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# Historical and Geographic Context for the Evolution of Climate Niche Breadth in Temperate Plants

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# HISTORICAL AND GEOGRAPHIC CONTEXT FOR THE EVOLUTION OF CLIMATE NICHE BREADTH IN TEMPERATE PLANTS

by

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# ABSTRACT OF THE DISSERTATION

In order to predict how species will respond to global climate change, scientists must understand the relationships between traits, fitness, environments and distributions. Niche theory provides a useful framework. Niche breadth describes the range of environmental conditions necessary for population growth. Among these conditions, climate is especially important. Climate niche breadth in turn may reflect a confluence of different forces. This dissertation presents a series of projects that assess the relative roles of historical, geographic and population processes that contribute to climate niche breadth in temperate plants.

The first project evaluates the predictive power of a classical hypothesis. If gene flow slows divergent adaptation, then range fragmentation should promote niche breadth. By quantifying the relationships between environmental difference, geographic distance and genetic isolation among European plants, I show that the effect of allopatry on niche breadth depends on the role of the geographic distance among populations.

The remaining projects focus in increasingly finer detail on the evolution of niche breadth in a taxonomically complex group. *Dodecatheon* sect. *Dodecatheon* grow in diverse habitats across North America. They have confounded taxonomists with polyploidy, hybridization and convergent adaptation. Currently recognized species are either widespread or rare microclimate specialists. First, with multilocus phylogenetics, I show that the difference in niche breadth among rare and widespread species is not simply due to differences in environmental tolerance. In eastern North America, geographic heterogeneity and paleoclimate history strongly contribute to taxonomic rarity. The next project focuses on this group. Both rare eastern species are considered

glacial relicts. I test this hypothesis by combining ecophysiological and population genetic data in a new phylogeographic framework. The analysis shows that the match between traits and habitats is largely due to local gene flow and selection rather than migration and habitat sorting. Finally, through morphometrics, cytology, population genetics and greenhouse experiments, I show that dynamic polyploidy permits local movement of alleles between rare and widespread taxa.

Overall, these results suggest that anthropogenic climate change may threaten biodiversity not by forcing impossible migrations, but by promoting hybridization and complicating taxonomy just as it has in the past.

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# DISSERTATION INTRODUCTION

In many ways, the earth's climate is its single most important feature for supporting life. The range of temperatures that occurs across the globe produces water in all three physical states. Among these, liquid water is necessary for metabolism. At its most basic level, life depends on climate. Life also depends on climate in much of its spectacular detail. Spatial and seasonal variation in solar irradiation generates global circulation of fluids. These patterns of circulation, molded by the relative positions of continents and their features, determine the geographic redistribution of energy and materials as temperature and precipitation. At a global scale, climate delineates biomes (Whittaker 1975). At continental scales, variation in solar energy, temperature and precipitation explain much of the variation in species richness among regions (Currie and Paquin 1987, Francis and Currie 2003). At regional scales, geographic variation in temperature and precipitation limit the distribution of individual species (Woodward 1987, Gaston 2003, Lomolino *et al.* 2006)). At local scales, these same features of climate can influence community structure and the performance of individual organisms (Oberle *et al.* 2009).

The tight correspondence between climate and contemporary patterns of biodiversity across spatial scales and levels of biological organization is remarkable, especially given strong evidence for repeated, dramatic climate change throughout recent geological history. During the last 2.5 million years, cyclical variations in the earth's orbital properties (Milankovitch cycles) interacted with earthbound feedbacks to melt and reform massive ice sheets every 40,000 to 100,000 years (Hays *et al.* 1976). During transitions between glacial and interglacial conditions, climate changed rapidly. How did

life persist? Answering this question may illustrate the properties of life that confer resilience to climate change. These insights, in turn, might apply to conserving biodiversity during anthropogenic climate change (Davis *et al.* 2005). My dissertation investigates how biodiversity, as represented by temperate plants, persisted through historical climate change. As an introduction, I will discuss two main sources of evidence for responses to historical climate change: the fossil record and the comparative method. As I discuss them, I will highlight the difficulty of inferring processes from these patterns. In particular, I argue that framing patterns in an appropriate geographic and historical context is critical for interpreting the effects of key processes. Finally, I will describe how one process, gene flow, may mediate diverse responses to climate change at different geographic, temporal and taxonomic scales.

**The fossil record:** The Pleistocene fossil record documents major changes in biodiversity as climate changed. Several species went extinct (Barnosky *et al.* 2004), very few originated and most shifted their geographic distributions dramatically (Roy *et al.* 1996). Distributions shifts are especially well documented in the fossil record during the 21,000 years since the Last Glacial Maximum (LGM). Since the LGM, global climate has warmed by 7°C on average, with more rapid changes in temperature and precipitation in some regions over short intervals (Kim *et al.* 2008). Fossil pollen deposited in ponds and in packrat middens shows continuous shifts in plant community composition and species distribution through space and time (Huntley and Webb 1988). Species tended to show individualistic responses, often forming transient communities that no longer occur (Williams *et al.* 2001). However, most species migrated poleward or up in elevation as climate warmed (Huntley 1991).

The individualistic patterns of migration in the Pleistocene fossil record suggest that species' properties play a fundamental role in the resilience of the relationship between climate and biodiversity. Two critical properties implicated in this pattern are climate niche breadth and dispersal ability. Climate niche breadth is the range of climatic conditions necessary to maintain stable population size (*i.e*. net growth rate ≥ 0) based on a species' inherent physiological tolerances (Hutchinson 1957). Species can potentially persist in geographic areas that meet these conditions. Theory suggests stable geographic distributions can occur under a range of demographic and population genetic conditions when environments are stable (Pease *et al.* 1989, Kirkpatrick and Barton 1997, García-Ramos and Kirkpatrick 1997). However, when environments change, such as they do during climate change, some populations may no longer experience conditions suitable for replacement. Meanwhile, some previously uninhabitable areas outside the species' range may develop conditions appropriate for population growth. Two responses can occur. Populations experiencing change may evolve different physiological tolerances, effectively shifting the species' climate niche. Populations may also disperse into new geographic areas while going locally extinct in others, effectively shifting the species' geographic distribution.

The prevalence of migration in the Pleistocene fossil record suggests that this response has predominated over climate niche evolution during most species' survival through historical climate change. This interpretation of the fossil record reinforces widely held assumptions about the evolutionary process. Darwin (1859) strongly argued that evolutionary change is gradual. Others have extended this argument by asserting that evolutionary processes (*i.e.* speciation and adaptation) occur at different temporal scales

than ecological processes (*i.e.* migration). Strong evidence for migration during the Pleistocene (and weak evidence for speciation) has been taken as evidence that rapid climate change overwhelmed potential evolutionary responses (Bennett 1997). Building on this interpretation, recent efforts to predict responses to anthropogenic global warming assume that species' climate tolerances are fixed, leaving migration as the only means for species survival (*e.g.* Thomas *et al.* 2004).

The conclusion that migration, rather than adaptation, mediates responses to climate change is based on categorical interpretation of responses evident in the fossil record. In this way, the conclusion is strongly biased by what the fossil record preserves best (Davis and Shaw 2001). Fossil pollen records information about distribution and sometimes abundance. Migration patterns can be reconstructed from these data in a straightforward way. However, changes in niche breadth can be more difficult to quantify. Fossil pollen poorly preserve characteristics associated with physiological tolerance, precluding direct estimates of niche breadth. However, several studies have indirectly estimated changes in climate niches through the fossil record by framing distribution data in an explicit geographic and temporal context (Pearman *et al.* 2008a). By statistically evaluating the association between geographic distribution and climate, they estimated the range of climatic conditions necessary to encounter a species. Then by contrasting the relationship between distribution and climate at different time periods they inferred changes in climate niches.

In some cases, the relationship between distribution and climate inferred from fossil pollen remains stable through time (Martinez-Meyer and Peterson 2006). In other cases, it does not. Pretince *et al.* (1991) found that the contemporary relationship

between distribution and climate for eastern hemlock (*Tsuga canadensis*) predicts a much larger area of occupancy for historical populations than what the fossil record actually documents. This pattern suggests that its climate niche breadth has increased since the LGM. Pearman *et al.* 2008b found a similar result for Common Juniper (*Juniperus communis*) but also noted a shift in climatic conditions it occupied relative to what was available on the landscape, indicating a directional shift in this species' climate niche. These results demonstrate how analyzing historical changes in an explicit geographic context can provide more precise inferences of species' responses to climate change.

**The comparative method:** The principle of descent with modification provides a powerful way to infer responses to climate change, even among species that left no fossil record. By comparing species ecological characteristics in the context of their phylogenetic relationship, one can quantify the evolutionary changes in these characters that has accrued relative to common ancestors (Harvey and Pagel 1991). Two early comparative studies suggested that evolutionary change in climate niches accrued very slowly. Ricklefs and Latham (1992) found that genera shared between eastern Asia and eastern North America had similar range sizes on each continent, despite having diverged during the late Miocene and Pliocene (Xiang *et al.* 2000). They interpreted this correlation as evidence that climate niche breadth had remained similar in these groups despite geographic differences among continents and the effects of Pleistocene climate change. Using a similar comparison across a biogeographic disjunction, Peterson *et al.* (1999) found that a climate niche inferred from species of birds, butterflies and mammals isolated on one side of the Isthmus of Tehuantepec in Mexico accurately predicted the distributions of sister species that occurred on the other side. Furthermore, they found

that the correspondence between sister species was stronger than the correspondence between species randomly chosen from the same family.

By documenting ecological similarity between long-diverged species, these early applications of the comparative method to climate niche evolution established a powerful paradigm: climate niche conservativism. Climate niche conservativism describes a tendency for species to retain ancestral ecological characteristics (Wiens and Graham 2005). As a categorical statement, climate niche conservativism simply reifies the principle of descent with modification. More quantitative approaches are necessary for a more nuanced understanding of evidence for the evolution of climate niches from neontological data in a comparative framework.

The nuance of inferring properties of climate niche evolution through the comparative method was highlighted in a recent debate between two of the field's most preeminent researchers. Losos (2008a) noted that comparative studies often assumed ecological similarity among related species in order to test more complex processes, such as community assembly. However, several studies have found that related species were ecologically dissimilar based on *a priori* criteria. In particular, a study of climate niches among Cuban anoles found no general relationship between phylogenetic distance and ecological similarity (Knouft et al 2006). Citing this study among others, Losos (2008a) argued that niche conservativism represented a pattern that must be tested against a null model in which ecological change was random through time. Rejecting simple phylogenetic signal could justify invoking more complex processes that constrain or promote ecological differentiation among relatives (Revell *et al.* 2008).

Wiens (2008) disagreed. He contended that niche conservativism is a ubiquitous feature of biological evolution. Therefore it is both pattern and process. In support of his argument, he invoked a larger biogeographic context for the same study on niche evolution in Cuban anoles. Even though phylogenetic relatedness does not predict ecological similarity among these species on Cuba, the entire clade is restricted to the tropics. At this scale, he argued, their niches are evolutionarily conserved. This kind of niche conservativism could contribute to the latitudinal gradient in species richness by restricting clades low latitudes where they originate (Wiens and Donoghue 2004). At macro scales, he argued, niche conservativism represents a process not simply a pattern among related organisms.

This debate shows features similar to the debate over contrasting interpretations of evidence for migration in the fossil record. In each case categorical assessments of a pattern reinforced assumptions about gradualism in the evolutionary process. In the fossil record, straightforward identification of range shifts was taken as evidence that climate change overwhelmed evolutionary responses. In the comparative approach, the ability to identify ecological similarity among species at an arbitrary level of relationship is taken as evidence that gradual changes in species ecological characteristics is a fundamental feature of their response to environmental change. Just as an explicit geographic context for identifying alternative responses to climate change was necessary to moderate the paradigm of exclusive migration in paleoecology, identifying the appropriate context for expected ecological similarity among related species may moderate the paradigm of niche conservativism in the comparative method.

To illustrate the importance of context in the comparative method, return to the disputed example of climate niche evolution in Cuban anoles cited above. Losos (2008a) and Wiens (2008) identified different patterns in the same study by interpreting the results in different spatial and temporal contexts. At spatial and temporal scales defined by the distribution and ancestry of the focal group, related species are not ecologically similar. Losos (2008a) concluded with the original authors that this pattern is consistent with a strong role for processes that promote ecological differences among species, such as habitat partitioning. At a larger spatial scale which includes environmental variation that the focal group does not currently experience and a temporal scale including a distant ancestor which may have dispersed into those contrasting environments, related species may show evidence for niche conservativism. If tested and found to be significant, the pattern of niche conservativism may be consistent with the influence of processes that maintain ecological similarity among relatives such as genetic constraint and stabilizing natural selection (Losos 2008b).

**Roles for gene flow:** The foregoing discussion focuses on species-level properties (climate niche breadth and dispersal ability) that mediate the resilience of biodiversity to climate change. However, species may not represent the most appropriate level of biological organization for identifying responses to climate change and for interpreting the responsible evolutionary and ecological processes. First, species are notoriously difficult to define. Second, responses to climate change (adaptation and migration) may be mediated by processes that operate at the population level (natural selection and local population dynamics). These issues are interrelated. Explicitly considering the role of gene flow in species biology illustrates how.

Understanding patterns and properties of gene flow is central to identifying species. Many species concepts state criteria for species recognition in terms of gene flow (Coyne and Orr 2004). The most prominent is the Biological Species Concept (Mayr 1942, 1996). In this concept, species are sets of actually or potentially interbreeding populations that are reproductively isolated from other such sets. Here, the relative magnitude of gene flow explicitly characterizes the relationships by which species are delineated. In the Cohesion Species Concept (Templeton 1989, 2000), species recognition depends on two inherent properties of organisms that facilitate gene flow in general: genetic exchangeability and ecological exchangeability. Other species concepts state criteria based on patterns that may be interpreted as products of historical patterns of gene flow (*e.g.* Cracraft 1983, Shaw 1998). In each species concept, gene flow plays a prominent role because it is a major evolutionary process responsible for maintaining similarity among populations through space and time. One important consequence of the cohesive effect of gene flow is the tendency for species to maintain stable geographic ranges. When environments, fitness and abundance vary spatially, gene flow from larger central populations can swamp local adaptation by peripheral populations to marginal habitats that would otherwise tend to continually expand the geographic range (Pease *et al.* 1989, Kirkpatrick and Barton 1997, Lenormand 2002). In this way, gene flow mediates two species properties that are critical for interpreting responses to climate change: niche breadth and geographic distribution.

However, climate change may influence patterns of gene flow by changing the configuration of the species' geographic range (Wiens 2004). Populations that were once connected by gene flow may become isolated. Populations that had been isolated may

come into secondary contact. In this way climate change may promote both the evolution of adaptive differentiation within species and the merger of incipient lineages (Jansson and Dynesius 2002). Both of these outcomes complicate attribution of responses to climate change to species-level properties. When adaptive diversification occurs within species or when differentiated lineages first merge, the physiological tolerances that define the niche may not be uniform across individuals, potentially compromising whether the concept of a species' ecological niche accurately depicts biological reality (Chase and Liebold 2003). Furthermore, both incipient diversification and hybridization frustrate attempts to identify species based on phylogenetic reconstructions of genetic variation among individuals. In my dissertation, I address responses to climate change that occur at the species-population interface by looking for the effects of gene flow on climate niche breadth in explicit geographic and historical contexts.

The first project investigates the effects of climate change on the earliest stages of evolutionary divergence—differentiation among populations within species. Species with evolutionarily conserved climate niches may respond to climate change by migrating. As populations migrate, the geographic configuration of environmental heterogeneity may force them into allopatry, reducing gene flow and promoting ecological divergence among isolated regions (Wiens 2004). The Pleistocene history of Europe provides a classical example. Many species with continuous distributions across northern Europe at present survived cooler conditions during glacial maxima by migrating south into peninsular refugia. Reduced gene flow among populations isolated

on different peninsulas promoted genetic differentiation that is evident in persistent geographic patterns of genetic structure in many species (Hewitt 1996).

