding, curator, pers. comm.). Specimens of *D. palustris* examined at the Ada Hayden Herbarium at Iowa State University (ISC) exhibit an apparent gradation in pubescence color from dark brown to white. Gray (1873) seems to have noticed similar variation in pubescence color. He cautioned that the pubescence color on bud scales of *D. occidentalis* might vary based solely on his observation that the pubescence on bud scales of *D. palustris* may be "occasionally pale." This suggests that Gray witnessed enough variation in the pubescence color of *D. palustris* to doubt the constancy of this trait within *D. occidentalis*.

Our observations of white pubescence on the bud scales of plants long considered *Dirca palustris* echo a report by Floden and Mayfield (2006). They described a population of *Dirca* in Kansas that differed from the published descriptions of *D. palustris* in several ways, including the presence of hoary pubescence on bud scales. Collectively, their observations and ours suggest either that traits of *D. palustris* may vary more than has been reported, or that the taxonomic rank of some populations ought to be reassessed.

In a species such as *Dirca palustris*, with patchy occurrence and apparently limited capacity for seed dispersal (Ward and Horn 1998), restriction of gene flow among populations may increase the opportunity for regional differentiation of traits. A question meriting further consideration is whether observed inconsistency in traits such as the color of pubescence on bud scales follows a clinal or more ecotypic pattern of variation within the extensive range of *D. palustris*. Identifying the pattern of variation in this trait and others might offer insight regarding the appropriate taxonomic treatment of apparently atypical populations. For instance, geographically gradual variation in the color of pubescence on bud scales from brown to white may validate a view that the species as one taxonomic unit should encompass varied bud scale phenotypes along a gradient. In contrast, a more isolated and abrupt variation in pubescence color and other traits might favor a case for taxonomic recognition.

It is clear that white pubescence on bud scales of *Dirca* should not be considered a reliable character to differentiate the three species. Our observations to date suggest that the population observed in Florida possesses the remaining characters of *D. palustris* presented in the key by Nesom and Mayfield (1995). These include "young twigs and both leaf surfaces completely glabrous; flowers and fruits pedicellate, the whole cluster often pedunculate; calyx margin merely crenulate-undulate, without distinct lobes; eastern United States" (Nesom and Mayfield 1995). This suggests that a classification as *D. palustris* or as a subspecies of *D. palustris* may be the most appropriate course of action for this population.

In light of the observation we report here, further research should be focused on variation among and within populations that long have been considered *Dirca palustris*. A comprehensive assessment of populations representing the entire range of *D. palustris* will

facilitate characterization of the extent and nature of phenotypic variation and resolve potential taxonomic confusion in the genus *Dirca*.

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Figures 1-2. Inflorescences of *Dirca palustris.* 1. White pubescence on bud scales of a plant in its native habitat in northwestern Florida. This pubescence color is inconsistent with the existing description of the species. 2. Brown pubescence on bud scales of a plant in its native habitat in southern Illinois. The description of *D. palustris* includes brown pubescence as a hallmark of the species.

CHAPTER 3. PHENOTYPIC AND GENOTYPIC DIVERSITY OF EASTERN LEATHERWOOD IN FIVE POPULATIONS THAT SPAN ITS GEOGRAPHIC DISTRIBUTION

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ABSTRACT.—Quantifying variation among indigenous populations of plants can facilitate the identification and conservation of novel populations that are threatened. *Dirca palustris* L. is a deciduous, understory shrub that occurs sporadically in North America. Geographic isolation among populations and a limited capacity for seed dispersal may lead to restricted gene flow and divergence among genotypes. To identify the extent of variation across its range, we characterized phenotypic and genotypic diversity among five populations from North Dakota southeast to Florida. Plants in North Dakota had the most inflorescences, the most flowers per inflorescence, and the shortest calyxes. Plants in Alabama and Illinois had the fewest inflorescences and the longest calyx tubes. Plants in North Dakota had annual stem elongation 95% and internode length 42% greater than means among the other populations. Color of pubescence on bud scales varied from all white in Florida to all brown in North Dakota, Wisconsin, and Illinois. Percentage open-sky radiation available to plants in Alabama and Florida was correlated positively with stem elongation and inflorescence count, suggesting that limited solar resources influence some traits. Moreover, we found evidence for tradeoffs between inflorescence length and both inflorescence count and stem elongation the previous year. Positive correlations existed among several floral and vegetative traits, implying that availability of resources influences various traits similarly. Use of inter-simple sequence repeats (ISSR) markers showed 63% polymorphism overall, with population-level polymorphism ranging from 20% for North Dakota to 36% for Alabama. Number of population-specific loci varied clinally from none in North Dakota to 14 in Florida. AMOVA revealed that 46% of molecular variance was within populations, whereas 54% was among populations. Phenotypic distances showed plants in North Dakota and Florida are phenotypically unique; genotypic distances showed the population in Florida is the most

distinct genetically. These peripheral populations, which are considered imperiled, should be priorities for conservation.

INTRODUCTION

Quantifying the extent of phenotypic and genotypic variation among natural populations is central to modern systematics (Schrader and Graves, 2002; 2004). Such knowledge also can direct focused efforts to conserve genetically diverse or distinct populations of species in danger of decline (Lesica and Allendorf, 1995). Evolutionary theory predicts intraspecific divergence in both phenotypes and genotypes within species occupying broad geographic ranges or occurring within scattered populations with limited potential for gene flow (Loveless and Hamrick, 1984; Lopez et al., 2007). Such divergence may result from reproductive isolation and directional selection or genetic drift. Small or isolated populations differentiated genetically from others of a species may be vulnerable to changes in climate and land use and could be important repositories of genetic variation for future adaptation (Davis and Shaw, 2001; Hampe and Petit, 2005).

Dirca palustris L. (eastern leatherwood) is a deciduous understory shrub that occurs sporadically from Nova Scotia west to North Dakota, and south to Oklahoma and Florida (Nevling, 1962; Peterson et al., 2009). Historically, the species likely occupied a more continuous area throughout eastern North America, and some of the populations that remain are those that escaped destruction during logging of old-growth forests (Schrader and Graves, 2004). Although ranked globally secure (G4), *D. palustris* is recognized as imperiled (S2) and critically imperiled (S1) in Florida and North Dakota, respectively, where the southernmost and northernmost populations occur (NatureServe, 2007). The discontinuous distribution of the

species, its broad geographic range, and the limited capacity for seed dispersal within the genus (Ward and Horn, 1998; Graves, 2008) led us to speculate that we would find phenotypic and genotypic differentiation among disparate populations of *D. palustris*. Noteworthy phenotypic differentiation of populations within the accepted range for *D. palustris* has been illustrated by Floden and Mayfield (2006) and Peterson et al. (2009), who reported populations of *Dirca* L. in Kansas and northern Florida, respectively, that differ in traits from the accepted description of *D. palustris*.

Although *in situ* studies of phenotypic diversity among indigenous populations are valuable to ecology, plastic responses of a genotype to heterogeneous environments make it difficult to separate genetic and environmental contributions to variation observed among natural populations. Regardless, taxonomists historically have relied on measurements collected from indigenous specimens, and there is good evidence that phenotypic differences can act as a partial surrogate for genetic differences (Waitt and Levin, 1998). Phenotypic divergence in vegetative traits among populations may, however, represent plastic responses of a common genotype to divergent environments rather than genotypic divergence itself (Dorken and Barrett, 2004). In contrast, expression of floral phenotypes is generally assumed to be under stronger genetic control (Lendvai and Levin, 2003; Ashman and Majetic, 2006), uncoupled from expression of vegetative traits (Waitt and Levin, 1998; Ashman and Majetic, 2006), and much less plastic than expression of vegetative traits (Waitt and Levin, 1998; Brock and Weinig, 2007). Although pollinator-mediated stabilizing selection is predicted to restrict intraspecific divergence in floral characters (Cresswell, 1998), variation in flower size and morphology can be found among indigenous populations of many species (Galen, 1999; Herrera, 2005; Pérez-Barrales et al., 2007). Traditional ecological theory does not predict

phenotypic plasticity as an explanation for such divergence, because stabilizing selection and strong genetic control presumably fix floral morphology for optimization of pollen transfer (Cresswell, 1998; Lendvai and Levin, 2003; Herrera, 2005; Pérez-Barrales et al., 2007).

Although floral phenotypes are considered more fixed than vegetative phenotypes, evidence suggests that plants in environments limited in resources may invest energy hierarchically to vegetative or reproductive functions, each at the expense of the other (Suzuki, 2001). The natural restriction of *D. palustris* to sites low in irradiance (Zasada et al., 2008), the slow growth rate of the species (Steyermark, 1963), and its strategy of flowering by using energy stored from previous seasons led us to ask whether vegetative and floral traits are environmentally influenced by, and might compete for, limited solar resources. We speculated that varying solar resources and differences in the magnitude of within-plant competition for allocation of stored energy would contribute to phenotypic differences among the populations. We considered this possibility because plastic responses or complex patterns of resource allocation for these traits could obscure genetically based divergence of phenotypic traits of *D. palustris*.

Although morphological differences may not represent genetic differences, genotypic divergence among populations within a species can be estimated by using genetic markers. Inter-simple sequence repeats (ISSR) markers can be particularly valuable in population-level genetic studies because they exhibit higher levels of polymorphism than other markers but maintain excellent reproducibility of banding patterns (Esselman et al., 1999). ISSRs have been utilized for both species- and population-level studies within the genus *Dirca*. Schrader and Graves (2004) used ISSR markers to resolve the interspecific phylogenetics of the three known species of *Dirca*, whereas Graves and Schrader (2008) used ISSR markers to resolve

the intraspecific phylogenetics of *D. occidentalis* Gray and to quantify genetic variation within its populations. We therefore explored the use of ISSR markers to elucidate patterns of genetic variation within and among geographically disparate populations of *D. palustris*.

Our broad goal was to characterize phenotypic variation within natural populations of *D. palustris* and relate the observed variation to environmental resource availability and to genetic differences among the populations. Our specific objectives were to 1) identify the nature and extent of variation in several floral and vegetative traits among five populations of *D. palustris* that span the geographic distribution of the species from its southernmost to its northernmost limits; 2) model relationships between traits for which variation obviously may be explained by phenotypic plasticity; and 3) assess genetic variation within, and divergence among, the same five populations by using ISSR molecular markers.