Most studies interpret genetic structure within species simply as evidence for historical patterns of migration (*e.g.* Petit *et al.* 2005). In this respect, they are similar to early paleoecological studies of the fossil pollen record. Just as an explicit geographic context for the fossil record illustrated evidence for niche evolution in some species, reexamining phylogeographic data can illustrate how historical patterns of gene flow may have promoted ecological differentiation within species. In Chapter 1, I present a simple, geographically explicit model for the evolution of realized niche breadth in allopatry. I then evaluate the model against a dataset of European plants with phylogeographic evidence for historical range fragmentation.

The remaining chapters focus in increasingly finer detail on the evolution on niche breadth in an ecologically diverse, taxonomically complex group. *Dodecatheon* sect. *Dodecatheon* L. (H. J. Thompson) (Primulaceae) is a clade of North American perennial herbaceous plants nested in the large genus *Primula* (Mast *et al.* 2004). While a suite of adaptations for buzz-pollination clearly distinguish *Dodecatheon* from other primroses, considerable variation within and among populations in basic floral characters has greatly complicated their taxonomy (Gray 1886). After careful biosystematic studies (Fassett 1944, Thompson 1953) and subsequent taxonomic revisions (Reveal 2009), the diversity in the section has been parsed into nine species. These species differ dramatically with respect to environmental tolerance and range size. Seven of the nine species in the section grow only in habitats with year-round moisture availability, including moist cliffs and mountain stream-sides. All of these species are rare, and each

occurs in a different small region of the continent. In contrast, the other two species in the section also grow in seasonally dry habitats, including forests, prairies and alpine meadows. These two species are very widespread. Their ranges collectively span most of northern North America. As such, both rare and common species co-occur in regions with starkly contrasting geographies and histories (Thorne 1993).

For my second chapter, I evaluate evidence for responses to climate change in the systematics of *Dodecatheon* sect. *Dodecatheon*. A previous study on the relationship between *Dodecatheon* and *Primula* (Mast *et al.* 2004) suggested two patterns that are pertinent to understanding responses to climate change in the group. First, it showed that widespread species were derived, suggesting an evolved increase in niche breadth. Second, it showed that the group includes deeply diverged and shallowly diverged species, suggesting that *Dodecatheon* sect. *Dodecatheon* may include both well-formed and poorly-formed lineages. However, the phylogeny did not resolve evolutionary relationships among widespread and rare eastern taxa, precluding any assessment of whether or not apparent changes in niche breadth occurred more than once. Moreover, the phylogeny was based on chloroplast DNA sequence variation from one individual per taxon, precluding detection and evaluation of gene-tree species-tree conflicts and the evolutionary processes that might generate them. By reconstructing and comparing chloroplast and nuclear gene genealogies from multiple individuals per taxon, I was able to more precisely resolve the evolutionary relationships among species. By assessing these relationships in the context of the geographic and paleoclimatic differences between eastern and western North America, I was able to evaluate the extent to which changes in range size likely reflected evolutionary changes in physiological tolerances. Then, by

assessing different gene-tree species-tree conflicts, I was able to identify a role for geographic heterogeneity in the outcome of secondary contact between lineages with incomplete reproductive isolation.

The third chapter focuses on support for alternative responses to climate change since the LGM among closely related *Dodecatheon* in eastern North America. Rare eastern species grow only on moist cliffs. This habitat is often cooler and more moist during stressful summer months than other nearby habitats, where the widespread species *D. meadia* often grows. Rare species also have thinner leaves than *D. meadia*. This trait mediates a tradeoff between light capture and water loss that influences photosynthetic performance in habitats that differ in light availability and water stress (Westoby *et al.* 2002). Reciprocal transplants between parapatric populations of the rare species, *D. frenchii,* and *D. meadia* have demonstrated that the leaf thickness difference among these taxa has a genetic basis (Voigt and Swayne 1955). They also suggest that this trait mediates divergent local adaptation to microclimate in each species respective habitat (Mohlenbrock 1987). Two scenarios could explain the match between traits and microclimate in this group. The first is consistent with primary roles for migration and ecological sorting. The rare species may be glacial relicts. Alternatively, the rare species may be ecotypes. This second scenario is consistent with primary roles for gene flow and natural selection. These two scenarios predict different rates of niche evolution relative to climate change, different patterns of distribution of genetic variation within and among taxa and different roles for regional versus local processes. By combining ecophysiological and population genetic data in an explicit geographic framework, I test the support for these alternative scenarios and the processes they imply.

In the final chapter, I investigate whether apparent intergradation between *D. frenchii* and *D. meadia* in southern Illinois facilitates ongoing gene flow between these taxa. In addition to the ecological differences among taxa, cytological work shows they have different ploidy levels (Olah and DeFilipps 1968). Ploidy level differences should limit gene flow among taxa. However, some populations have highly variable morphology, complicating taxonomic determination (Fassett 1944). Moreover, bizarre meiotic behavior in both taxa suggests the possibility for repeated changes in ploidy level. With a morphometric analysis, I quantify the morphological characteristics of an intergrading population in the context of differences between typical populations of each species. Then, to investigate the role of this intergrading population in the evolution of the group, I compare its fitness to the fitness of nearby typical populations. By assessing patterns of population genetic differentiation among populations of different geographic configuration, I assess support for local interspecific gene flow mediated by the intergrading colony. Finally, with a limited cytological analysis, I interpret the role of ploidy evolution in the group.

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# CHAPTER 1

## The effect of allopatry on climate niche breadth among European plants depends on both history and the geographic distance among isolated portions of the range.

## INTRODUCTION

Evolutionary diversification proceeds as genetic differences accumulate among populations. The geographic context for this process has framed a large body of research in evolutionary biology (Darwin 1859, Wagner 1868**,** Jordan 1905). Mayr (1963) is widely credited with synthesizing earlier ideas into a powerful argument that diversification is most likely to occur when a species' range is geographically discontinuous. While Mayr focused on the evolution of reproductive isolation, a more recent focus on lineage formation (de Quieroz 1999) has highlighted the ecological and microevolutionary forces that promote range fragmentation itself (Wiens 2004). Arguments for allopatric diversification generally rest on four premises. First, selective environments change across the range (Toebler 1970, Gould and Johnston 1972, Gaston 2003). Second, gene flow across the range limits differentiation (Malécot 1950, Endler 1977, Slatkin 1988, Lenormand 2002). Third, range fragmentation interrupts the pattern of gene flow (Wright 1969, Templeton *et al.* 1995). Fourth, freedom from the homogenizing effects of gene flow due to allopatry allows populations in relatively isolated regions to respond independently to different selective pressures, ultimately resulting in speciation (Dobzhansky 1946, Schluter 2001, Lozier and Mills 2009). If we focus on the earliest stages of divergence, we can formally represent this argument for allopatric diversification with the path model in Figure 1.1.

The model focuses on the causes of ecological variation among populations, or realized niche breadth. It focuses on two contributing factors. The first is simply geographic distance. Localities which are further apart may have more strongly contrasting environments without demonstrating evolved differences among populations

(premise 1, arrow 1) (Endler 1986). In the context of the model, relative evolutionary differentiation depends on gene flow. Populations experiencing higher gene flow should be more ecologically similar given their geographic distance (premise 2, arrow 2) (Moore *et al.* 2007). Two factors, in turn, influence gene flow. When dispersal is limited, more distant populations experience less gene flow (premise 3, arrow 3) (Wright 1943). Therefore, geographic distance may promote niche breadth indirectly, by limiting the spatial scale of the homogenizing effects of gene flow among populations (arrow 3 x arrow 2) (Garant *et al.* 2007). These processes are ongoing and they may explain niche breadth for species with any range form. This model focuses on the effects of allopatry, represented here as a factor that reduces gene flow independent of geographic distance (arrow 4, premise 4). For instance, populations that are isolated by a vicariant event through a formerly continuous distribution may become progressively divergent through time, although the geographic distance separating them does not change (arrow 4 x arrow 2) (*e.g.* Knowlton *et al.* 1993). The evolutionary effects in this model are a function of gene flow at loci responsible for adaptation to contrasting environments. Measuring gene flow at causative loci is difficult (McCracken *et al*. 2009). Many studies on this topic use neutral genetic differentiation as a proxy for the net effects of gene flow given population size (arrow 5) (McKay and Latta 2002). Two observed correlations can support whether neutral variation is a reliable indicator of gene flow in the context of this model: a correlation between neutral genetic differentiation and geographic distance, (arrow 3 x arrow 5) and hierarchical genetic structure among geographically cohesive regions (arrow 4 x arrow 5) (Hutchison and Templeton 1999).

The argument for allopatry is so persuasive and the elements of the model enjoy such strong support that more complex models of diversification often assume this geographic mode of divergence (*e.g.* Hubbel 2001, Jansson and Dynesius 2002). Nevertheless, the model has many critics. Some argue that it cannot explain diversity in certain taxa, such as plants (Ehrlich and Raven 1969). Others question the assumption that geography is the primary factor by arguing that other factors, including genetic architecture and demography, can play important roles in divergence (Carson and Templeton 1984, Holt and Gomulkiewicz 1997). Several studies defend the allopatric model against these criticisms by questioning the plausibility of alternative divergence models or by showing low frequency of other geographic modes (Coyne and Orr 2004).

Most studies that evaluate the allopatric diversification model focus on newly diverged species (Lynch 1989, Savolianen et al. 2006). Here we focus on the necessarily earlier stage: divergence among populations. Each species in this study has documented regional population genetic structure reflecting allopatry. We first test whether correlations between geographic distance, genetic differentiation and realized niche breadth correspond to those predicted by the allopatric diversification model (Fig. 1.1). Then, among species that fit, we quantify the explanatory power of the model and attribute portions of this explanatory power to effects associated with allopatry. Finally, given the focus on the evolution of species' range limits during diversification (Wiens 2004, Gaston 2009), we test whether the model explains more realized niche breadth for ecological variables that limit species' geographic distributions than for other variables.

## MATERIALS AND METHODS

To test the allopatric diversification model, we assembled a database of phylogeographic studies on European plants. Europe has a well-known glacial history that has fragmented the ranges of many species (Hewitt 2000). Among these, plants have been intensively studied (Taberlet *et al*. 1998, Schönswetter *et al.* 2005, Petit *et al*. 2005). Considering that they are the basis for an early criticism of the allopatric diversification model (Ehrlich and Raven 1969), they provide an interesting test for its predictions. In order to focus on plants with the strongest population genetic support for an allopatric history, we generated a database of phylogeographic studies that met two criteria. First, we required that the authors support their discussion of allopatry with a statistical test for hierarchical genetic structure among geographically cohesive regions within the species range (*e.g.* AMOVA, STRUCTURE, distance trees). Second, we required that they test over at least five putatively unlinked loci. As the number of loci increases, so does confidence that patterns of genetic structure reflect general demographic events (Takahata *et al*. 2001, Templeton 2004, Maddison and Knowles 2006). We chose five loci as a compromise between data quality and quantity. After applying these criteria, our dataset included 44 species (Table 1.1).

The allopatric diversification model pertains to the relationships between three measured variables: geographic distance, neutral genetic differentiation, and realized niche breadth. For each species, we estimated these variables using locality data for the populations that showed the signature of an allopatric history. For geographic distance, we projected the coordinates of localities onto the European Equidistant Conic projection with ArcGIS v 9.0 and computed pairwise distances in meters. For neutral genetic

differentiation, we used the results of authors' tests for allopatry to compute a binary matrix in which 1's correspond to pairs of sampled localities in genetically differentiated regions and 0's correspond to pairs of sampled localities within a region. For realized niche breadth within species, we characterized climatic conditions at each sampled locality using ArcGIS v 9.0. Climate often limits plant distributions (Woodward 1987, Gaston 2003, Lomolino *et al.* 2006, Angert *et al.* 2008), and populations of many plant species are locally adapted to climatic conditions (Leimu and Fischer 2008) (*e.g.* Macel *et al.* 2007). We quantified realized niche breadth as the semivariance (Fortin and Dale 2005) among all pairs of populations along each of 19 variables representing the central tendency, seasonal variation and extremes of temperature and precipitation (Hijmans *et al.* 2005). Because seasonal variation (Janzen 1967, Ghalambor *et al*. 2006) and extremes of water stress (Pither 2003) may be particularly important in limiting plant distributions, we hypothesized that allopatry would explain more realized niche breadth with respect to climate variables measuring seasonal variation and extremes than with respect to variables measuring central tendency (Figure 1.2). We were also interested in multivariate niche breadth. For the subset of species represented by more localities than the number of climatic variables measured (i.e., ≥20 sampled localities), we expressed climate using 19 orthogonal axes resulting from a principal component analysis and calculated multivariate semivariance between sampled localities as the sum of the semivariances across all 19 principal components (Wagner 2003).

We evaluated the allopatric diversification models through a two step process. We first tested whether the data for each species fit the causal model type based on two criteria. The first and more general criterion is that at least one predictor variable (*i.e*.

geographic distance or genetic differentiation) correlates significantly with the response, indicating sufficient sampling to infer some effect (Legendre and Legendre 1998). Among cases that met this criterion, we tested whether the correlational structure of the data was consistent with that implied by the model. Because we are evaluating the model with distance matrices, we could not formally evaluate model fit using standard techniques of Structural Equation Modeling (Grace 2006). Rather, we evaluated whether a linear matrix model with realized niche breadth as the response and both geographic distance and neutral genetic differentiation predictors was consistent with the expected correlations. This model included only measured variables and no latent variables. All the partial correlations in this measurement model were predicted to be positive because the conceptual model included an endogenous latent variable (gene flow) associated with exclusively negative effects (Fig. 1.1) (Sharpe and Roberts 1997).

Among cases that met both model fit criteria, we further evaluated the model by identifying its overall explanatory power and quantifying the proportion attributable to allopatry. To do this, we compared the $R^2$ of the measurement model including both predictors to that of models including only one or the other (Legendre *et al.* 1994, Bring 1995). The largest proportion of niche breadth attributable to allopatry is the $R^2$ of a model including only genetic differentiation. This attribution of explanatory to allopatry includes effects that covary with geographic distance among relatively isolated regions. The smallest proportion of niche breadth attributable to allopatry is the difference between the $R^2$ of a model including both geographic distance and genetic differentiation and a model including only geographic distance. The remaining proportion corresponds to the explanatory power of allopatry, independent of distance.

Prior to all tests, we applied the box-cox procedure to models including both predictors to select an optimal power transformation. Preliminary analyses indicated that results based on these transformations and Pearson's correlation coefficients were very similar to those based on non-parametric Spearman's Rank correlation coefficients. We tested the statistical significance of all correlations against 10,000 permutations of the response matrix as a one-tailed test with a significance threshold of 0.05 following the procedure of Legendre et al. 1994 as implemented in the R package 'ecodist' (Goslee and Urban 2007). In order to test for stronger effects of allopatry on divergence with respect to potentially range-limiting climate variables versus others, we used a Mann-Whitney U test on the mean effects across all species that supported the causal model structure (Fig. 1). We also tested whether more species met our model fit criteria for range-limiting variables with a Mann-Whitney U test. All statistical tests were implemented in R v.9.0.

## RESULTS

Most species showed a significant correlation between either geographic distance or genetic differentiation and realized niche breadth. Across all 836 cases (44 species × 19 climate variables), 79.3% met this weak criterion for model fit. Among the models with at least one significant effect, less than half (40.3%) met the strong criterion that both correlation coefficients in the measurement model were positive.

In the cases where the allopatric diversification model fit the data, it explained a modest amount of the realized niche breadth. Across those 267 models, the mean total model $R^2$ was 0.311. Models including only genetic differentiation had a mean $R^2$ of 0.188. The maximum explanatory power attributable to allopatry, without controlling for

effects that covary with the geographic distance among isolated populations, represented 57.5% of the explanatory power of these models on average. However, the minimum explanatory power of allopatry, measured by excluding the explanatory effect of geographic distance, was only 0.025, representing less than 12.5% of the total explanatory power of the models.

Rates of model fit, total explanatory power and the relative proportion attributable to various effects differed among climate variables (Fig. 1.2). More species met our criteria for model fit when applied to niche breadth for range-limiting variables (Mann-Whitney U test, W = 16.5, p=0.022). Models explained more niche breadth with respect to variables that are likely to limit species ranges (Mann-Whitney U test, W = 74, p=0.017). The mean $R^2$ of models including both geographic distance and regional genetic differentiation was 0.346 for variables measuring extremes or seasonal variation in climate compared to 0.257 for variables measuring central tendencies. Part of this difference was due to greater maximum explanatory power attributable to allopatry (Mann-Whitney U test, W = 76, p=0.010). The mean $R^2$ of models including only genetic differentiation was 0.223 for extreme and seasonality variables, while it was only 0.135 for variables measuring central tendency. However, the minimum explanatory power attributable to allopatry, with geographic distance excluded, did not differ between categories of variables (p=0.24).

Of the 23 species for which we could estimate multivariate niche breadth, all were sufficiently sampled to meet our weak model fit criterion. Relative to the univariate models, a higher proportion of species (15/23) also satisfied the stronger criterion for model fit. The model explained slightly more climate niche breadth, although somewhat

less of this explanatory power was attributable to the effects of allopatry (Fig. 2). The mean total model $R^2$ was 0.364, while the maximum and minimum proportions of the explained variation attributable to allopatry were 37.9% and 5.8%, respectively.

## DISCUSSION

Our analysis produced four main results. First, relatively few cases met our criteria for model fit. Second, among cases that fit, the model explained a modest amount of realized niche breadth. Third, the proportion of explained niche breadth attributable to allopatry depended on whether or not we excluded the explanatory power attributable to geographic distance. Finally, as predicted, rates of model fit, explanatory power and the proportion attributable to allopatry were greater for niche breadth with respect to range-limiting variables. We will discuss each of these results in turn.

The low proportion of cases for which the model fit the data suggests that a simple representation of allopatric diversification may not capture all of the pertinent processes. For instance, where conditions for reproduction are so poor that local populations cannot replace themselves, dispersal from more suitable portions of the species range can boost numbers and increase the probability of novel adaptation (Holt and Gomulkiewicz 1997). If these demographic effects are strong, gene flow can promote population divergence, effectively switching the sign of arrow four in the causal model (Fig. 1.1) from positive to negative. Demographic and genetic rescue are two mechanisms among many by which increased gene flow may promote population persistence and divergent adaptation (Garant *et al.* 2007). In this dataset, many cases fail the strong criterion for model fit because the multiple regression coefficient associated

with genetic differentiation is negative (data not shown). This may suggest a role for demographic effects on the evolution of niche breadth in some species.

When the model fits the data, it explains a modest amount of niche breadth. This may reflect error in our measurement of realized niche breadth, or the poor explanatory power of a simple binary measure of genetic differentiation. However, it may reflect limited power of allopatry to explain ecological variation within species (Allmon 1992). Early verbal arguments for the importance of allopatry focused on the evolution of reproductive isolation (Dobzhansky 1946, Mayr 1963). Reproductive isolation may be important for promoting ecological differentiation due to character displacement (Servedio and Noor 2003). The taxa we study presumably lack evidence for strong reproductive isolation, limiting the effectiveness of this mechanism for the evolution of ecological diversity among close relatives. Moreover, ecological character displacement occurs upon secondary contact (Brown and Wilson 1956**,** Rundell and Price 2009). Under these conditions, ecological differentiation may decrease with the geographic distance among populations (Goldberg and Lande 2006). This pattern would fail our strong criterion for model fit.

Of the niche breadth explained, we found that the maximum proportion attributable to allopatry was relatively large. However, if we excluded the explanatory power of geographic distance, the minimum explanatory power attributable to allopatry was relatively small. Interpreting the attribution of explanatory power from commonality analyses like ours is not always straight-forward (Legendre *et al.* 2008, Tuomisto and Ruokolainen 2008). However, a conventional heuristic distinction may apply in this case (e.g. Duivenvoorden *et al.* 2002, Telles and Diniz-Filho 2005). In the context of our

model, geographic distance predominately influences ongoing processes, such as dispersal and natural selection. The explanatory power of the model that is independent of geographic distance may measure the effects of historical events, such as range fragmentation. Under this interpretation, range fragmentation, as a historical event, explains a relatively small proportion of niche breadth. However, ongoing processes that depend on range fragmentation appear to explain a relatively large proportion of niche breadth.

The different patterns that we observed for explained niche breadth for range-limiting variables compared to others might illustrate how historical and ongoing processes interact to promote ecological diversification. Specifically, we found that the model fits more frequently and it explains more niche breadth with respect to range-limiting variables. The increase in explanatory power was attributable to a larger proportion of explained niche breadth associated with both allopatry and geographic distance. This outcome could occur if gene flow has a cohesive effect not just on traits, but on the species' geographic range itself (Bridle and Vines 2006). Theoretical models predict that when abundance and fitness vary along a spatial environmental gradient, stable geographic distributions can occur if gene flow from larger, more central populations swamps local adaptation to limiting conditions by smaller populations at the margins of the geographic range (Kirkpatric and Barton 1997). Range fragmentation along this gradient could improve chances that marginal populations adapt to extreme environmental conditions by reducing the swamping effects of gene flow (García-Ramos and Kirkpatrick 1997). It may also initiate colonization of new environments in different directions along the gradient by relatively isolated populations. In this way, the observed

increase in niche breadth resulting from this process cannot be uniquely attributed to either the historical event of range fragmentation, or the ensuing dispersal of isolated populations with progressively divergent adaptation. Moreover, this kind of "run-away vicariance" would occur predominately for range-limiting environmental variables. Our results are entirely consistent with this subtle prediction for the evolution of ecological tolerance and species geographic range limits following range fragmentation.

Alternative interpretations of the attribution of explanatory power in our model may be plausible (Räsänen and Hendry 2008). For instance, if different environments sort out maladapted individuals, selection can accentuate genetic differentiation among regions, effectively reversing arrow two (Fig. 1.1) (Barton and Bengtsson 1986). This alternative process of Isolation by Adaptation (Nosil *et al*. 2009) is indistinguishable from the effect of gene flow on realized niche breadth (Bring 1995, Legendre and Legendre 1998). However, recent simulation studies have found very restrictive conditions for identifying Isolation by Adaptation at neutral loci (Thibert-Plante and Hendry 2009). Therefore this alternative interpretation of the attribution of explanatory power may only weakly apply to our analysis.

In conclusion, we showed that a simple representation and analysis of a classical evolutionary hypothesis can reveal non-intuitive results. Specifically, allopatry may initiate a self-reinforcing process of geographic separation by promoting divergent adaptation to range-limiting conditions. Furthermore, our approach shows how comparative phylogeography can illustrate important features of adaptive diversification, beyond simply documenting shared migration patterns. Intraspecific genetic structure may be associated with patterns of local adaptation. We would predict that reciprocal

transplant experiments on species with stronger effects of allopatry would demonstrate stronger local adaptation to climate, especially for range-limiting conditions.

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**TABLE 1.1**: Summary of studies included in the analysis.

| Species | Family | Marker Type | # Loci | # Pops | # Regions | Method | Reference |
|---|---|---|---|---|---|---|---|
| *Androsace alpina* | Primulaceae | AFLP | 218 | 53 | 4 | AMOVA, PCA | Schönswetter, P., et al. 2003. Plant Biology 5: 623-630. |
| *Androsace brevis* | Primulaceae | AFLP | 116 | 8 | 2 | AMOVA | Schönswetter, P., et al. 2003. Botanical Journal of the Linnean Society 141:437-446. |
| *Androsace wulfeniana* | Primulaceae | AFLP | 119 | 4 | 3 | AMOVA | Schönswetter, P., et al. 2003. Botanical Journal of the Linnean Society 141:437-446. |
| *Anthyllis montana* | Fabaceae | AFLP | 1211 | 16 | 2 | AMOVA, etc. | Kropf, M., et al. 2002. Molecular Ecology 11:447-463. |
| *Arabidopsis lyrata* | Brassicaceae | Microsatellite | 18 | 26 | 2 | AMOVA, STRUCTURE | Gaudeul, M., et al. 2007. American Journal of Botany 94:1146–1155. |
| *Arabis alpina* | Brassicaceae | AFLP | 254 | 57 | 3 | AMOVA, STRUCTURE | Ehrich, D., et al. 2007. Molecular Ecology 16:2542-2559. |
| *Armeria pungens* | Plumbaginaceae | AFLP | 223 | 23 | 2 | AMOVA | Piñero, R., et al. 2007. Molecular Ecology 16:2155-2171. |
| *Bordera pyrenacia* | Dioscoreaceae | Microsatellite | 18 | 15 | 2 | AMOVA, STRUCTURE | Segarra-Moragues, J.G. et al. 2007. Journal of Biogeography. 34:1893–1906. |
| *Bupleurum stellatum* | Apiaceae | AFLP | 287 | 24 | 2 | AMOVA, PCA | Schönswetter, P., et al. 2005. Taxon 54:725–732. |
| *Campanula alpina* | Campanulaceae | AFLP | 176 | 36 | 4 | AMOVA, STRUCTURE | Ronikier, M., et al. 2008. Molecular Ecology 17:1763–1775. |
| *Carex curvula* | Cyperaceae | AFLP | 115 | 37 | 4 | AMOVA | Puscas, M., et al. 2008. 17:2417–2429. |
| *Comastoma tenellum* | Gentianaceae | AFLP | 130 | 30 | 3 | AMOVA, etc. | Schönswetter, P., et al. 2004. Journal of Biogeography. 31:1673–1681. |
| *Dryas octopetala* | Rosaceae | Allozyme | 6 | 8 | 3 | AMOVA, etc. | Philipp, M., and H.R. Seigesmund. 2003. Molecular Ecology 12:2231-2242 |
| *Dryopteris cristata* | Dryopteridaceae | RAPD | 361 | 12 | 3 | AMOVA | Landergott, U., et al. 2001. Heredity 87:344-355. |
| *Erinus alpinus* | Scrophulariaceae | AFLP | 525 | 22 | 2 | AMOVA, etc. | Stehlik, I., et al. 2002. Biological Journal of the Linnean Society 77:87-103. |
| *Eritrichium nanum* | Boraginaceae | AFLP | 806 | 18 | 3 | AMOVA, etc. | Stehlik, I., et al. 2001.Molecular Ecology 10:357-370. |
| *Eryngium campestre* | Apiaceae | AFLP | 180 | 29 | 3 | AMOVA, STRUCTURE | Bylebyl, K., et al. 2008. Molecular Ecology 17:3379–3388. |
| *Fraxinus excelsior* | Oleaceae | Microsatellite | 5 | 33 | 4 | STRUCTURE | Heuertz, M., et al. 2004. Evolution 58:976–988. |
| *Hypochaeris radicata* | Asteraceae | AFLP | 517 | 37 | 5 | Structure | Ortiz, M.Á., et al. 2008. Molecular Ecology 17:3654–3667. |
| *Hypochaeris salzmanniana* | Asteraceae | AFLP | 546 | 13 | 2 | AMOVA, etc. | Ortiz, M.Á., et al. 2007. Molecular Ecology 16:541-552 |
| *Hypochaeris uniflora* | Asteraceae | AFLP | 87 | 77 | 3 | AMOVA, etc. | Mráz, P., et al. 2007. Journal of Biogeography 34:2100–2114. |
| *Iris aphylla* | Iridaceae | AFLP | 501 | 25 | 3 | KRIGING/ AMOVA | Wróblewska, A. 2008. Plant Systematics and Evolution 272:49–65. |
| *Juniperus thurifera* | Cupressaceae | AFLP | 326 | 19 | 2 | AMOVA | Terrab, A., et al. 2008. Molecular Phylogenetics and Evolution 48:94–102. |
| *Minuartia biflora* | Caryophyllaceae | AFLP | 171 | 14 | 2 | AMOVA | Schönswetter, P., et al. 2006. Molecular Ecology 15:709-720. |
| *Mycelis muralis* | Asteraceae | Microsatellite | 12 | 17 | 3 | AMOVA, etc. | Chauvet, S. et al. 2004. Molecular Ecology 13:1391-1407. |
| *Papaver alpinum* | Papaveraceae | AFLP | 351 | 7 | 2 | AMOVA | Kropf, M., et al. 2006. New Phytologist 172:169-185 |
| *Phyteuma globulariifolia* | Campanulaceae | AFLP | 257 | 69 | 4 | AMOVA, PCA | Schönswetter, P., et al. 2002. Molecular Ecology 11:2637-2647. |
| *Pinus cembra* | Pinaceae | Allozyme | 28 | 5 | 2 | UPGMA, PCA | Belokon, M.M., et al. 2005. Russian Journal of Genetics 41:1538–1551. |
| *Pinus pinaster* | Pinaceae | Allozyme | 18 | 12 | 3 | Chord distance tree | Salvador, L., et al. 2000. Theoretical and Applied Genetics 100:89–95. |
| *Polytrichium juniperinum* | Polytrichaceae | Allozyme | 20 | 11 | 2 | UPGMA | Van der Velde, M. and R. Bijlsma. 2003. Biol. J. Lin. Soc. 78:203-213. |
| *Pritzelago alpina* | Brassicaceae | AFLP | 809 | 14 | 4 | AMOVA, etc. | Kropf, M., et al. 2003. Molecular Ecology 12: 931-949. |
| *Quercus ilex* | Fagaceae | Allozyme | 8 | 57 | 5 | MDS | Michaud, H., et al. 1995. Heredity 74:590-606. |
| *Ramonda myconi* | Gesneriaceae | RAPD | 69 | 19 | 5 | SAMOVA | Dubreuil, M., et al. 2008. American Journal of Botany 95:577–587. |
| *Ranunculus glacialis* | Ranunculaceae | AFLP | 192 | 75 | 4 | AMOVA, PCA | Schönswetter, P., et al. 2004. Biological Journal of the Linnean Society 81:183-195. |
| *Ranunculus pygmaeus* | Ranunculaceae | AFLP | 207 | 23 | 2 | AMOVA | Schönswetter, P., et al. 2006. Molecular Ecology 15:709-720. |
| *Rumex nivalis* | Polygonaceae | AFLP | 205 | 23 | 6 | AMOVA, etc. | Stehlik, I. 2002. American Journal of Botany 89:2007–2016. |
| *Saponaria pumila* | Caryophyllaceae | AFLP | 233 | 33 | 3 | AMOVA, etc. | Tribsch, A., et al. 2002. American Journal of Botany 89:2024–2033. |
| *Senecio gallicus* | Asteraceae | RAPD | 103 | 9 | 2 | AMOVA, etc. | Comes, H.P. et al. 2000. Molecular Ecology 9:61-76. |
| *Sesleria paniculata* | Poaceae | RAPD | 334 | 25 | 3 | AMOVA, etc. | Reisch, C. 2002. Dissertation. Universität Regensburg. |
| *Silene rupestris* | Caryophyllaceae | AFLP | 350 | 13 | 3 | AMOVA, etc. | Kropf, M., et al. 2006. New Phytologist 172:169-184 |
| *Sorbus aucuparia* | Rosaceae | Allozyme | 10 | 17 | 2 | UPGMA | Raspe, O., and A.-L. Jacquemart. 1998. Heredity 81: 537-545. |
| *Trollius europaeus* | Ranunculaceae | AFLP | 128 | 16 | 3 | AMOVA, etc. | Despres, L. et al. 2002. Molecular Ecology 11: 2337-2347. |
| *Veronica alpina* | Plantaginaceae | AFLP | 135 | 51 | 4 | AMOVA, STRUCTURE | Albach, D.C., et al. 2006. Molecular Ecology 15:3269-3286. |
| *Veronica bellidoides* | Plantaginaceae | AFLP | 207 | 30 | 2 | AMOVA, STRUCTURE | Albach, D.C., et al. 2006. Molecular Ecology 15:3269-3286. |

**FIGURE 1.1:** Causal model representing evolution of ecological differences among populations (realized niche breadth) in allopatry. Variables in rectangles are measured. Variables in ovals are unmeasured and are presented to illustrate the relationship between theoretical expectations and the measurement model. Arrow numbers identify signed expected correlations as defined in the text.