MATERIALS AND METHODS

PHENOTYPIC VARIATION

We chose five populations that span the geographic distribution of *D. palustris* to study in spring and summer 2008 (Fig. 1). The populations were along the Little Pembina River, Pembina Co., North Dakota (48°57.892'N, 98°06.031'W); in Jersey Valley Co. Park, Vernon Co., Wisconsin (43°41.688'N, 90°48.220'W); in the Bell Smith Ecological Area, Shawnee National Forest, Pope Co., Illinois (37°30.855'N, 88°39.718'W); in Buck's Pocket State Park, Marshall Co. and DeKalb Co., Alabama (34°28.492'N, 86°02.665'W); and in Torreya State Park, Liberty Co., Florida (30°34.632'N, 84°56.747'W) (Fig. 1). We defined a population as a group of individuals within 3 km of each other within each site.

We measured floral traits on 30 plants within each population between 14 February, during anthesis of plants in Florida, and 4 May, during anthesis of plants in North Dakota. Plants within each population were selected to represent the range of plant sizes observed and differences among plants in exposure to insolation. Five inflorescences with pre-senescent stigmas and anthers with bright yellow, grainy pollen were selected on each plant. We counted the number of flowers per inflorescence and measured length and width of each inflorescence by using handheld calipers (Fig. 2). One flower per inflorescence was selected to measure the length and width of the calyx tube and the extension of the pistil and stamens (Fig. 2). We counted the relative abundance of inflorescences arising from the terminal 30 cm of three main branches selected on each plant. Branches were selected by blindly reaching out to mid-canopy, and repeating this procedure three times at equidistant points around each plant.

We measured vegetative traits on the same 30 plants selected for measurements of floral traits. Total height from the soil surface to the highest point of the plant and total canopy width at the widest dimension were recorded, and a height-to-width ratio was calculated for each plant. The diameter of the trunk at its widest dimension was measured at the soil surface with calipers and relative trunk shape was calculated as trunk diameter divided by plant height. To assess the relative growth rate of each plant during the previous two seasons, we determined the average stem elongation from 2006 and 2007 on the three branches previously selected to obtain inflorescence counts. Relative branching of each plant was documented as the number of first-order, second-order, and third-order branches arising from the terminal 30 cm of each selected branch. We used calipers to measure the internode

between the terminal and first subterminal inflorescence. The color of pubescence on bud scales of each plant was recorded as either brown or white.

We returned to the site of each population when fruits were mature to estimate photosynthetically active radiation (PAR) received by each plant during the growing season. These measurements were used to test the hypothesis that variation in insolation among populations is related to variation observed in floral and vegetative traits. Measurements were taken between 11 April (Florida) and 4 July (North Dakota), when leaves of *D. palustris* and those in the overstory were fully expanded. For each plant, PAR was measured during 1000 to 1200 HR and during 1500 to 1700 HR, according to local time zones. Three measures were taken over the center of each plant during both time periods by using a quantum meter with an LI-190 sensor (LI-COR Inc., Lincoln, NE). To obtain a single value for percentage of open-sky radiation available to each plant, measures were converted to percentage of PAR recorded in a location open to the sky at each site, and averaged for each plant over the two time periods. Additionally, pH, percentage total nitrogen, and percentage organic matter of soil at each site were determined from a composite soil sampling comprising ten subsamples. Annual precipitation and mean daily minimum and maximum temperatures were gathered from the National Climatic Data Center website (U.S. Department of Commerce, 2007).

Data were analyzed with the Statistical Analysis System 9.1.3 (SAS Institute Inc., Cary, NC). The general linear models (GLM) procedure was used with a subsampling design to account for multiple observations per plant. The LSMEANS option of the GLM procedure was used with Tukey adjustment for multiple comparisons to generate means-separation statistics. We calculated phenotypic distances (Euclidean distances; Sneath and

Sokal, 1973) among the populations by using 14 measures of growth and morphology (Table 1 and Table 2, excluding plant height and trunk diameter). The regression (REG) procedure and F-tests were used to test the significance of linear and quadratic models for correlations between variables or between a variable and percentage open-sky radiation. Before regression, individual measurements were averaged for each plant such that the 30 plants per population constituted the observational units for regression. We also calculated environmental distances (Euclidean distances; Sneath and Sokal, 1973) among the populations by using all parameters from Table 4 except minimum and maximum percentage open-sky radiation as unweighted environmental measures for each study site. Phenotypic distances were compared with environmental and geographic (km) distances by using the Mantel test (Mantel, 1967) option in GenAlEx 6.1 (Peakall and Smouse, 2006).

GENOTYPIC VARIATION

From each population we collected fully expanded leaves from 19 mature *D. palustris* selected to represent the spatial distribution of plants within the population. To reduce the likelihood of sampling ramets (Graves, 2008), leaves were collected only from plants >10 m from the nearest sampled neighbor. Laminal tissue desiccated with silica gel was ground, and DNA was extracted. We used five fluorescent 3'-anchored ISSR primers [$(CA)_6RG$, $(GTG)_3GC$, $(AC)_8G$, $(CAC)_3RC$, and $(CTC)_3SG$] previously optimized for analysis of genomic DNA from the genus *Dirca* by Schrader and Graves (2004). Three replicate PCR reactions for each primer were carried out as described in Graves and Schrader (2008). An Applied Biosystems 377 DNA Sequencer separated the amplified DNA fragments by electrophoresis according to number of base pairs and produced gel images of resulting

fluorescent bands. We used Genographer 2.1.4 (Benham et al., 1999) to analyze gel images for the presence or absence of a band at each locus. Only loci between 100 and 500 base pairs with unambiguous character and consistent amplification were scored, and only bands present in at least two of three replicate gel images were considered present. After scoring, matrices representing presence (1) and absence (0) of bands produced by each of the five primers were combined for analyses of genetic variation. Samples that produced ambiguous loci or that failed to amplify were removed before analysis.

Partitioning of molecular variance within and among populations was performed with the analysis of molecular variance (AMOVA) procedure in GenAlEx 6.1 (Peakall and Smouse, 2006). A consensus allele profile was generated for each population based on the presence or absence of each allele in ≥50% of samples from each population (Graves and Schrader, 2008). Genetic distance (Euclidean distance; Sneath and Sokal, 1973) was calculated between each pair of populations by using these consensus allele profiles. Genetic distances were compared with environmental (Euclidean), geographic (km), and phenotypic (Euclidean) distances by using the Mantel test (Mantel, 1967) option in GenAlEx 6.1 (Peakall and Smouse, 2006).

RESULTS

PHENOTYPIC VARIATION

Floral and vegetative traits differed among the populations. Plants in North Dakota were most floriferous with 141% more inflorescences on selected branches than plants in Illinois, which were least floriferous (Table 1). Flower count per inflorescence was greater in North Dakota than in the other populations, and the length and width of inflorescences varied

among populations (Table 1). Length of calyxes ranged from 5.9 to 8.0 mm among populations, width of calyxes ranged from 2.4 to 3.0 mm, and extension of pistils and stamens varied among populations (Table 1). Plants in North Dakota and Wisconsin were taller than plants in Florida and Alabama, and plants in North Dakota and Illinois had the most upright growth form (Table 2). Plants in Wisconsin had the greatest trunk diameter (Table 2). Plants in Alabama and Wisconsin had 18% greater branch counts than plants in the other populations (Table 2). Plants in North Dakota showed mean stem elongation 95% greater and internode length 42% greater than means across the other populations (Table 2). The color of pubescence on bud scales of sampled plants as well as others we observed in each population was brown in North Dakota, Wisconsin, and Illinois, whereas several plants in Alabama and all plants in Florida had white pubescence (Table 2).

Populations in Florida and North Dakota were the most phenotypically distinct among the five; their phenotypic distances across all pairwise comparisons of populations averaged 46% greater and 37% greater, respectively, than the mean distances of the three other populations (Table 3). The populations in Wisconsin and Alabama were the least phenotypically distinct, with average distances 31% less and 28% less, respectively, than the mean distances of the other populations (Table 3). Among pairwise comparisons, the populations constituting the most phenotypically dissimilar pair were in North Dakota and Florida, whereas the most phenotypically similar populations were in Wisconsin and Alabama (Table 3).

We found evidence both for a relationship between insolation and investment in floral and vegetative structures, and for competition among floral and vegetative structures for allocation of resources. Among populations, minimum and maximum insolation received by

plants averaged 1.7 and 31.5% of open-sky radiation (Table 4). Although the mean percentage of open-sky radiation received by plants did not exceed 12% among the populations, the percentage of open-sky radiation received by plants in Alabama and Florida was more than twice the mean percentage received by plants elsewhere (Table 4). Regression analysis showed that annual stem elongation and inflorescence count on selected branches correlated positively with percentage open-sky radiation in Alabama and Florida (Fig. 3), the two sites with greatest insolation. Among the populations, inflorescence length correlated negatively with inflorescence count on selected branches and stem elongation during the previous year (Fig. 4). Inflorescence count correlated positively with stem elongation during the previous year, length of internodes, and number of first-order branches arising from selected stems (Fig. 5).

Soils supporting the five populations differed in pH, percentage total nitrogen, and percentage organic matter (Table 4). The population in Florida received the greatest annual precipitation, whereas that in North Dakota received the least (Table 4). Historical mean daily minimum and maximum temperatures were highest in Florida and lowest in North Dakota (Table 4). Mantel correlation tests revealed a correlation between phenotypic and environmental distances and yielded suggestive, but inconclusive, evidence ($P = 0.087$) for a correlation between phenotypic and geographic distances (Table 5). Geographic distance also correlated with environmental distance (Table 5).

GENOTYPIC VARIATION

Total polymorphism was 63% among 293 loci. Eighty-four samples were amplified from the five populations (Table 6). The number of loci per population ranged from 230 for

North Dakota to 264 for Alabama and Florida (Table 6). North Dakota had no population-specific loci, whereas Florida had 14 (Table 6). Percentage of polymorphic loci varied from 20 for North Dakota to 36 for Alabama (Table 6). AMOVA showed genetic variation among populations ($P < 0.001$) and revealed that 54% of the total molecular variance was among populations and 46% was within populations.

Plants in Florida and Wisconsin were the most and least genetically distinct, respectively. The mean genetic distance between the population in Florida and the four other populations was 69.5, whereas the mean genetic distance between the population in Wisconsin and the four others was 37.8 (Table 3). Among pairwise comparisons, the populations in North Dakota and Florida were most dissimilar, whereas the populations in North Dakota and Wisconsin were most alike (Table 3). Mantel correlation tests showed that genetic distance correlated positively with environmental distance, but not with geographic distance (Table 5). We found suggestive, but inconclusive, evidence ($P = 0.071$) for a positive correlation between genetic and phenotypic distances (Table 5).