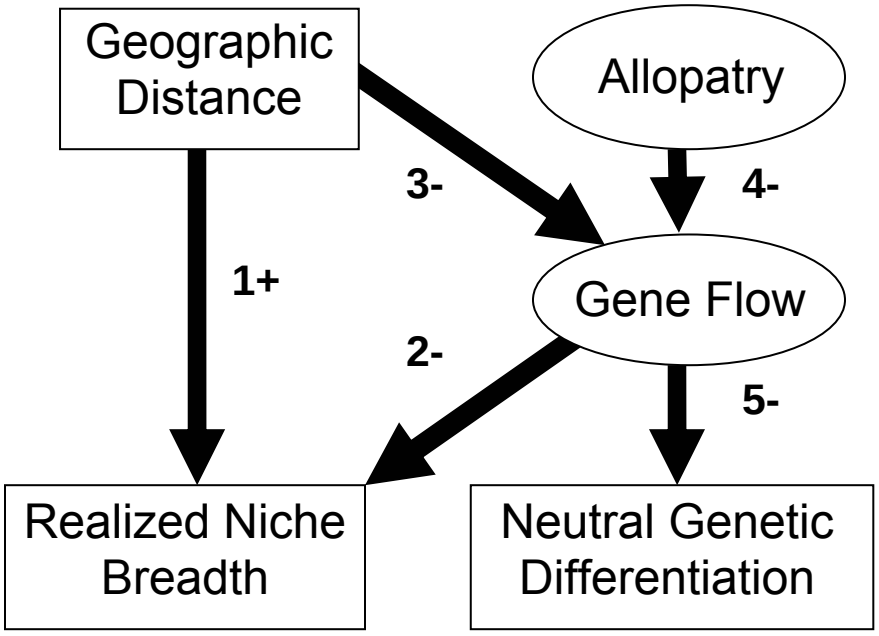
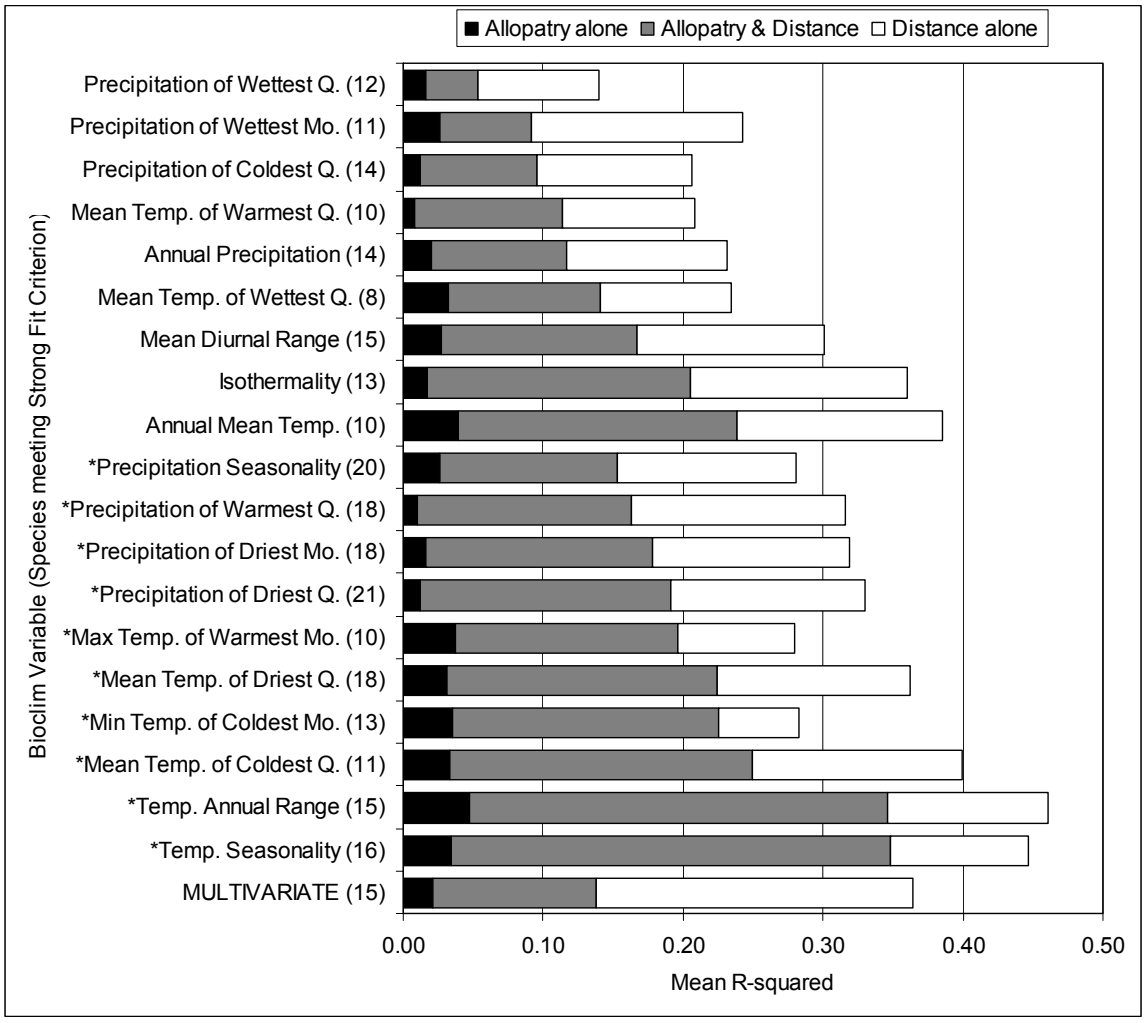


**FIGURE 1.2:** Attribution of explanatory power to different causes of realized niche breadth for 19 bioclimatic variables and multivariate realized climate niche breadth. Values represent means across species that met both criteria for model fit (Materials and Methods), with the number of species given in parenthesis for each climate variable. Asterisks indicate variables likely to limit plant species' distributions.



# CHAPTER 2

## Multi-locus phylogenetics indicate that environmental tolerance, geographic heterogeneity and history contribute to different forms of rarity in *Dodecatheon* sect. *Dodecatheon*.

## INTRODUCTION

Understanding rarity is a major goal of population, community and conservation biologists. While defined in different ways, rare species tend to have more narrow environmental tolerances, more restricted geographic ranges and lower abundances (Rabinowitz 1981). These three aspects of rarity are interrelated (Gaston 1997). Niche theory illustrates how (Brown 1984, Thompson *et al.* 1998, Thompson *et al.* 1999). Environmental tolerance, if defined as the range of conditions necessary to maintain stable population size, is equal to fundamental niche breadth (Hutchinson 1957). Given fundamental niche breadth, the maximum spatial extent of populations depends on the geographic configuration of limiting environmental conditions. This potential range is a geographic projection of the fundamental niche across the region where that species occurs (Jackson and Overpeck 2000). The actual range size and local abundance of a species depend on historical events that displace local populations from resource-based equilibrium sizes (VanDerWal *et al.* 2009). A classical example would be the introduction of a strong competitor (Gause 1932). This negative interaction can produce a more constricted realized niche and realized distribution than would otherwise occur. Other historical events that reduce potential range filling and local abundance include speciation (Paul *et al.* 2009, Chown 1997), introduction into a new region (Broennimann *et al.* 2007) and changes in the configuration of limiting environmental conditions across the region (Pearman *et al.* 2008).

Given these relationships, rarity emerges as an interaction between traits that determine environmental tolerance, geographic constraints and historical contingency. Determining their relative contributions can be difficult. A large body of research

focuses on traits that contribute to rarity among species (Gaston 1993, Gaston and Kunin 1997). However, many of these have been frustrated by the possibility that traits are correlated with phylogenetic relatedness (Felsenstein 1985a). While several studies correct for phylogenetic correlations by contrasting rare and common species within genera (Lavergne *et al.* 2004, Cole 2003), few studies have taken advantage of an explicit phylogenetic framework for testing hypotheses for the origins of rarity (*e.g.* Quattro *et al.* 2001). Even fewer studies have considered how traits contribute to rarity in the context of geographic and historical differences among study regions (*e.g.* Swihart *et al.* 2006). Several recent studies on introduced ants demonstrate that environmental tolerances may increase, decrease or remain the same as species expand their ranges into new landscapes (Fitzpatrick *et al.* 2007, Steiner *et al.* 2008, Roura-Pascual *et al.* 2006)

An excellent system for an integrative study of causes for rarity is *Dodecatheon* sect. *Dodecatheon.* This North American clade of perennial herbaceous plants is nested in the large genus *Primula* (Mast *et al.* 2004). For consistency with the historical literature, we will use the traditional taxonomy here, despite the fact that recognizing this rank renders *Primula* paraphyletic (Mast and Reveal 2007). A suite of adaptations for buzz-pollination clearly distinguish *Dodecatheon* from other primroses. Among them are the pendant flowers and connate anthers which inspired the common name "Shooting Stars." However, taxonomic characters that are use useful in other primroses have become reduced or modified in *Dodecatheon.* Consequently, the taxonomy of the group is notoriously complex (Grey 1883). After careful biosystematic studies (Fassett 1944, Thompson 1953) and subsequent taxonomic revisions (Reveal 2009), the diversity in the section has been parsed into nine species. These species differ dramatically with respect

to environmental tolerance and range size. Seven of the nine species in the section grow only in habitats with year-round moisture availability, including moist cliffs and mountain stream-sides. All of these species are rare, and each occurs in a different small region of the continent (Figure 2.1). In contrast, the other two species in the section also grow in seasonally dry habitats, including forests, prairies and alpine meadows. These two species are very widespread. Their ranges collectively span most of northern North America. As such, both rare and common species co-occur in regions with starkly contrasting geographies and histories (Thorne 1993). In western North America, the Rocky Mountains generate dramatic habitat heterogeneity over short geographic distances. This geographic complexity may have moderated the effects of Pleistocene climatic oscillations on biological communities (Thompson *et al.* 1993, Reveal 1979). By contrast, eastern North America is relatively flat and repeated glaciations there dramatically impacted species' distributions (Williams *et al.* 2001).

A previous systematic study including members of this section indicated that its common ancestor occurred in western North America and that both widespread and eastern taxa are derived (Mast *et al.* 2004). However, it lacked the resolution to determine systematic relationships among common and rare eastern species. These relationships are critical for understanding the roles of environmental tolerance, geography and history as causes for rarity in the group. If widespread species have an exclusive single origin, then transition from rarity likely occurred when the common ancestor of the widespread species adapted to periodic drying. Moreover, rare species would have likely preceded common species in both regions, indicating that time for dispersal has not limited their distribution relative to widespread species. This

biogeographic pattern would suggest that traits, rather than geographic or historical circumstances, cause the difference in rarity among species. In this case, rarity in the section would be a plesiomorphic condition associated with restriction to moist habitats.

Alternatively, if widespread species are not exclusively derived, then the causes for rarity in the group may be more complex than narrow environmental tolerance *per se*. The first full revision of the genus suggested one such possibility. Thompson (1953) synonymised the eastern rare species *D. amethystinum* under the widespread western species *D. pulchellum*. Subsequent workers explained this relationship by noting that habitats near glaciers where *D. pulchellum* grows in Alaska must have been widespread in North America at times during Pleistocene (Ugent *et al.* 1982). Specifically, they suggested that *D. pulchellum* migrated into eastern North America along the retreating Laurentide ice sheet following the last glacial maximum. According to this scenario, *D. amethystinum* is an allopatric variety of this widespread western species. As such, the rarity of *D. amethystinum* is a derived condition and its cause may involve a combination of changes in environmental tolerance, geographic differences between eastern and western North America and historical contingencies. We evaluate alternative causes for rarity in *Dodecatheon* sect. *Dodecatheon.*with a well-sampled multi-locus phylogenetic analysis.

## MATERIALS AND METHODS

**Taxon sampling**: We sampled 8 of the 9 currently recognized species in *Dodecatheon* Sect. *Dodecatheon* (Table 2.1). The other species in the section, *D. poeticum,* is rare species from the Pacific Northwest which probably originated as an

allopolyploid hybrid with the other section of the genus (Thompson 1953, Mast *et al.* 2004). Because allopolyploidy greatly complicates phylogeny reconstruction (Linder and Rieseberg 2004, Guggisberg *et al.* 2009), we excluded this species from our study. We attempted to include some of the genetic variation within each species by sampling individuals from different localities. These localities represented most of the geographic range for each species. For the widespread species, we also sampled different infraspecific taxa. For *D. pulchellum* we sampled 6 of the 7 currently recognized varieties, excluding recently described var. *distolum*. We also recognized an individual as var. *watsonii*, a high elevation endemic (Suttill and Allen 1992), despite the fact that this taxon was recently synonymized under var. *pulchellum* (Reveal 2009). For *D. meadia* we recognized an undescribed variety with enlarged anther connectives and magenta flowers. This variety occurs in the western portion of this species' range, in habitats where plants had been traditionally described as var. *brachycarpum* (Fassett 1944).

For outgroups, we included *Primula parryi*, a species from the sister subgenus to *Dodecatheon* (Mast *et al.* 2004), as well as *D. frigidum* and *D. alpinum* which are members of *Dodecatheon* sect. *Pupureo-tubulosa*. Our samples of *P. parryi* and *D. frigidum* came from recently collected herbarium material. All other samples were collected in the field and dried in silica gel. Vouchers for most specimens collected by B. Oberle are deposited in the herbarium of the Missouri Botanical Garden. Photographic vouchers of these and the other specimens, along with detailed morphological notes, are available upon request.

**Molecular procedures:** We extracted DNA from all samples using Viogene plant DNA miniprep kits following the manufacturer's protocol. We analyzed both chloroplast and nuclear DNA regions. To assess cpDNA variation, we focused on two adjacent non-coding regions: *trnQ*$^{(UUG)}$-5'*rps16* and 3'*rps16*–5'*trnK*$^{(UUU)}$. We amplified these regions separately using the primers described by Shaw *et al*. (2007). For both cpDNA regions, we conducted PCR in 20 μL total volume reactions consisting of two units KlentaqLA polymerase, 1x Klentaq PCR buffer pH 7.9, 0.5 M betaine, 0.2 μM each primer, 0.2mM dNTPs, 2.5 mM $MgCl_2$ and approximately 1 ng total genomic DNA. Our amplification profile consisted of an initial denaturation at 94° for 1m, followed by 33 cycles of 93° for 20s, 58° for 1m and 68° for 1m 20s, ending with a final extension at 68° for 5m. We quantified the DNA concentration of 5 μL of PCR product via agarose gel electrophoresis and ethidium bromide staining by comparing sample brightness to the brightness of a DNA mass ladder with standard concentration. We then purified the remaining PCR product by adding 3 U Exonuclease I (New England Biolabs) and 0.015 U Shrimp Alkaline Phosphatase (Promega), and then incubating samples at 37° for 30m followed by 80° for 20m.