DISCUSSION

PHENOTYPIC VARIATION

Floral and vegetative traits among the populations of *D. palustris* (Table 1 and Table 2) have diverged, with populations in Florida and North Dakota the most distinct (Table 3). Differences in floral traits could represent genetic divergence, as the reproductive phenotype is under strong genetic control (Cresswell, 1998). Although vegetative traits are recognized as more plastic than floral traits, considerable differences in vegetative measures also suggest genetic divergence (Waitt and Levin, 1998). For instance, whereas the qualitative difference

in color of pubescence on bud scales (Peterson et al., 2009) represents a discrete signal of divergence, the less discrete differentiation of quantitative traits may likewise represent evolutionary divergence. Considering all phenotypic measures, populations at the northern and southern extremes of the distribution differed the most from each other and from the other populations used for comparison (Table 3). Collectively, these results indicate that geographically disparate populations of *D. palustris* that may be reproductively isolated have diverged.

Despite the ecological value of *in situ* studies, they cannot resolve the extent to which phenotypic differences are the result of genetic differentiation versus plasticity (Dorken and Barrett, 2004; Brock and Weinig, 2007). Several of the differences we observed among populations may be explained to some degree by environmental influences and tradeoffs in allocation of stored resources to plant growth (Fig. 3-5). The positive correlations between percentage open-sky radiation and both annual stem elongation and inflorescence count (Fig. 3) are evidence that resource limitation explains some of the phenotypic variation we found. The lack of such correlations in North Dakota, Wisconsin, and Illinois may be a result of the low range of insolation found within these populations (Table 4), rather than evidence that these plants are less plastic than those in Alabama and Florida.

Additional evidence that *D. palustris* is limited in resources available for growth in habitats where it is indigenous are the negative correlations between length of inflorescences and both inflorescence count and stem elongation during the previous year (Fig. 4). A tradeoff seems to exist between inflorescence size and inflorescence count, a conclusion consistent with theory predicting that a plant with finite resources may produce either more inflorescences or larger inflorescences, but not both (Worley and Barrett, 2001). Different

patterns of allocation to organ development may produce population means suggestive of genetic divergence, when such differences in fact represent plastic responses to resource availability (Dorken and Barrett, 2004). The Mantel correlation shows that phenotypic and environmental distances are positively correlated (Table 5), which suggests that environmental differences influence the average phenotype of each population. Genetic differentiation in response to environmental selection pressure or plastic responses to contrasting environments may account for this observation.

GENOTYPIC VARIATION

Although not all possible sources of apparent divergence in phenotypic traits are genetic, we found clear genotypic differences among the five populations of *D. palustris* (Table 3 and Table 6). AMOVA showed that genotypic differentiation among the five populations accounted for more of the total molecular variance than diversity within populations, evidence for historically limited gene flow among widely separated gene pools. The correlation between genotypic and environmental distance, but not between genotypic and geographic distance (Table 5) suggests that genetic differentiation among populations is shaped more by environmental selection pressures than by the influences of geographic distance alone. However, we also found that geographic distance and environmental distance were themselves correlated; the absence of evidence for a correlation between genetic and geographic distances may therefore be a product of limited statistical power. Although merely suggestive, the positive correlation we found between phenotypic and genotypic distance is evidence that some of the divergence in phenotypic traits among the five populations is genetic (Table 5).

Our findings offer some information on the phylogeography and conservation value of several populations of *D. palustris*. For instance, the northward expansion of *D. palustris* to North Dakota following the last glaciation seems to be associated with loss of allelic richness (Table 6) as a consequence of genetic bottlenecks, founder effects, or strong directional selection (Lacy, 1987; Broyles, 1998). In contrast, the population in Florida, which is at the southern limit of the range of *D. palustris,* is distinguished genetically by its population-specific loci. Populations like the one in North Dakota, which may be best-adapted to conditions at the leading edge of range shifts as a consequence of directional selection during migration (Lacy, 1987), may be important for northward expansion of *D. palustris* in response to regional climate change (Davis and Shaw, 2001). Genetically diverse populations at the trailing edge of range shifts, like the one in Florida (Table 6), represent important repositories for genetic diversity of a species (Hampe and Petit, 2005). Our findings, which show the population in Florida is most phenotypically distinct and the populations in North Dakota and Florida are the most genetically dissimilar (Table 3), demonstrate that these populations at the northern and southern distributional extremes of *D. palustris* represent priorities for conservation (Hampe and Petit, 2005).

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TABLE 1.—Floral traits of *Dirca palustris* in five populations. Population means are of 30 replicate plants, with five observations per plant for inflorescence measures and three observations per plant for number of inflorescences on selected branches.

	Population				
Measure	North Dakota	Wisconsin	Illinois	Alabama	Florida
Inflorescences per branch	13.5 a[z]	10.5 b	5.6 c	9.7 b	9.4 b
Flowers per inflorescence	3.2 a	3.0 b	3.0 b	3.0 b	3.0 b
Length of inflorescence (mm)	8.8 c	9.3 b	10.6 a	10.7 a	9.4 b
Width of inflorescence (mm)	5.6 b	5.6 b	5.6 b	5.8 b	6.3 a
Length of calyx (mm)	5.9 c	6.6 b	7.8 a	8.0 a	6.4 b
Width of calyx (mm)	2.4 cd[y]	2.5 c	2.4 d	2.6 b	3.0 a
Extension of pistil (mm)	3.4 c	4.0 b	4.6 a	3.8 b	4.1 b
Extension of stamens (mm)	2.9 ab[y]	2.7 ab	2.8 ab	2.6 b	2.9 a

[z]Means followed by the same letter within rows are not different at $P \leq 0.05$, according to Tukey's Honestly Significant Difference test.

[y]Identical means followed by different letters within rows are explained by rounding effects.

TABLE 2.—Vegetative traits of *Dirca palustris* in five populations. Population means are of 30 replicate plants. Measures of branches on selected stems, stem elongation, and length of internodes are from three observations per plant.

	Population				
Measure	North Dakota	Wisconsin	Illinois	Alabama	Florida
Plant height (cm)	160 a[z]	161 a	147 ab	127 b	126 b
Plant height-to-width ratio	1.2 a	1.0 b	1.2 a	1.0 b	1.0 b
Trunk diameter (mm)	34.9 b	43.1 a	33.2 b	29.4 b	27.1 b
Trunk shape ($mm \cdot cm^{-1}$)[y]	0.22 b	0.27 a	0.23 b	0.23 b	0.21 b
Branches on selected stems	6.8 b	7.4 ab	6.5 b	8.0 a	6.2 b
Stem elongation (mm)[x]	87.5 a	47.3 b	35.0 c	43.2 bc	54.2 b
Internode (mm)	28.3 a	19.8 b	21.3 b	20.5 b	18.4 b
Pubescence on bud scales	brown	brown	brown	brown[w]	white

[z]Means within each row followed by the same letter are not different at $P \leq 0.05$ according to Tukey's Honestly Significant Difference test.

[y]Calculated as trunk diameter divided by plant height.

[x]Represents the average of stem elongation during 2006 and 2007.

[w]Several plants in the population in Alabama had white pubescence on bud scales, although all plants sampled had brown-pubescent bud scales.

TABLE 3.—Phenotypic, environmental, geographic, and genetic distances calculated from pairwise comparisons of five populations of *Dirca palustris*. Values for phenotypic, environmental, and genetic distances are unitless Euclidean distances. Values for geographic distances are km.

	Distance			
Population comparison	Phenotypic	Environmental	Geographic	Genetic
Florida - Alabama	2.2	2.1	444	67
Florida - Illinois	2.6	2.1	842	68
Florida - Wisconsin	2.0	3.8	1545	69
Florida - North Dakota	2.9	4.7	2324	74
Alabama - Illinois	1.2	1.9	412	41
Alabama - Wisconsin	0.8	2.1	1103	34
Alabama - North Dakota	2.1	3.3	1891	43
Illinois - Wisconsin	1.5	2.0	710	29
Illinois - North Dakota	2.3	3.0	1483	40
Wisconsin - North Dakota	1.8	1.3	811	19

TABLE 4.—Environmental conditions at sites supporting five populations of *Dirca palustris*.

Measure	Population				
	North Dakota	Wisconsin	Illinois	Alabama	Florida
Open-sky radiation (%)[z]					
Minimum	2.5	0.8	0.5	1.8	2.8
Mean	4.8	4.4	2.5	11.6	9.2
Maximum	7.4	19.6	25.3	79.4	25.6
Soil[y]					
pH	5.2	6.0	5.6	6.0	7.4
Nitrogen (%)	0.1	0.3	0.2	0.3	0.3
Organic matter (%)	1.0	4.4	2.4	4.5	6.1
Climate[x]					
Annual precipitation (cm)	47.2	83.9	117.2	136.0	143.1
Mean daily minimum (°C)	-3.5	0.7	7.2	9.4	12.9
Mean daily maximum (°C)	9.4	11.9	19.3	21.9	25.6

[z]Mean percentage open-sky radiation within each population is the average of three measurements recorded over each of 30 replicate plants. Minimum percentage open-sky radiation within each population is the single-lowest average of six measurements over a plant. Maximum percentage open-sky radiation within each population is the single-highest average of six measurements over a plant.

[y]Soil measurements were obtained from composite soil samples comprising 10 subsamples collected at each study site.

[x]Data are from the National Climatic Data Center (U.S. Department of Commerce, 2007).

TABLE 5.—Correlations among genetic, phenotypic, geographic, and environmental distance matrices according to the Mantel test. Numbers represent R_{xy} values for comparisons between each pair of distance matrices. Numbers in parentheses are upper-tail *P* values representing the likelihood of observing an equal or greater correlation strictly by chance.

Distance measure	Genetic	Phenotypic	Geographic
Phenotypic	0.66 (0.071)		
Geographic	0.33 (0.216)	0.50 (0.087)	
Environmental	0.63 (0.010)	0.56 (0.048)	0.89 (0.013)

TABLE 6.—Comparisons of loci produced using five 3'-anchored ISSR primers to amplify genomic DNA collected from five populations of *Dirca palustris*. Values for each population depict the number of samples that amplified, the number of loci produced, the number of population-specific loci, and the percentage of polymorphic loci.

	Population				
	North Dakota	Wisconsin	Illinois	Alabama	Florida
No. of samples	17	19	14	18	16
No. of loci	230	254	249	264	264
No. of population-specific loci	0	1	3	5	14
% of polymorphic loci	20	32	24	36	31

Figure Captions

FIG. 1.—Locations (black circles) of five populations of *Dirca palustris* assessed. Populations are in North Dakota (along Little Pembina River; Pembina Co.), Wisconsin (Jersey Valley County Park; Vernon Co.), Illinois (Bell Smith Ecological Area, Shawnee National Forest, Pope Co.), Alabama (Buck's Pocket State Park; Marshall Co. and DeKalb Co.), and Florida (Torreya State Park; Liberty Co.).