To assess variation from the nuclear genome, we amplified a portion of the NADP-dependant isocitrate dehydrognease (*idh*) gene. This low-copy nuclear gene family is sufficiently variable to infer the phylogenetic relationships of a recently-evolved species complex in Polemonaiceae (Weese and Johnson 2005). Following a preliminary analysis of sequences amplified by primers *idh*751f and *idh*1117r as described by Weese and Johnson (2005), we redesigned the reverse primer for more consistent amplification in *Dodecatheon* (*idh*823r: 5'-TCC AAT TTC GCT CTG TCA TC-3'). Using our new

primer in combination with *idh*751f, we PCR amplified this region for every sample in 40 μL total volume reactions consisting of 4 U KlentaqLA polymerase, 1x Klentaq PCR buffer pH 7.9, 0.25 M betaine, 0.4 μM each primer, 0.2mM dNTPs, 2.5 mM $MgCl_2$ and approximately 4 ng total genomic DNA. Our amplification profile consisted of an initial denaturation at 96° for 1m30s, followed by 11 cycles of 96° for 10s, 58° for 30s and 68° for 2m30s, then 11 cycles with annealing at 56° and another 11 cycles with annealing at 54°, ending with a final extension at 68° for 30 minutes. We excised the single brightest band produced by each reaction from 2% (w/v) agarose gels using a sterile razor blade. We purified DNA from excised bands using Qiagen PCR clean-up kits following the manufacturer's protocol. We then cloned samples into a pGEM-T easy vector system (Promega). Using the standard vector primers SP6 and T7, we PCR amplified inserts directly from 6-20 colonies in 30 μL total volume reactions consisting of 1 U GoTaq (Promega), 1x GoTaq clear buffer, 0.2 μM each primer, 0.2mM dNTPs and 2.5 mM $MgCl_2$ using a cycle of 94° for 5m followed by 35 cycles at 94° for 30s, 50° for 35s 72° for 1m45s ending with a final extension at 72° for 5m. We purified all colony PCRs using GeneAid PCR purification kits.

We cycle-sequenced both cpDNA and cloned *idh* DNA templates with ABI BigDye v 1.1 chemistry following the manufacturers protocol modified for 10 μL reactions. Both the *trnQ*$^{(UUG)}$-5'*rps16* region and *idh* were too long to reliably sequence with external primers only. For some samples, we also sequenced from internal primers for these regions (*Q*if: 5'-CGT TCT ATT GAG GAA AGT TAT TTA-3', *Q*ir1: 5'-AGA ATA GTT CCT ATC TAT ATC TAT C-3', *idh*if1: 5'TCT GTT CTG GTC GGT TCT TTG TC-3', *idh*ir1: 5'-TGA ACC CTG TAA TGA CGT GTA AC-3'). Prior to

sequencing, we removed unincorporated BigDye by centrifuging sequencing reactions through Sephadex gel (GE). We then sequenced all samples on an ABI 3130xl genetic analyzer. The first 20-30 bp of sequence at both the 5' and 3' of each fragment was unreliable and we excluded these characters from all subsequent analyses.

**Phylogenetic analyses:** We produced preliminary alignments for each region with Clustal W using the default parameters in Mega 4.0 (Tamura *et al.* 2007) followed by manual correction. Small portions of each region showed evidence of multiple overlapping insertions and deletions resulting in ambiguous alignment (Table 2.2). We excluded these characters from all subsequent analyses. For all DNA regions, we coded unambiguous gaps as present or absent using the simple method described by Simmons and Ochoterena (2000) as implemented the software FastGap V 1.1 (Borchsenius 2009) and appended these binary characters to the end of the 4-state nucleotide data matrix. To identify gene boundaries, we compared our nucleotide alignments to annotated sequences of other species in the Ericales downloaded from Genbank. For the 3'*rps16*–5'*trnK*$^{(UUU)}$ cpDNA region, we excluded a 3' portion of the *rps16* coding sequence that was monomorphic. Our alignment of cloned *idh* sequences included part of exon K, all of exons L and M in addition to part of the M-N spacer. The beginning of each intron in the consensus sequence began with GT and ended with AG.

Previous work on the *idh* gene family had identified ancient gene duplication (Weese and Johnson 2005). Among the clones from a single individual, we often recovered highly divergent copies (7-10% sequence divergence). Preliminary analyses of divergent sequences from the same individuals produced reciprocally monophyletic groups with congruent topologies. This pattern is consistent with duplication of this gene

before the diversification of *Dodecatheon*. However, copies from one group did not occur among the sequenced clones from some individuals. Because the two groups of sequences were easily distinguished, we only analyzed sequences from the group that we recovered from every individual (Table 2.1), which we hereafter refer to as *idh*A. We combined identical sequences from the same individual for subsequent analyses. To compare *idh*A variation within individuals to overall variation in the dataset, we computed the mean pairwise sequence distance among all distinct *idh*A haplotypes from each polymorphic individual to the grand mean of all pairwise sequence distances under a global best-fit substitution model (see below).

In order to infer the evolutionary history for *Dodecatheon* sect. *Dodecatheon* we reconstructed two gene trees: one for both cpDNA markers and one for *idh*A. A combined cpDNA analysis is appropriate because the chloroplast genome is maternally inherited as an unrecombining unit in most angiosperms, including Primulaceae (Corriveau and Coleman 1988). Preliminary analyses of each cpDNA region produced congruent topologies, indicating little conflict. However, preliminary comparisons of cpDNA trees with *idh*A trees demonstrated several conflicts, precluding a global analysis. For both regions, we reconstructed gene trees in both parsimony and Bayesian frameworks. For the parsimony analyses, we conducted heuristic searches using beta 10 version of PAUP* 4.0 (Swofford 1999) with TBR branch swapping, character states weighted equally, gaps / polymorphisms treated as missing data / uncertainties, Steepest Descent ON, Mulpars ON, and Collapse branches option ON for branches with a minimum length of zero, and 200 random sequence addition replicates. We calculated

support for branches with 1000 bootstrap replicates (Felsenstein 1985b) under the same settings, except for using only 50 random sequence additions per bootstrap replicate.

For the Bayesian reconstructions we partitioned each alignment. The cpDNA analysis consisted of separate partitions for the partial *trnQ*$^{(UUG)}$-5'*rps16* sequence, the partial 3'*rps16*–5'*trnK*$^{(UUU)}$ sequence and all indels. For the *idh*A alignment, the putative coding sequence was small (Table 2.3) and some sequences showed evidence of pseudogenization (indels out of reading frame and substitutions at the boundaries of introns). Therefore, we did not attempt to partition this dataset into individual exons or codon positions. Instead, the *idh*A analysis consisted of separate partitions for the concatenated exon sequences, the concatenated intron sequences and all indels. For each nucleotide partition we selected an optimal model of sequence evolution using the Akaike Information Criterion as calculated by MrModeltest v 2.3 (Nylander 2004) (Table 2.3). For the indel partitions we specified simple F-81-like models. We approximated posterior probabilities of trees and parameters for the selected substitution models using Mr. Bayes v 2.1.3 (Hulsenbeck and Ronquist 2001). Our search of parameter space consisted of two independent runs with four linked Monte Carlo Markov chains sampling every 100 generations. We assessed stationarity by plotting the log-likelihood against the number of generations and by confirming that the ESS for each parameter was greater than 100 using Tracer v1.4.1 (Rambaud and Drummond 2007). Once searches had completed, we computed consensus trees across both independent runs after discarding the first 25% of the trees as burn-in.

For a general assessment of relationships among gene sequences and species, we interpreted strongly supported clades in both the cpDNA gene tree and the *idh*A gene

tree. We also compared the structures of unconstrained trees for each region. In order to formally test whether widespread species had an exclusive single origin, we conducted identical Bayesian searches that were constrained to reconstruct monophyletic gene trees for widespread species. If trees generated by unconstrained runs provide a consistently better fit to the data than the constrained trees, then we can reject the hypothesis that rarity is an exclusively plesiomorphic condition in *Dodecatheon* sect. *Dodecatheon*. To test whether the unconstrained topology was significantly better than the constrained topology for each region, we used parsimony-based Templeton's tests (Templeton 1983) and likelihood-based Shimodaira–Hasegawa tests (Shimodaira and Hasegawa 1999). For both tests, we compared both the consensus tree from the unconstrained run and the last 100 trees sampled from the posterior distribution during the unconstrained run to the last 100 trees sampled from the posterior distribution during the constrained run. Although we inferred gene trees by applying multiple substitution models to a partitioned dataset, using multiple substitution models to calculate the likelihood of character changes along a test tree is difficult. Therefore, to implement the S-H test, we used MrModeltest v 2.3 to select a single best-fit substitution model for each alignment with gap presence versus absence coded as A versus C. We then calculated the likelihoods of character changes relative to these global substitution models and tested the significance of differences between topologies through 1000 resampling estimated log-likelihood (RELL) replicates. We implemented the Templeton's Tests and the S-H tests in PAUP*.

## RESULTS

**Chloroplast variation and gene tree:** Rates of polymorphism at the chloroplast spacers were moderate (Table 2.2). Both Parsimony and Bayesian analyses reconstructed congruent gene trees for the combined chloroplast spacers (Figure 2.2). The monophyly of *Dodecatheon* sect. *Dodecatheon* was strongly supported (pp = 1.00 / bp = 99). However, basal relationships in the clade were not. Bayesian analysis reconstructed a grade of sequences from rare western species with widespread and eastern species derived. However, none of these relationships exceeded 60% bootstrap support. Within the basal grade, the two species with the most extreme rarity (*D. austrofrigidum* and *D. utahense*) were strongly supported as monophyletic and sister to each other . However, sequences from the other two rare western taxa, *D. dentatum* and *D. ellisiae*, did not form monophyletic groups.

A weakly supported derived clade included sequences from widespread and eastern species. This clade consisted of two strongly supported groups. The first included two sequences from northwestern accessions of widespread *D. pulchellum*. Every other accession from *D. pulchellum* along with all accessions from all eastern species formed a large, strongly supported polytomy. Some accessions from eastern species occurred in two clades that were derived within the polytomy. However, each of these clades included sequences from all three eastern species. As such, neither widespread nor rare eastern species showed evidence for monophyletic gene trees at the combined chloroplast DNA markers.

**Nuclear variation and gene tree:** Among the 47 accessions, we recovered 118 distinct *idh*A haplotypes among over 300 sequenced clones (Table 2.1). Rates of

polymorphism were high for both nucleotide substitutions and indels (Table 2.3). Two of the outgroup taxa had large deletions (*P. parryi* 87 bp, *D. frigidum* 73 bp). We recovered up to 6 distinct *idh*A haplotypes from some accessions (Table 2.1). Among the 39 polymorphic accessions, mean pairwise sequence distance among different *idh*A haplotypes was 0.007, compared to 0.035 across all analyzed sequences. Four individuals produced *idh*A haplotypes with sequence distance greater than 0.015, and two produced *idh*A haplotypes with sequence distances greater than 0.04.

Parsimony and Bayesian analyses reconstructed congruent gene trees for *idh*A (Fig. 2.3). Basal relationships were unresolved, including the relationships between *Dodecatheon* sect. *Dodecatheon* and the outgroup taxa. Three distinct, strongly supported clades of sequences (pp = 1.0 / bp > 0.98) occurred within the focal group. The first consisted exclusively of sequences from rare western species. This clade showed strong taxonomic structure. Sequences from all four species form strongly supported clades (pp = 1.0/bp > 85). However, sequences from *D. austrofrigidum* were nested within a clade of sequences from the other rare Pacific Northwestern species *D. dentatum*. The second major clade consisted of haplotypes from some Pacific Northwestern accessions of the widespread species *D. pulchellum*. This clade included every haplotype from *D. pulchellum* var. *cusickii* and *D. pulchellum* var. *pulchellum* from that region, along with a single haplotype from *D. pulchellum* var. *monanthum* (Oberle260). However, there was no structure among these infraspecific taxa in this clade.

The final major clade in the *idh*A gene tree included haplotypes from the remaining accessions of *D. pulchellum* along with haplotypes from all eastern accessions.

This clade showed some geographic structure. Most haplotypes from eastern accessions formed a strongly supported clade (pp = 1.00 / bp = 99). Relationships within this large clade were weakly resolved, with little apparent structure among the three eastern species. Two other clades consisted largely of haplotypes from accessions collected in the Intermountain Southwest. The final clade consisted of haplotypes from a broad geographic area and from several taxa, including *D. pulchellum* var. *macrocarpum* (Oberle 252), *D. austrofrigidum* (Chambers 6299) and *D. pulchellum* var. *monanthum* (Oberle 260) from the Pacific Northwest, *D. pulchellum* var. *pulchellum* from the Front Range of the Rocky Mountains (Oberle 319), the undescribed variety of *D. meadia* from Missouri (Oberle 334) and *D. amethystinum* from Pennsylvania (Oberle 340) and from Iowa (Oberle 350). Three accessions produced haplotypes that were exclusively resolved into this clade: *D. pulchellum* var. *macrocarpum*, *D. pulchellum* var. *pulchellum* (Oberle 319) and *D. amethystinum* (Oberle 340). The other haplotypes in this clade came from the accessions that produced highly divergent haplotypes (mean pairwise sequence distance among haplotypes > 0.015). In each case, the other haplotypes from these accessions were resolved into clades that were more geographically and taxonomically cohesive (Fig. 2.3).

**Comparisons of chloroplast and nuclear gene trees:** The overall topologies of the chloroplast and nuclear gene trees shared basic similarities, although support for relationships among major groups differed, as did the memberships of those groups. Relationships between *Dodecatheon* sect. *Dodecatheon* and outgroup taxa were strongly supported in the cpDNA gene tree but were unresolved in the nuclear gene tree.

Within the focal group, the three major clades of haplotypes in the nuclear gene tree corresponded to different parts of the cpDNA gene tree. The first major clade in the nuclear gene tree and the basal grade of the cpDNA both included haplotypes from rare western taxa. However, support for taxa and the inferred relationships among them differed. In the nuclear gene tree, haplotypes from different species resolved into different well supported clades, with the exception of haplotypes from *D. dentatum* which were rendered paraphyletic by a strongly supported clade of *D. austrofrigidum* haplotypes. In the cpDNA gene tree, only haplotypes from *D. austrofrigidum* and *D. utahense* formed clades which were resolved as sister. The second major major clade in the nuclear gene tree included haplotypes from both accessions of *D. pulchellum* that produced divergent sequences at the cpDNA loci. However, several other accessions produced divergent *idh*A haplotypes that did not produce divergent cpDNA sequences. The final major clade in the nuclear gene tree includes haplotypes from accessions that form the derived polytomy in the cpDNA gene tree. The nuclear gene tree provided more resolution. While groups of sequences from eastern species tended to form derived clades in the cpDNA gene tree, they form a very well supported clade in the nuclear gene tree. Furthermore, the widespread western species in the derived clade shows little variation at the chloroplast loci, but considerable geographically structured variation at the nuclear marker.

**Topology tests:** Topology tests comparing these gene trees to constrained gene trees strongly reject the monophyly of sequences from widespread species. The unconstrained majority rule consensus reconstructions provided a better fit to the data than a representative sample of reconstructions constrained to have all haplotypes from

widespread taxa as a monophyletic group at both the cpDNA regions (Templeton's tests, p-values < 0.01, S-H tests p-values < 0.01) and the nuclear region (Templeton's tests, p-values <0.001, S-H tests p-values < 0.001). When representing the unconstrained topologies by a sample of trees from the posterior probability distribution, all unconstrained trees provided similar fits to the data (cpDNA: Templeton's tests, p-values > 0.1; S-H tests p-values > 0.5; *idh*A: Templeton's tests, p-values > 0.05, S-H tests p-values > 0.5) which were significantly better than constrained topologies (cpDNA: Templeton's tests, p-values < 0.01, S-H tests p-values < 0.01; *idh*A: Templeton's tests, p-values < 0.001, S-H tests p-values < 0.001).

## DISCUSSION

Our analysis of chloroplast and nuclear gene trees clearly shows that genes from widespread species are not monophyletic in *Dodecatheon* sect. *Dodecatheon*. Consequently, rarity is not likely to be an exclusively plesiomorphic condition in this group. As such, the evolution of drought tolerance alone is unlikely to explain differences in range size and abundance among species. Instead, environmental tolerances, geographic constraints and historical contingencies may have contributed to rarity in different ways for different species.

**Rare western species:** The four rare western species retain sequences that diverged early in the evolution of the group. The chloroplast gene tree reconstructs them as a basal grade. Better resolution in the nuclear gene tree groups them into one of three main lineages in *Dodecatheon* sect. *Dodecatheon*. However, some Pacific Northwestern accessions of the widespread species *D. pulchellum* retain chloroplast and nuclear gene

sequences that diverged at the same time. This pattern suggests that the ancestors of rare and widespread species co-occurred in western North America during the early stages of diversification of the group.

If both lineages have occupied the landscape for the same amount of time, why is one lineage rare today while the other is not? Different paleoclimatic histories for the characteristic habitats of each could contribute. Rare western shooting stars are confined to moist habitats and are more abundant at higher elevations. During Pleistocene glacial maxima, moist cool habitats were more prevalent at low altitudes in western North America (Spaulding *et al.* 1983, Thompson et al. 1993), and these plants may have been more widespread. However, given their apparent failure to adapt to drying conditions, they appear to have survived climate change by migrating into moist, high elevation refugia. Because altitudinal climate gradients are steep compared to latitudinal gradients (Colwell *et al.* 2008), altitudinal habitat tracking can occur within a spatially restricted area (Jansson and Dynesius 2002). Repeated cycles of local altitudinal migration during the Pleistocene may have generated the geographic structure among species that is evident in the nuclear gene tree. Similar patterns occur among other high elevation species in western North America, from stone crops (DeChaine and Martin 2005) and primroses (Kelso *et al.* 2009) to flightless grasshoppers (Knowles *et al.* 2007). As such, these four western species may be rare because they are geographically isolated glacial relicts (Holmgren 1994). *D. pulchellum* likely differs for two reasons. It can also occur in habitats that dry (it has broader realized niche breadth) and because dry habitats are more prevalent in the region during the current interglacial.

However, gene tree species tree conflicts involving *D. austrofrigidum* complicate this interpretation. The first conflict involves highly divergent *idh*A sequences from one accession of this species. The second conflict occurs with respect to the inferred sister species for *D. austrofrigidum* in the cpDNA gene tree versus the nuclear gene tree. We suspect that both conflicts reflect hybridization. With respect to the highly divergent sequences, one is resolved into a clade of other sequences from *D. austrofrigidum* while the second sequence occurs among a clade including sequences from the *D. pulchellum* var. *macrocarpum*. Given the fact that these accessions were collected only 100 km apart, we suspect that this instance of conflict involves recent genetic introgression from *D. pulchellum* into *D. austrofrigidum.* The second conflict may involve more ancient hybridization. In the cpDNA tree *D. utahense* is sister while in the nuclear gene tree *D. austrofrigidum* is nested within *D. dentatum*. The ancestor of *D. austrofrigidum* may have captured a chloroplast from the ancestor of *D. utahense* earlier in the Pleistocene when ranges would have been different. However, most of its *idh*A variation was derived from *D. dentatum* which grows nearby. Together patterns indicate that *D. austrofrigidum* may need additional systematic and population genetic attention.

**Rare eastern species:** Causes for rarity among eastern species differ. Neither eastern species appears to be rare for the same reasons as rare western species. In both chloroplast and nuclear gene trees, sequences from rare eastern species are derived from the same clades as sequences from widespread species, suggesting close evolutionary relationships. The precise relationships appear to differ, as do the most likely causes of rarity.

One rare eastern species, *D. frenchii*, shows no evidence for genetic differentiation from the widespread eastern species *D. meadia.* The lack of differentiation among these taxa is surprising. Reciprocal transplant experiments have demonstrated local adaptation to their respective habitats (Voigt and Swayne 1955). Furthermore, cytological studies found different ploidy levels for these taxa, which should restrict gene flow (Olah and DeFilipps 1968). However, given that all sequences from these taxa occur in the same recently derived clade, the apparent difference in environmental tolerance between them is not a property of two distinct lineages with different niche breadths. Rather it appears to reflect convergent adaptation by a single lineage, producing a very well-marked ecotype. Given that fine-scale population genetic data suggest local gene flow among taxa (Chapter 4), and that a range-wide phylogeographic analysis fails to find genetic structure for more polymorphic markers (Chapter 3), inaccurate taxonomy appears to be the most likely cause for the rarity of *D. frenchii*.

The final rare eastern species, *D. amethystinum* also shares a close relationship with widespread species. However, the causes for its rarity may be more complex. Accessions of *D. amethystinum* collected within the range of *D. meadia* have chloroplast and nuclear sequences that are similar to sequences from *D. meadia*. However, an accession that was collected outside the range of *D. meadia* only produced nuclear haplotypes that were closely related to haplotypes from western *D. pulchellum*. An accession from *D. amethystinum* collected at the margin of the distribution of *D. meadia* includes divergent haplotypes, one more closely related to haplotypes from *D. pulchellum* and the other more closely related to haplotypes from *D. meadia.* While these three taxa

are so closely related that we cannot exclude lineage sorting as the cause for patterns of allele sharing, several lines of evidence suggest that non-random evolutionary forces play a role. Specifically, the relationship between *D. amethystinum* and *D. pulchellum* is consistent with a previous taxonomic and biogeographic hypotheses: that populations of *D. amethystinum* reflect post-glacial migration of *D. pulchellum* into eastern North America (Ugent *et al.* 1982).

If this biogeographic hypothesis is correct, and *D. amethystinum* is derived from *D. pulchellum,* which is widespread, why is *D. amethystinum* rare? Different geographic constraints and historical events could contribute. First, eastern and western North America have different patterns of habitat heterogeneity. Habitats where *D. pulchellum* performs well may be abundant in western North America but rare in eastern North America. In other words, the size of the potential distribution for this species may differ among regions. Second, *D. amethystinum* may be rare because it migrated so recently that it has not had time to expand its distribution. However, the great disjunction between upper Midwestern and eastern populations of this species suggest that dispersal limitation does not restrict potential range filling. Instead, rarity of *D. amethystinum* appears to reflect competition and hybridization with the widespread eastern species *D. meadia*. Because these taxa are so closely related, *D. meadia* may be competitively and reproductively excluding *D. amethystinum* from parts of its potential distribution.

Of course, these explanations presuppose evolutionary distinction between *D. amethystinum* and *D. meadia* which our data only weakly demonstrate. However, a separate phylogeographic analysis of genome-wide dominant markers is consistent with this interpretation of the nuclear gene tree (Chapter 3). Allopatric populations of *D.*

*amethystinum* are the most genetically distinct eastern shooting stars. Populations collected further south and east share progressively more variation with *D. meadia.* Alternatively, coalescent simulations might statistically distinguish patterns of allele sharing due to lineage sorting from allele sharing due to hybridization in this group (Joly *et al.* 2009). However, these methods depend on accurate specification of historical effective population size (Liu and Pearl 2007). Given that our study was designed examine support for alternative origins of rarity across this section of the genus, and that rarity and effective population size may be related, effectively using coalescent simulations to explore relationships between these taxa would require different sampling and assumptions.

**Widespread species:** Our analysis also identified genetic diversity within widespread species. This is particularly true for some Pacific Northwestern accessions of *D. pulchellum*. Two accessions retain chloroplast sequences that diverged early in the history of *Dodecatheon* sect. *Dodecatheon*. Many more retained divergent *idh*A haplotypes. In the nuclear gene tree all accessions of *D. pulchellum* var. *cusickii* have divergent sequences. Traditionally, dense pubescence distinguishes this taxon, which tends to grow in drier habitats compared to *D. pulchellum* var. *pulchellum* (Thompson 1953). Common garden experiments demonstrated that the morphological differences between these taxa have a genetic basis and *D. pulchellum* var. *cusickii* is almost exclusively diploid (Suttill and Allen 1992). Nevertheless, the varieties tend to share related haplotypes at both nuclear and chloroplast loci. An accession from a third variety collected in the same region had two highly divergent *idh*A haplotypes, one characteristic of plants in the Pacific Northwest and the other characteristic of *D. pulchellum* in other

regions. Given that some populations in the region are polymorphic for pubescence (B. Oberle, pers. obs), we suspect that if early diverged and more recently derived lineages of *D. pulchellum* co-occur in the region, they may be hybridizing.

Our analysis also identified some diversity in *D. meadia* although it was less marked. One accession which we had determined as the undescribed variety retained *idh*A sequences more closely related to sequences from *D. pulchellum* and *D. amethystinum*. Like these two taxa, plants of the undescribed variety tend to have violet or magenta corolla lobes. They also occur geographically closer to *D. pulchellum* than other eastern *Dodecatheon*. We suspect that these plants may also have a hybrid origin between eastern and western groups of widespread *Dodecatheon.* Although these infraspecific taxa show some distinction, evidence for extensive hybridization suggests that species-level recognition is unwarranted.

**Conservation and diversification:** Our results suggest that phylogenetic and landscape approaches can improve the understanding of rarity in general, and improve conservation strategies for rare species in *Dodecatheon* sect. *Dodecatheon* in particular. In our system, we show that one rare species, *D. frenchii*, has no evolutionary distinctiveness. Another, *D. amethystinum*, is very closely related to widespread species. These taxa should not be conservation priorities. However, rare western species are distinct and one has an extreme limited distribution. *D. utahense*, which occurs in a single valley near a growing metropolitan area, has distinct sequences at both chloroplast and nuclear loci. Until recently, plants in this population were considered a variety of *D. dentatum* (Holmgren 1994). Our results show that this population merits species-level recognition. Given local pressure for development, we encourage more aggressive

conservation efforts. Moreover, our analysis suggests that failure to adapt to drying and warming climates since the last glacial maximum contributes to the limited distributions of all rare western taxa. This pattern of evolutionary niche stability may make these taxa especially vulnerable to extinction with anthropogenic global climate change (Wiens and Graham 2005). We expect global climate change to pose the greatest threat to *D. ellisiae*, which only occurs on the highest mountains in southwestern North America. Given limited dispersal ability among shooting stars, we would expect that *ex situ* conservation or assisted dispersal further north may be necessary for this species. However, any conservation strategy should consider the strong possibility for differentiation and local adaptation among populations on different mountains.

Finally our analysis reinforces that geographic and historical contexts are important for understanding diversification (Donoghue 2008). In western North America, landscape heterogeneity appears to have been sufficient to maintain differences between rare and widespread species, despite limited hybridization. This heterogeneity also appears to have to promote diversification among ecologically similar species as climate-forced range dynamics isolated populations in different regions (Hewitt 1996, Jansson and Dynesius 2002, Wiens 2004). This process could promote diversification in many groups, from closely related *Primula* in the same region (Kelso *et al.* 2009), to salamanders in eastern North America (Kozak and Wiens 2006). However, the pattern differs dramatically for shooting stars in eastern North America. Some of the same ecophysiological variation occurs among shooting star populations in eastern North America (Thompson 1953, Holmgren 1994). However, this ecological variation is not associated with evolutionary distinction. Different histories and spatial patterns of

environmental heterogeneity may have contributed. The nested position of eastern taxa suggests that representatives of this genus migrated more recently into this region. If barriers to gene flow evolve gradually, eastern species may be less reproductively isolated (Cavender-Bares *et al.* 2009). Indeed, we find abundant evidence for rampant gene flow among eastern taxa in this and other datasets. Furthermore, geographic heterogeneity is less pronounced in eastern North America. Given that local microclimate gradients are shorter relative to the magnitude of climate change during the Pleistocene in eastern North America, locally adapted populations would have limited ability to persist by local migration. Instead, population persistence may have been facilitated by gene flow among populations adapted to different climatic conditions.

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**TABLE 2.1**: Collection information and number with number of clones sequenced and *idh*A haplotypes recovered.

| Taxon | Collector | Clones | Haplotypes | Locality |
|---|---|---|---|---|
| *P. parryi* (Outgroup) | Gust 187 | 12 | 1 | Grand Co., CO |
| *D. frigidum* (Outgroup) | Parker 7873 | 12 | 2 | Debauch Mt., AK |
| *D. alpinum* (Outgroup) | Oberle 325 | 3 | 1 | Lake Blanche, Salt Lake Co., UT |
| *D. alpinum* (Outgroup) | Oberle 330a | 15 | 2 | Fish Creek, Greenlee Co., AZ |
| *D. austrofrigidum* | Chambers 5436 | 2 | 1 | Trask River, Tillmook Co., OR |
| *D. austrofrigidum* | Chambers 6299 | 3 | 2 | Diamond Falls, Tillamook Co., OR |
| *D. austrofrigidum* | Chambers 6300 | 10 | 5 | Kilchis Falls, Tillamook Co., OR |
| *D. dentatum* | Oberle 253 | 6 | 2 | Elowah Falls, Multnomah Co., OR |
| *D. dentatum* | Oberle 261.6 | 6 | 1 | Eagle Creek, Shoshone Co., ID |
| *D. dentatum* | Oberle 265 | 11 | 3 | Ashnola River, BC |
| *D. dentatum* | Oberle 266 | 10 | 2 | Camas Land, Chelan Co., WA |
| *D. ellisiae* | Oberle 318 | 5 | 2 | Manzano Mts., Torrance Co., NM |
| *D. ellisiae* | Oberle 328 | 9 | 4 | Mt. Lemmon, Pima Co., AZ |
| *D. ellisiae* | Oberle 329 | 4 | 3 | Mt. Graham, Graham Co., AZ |
| *D. ellisiae* | Oberle 330.1 | 3 | 2 | Fish Creek, Greenlee Co., AZ |
| *D. utahense* | Oberle 323.1 | 13 | 1 | Mossy Falls, Salt Lake Co., UT |
| *D. utahense* | Oberle 323.2 | 1 | 1 | Mossy Falls, Salt Lake Co., UT |
| *D. pulchellum* var. *cusickii* | Oberle 255 | 7 | 1 | Eagle Cap, Wallowa Co., OR |
| *D. pulchellum* var. *cusickii* | Oberle 256 | 6 | 2 | Kamiak Butte, Whitman Co., WA |
| *D. pulchellum* var. *cusickii* | Oberle 264 | 12 | 4 | Osoyoos, BC |
| *D. pulchellum* var. *macrocarpum* | Oberle 252 | 5 | 2 | Kingston Prairie, Linn Co., OR |
| *D. pulchellum* var. *monathum* | Oberle 260 | 3 | 2 | Blue Mountains, Garfield Co., WA |
| *D. pulchellum* var. *monathum* | Oberle 326 | 4 | 2 | Mt. Nebo, Utah Co., UT |
| *D. pulchellum* var. *pulchellum* | Oberle 263 | 10 | 6 | Christina Lake, BC |
| *D. pulchellum* var. *pulchellum* | Oberle 319 | 2 | 2 | Pike's Peak, El Paso Co., CO |
| *D. pulchellum* var. *pulchellum* | Matheson-Price | 10 | 5 | Beck's Creek, Emery Co., UT |
| *D. pulchellum* var. *shoshonense* | Reveal 8850 | 3 | 2 | Long Valley, Mono Co., CA |
| *D. pulchellum* var. *shoshonense* | Reveal 8876 | 8 | 6 | Ash Meadows, Nye Co., NV |
| *D. pulchellum* var. *watsoni* | Kelso 07-100 | 7 | 2 | Island Lake, Elko Co., NV |
| *D. pulchellum* var. *zionense* | Oberle 321.1 | 9 | 1 | Cottonwood Canyon, Uintah Co., UT |
| *D. pulchellum* var. *zionense* | Oberle 321.2 | 5 | 3 | Cottonwood Canyon, Uintah Co., UT |
| *D. meadia* | Oberle 292 | 6 | 3 | Lake Oconee, Green Co., GA |
| *D. meadia* | Oberle 295 | 2 | 2 | Bayou L'Ivrogne, Natchitooches Pa., LA |
| *D. meadia* | Oberle 302 | 12 | 2 | Shope Creek, Buncombe Co., NC |
| *D. meadia* | Oberle 313 | 4 | 4 | Pounds Escarpment, Gallatin Co., MO |
| *D. meadia* | Oberle 349 | 4 | 1 | Hogback Prairie, Crawford Co., WI |
| *D. meadia* var. nov | Oberle 296 | 7 | 4 | Wild Basin, Travis Co., TX |
| *D. meadia* var. nov | Oberle 297 | 4 | 2 | Pontotoc Ridge, Pontotoc Co., OK |
| *D. meadia* var. nov | Oberle 334 | 6 | 3 | Taberville Prairie, St. Clair Co., MO |
| *D. amethystinum* | Oberle 332 | 7 | 3 | Clark's Hill, Osage Co., MO |
| *D. amethystinum* | Oberle 340 | 2 | 1 | Catawissa Bluffs, Colmbia Co., PA |
| *D. amethystinum* | Oberle 341 | 6 | 3 | Ray Norbut SFWA, Pike Co., IL |
| *D. amethystinum* | Oberle 350 | 7 | 3 | North Beark Creek, Winneshiek Co., IA |
| *D. frenchii* | Oberle 294 | 4 | 3 | Cane Creek Canyon, Colbert Co., AL |
| *D. frenchii* | Oberle 300 | 5 | 2 | Dismal Hollow, Newton Co., AR |
| *D. frenchii* | Oberle 310 | 9 | 2 | Carter Caves, Cater Co., KY |
| *D. frenchii* | Oberle 312 | 5 | 2 | Oil Creek, Perry Co., IN |
| *D. frenchii* | Oberle 317 | 5 | 3 | Hickory Canyons, Ste. Genevieve Co., MO |