FIG. 2.—Diagram of a typical inflorescence of *Dirca palustris.* Measurements recorded were length of calyx (LC), width of calyx (WC), extension of stamens (ES), extension of pistil (EP), length of entire inflorescence (LI), and width of entire inflorescence (WI). Measurements of individual flower parts were recorded from one flower within each inflorescence.

FIG. 3.—Relationship between percentage of open-sky radiation and annual stem elongation and inflorescence count on selected branches of plants of *Dirca palustris* in Alabama and Florida. Each point represents the mean value for a plant. Regression functions are: stem elongation = 121.8 (% open sky) + 36.3, $r^2 = 0.36$; inflorescence count = -26.1 (% open sky^2) + 33.6 (% open sky) + 6.6, $r^2 = 0.33$.

FIG. 4.—Relationships of inflorescence length to inflorescence count on selected branches and to annual stem elongation of *Dirca palustris* during the previous year. Data are from all five populations; each point represents the mean value for a plant. Regression functions are:

length = -0.11 (inflorescence count) + 10.8, $r^2 = 0.17$; length = -0.01 (stem elongation) + 10.42, $r^2 = 0.12$.

FIG. 5.—Relationships of inflorescence count to stem elongation during the previous year, internode length, and branch counts on selected stems of *D. palustris*. Data are from all five populations; each point represents the mean value for a plant. Regression functions are: count = -0.001 (stem elongation^2) + 0.23 (stem elongation) + 2.0, $r^2 = 0.36$; count = 0.24 (internode) + 4.8, $r^2 = 0.13$; count = 1.9 (first-order branches) + 1.5, $r^2 = 0.18$.

FIG. 1

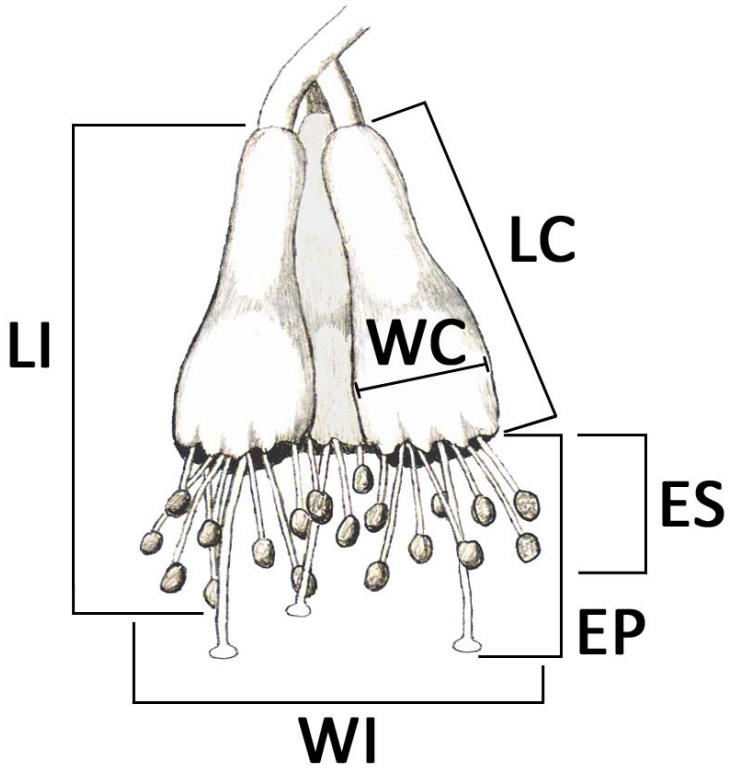

FIG. 2

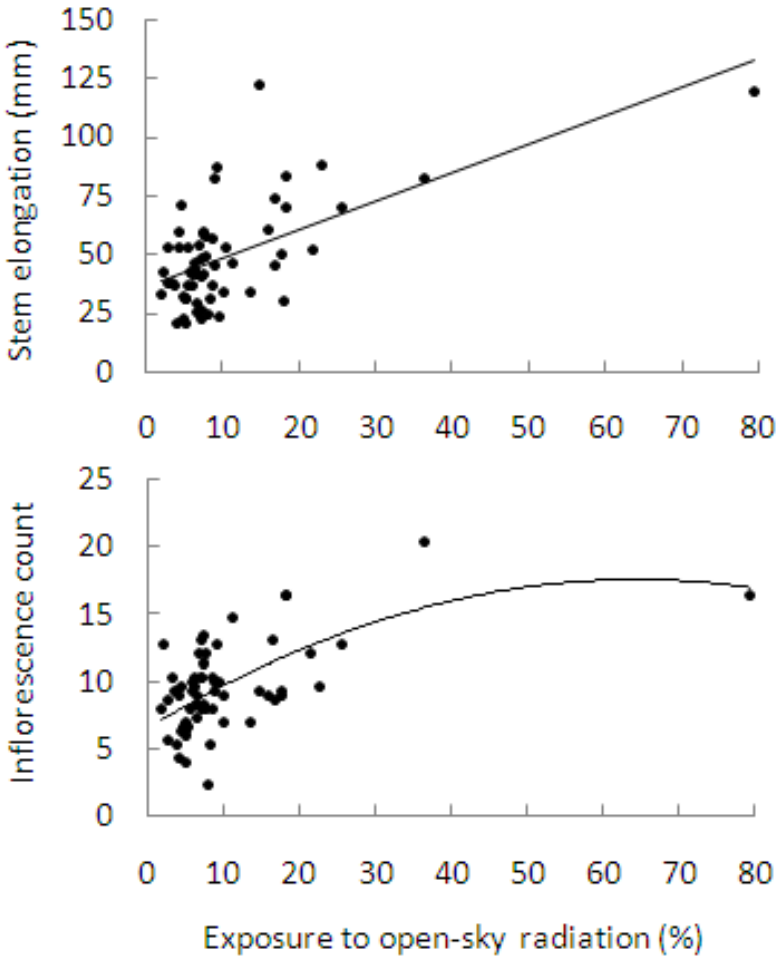
Stem elongation (mm)
150
125
100
75
50
25
0
0 10 20 30 40 50 60 70 80
Inflorescence count
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80
Exposure to open-sky radiation (%)

FIG. 3

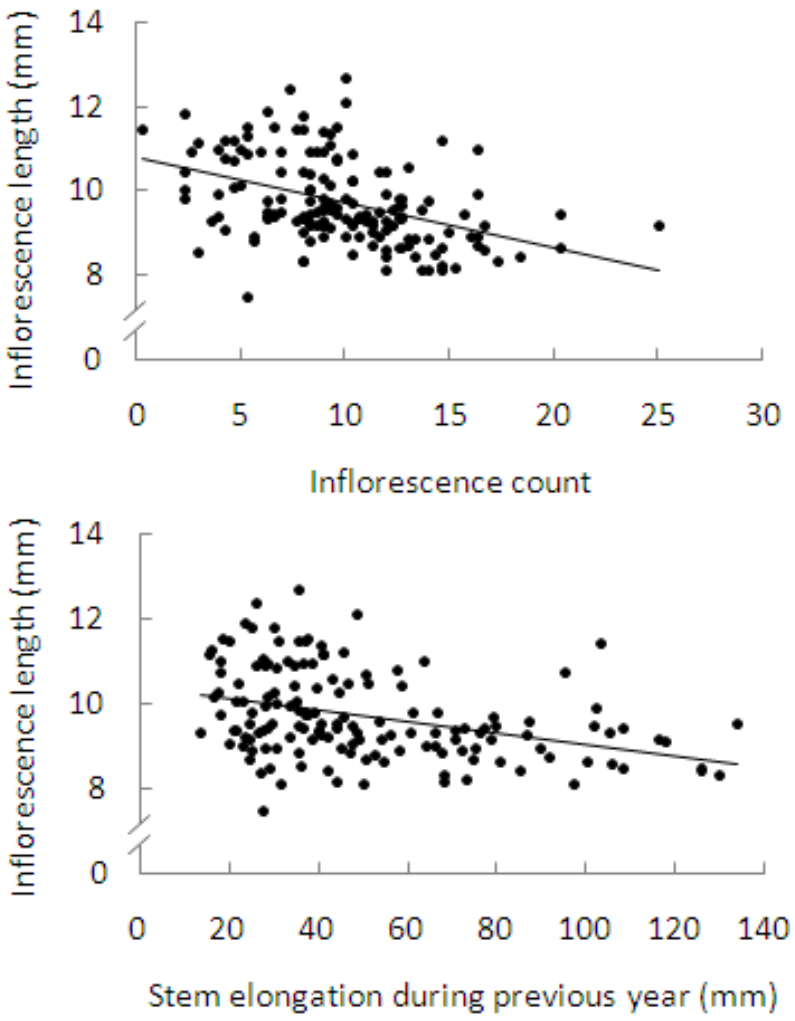
Inflorescence length (mm)
14
12
10
8
0
0
5
10
15
20
25
30
Inflorescence count
Inflorescence length (mm)
14
12
10
8
0
0
20
40
60
80
100
120
140
Stem elongation during previous year (mm)

FIG. 4

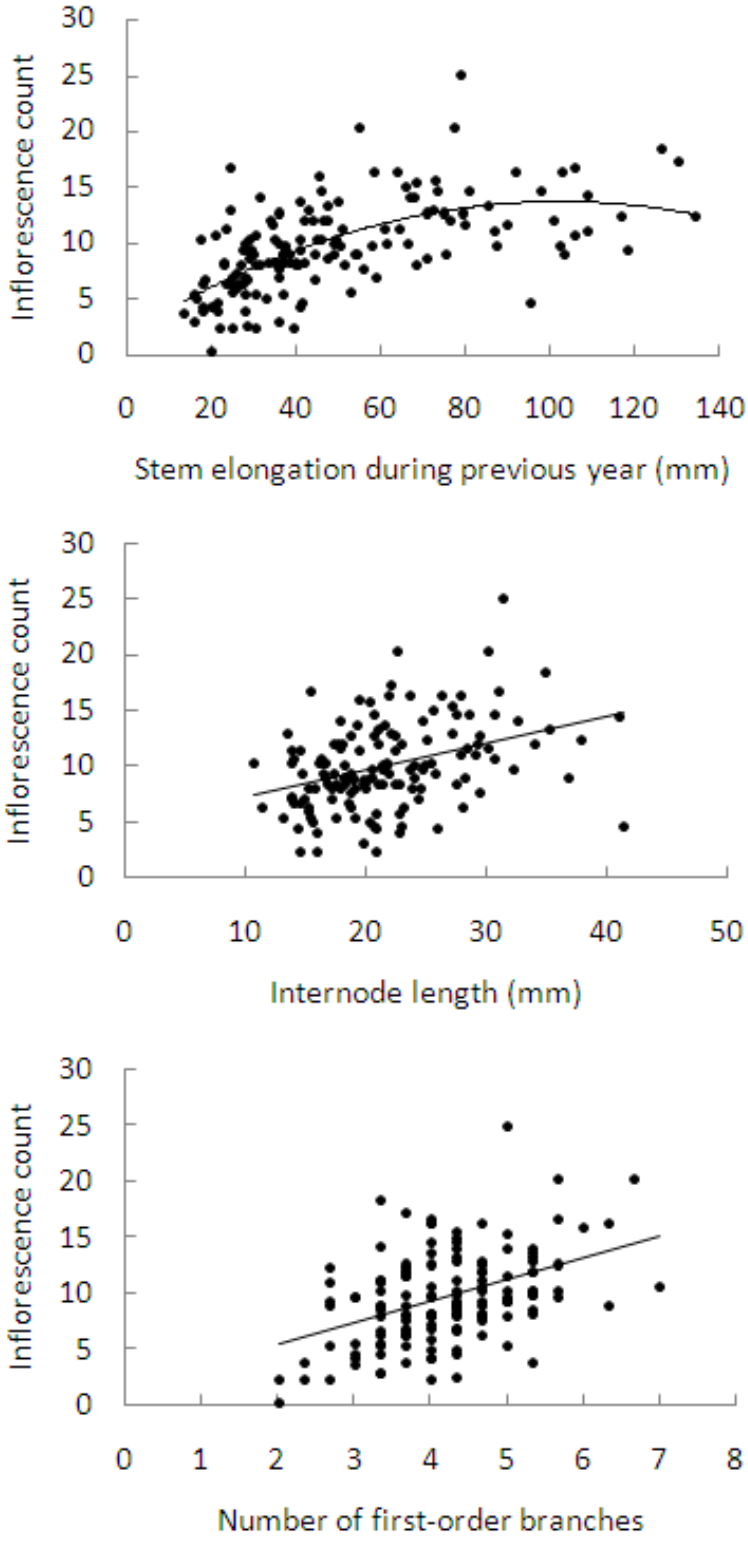
Inflorescence count
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
Stem elongation during previous year (mm)
Inflorescence count
30
25
20
15
10
5
0
0
10
20
30
40
50
Internode length (mm)
Inflorescence count
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
Number of first-order branches

FIG. 5

CHAPTER 4. VARIATION IN DEVELOPMENT AND RESPONSE TO ROOT-ZONE PH AMONG SEEDLINGS OF *DIRCA PALUSTRIS* (THYMELAEACEAE) FROM THREE PROVENANCES

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ABSTRACT. Horticulturists have not promoted use of *Dirca palustris* L. (eastern leatherwood) despite its suite of traits valued by gardeners and landscapers. Horticultural production of *D. palustris* may be hindered by slow shoot growth and sensitivity of plants to edaphic conditions. Because of discrepancies in reported tolerances of *D. palustris* to root-zone pH, we assessed whether pH of soils supporting indigenous populations in Florida, Maine, and North Dakota corresponded to responses of seedlings from the three provenances to root-zone pH of 4.5 to 7.3 in soilless media. Regression showed that root zones at pH 5.8 promoted maximum stem length of seedlings from Florida and North Dakota, whereas root zones at pH 4.5 led to maximum stem length of seedlings from Maine. Root-zone pH 5.6 and 5.5 fostered maximum root and shoot dry weight, respectively, for seedlings from Florida, whereas root zones at pH 4.5 promoted maximum root and shoot dry weights of seedlings from Maine and North Dakota. Averaged over provenance, relative leaf greenness (SPAD) decreased by 62%, and foliar N, Fe, Mn, and Zn decreased by 49%, 70%, 95%, and 48%, respectively, as root-zone pH increased from 4.5 to 7.3. Foliar P decreased at both low and high pH. The pH of soils where seeds were collected did not predict optimal root-zone pH for stem length or biomass accrual in soilless media; genotypes from soils with a pH of 7.4 in North Dakota did not exhibit greater tolerance to high pH than genotypes from Maine or Florida, where pH of indigenous soil was 6.1 and 5.2, respectively. Averaged over pH treatments, seedlings from Florida showed the greatest stem length and formed the most shoot biomass, whereas seedlings from North Dakota had stouter stems, greater root biomass, and greater root-to-shoot ratios than did seedlings from Florida and Maine. Our results illustrate that acidic media facilitate horticultural production of *D. palustris,* that further evaluation of provenance differences could facilitate selection of genotypes for horticulture,

and that tolerances of genotypes to root-zone pH do not strictly correspond to the pH of soils on which they were indigenous.

Introduction

Dirca palustris L. occurs sporadically as an understory shrub in rich mesic forests from Nova Scotia west to North Dakota, and south to Oklahoma and Florida (Nevling, 1962; Peterson et al., 2009). Known commonly as eastern leatherwood, the species has received little attention from horticulturists despite its suite of traits valued by gardeners and landscapers, including its flowers in early spring, unique and upright growth habit, rich yellow foliage in autumn, and shade tolerance (Anderson, 1933; Del Tredici, 1984; Esson, 1949). Our observations indicate that limitations to commercial production of *D. palustris* include its sensitivity to edaphic conditions and slow shoot growth (5-10 cm annually). Overcoming these challenges could facilitate the use of *D. palustris* as a commercially viable shrub for horticulture. Our broad goal is to determine how horticulturists can manage the root-zone environment during production and use of *D. palustris* to promote growth and plant health.

Responses of *D. palustris* to pH of substrates used for horticultural production are unknown, and discrepancies exist in the reported pH affinities of the species in the wild. Del Tredici (1984) reported that *D. palustris* performs best in moist areas that have high limestone content, and Cooperrider (1962) found occurrence of the species in eastern Iowa to be limited to sites over limestone bedrock. Clark (1971) asserted that *D. palustris* occurs over circumneutral or basic soils in the southeastern United States. Nevling (1962) characterized *D. palustris* as a facultative calciphile, whereas Anderson (1933) claimed it

grows in a range of environments but avoids limestone. A survey of *D. palustris* in South Carolina demonstrated that it occurs on acidic soils (Ward and Horn, 1998), and Dirr (1997) stated that the species prefers acidity. Conflicting information on soil-pH tolerances of *D. palustris* might indicate either that the species is inherently adapted to a wide range of soil pH, or that ecotypic adaptation to localized soil pH has occurred (Dawson et al., 2007; Rajakaruna, 2004; Snaydon, 1970). If the latter is true, genotypes of *D. palustris* from disparate provenances may respond differently to root-zone pH, which could influence strategies for selecting and using genotypes for horticulture. Researchers have demonstrated that genotypes of the same species from contrasting edaphic environments may vary in response to root-zone pH (Anderson and Ladiges, 1978; Dawson et al., 2007; Kerley et al., 2002; Snaydon, 1970). Nevertheless, the degree to which the pH of soil where a genotype is indigenous predicts responses of propagules of that genotype to pH of horticultural substrates likely varies among species according to their evolutionary histories (Bounejmate and Robson, 1992; Snaydon, 1970; Symonds et al., 2001).

We evaluated responses of seedlings of *D. palustris* from diverse provenances to pH of a soilless medium. We studied seedlings indigenous to Florida, Maine, and North Dakota because our assessments of wild populations showed that the pH of soils supporting these populations differs. Our specific objectives were to 1) evaluate growth of first-year seedlings of *D. palustris* in soilless media across a range of pH, 2) assess whether pH of soils where the genotypes were native corresponds to early responses of seedlings to pH in a horticultural substrate, and 3) characterize phenotypic differences of horticultural utility among seedlings from the three provenances.

Materials and Methods

Plant materials and handling

Dirca palustris was propagated from seeds harvested within three populations in 2007. Drupes were collected in April from Torreya State Park in Liberty Co., FL (30°34'632"N, 84°56'747"W) from a minimum of 30 maternal parents. In July, drupes were collected near Sherman Mills in Aroostook Co., ME (45°56'056"N, 68°19'357"W) and near the Little Pembina River in Cavalier Co., ND (48°57'892"N, 098°06'031"W) from a minimum of 10 maternal parents and 30 maternal parents, respectively. A composite soil sample comprising ten subsamples was collected from each site where drupes were obtained. Soil pH was measured by the Soil and Plant Analysis Laboratory, Iowa State University, Ames, IA, using the 1:1 soil:water suspension method. Drupes were sown in flats filled with a soilless, peat moss-based medium (Fafard® 52, Fafard®, Inc., Agawam, MA) within one week after collection. Flats were held in a minimally heated greenhouse until seeds germinated during spring 2008, after exposure to warm and cold stratification under ambient conditions.

Plants from each provenance were randomly assigned to one of five pH treatments when seedlings had three to four true leaves. The experiment was conducted on a shaded bench in a glass-glazed greenhouse where photosynthetically active radiation (PAR) at canopy level at solar noon was measured on 10 dates during the experiment. Mean PAR was 245 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (SE = 11.6). Temperatures in the greenhouse were logged with an iButton Data Logger (Maxim Integrated Products, Sunnyvale, CA). The mean daily temperature during the experiment was 22 °C; mean daily minimum and maximum were 17 and 32.5 °C.

Treatments and experimental design

We adjusted pH of a soilless, peat moss-based medium (Fafard® 52) to 4.5, 5.3, 5.9, 6.5, and 7.3 with 1-M sulfuric acid (H_2SO_4) or reagent-grade calcium carbonate ($CaCO_3$) (Table 1). These chemicals were chosen because injection of H_2SO_4 into irrigation water and addition of $CaCO_3$ to container substrates are common methods for lowering or raising pH of horticultural media. Distilled water was then added to each adjusted medium to ensure uniform moisture content among treatments. Seedlings from each provenance were transplanted singly into square band pots with a diameter of 6 cm and a height of 12.5 cm (Anderson Die & Mfg. Co., Portland, OR) filled with the media.

The experiment was designed as a two-way factorial, randomized complete block with three blocks. Six replicates were used for each combination of provenance and root-zone pH within each block (N=270; 90 per block). Seedlings were irrigated with tap water to container capacity every 5 to 9 d. Root-zone pH was measured every 7 to 14 d (Cavins et al., 2000). Leachate from two pots per treatment per block was collected and its pH determined with an IQ160 pH meter (IQ Scientific Instruments, Carlsbad, CA) with an IFSET probe.