**TABLE 2.2**: Parameters for Parsimony-based analyses. CI = consistency index, RI = retention index, TL = tree length

| Character metrics | trnQ(UUG)-rps16 | rps16-trnK(UUU) | Total | *idh* A introns | *idh* A exons | Total |
|---|---|---|---|---|---|---|
| Aligned length | 1091 | 836 | 1927 | 635 | 275 | 910 |
| Variable | 79 | 59 | 138 | 204 | 45 | 249 |
| Pars. Inform. | 34 | 27 | 61 | 148 | 19 | 167 |
| Gaps | 21 | 6 | 27 | 44 | 1 | 45 |
| Pars. Inform. Gaps | 12 | 2 | 14 | 29 | 1 | 30 |
| excluded | 22 | 41 | 63 | 15 | 0 | 15 |
| Tree metrics | | | cpDNA | | | *idh* A |
| CI | | | 0.91 | | | 0.88 |
| RI | | | 0.94 | | | 0.98 |
| TL | | | 185 | | | 365 |

**TABLE 2.3**: Substitution models and parameters for Bayesian and Likelihood analyses. AIC calculated relative to 24 models of sequence evolution as estimated by MrModeltest v 2.3 Parameter estimates for partitioned datasets based on two independent runs in MrBayes v 3.1.2 at 20001 samples, first 5000 discarded as burn-in. Parameter estimates for "global" datasets estimated directly by MrModeltest v 2.3.

| | | | Substitution Models | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Base freq. | | | | Substitution Rates | | | | | | Among site var. | | | |
| Dataset | Model | AIC | A | C | G | T | A<->C | A<->G | A<->T | C<->G | C<->T | G<->T | κ | Γ | I | tree length |
| trnQ(UUG)-rps16 | GTR+Γ | 3987.136 | 0.354 | 0.119 | 0.143 | 0.384 | 0.157 | 0.196 | 0.031 | 0.048 | 0.415 | 0.154 | - | 24.28 | - | 0.149598 |
| rps16-trnK(UUU) | GTR | 2907.388 | 0.298 | 0.133 | 0.119 | 0.45 | 0.261 | 0.138 | 0.045 | 0.142 | 0.256 | 0.157 | - | - | - | 0.149598 |
| | | | | | | | | | | | | | | | | |
| *idh* A introns | GTR+I | 5363.225 | 0.254 | 0.14 | 0.206 | 0.399 | 0.104 | 0.34 | 0.078 | 0.17 | 0.219 | 0.089 | - | - | 0.223 | 0.67333 |
| *idh* A exons | K80 | 1476.65 | - | - | - | - | - | - | - | - | - | - | 5.574 | - | - | 0.67333 |
| | | | | | | | | | | | | | | | | |
| Global cpDNA | GTR+Γ | 7512.062 | 0.338 | 0.123 | 0.128 | 0.411 | 2.589 | 1.11 | 0.197 | 0.423 | 2.025 | 1 | - | 0.813 | - | - |
| Global *idh* A | GTR+I | 7920.15 | 0.268 | 0.167 | 0.217 | 0.348 | 2.765 | 3.024 | 0.808 | 1.322 | 2.746 | 1 | - | - | 0.183 | - |

**FIGURE 2.1**: Range map for species in *Dodecatheon* sect. *Dodecatheon*.

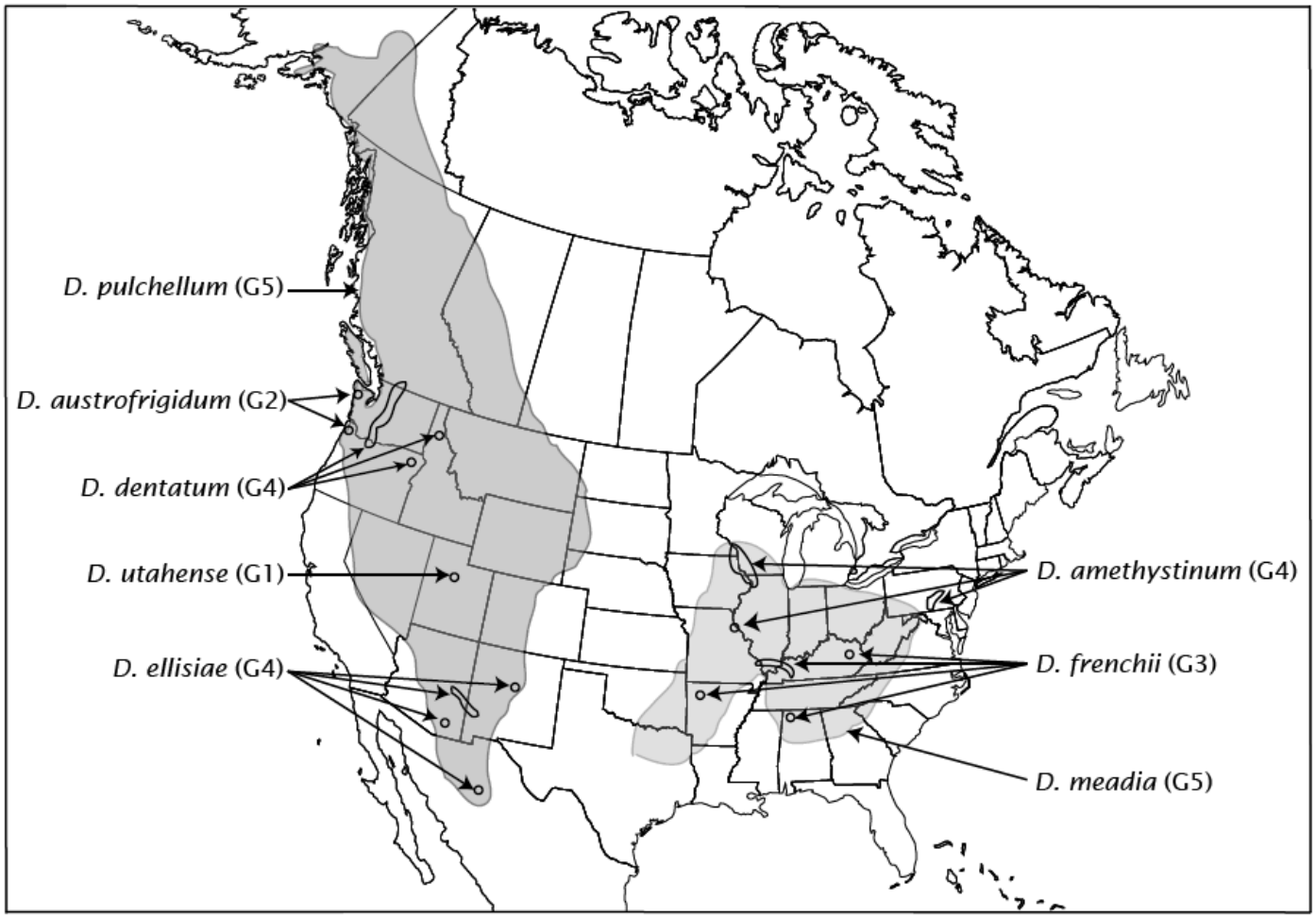


**FIGURE 2.2**: Consensus phylogram from Bayesian analysis of cpDNA sequences. First number above before each node represents posterior probability. Second number represents maximum parsimony based-bootstrap proportion for corresponding branches.

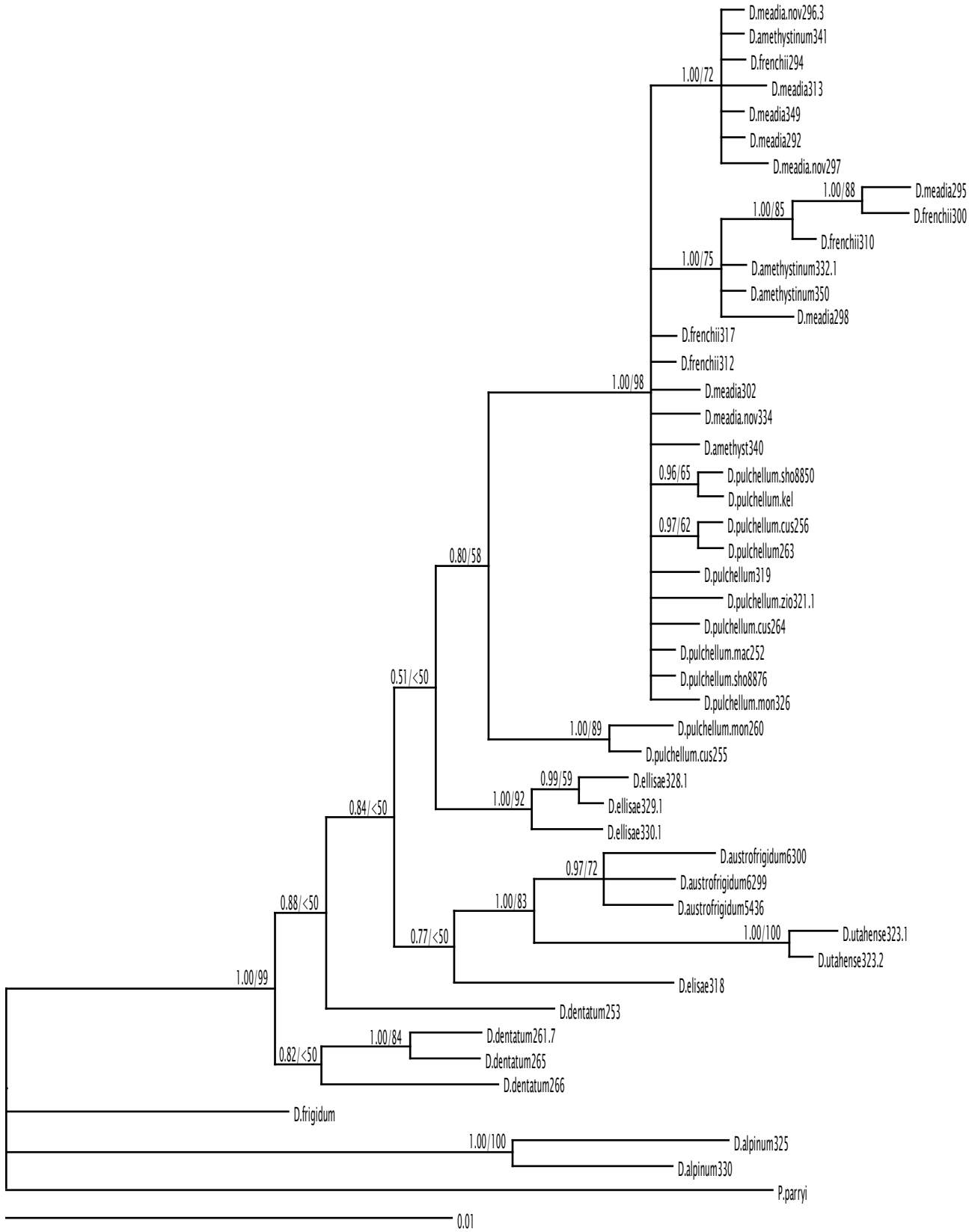
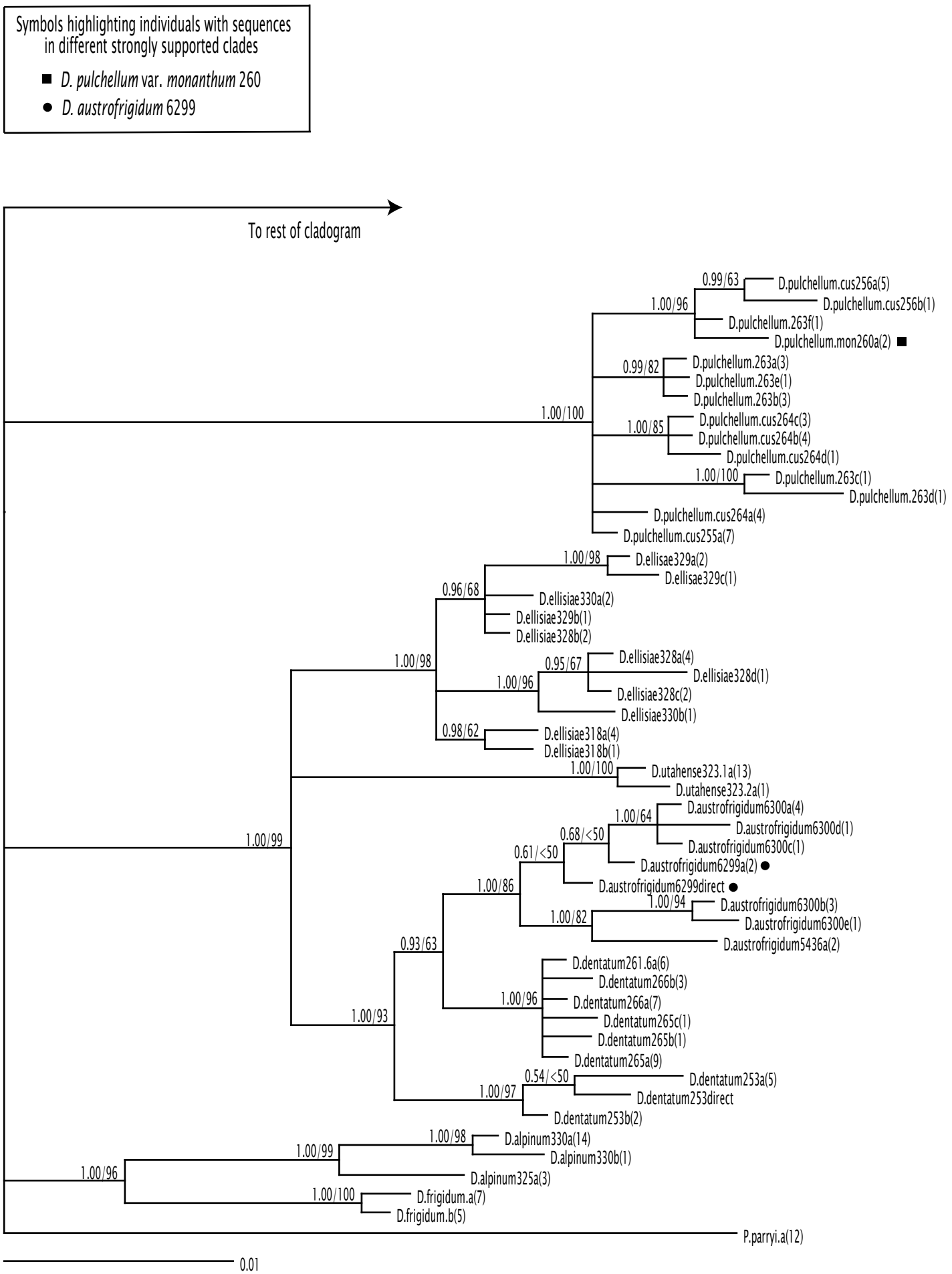
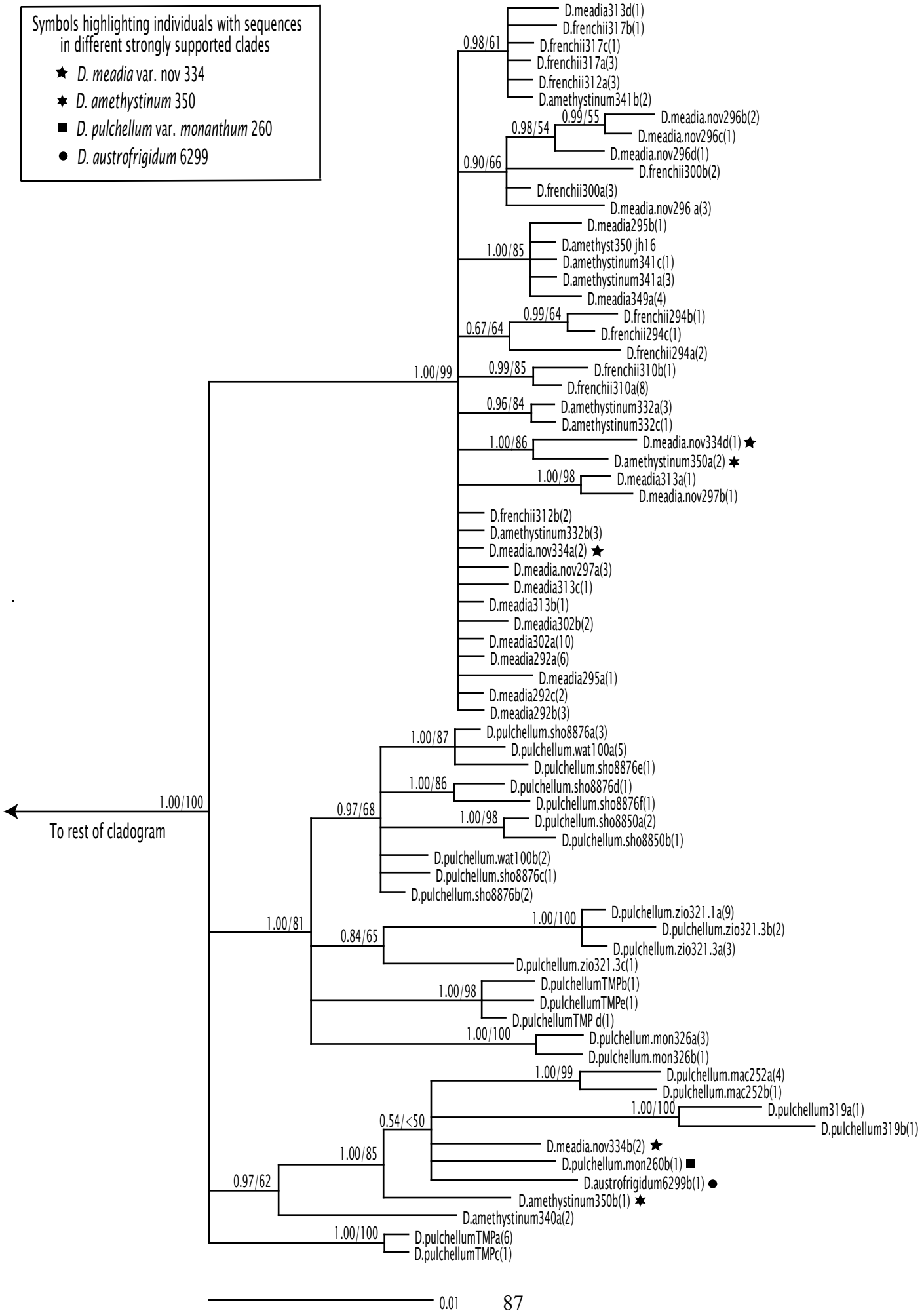