Harvest and data analysis

Responses of plants were assessed after 15 weeks. Relative greenness, a presumed measure of relative chlorophyll content (Netto et al., 2005), was measured on the youngest fully expanded leaf of each plant with a handheld SPAD-502 Chlorophyll Meter (Konica Minolta Sensing, Tokyo, Japan). Length of the single stem of every seedling was determined from the cotyledon scars to the youngest node. Stem diameter was measured immediately below the cotyledon scars with handheld calipers. Shoot and root tissue was harvested from

every plant, media were washed from the roots, and both shoots and roots were dried at 67 °C for 5 to 7 d. Roots included all biomass below the cotyledon scars. Shoots and roots were weighed, and a root-to-shoot ratio was calculated for each seedling.

Leaves of plants from each treatment were analyzed for nutrient content by the Soil and Plant Analysis Laboratory, Iowa State University, Ames, IA. Because we had limited material for analysis, one sample was created for each combination of provenance and pH by combining leaves from multiple plants until there was enough biomass for the analyses. This allowed three analyses from each of the five pH treatments, with one sample from each of the three seedling provenances (N=15). Total nitrogen (N) was measured by combustion analysis, and iron (Fe), manganese (Mn), zinc (Zn), and phosphorus (P) were measured by microwave digestion.

Data were analyzed with the Statistical Analysis System 9.1.3 (SAS Institute Inc., Cary, NC). Treatment responses were natural log-transformed when necessary to normalize variances. The regression (REG) procedure and F-tests were used to test the significance of linear and quadratic models for responses of seedlings to root-zone pH. The LSMEANS option of the general linear models (GLM) procedure was used with Tukey adjustment for multiple comparisons to generate means-separation statistics. Main effects of root-zone pH and seedling provenance were tested, as were their interactions.

Results

The pH of soils supporting the populations from which drupes were collected varied from acidic (5.2 in Florida and 6.1 in Maine) to basic (7.4 in North Dakota). In containers, acidic root zones enhanced stem length and root and shoot dry weights of seedlings from all

three provenances, though interactions existed between root-zone pH and provenance for all three responses (interactions: $P = 0.0002$, $P < 0.0001$, and $P = 0.0122$, respectively). Stem length of seedlings from Maine decreased linearly with increasing pH, whereas stem length of seedlings from Florida and North Dakota was best described by quadratic functions with predicted maxima at pH 5.8 (Fig. 1). Both root (Fig. 2A) and shoot (Fig. 2B) dry weight of seedlings from Maine and North Dakota decreased linearly with increasing pH. Quadratic functions best described responses of seedlings from Florida, with predicted maxima at pH 5.6 and pH 5.5, for root (Fig. 2A) and shoot (Fig. 2B) dry weight, respectively. Root-to-shoot ratios of seedlings from Maine and North Dakota increased linearly with increasing root-zone pH, whereas a quadratic function described the root-to-shoot ratios of seedlings from Florida (Fig. 2C).

Relative greenness of leaves on plants from all provenances decreased linearly with increasing pH (Fig. 3), and analysis of variance showed no evidence of differences among provenances in this response ($P = 0.1942$). Foliar concentrations of N, Fe, and Zn decreased linearly with increasing root-zone pH (Table 2), whereas concentration of Mn decreased quadratically with increasing root-zone pH. In contrast, regression of leaf P concentration with pH revealed a quadratic response with a predicted maximum P concentration at pH 5.5 (Table 2).

Differences in growth were found among seedlings from the three provenances, averaged across pH treatments (Table 3). Seedlings from Florida formed stems 48% longer than seedlings from North Dakota, and 211% longer than seedlings from Maine (Table 3). Seedlings from North Dakota and Maine had the thickest and thinnest stems, respectively (Table 3). Mean shoot dry weight of seedlings from Florida was 25% greater than that of

seedlings from North Dakota, and 129% greater than that of seedlings from Maine (Table 3). Root dry weight of seedlings from North Dakota averaged 53% and 134% greater than mean root dry weights of seedlings from Florida and Maine, respectively (Table 3). Root-to-shoot ratios of seedlings from North Dakota exceeded the root-to-shoot ratios of seedlings from Florida and Maine by 93% and 12%, respectively (Table 3).

Discussion

Seedlings of *D. palustris* were sensitive to the pH of a soilless, horticultural medium under greenhouse conditions. Regression statistics predicted that stem length and root and shoot dry weights of seedlings from all three provenances were maximized by root-zone pH <6.0; some measures of growth were maximal at pH 4.5, the lowest root-zone pH we evaluated (Fig. 1, Fig. 2A and B). These results demonstrate that use of acidic media facilitates cultivation of this species. Furthermore, the positive relationship between root-to-shoot ratio and root-zone pH (Fig. 2C) indicates that low-nutrient stress of seedlings increased as pH increased. This conclusion is based on numerous reports that relative allocations of biomass to roots increase as the nutrients available to a plant decrease (Gutschick and Kay, 1995; Huante et al., 1995). Also supporting this conclusion are data on relative greenness of leaves (Fig. 3) and foliar concentrations of N, Fe, Mn, Zn, and P (Table 2), which were greatest among plants in acidic media. These nutrient analyses support the assertion that root zones with pH <6.0 promote the health of *D. palustris*. However, curling and puckering was noted on lamina of some seedlings from all three provenances when grown in media at pH 4.5. This symptom and the high Mn concentrations in leaves of plants in media at pH 4.5 (Table 2) suggest that seedlings from all three provenances may be

sensitive to Mn availability in highly acidic media (Handreck and Black, 2002). Collectively, these results do not support the contention that *D. palustris* is insensitive to root-zone pH, nor the possibility that extensive local adaptation to root-zone pH has occurred. Some researchers have stated that the species is restricted primarily to limestone soils (Clark, 1971; Cooperrider, 1962: Del Tredici, 1984), whereas others have reported that *D. palustris* is typically found on or prefers acidic soils (Anderson, 1933; Dirr, 1997; Ward and Horn, 1998). Our results provide clarification for horticulturists by demonstrating that seedlings of *D. palustris* in horticultural media prefer acidic root zones.

Despite their general preference for acidic media, seedlings from the three provenances varied in some ways in their responses to the wide range of root-zone pH we used. Stem length and root and shoot dry weight of seedlings from Florida were diminished by both low-pH and high-pH root zones (Fig. 1, Fig. 2A and B). In contrast, stem length and root and shoot dry weight of seedlings from Maine were greatest at pH 4.5 and diminished with increasing pH (Fig. 1, Fig. 2A and B). Responses of seedlings from North Dakota were less consistent. Stem length was reduced by both low-pH and high-pH root zones (Fig. 1), whereas dry weights were greatest in the low-pH root zone of 4.5 (Fig. 2A and B). The reason for these differential responses to root-zone pH among the provenances is unclear, though they may represent divergent tolerances to micronutrient limitation or toxicity. Regardless, the varied responses of seedlings from the three provenances to root-zone pH suggest that comprehensive assessments of edaphic tolerances may facilitate horticultural selection for specific responses to root-zone pH.

The pH of soils that supported the indigenous plants from which we collected seeds did not predict optimal root-zone pH for stem length or biomass accrual in soilless media.

This contrasts with previous work in which populations within a species occurring on soils of divergent pH demonstrated localized adaptation to disparate root-zone conditions (Kerley et al., 2002; Snaydon, 1970). In our experiment, responses of seedlings of *D. palustris* to root-zone pH were independent of indigenous soil pH at the three provenances. Although the populations from which seeds were collected in North Dakota and Maine were supported by slightly basic soils (pH 7.4) and acidic soils (pH 6.1), respectively, seedlings from these provenances produced greatest biomass in strongly acidic media at pH 4.5 (Fig. 2A and B). In contrast, soil supporting the population in Florida was the most acidic of the three provenances (pH 5.2), but regression predicted a root-zone pH near 5.5 would promote the greatest dry weight of seedlings from Florida (Fig. 2A and B). This root-zone pH is higher than that which maximized dry weights among seedlings from both North Dakota and Maine. Similarly, stem-length responses of seedlings from the three provenances did not correspond to indigenous soil pH. Stem length was maximal for seedlings from Maine when grown at pH 4.5 in the root zone, whereas seedlings from Florida and North Dakota had the longest stems at a pH near 5.8 (Fig. 1). We conclude that efforts to select genotypes for specific adaptation to acidic or basic root zones should not be restricted to populations occurring on soils with the pH range of interest.

Evidence from *D. palustris* in the wild and results from other species demonstrate that our results should not be extrapolated to the ecology of *D. palustris* in its native habitats or horticultural landscapes. Although we observed that *D. palustris* preferred an acidic horticultural substrate, the wild population on a basic soil in North Dakota grows more vigorously than the population in Florida on an acidic soil (Peterson, 2009). We recognize that other factors might explain the difference in growth of plants in these populations.

Nonetheless, it is apparent that *D. palustris* can grow vigorously in field soils that are mildly basic. Our use of a soilless medium may account for the relatively poor response we observed among seedlings as pH increased (Fig. 1, Fig. 2A and B). Plants often tolerate a higher root-zone pH in mineral soils than in soilless media, due in part to the greater cation exchange capacity and micronutrient availability of many field soils (Handreck and Black, 2002). Our results are consistent with those of Symonds et al. (2001), who found that growth of species of *Eucalyptus* Labill. indigenous to either acidic or alkaline soils was maximized by an acidic container medium. Trials in horticultural landscapes could extend our knowledge of the responses of *D. palustris* to soil pH.

Phenotypic differences were observed among seedlings from the three provenances (Table 3), suggesting that further evaluation of provenance differences may facilitate selection of genotypes for horticulture. Annual stem elongation should be a selection trait of primary interest because the species typically grows slowly (Steyermark, 1963). The population in Florida is promising in this regard; seedlings from Florida showed the greatest stem length and shoot dry weight (Table 3). Other traits of potential concern to those interested in selecting genotypes for horticulture include stem diameter, root biomass, and root-to-shoot ratios. Seedlings from the population in North Dakota had stouter stems, greater root dry weight, and greater root-to-shoot ratios than did seedlings from Florida or Maine (Table 3). Adaptation for enhanced foraging of soil among plants from North Dakota represents a plausible explanation for the greater allocation of biomass to root-system development by seedlings from this provenance. Low available soil moisture can favor the evolution of increased root-to-shoot ratios (Markesteijn and Poorter, 2009; Zhang et al., 2005), and mean annual precipitation near the population of *D. palustris* in North Dakota is

less than 40% of the annual precipitation near the population in Florida (U.S. Department of Commerce, 2007). The horticultural implications of these provenance differences merit further investigation. If the trait remains stable in different environments and as plants age, the high root-to-shoot ratio of seedlings from North Dakota may impart increased drought avoidance and an enhanced capacity to sequester nutrients in horticultural landscapes.

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Table 1. Addition of 1-M H_2SO_4 and reagent-grade $CaCO_3$ to a soilless, peat moss-based medium to achieve pH treatments.

Amendment	Amount added L^{-1} medium	Mean pH[z]	SE of mean pH
1-M H_2SO_4	35 mL	4.5	0.06
1-M H_2SO_4	23 mL	5.3	0.06
1-M H_2SO_4	14 mL	5.9	0.07
None	- -	6.5	0.07
$CaCO_3$	5 g	7.3	0.05

[z]Values represent average measures of leachate pH taken every 7 to 14 d.

Table 2. Mean foliar nutrient concentration of *Dirca palustris* after 15 weeks of growth. Means are of three replicates for each pH treatment, one from each of three provenances. Regression functions for foliar nutrients are: % N = -0.6 (pH) + 6.4, $r^2 = 0.87$; $mg \cdot kg^{-1}$ Fe = -64.5 (pH) + 520.6, $r^2 = 0.51$; $mg \cdot kg^{-1}$ Mn = 287.2 (pH^2) - 4104.6 (pH) + 14720, $r^2 = 0.86$; $mg \cdot kg^{-1}$ Zn = -10.2 (pH) + 115.2, $r^2 = 0.16$; $mg \cdot kg^{-1}$ P = -478.5 (pH^2) + 5218.3 (pH) – 11301, $r^2 = 0.59$.

Root-zone pH	N (%)	Fe ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Zn ($mg \cdot kg^{-1}$)	P ($mg \cdot kg^{-1}$)
4.5	3.5	247	1,979	65	2,381
5.3	3.0	186	1,313	65	3,221
5.9	2.5	103	284	52	2,684
6.5	2.1	91	165	59	2,271
7.3	1.8	74	101	34	1,369
Model					
Linear	<0.0001	0.0002	<0.0001	0.0492	0.0251
Quadratic	NS[z]	NS	0.0092	NS	0.0059

[z]Not significant at $P \leq 0.05$.

Table 3. Mean stem length, stem diameter, shoot dry weight, root dry weight, and root-to-shoot ratio of seedlings of *Dirca palustris* from three provenances after 15 weeks of growth. Provenance means are of 90 replicates, 18 each of five pH treatments (N=270). Interactions between provenance and root-zone pH existed for some variables; an assessment of the sources of the interactions showed that they did not preclude consideration of provenance main effects. Means for the main effect of provenance therefore are shown.[z]

	Stem (mm)		Dry weight (mg)		
Provenance	Length	Diameter	Shoot	Root	Root-to-shoot ratio
Florida	56 a[y]	2.2 b	188 a	245 b	1.6 c
Maine	18 c	2.1 c	82 c	161 c	2.6 b
North Dakota	38 b	2.4 a	151 b	376 a	2.9 a

[z]Interaction means for stem length are depicted in Fig. 1, and interaction means for shoot dry weight, root dry weight, and root-to-shoot ratio are depicted in Fig. 2. Interaction means for stem diameter (mm) of seedlings grown in root-zone pH 4.5, 5.3, 5.9, 6.5, and 7.3: Florida: 2.3, 2.5, 2.3, 2.3, and 1.7, respectively; Maine: 2.5, 2.0, 2.1, 1.9, and 1.8, respectively; North Dakota: 2.9, 2.5, 2.4, 2.2, and 2.0, respectively.

[y]Means within each column followed by the same letter are not different at $P \leq 0.05$ according to Tukey's Honestly Significant Difference test.

Fig. 1. Interaction of root-zone pH and provenance for stem length of seedlings of *Dirca palustris*. Symbols represent means of 18 single-plant replications ± SE. Regression models were determined based on log-transformed data, but plot depicts untransformed data for clarity. Regression functions are: Florida: stem length = -13.1 (pH^2) + 152.7 (pH) – 376.0, r^2 = 0.93; Maine: stem length = -4.3 (pH) + 43.0, r^2 = 0.57; North Dakota: stem length = -5.7 (pH^2) + 66.0 (pH) – 148.2, r^2 = 0.88.

Fig. 2. Interactions of root-zone pH and provenance for root dry weight, shoot dry weight, and root-to-shoot ratio of seedlings of *Dirca palustris*. Symbols represent means of 18 single-plant replications ± SE. Regression models were selected based on log-transformed data, but plots depict untransformed data for clarity. Regression functions for root dry weight are: Florida: weight = -37.7 (pH^2) + 418.2 (pH) – 874.3, r^2 = 0.87; Maine: weight = -18.5 (pH) + 269.9, r^2 = 0.43; North Dakota: weight = -59.3 (pH) + 725.2, r^2 = 0.95. Functions for shoot dry weight are: Florida: weight = -48.5 (pH^2) + 524.2 (pH) – 1171.8, r^2 = 0.92; Maine: weight = -37.0 (pH) + 299.8, r^2 = 0.77; North Dakota: weight = -46.5 (pH) + 424.6, r^2 = 0.92. Functions for root-to-shoot ratio are: Florida: ratio = 0.3 (pH^2) – 2.9 (pH) + 8.9, r^2 = 0.93; Maine: ratio = 0.8 (pH) – 1.8, r^2 = 0.91; North Dakota: ratio = 0.6 (pH) – 0.4, r^2 = 0.92.

Fig. 3. Relative greenness of leaves of *Dirca palustris* grown in a soilless medium adjusted to five pH levels. The unitless values were obtained as measures from a SPAD-502 Chlorophyll Meter. Symbols represent means of 54 single-plant replicates, 18 from each of Florida, Maine, and North Dakota. For all means, SE bars fell within the areas of the filled symbols. The regression function is: SPAD = -10.1 (pH) + 91.8, r^2 = 0.99.

Fig. 1

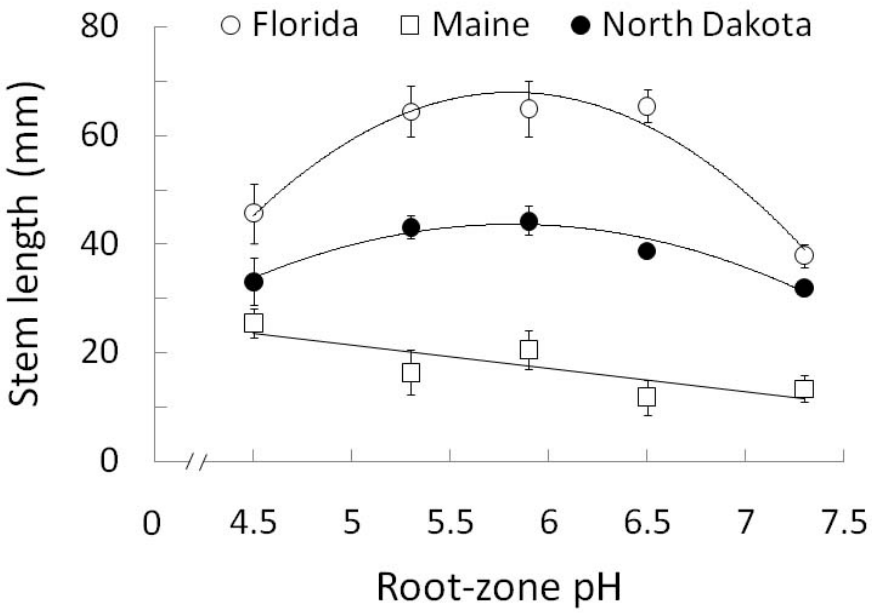

Florida
Maine
North Dakota
Stem length (mm)
80
60
40
20
0
0
4.5
5
5.5
6
6.5
7
7.5
Root-zone pH

Fig. 2

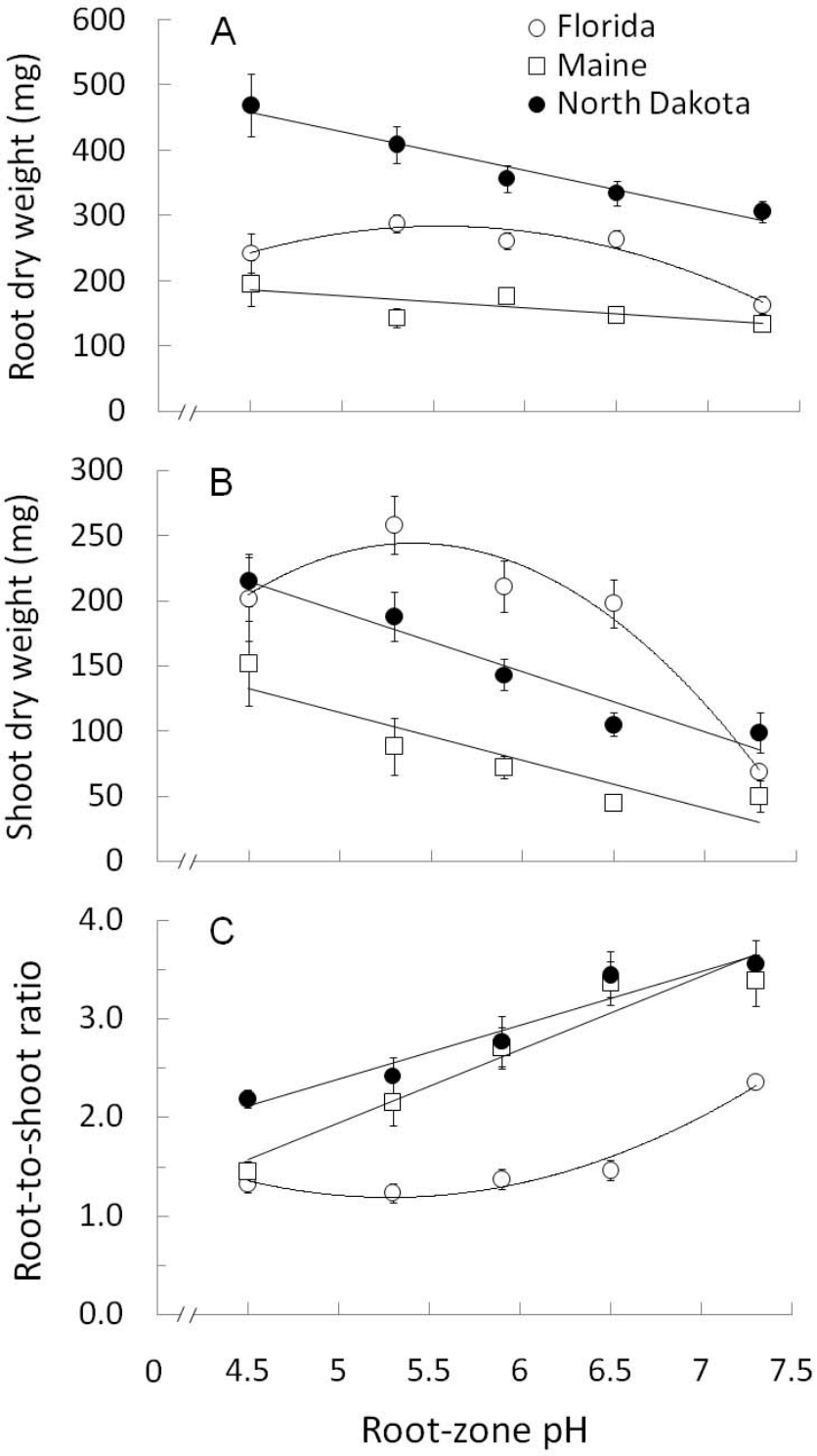
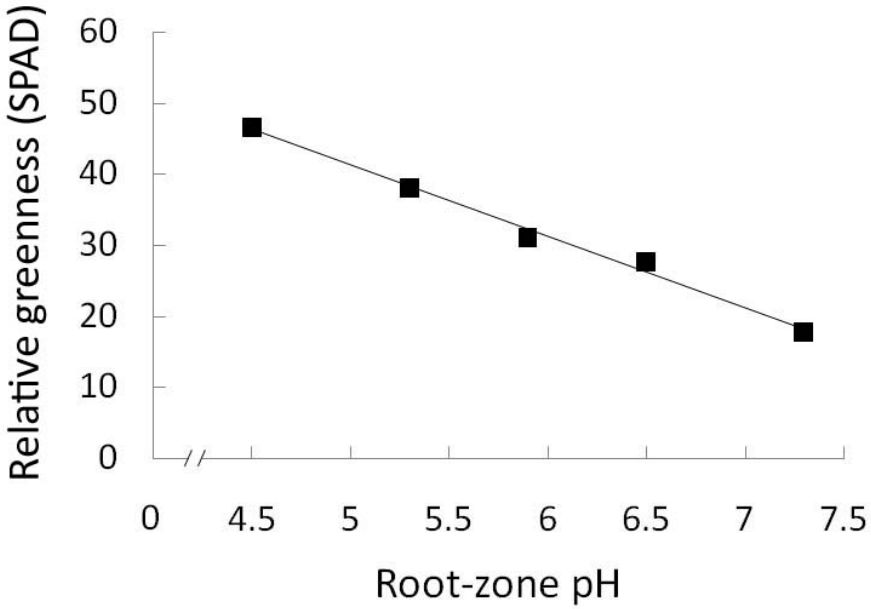

A
B
C
Florida
Maine
North Dakota
Root dry weight (mg)
Shoot dry weight (mg)
Root-to-shoot ratio
Root-zone pH
600
500
400
300
200
100
0
250
150
50
4.0
3.0
2.0
1.0
0.0
4.5
5
5.5
6
6.5
7
7.5

Fig. 3


Relative greenness (SPAD)
60
50
40
30
20
10
0
0
4.5
5
5.5
6
6.5
7
7.5
Root-zone pH

CHAPTER 5. GENERAL CONCLUSIONS

Many native plants have not been formally evaluated for phenotypic traits or physiological tolerances valuable to horticulture, despite recent interest in native landscapes. As natural genetic resources represent both the historical and modern foundations for horticultural improvements, the status of these indigenous genetic resources should receive greater consideration in the discipline. Evaluations of indigenous species for horticulture may be strengthened when they include assessments of variation in important traits among plants from disparate provenances. The preceding manuscripts demonstrate some benefits of interdisciplinary research that incorporates elements of both horticulture and ecology to preserve, identify, and utilize indigenous genetic resources. Consideration of the diversity of largely overlooked native species as potential ornamentals could offer greater ecological benefits than the development of nonnative species for horticulture.

Dirca palustris represents a good example of an indigenous species with ornamentally promising characteristics that is nonetheless overlooked in horticulture. The horticultural potential of scattered populations throughout the eastern United States and adjacent Canada has not been comprehensively assessed, despite the geographic, edaphic, and environmental diversity of the species. The results presented in the preceding manuscripts demonstrate that *D. palustris* is phenotypically and genotypically diverse, and that the identification of horticulturally superior seed sources merits further investigation. For instance, plants in a population in Florida were genetically and phenotypically distinct among the five populations discussed in chapter 3. The population in North Dakota was also phenotypically distinct, with more inflorescences, more flowers per inflorescence, and

greater annual stem elongation than plants within the other populations. I recommend the use of comprehensive provenance trials to elucidate the extent to which observed differences represent genetic divergence among provenances.

Regarding the natural habitats of *D. palustris,* several authors described the species as one largely restricted to limestone or alkaline soils, whereas others claimed it is typically found on or prefers acidic soils. The broad geographic distribution of *D. palustris*, the diverse edaphic habitats in which it is reported to persist, and the potential for reproductive isolation among populations of the species led me to speculate that provenances differing in soil pH might support genotypes with different preferences for root-zone pH. An experiment in the greenhouse in which seedlings from Florida, North Dakota, and Maine were cultured in soilless media of varying pH elucidated the pH preference of the species (chapter 4). Although the provenances from which seeds were collected represented diverse soil pH, seedlings of *D. palustris* were nonetheless sensitive to root-zone pH and preferred acidic media. Seedlings from the three provenances differed somewhat in response to root-zone pH, but differences in development according to provenance were more pronounced. This provenance variation demonstrates potential horticultural gains that may be made by selection of genotypes for increased shoot or root growth, depending on specific landscape use. Ecologically, the results of this experiment suggest that despite its reported affinity for limestone or alkaline soils in nature, the species ultimately prefers acidic root zones.

Additional ecological conclusions can be drawn from the work described in this thesis. The manuscripts in chapters 2, 3, and 4 present phenotypic differences among several populations within the range of *D. palustris,* whereas the manuscript in chapter 3 presents genotypic differences. Although a hallmark of *D. palustris* is the brown pubescence on bud

scales of plants throughout its range in eastern North America, the identification of white-pubescent plants in Florida demonstrates the need to quantify phenotypic variation within the range of *D. palustris.* A greater understanding of the extent and patterns of phenotypic variation can resolve potential taxonomic confusion in the genus *Dirca.* Results presented in chapter 3 demonstrate that the broad distribution of the species coupled with likely reproductive isolation has enabled considerable differentiation among populations. From a conservation perspective, the population in North Dakota could be best-adapted to conditions at the leading edge of range shifts, and may be important for northward expansion of *D. palustris* in response to global climate change. In contrast, the population in Florida, with its high number of population-specific loci and high degree of polymorphism, represents an important source of genetic diversity in *D. palustris*. This work leads me to conclude these peripheral populations of *D. palustris*, listed as endangered within their respective states, should represent priorities for conservation.

Suggestions for Further Research

Future research focused on *Dirca palustris* might include additional evaluation of provenance differences through in situ or common-garden studies. Extensive genotypic and phenotypic variation is probably present in the range of *D. palustris* as a consequence of the apparent reproductive isolation and diverse habitats in which the species is indigenous. Given the results reported here, the population of *Dirca* described in Florida may merit special taxonomic recognition following further evaluation. Only more intensive surveys of variation throughout the range of *D. palustris* will identify conclusively the appropriate taxonomic treatment for this population. Additional questions of ecological interest relate to

the factors responsible for both the broad range of *D. palustris* in the absence of obvious seed-dispersing agents, and the apparent contradiction between the widespread nature of the species and its scattered distribution restricted largely to mesic, forested, north-facing slopes. Finally, both the early-spring flowering schedule of the species, which may limit sexual reproduction, and confirmation that the genus is capable of asexual reproduction via rhizomes, leads to questions regarding the prevalence of sexual and asexual modes of reproduction within populations of *D. palustris*.

APPENDIX. INFLUENCE OF ROOT-ZONE PH ON GROWTH OF *DIRCA PALUSTRIS* IN HYDROPONIC CULTURE

Seedlings of *Dirca palustris* from Florida, North Dakota, and Maine were cultured in hydroponic media of 10% Hoagland Solution no. 1. Five pH treatments of 4, 5, 6, 7, and 8 were achieved using 1-N hydrochloric acid and 1-N sodium hydroxide. When seedlings had three to four true leaves, they were transplanted from flats of soilless media to metal containers holding the oxygenated liquid media. The experiment was arranged as a split-plot design with pH on the level of the main plot and provenance on the level of the split plot. Each combination of medium pH and seedling provenance had eight replicates. After 12 weeks, plants were harvested and data on their performances were collected. Data were analyzed with the Statistical Analysis System (SAS/STAT®, Version 8.2, 1999-2000). The general linear models (GLM) procedure was used with a split-plot design to account for multiple levels for provenance within each replicate of pH treatments. Seedlings overall did not respond well to culture in the liquid medium. The properties of the medium, higher than optimal temperatures in the greenhouse, damage to plants by spider mites, or unidentified mechanical damage to the seedlings during transplanting may explain the observed response of seedlings. Analysis of variance demonstrated no effect of root-zone pH on growth of seedlings of *D. palustris* ($P \geq 0.160$ for the three responses in Table 1). Because of this, the interaction between provenance and root-zone pH was not pursued. Table 1 summarizes the results of the experiment. Averaged over pH treatment, analysis of variance showed provenance differences for shoot dry weight and root dry weight ($P < 0.0001$), but not for stem length ($P = 0.461$).

Table 1. Stem length, shoot dry weight, and root dry weight of seedlings of *Dirca palustris* after 12 weeks of growth in liquid media adjusted to pH 4.0, 5.0, 6.0, 7.0, and 8.0.

		Provenance		
Response	Root-zone pH	Florida	Maine	North Dakota
Stem length (mm)	4	34.7	13.8	19.6
	5	35.3	13.3	27.1
	6	35.1	11.3	30.8
	7	39.7	12.8	27.5
	8	36.9	11.2	22.7
Shoot dry weight (mg)	4	86.4	42.8	59.6
	5	80.9	40.9	99.8
	6	84.8	38.0	116.8
	7	90.8	40.0	81.5
	8	93.5	29.0	70.2
Root dry weight (mg)	4	178.3	117.6	206.6
	5	157.1	125.4	248.8
	6	162.4	132.0	268.7
	7	152.9	108.8	225.8
	8	183.8	79.5	222.8

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