D.meadia.nov296.3
D.amethystinum341
D.frenchii294
1.00/72
D.meadia313
D.meadia349
D.meadia292
D.meadia.nov297
1.00/88
D.meadia295
D.frenchii300
1.00/85
D.frenchii310
1.00/75
D.amethystinum332.1
D.amethystinum350
D.meadia298
D.frenchii317
D.frenchii312
1.00/98
D.meadia302
D.meadia.nov334
D.amethyst340
0.96/65
D.pulchellum.sho8850
D.pulchellum.kel
0.97/62
D.pulchellum.cus256
D.pulchellum263
0.80/58
D.pulchellum319
D.pulchellum.zio321.1
D.pulchellum.cus264
D.pulchellum.mac252
0.51/<50
D.pulchellum.sho8876
D.pulchellum.mon326
1.00/89
D.pulchellum.mon260
D.pulchellum.cus255
0.99/59
D.ellisae328.1
1.00/92
D.ellisae329.1
0.84/<50
D.ellisae330.1
D.austrofrigidum6300
0.97/72
D.austrofrigidum6299
D.austrofrigidum5436
0.88/<50
1.00/83
1.00/100
D.utahense323.1
0.77/<50
D.utahense323.2
1.00/99
D.elisae318
D.dentatum253
1.00/84
D.dentatum261.7
0.82/<50
D.dentatum265
D.dentatum266
D.frigidum
1.00/100
D.alpinum325
D.alpinum330
P.parryi
0.01

**FIGURE 2.3**: Consensus phylogram from Bayesian analysis of *idh*A sequences. First number above before each node represents posterior probability. Second number represents maximum parsimony based-bootstrap proportion for corresponding branches. The name of each OTU includes the accession name followed by a letter indicating the haplotype identity. Number in parentheses represents the number of clones with that haplotype.


Symbols highlighting individuals with sequences in different strongly supported clades
■ D. pulchellum var. monanthum 260
● D. austrofrigidum 6299
To rest of cladogram
0.99/63
D.pulchellum.cus256a(5)
1.00/96
D.pulchellum.cus256b(1)
D.pulchellum.263f(1)
D.pulchellum.mon260a(2) ■
0.99/82
D.pulchellum.263a(3)
D.pulchellum.263e(1)
D.pulchellum.263b(3)
1.00/100
D.pulchellum.cus264c(3)
1.00/85
D.pulchellum.cus264b(4)
D.pulchellum.cus264d(1)
1.00/100
D.pulchellum.263c(1)
D.pulchellum.263d(1)
D.pulchellum.cus264a(4)
D.pulchellum.cus255a(7)
1.00/98
D.ellisae329a(2)
D.ellisae329c(1)
0.96/68
D.ellisiae330a(2)
D.ellisiae329b(1)
D.ellisiae328b(2)
1.00/98
D.ellisiae328a(4)
0.95/67
D.ellisiae328d(1)
1.00/96
D.ellisiae328c(2)
D.ellisiae330b(1)
0.98/62
D.ellisiae318a(4)
D.ellisiae318b(1)
1.00/100
D.utahense323.1a(13)
D.utahense323.2a(1)
D.austrofrigidum6300a(4)
1.00/64
D.austrofrigidum6300d(1)
1.00/99
0.68/<50
D.austrofrigidum6300c(1)
0.61/<50
D.austrofrigidum6299a(2) ●
D.austrofrigidum6299direct ●
1.00/86
1.00/94
D.austrofrigidum6300b(3)
1.00/82
D.austrofrigidum6300e(1)
D.austrofrigidum5436a(2)
0.93/63
D.dentatum261.6a(6)
D.dentatum266b(3)
1.00/96
D.dentatum266a(7)
1.00/93
D.dentatum265c(1)
D.dentatum265b(1)
D.dentatum265a(9)
0.54/<50
D.dentatum253a(5)
1.00/97
D.dentatum253direct
D.dentatum253b(2)
1.00/98
D.alpinum330a(14)
1.00/99
D.alpinum330b(1)
1.00/96
D.alpinum325a(3)
1.00/100
D.frigidum.a(7)
D.frigidum.b(5)
P.parryi.a(12)
0.01


Symbols highlighting individuals with sequences in different strongly supported clades
★ D. meadia var. nov 334
✭ D. amethystinum 350
■ D. pulchellum var. monanthum 260
● D. austrofrigidum 6299
D.meadia313d(1)
D.frenchii317b(1)
0.98/61
D.frenchii317c(1)
D.frenchii317a(3)
D.frenchii312a(3)
D.amethystinum341b(2)
0.99/55
D.meadia.nov296b(2)
0.98/54
D.meadia.nov296c(1)
D.meadia.nov296d(1)
0.90/66
D.frenchii300b(2)
D.frenchii300a(3)
D.meadia.nov296 a(3)
D.meadia295b(1)
1.00/85
D.amethyst350 jh16
D.amethystinum341c(1)
D.amethystinum341a(3)
D.meadia349a(4)
0.99/64
D.frenchii294b(1)
0.67/64
D.frenchii294c(1)
D.frenchii294a(2)
1.00/99
0.99/85
D.frenchii310b(1)
D.frenchii310a(8)
0.96/84
D.amethystinum332a(3)
D.amethystinum332c(1)
1.00/86
D.meadia.nov334d(1) ★
D.amethystinum350a(2) ✭
1.00/98
D.meadia313a(1)
D.meadia.nov297b(1)
D.frenchii312b(2)
D.amethystinum332b(3)
D.meadia.nov334a(2) ★
D.meadia.nov297a(3)
D.meadia313c(1)
D.meadia313b(1)
D.meadia302b(2)
D.meadia302a(10)
D.meadia292a(6)
D.meadia295a(1)
D.meadia292c(2)
D.meadia292b(3)
1.00/87
D.pulchellum.sho8876a(3)
D.pulchellum.wat100a(5)
D.pulchellum.sho8876e(1)
1.00/86
D.pulchellum.sho8876d(1)
D.pulchellum.sho8876f(1)
1.00/100
To rest of cladogram
0.97/68
1.00/98
D.pulchellum.sho8850a(2)
D.pulchellum.sho8850b(1)
D.pulchellum.wat100b(2)
D.pulchellum.sho8876c(1)
D.pulchellum.sho8876b(2)
1.00/81
1.00/100
D.pulchellum.zio321.1a(9)
D.pulchellum.zio321.3b(2)
0.84/65
D.pulchellum.zio321.3a(3)
D.pulchellum.zio321.3c(1)
1.00/98
D.pulchellumTMPb(1)
D.pulchellumTMPe(1)
D.pulchellumTMP d(1)
1.00/100
D.pulchellum.mon326a(3)
D.pulchellum.mon326b(1)
1.00/99
D.pulchellum.mac252a(4)
D.pulchellum.mac252b(1)
1.00/100
D.pulchellum319a(1)
D.pulchellum319b(1)
0.54/<50
D.meadia.nov334b(2) ★
1.00/85
D.pulchellum.mon260b(1) ■
0.97/62
D.austrofrigidum6299b(1) ●
D.amethystinum350b(1) ✭
D.amethystinum340a(2)
1.00/100
D.pulchellumTMPa(6)
D.pulchellumTMPc(1)
0.01

# CHAPTER 3

## Integrated phylogeographic and ecophysiological data suggest different patterns of gene flow mediated alternative responses to historical climate change in eastern North American *Dodecatheon*.

## INTRODUCTION

The match between organism and environment is among the most remarkable patterns in nature (Darwin 1859). This is especially true with respect to climate. Climate limits the geographic range of many species (Woodward 1987, Gaston 2003, Lomolino *et al.* 2006) and many populations are locally adapted to climatic conditions (Clausen *et al.* 1940, Leimu and Fischer 2008). These relationships are pervasive despite dramatic global climate change in recent geological history. During the Pleistocene to Holocene transition, global temperature increased by 7°C and shifts in regional patterns of precipitation transformed grasslands into deserts (Kim *et al.* 2008). Understanding how species survived climate change since the last glacial maximum could provide insights into basic ecological and evolutionary processes. It may also improve predictions for how they might respond to anthropogenic global climate change (Davis and Shaw 2001, Wiens and Graham 2005).

Climate change threatens species when conditions across the species' range become unsuitable for population replacement. Under these circumstances, species may survive by shifting their ranges to track suitable climates and by evolving to tolerate new conditions. Migration and adaptation are not mutually exclusive (Davis *et al.* 2005). However, these responses differ in several important respects. The first key difference pertains to the rate of change in climate tolerance relative to the rate of climate change. If species successfully track habitats with similar climate, then stabilizing selection can reduce the rate of change of climate tolerance (Eldridge 1989). However, adaptation occurs when the rate of change in climate tolerance is associated with the rate of climate change. The second key difference pertains to adaptive genetic variation (Jump and

Peñuelas 2005). If species lack genetic variation for climate adaptive traits, then evolution of climate tolerance is impossible and species must habitat track. In contrast, species with additive genetic variation may adapt to changing climates, provided that generation times are short and any genetic constraints are weak (Kelley *et al.* 2003, Etterson 2004). The final key difference is the spatial scale of the processes that match traits to environments. If habitat tracking predominates, then the match between traits and environments results from the regional processes of migration and ecological sorting of species (Ackerly 2003). If adaptive evolution predominates, then the match between traits and environments results from local natural selection among alternative genes.

Because of these differences, alternative responses to climate change may leave different signatures in the relationships between trait variation, genetic variation and geographic distributions. We explore how to interpret these signatures among eastern North American *Dodecatheon* (Primulaceae). First, we describe the regional geographic and historical context for our study. Then we describe our focal taxon and an important adaptive trait that varies among habitats across the study region. Finally we present alternative scenarios for how *Dodecatheon* survived warming since the last glacial maximum that involve different roles for migration and adaptation. Based on these alternative scenarios we generate predictions for expected relationships between trait variation and genetic variation. We then test support for these scenarios, and the responses they entail, by integrating ecophysiological and phylogeographic data at a regional scale.

## STUDY SYSTEM

**Region:** Eastern North America is well suited to studying the effects of past climate change. Pleistocene glacial cycles have strongly impacted topography and biological communities across the region. In unglaciated areas, complex landforms generate microclimate heterogeneity. Extremes along local climate gradients can provide refuges for populations adapted to conditions that prevailed at different times during the glacial-interglacial cycle (Thorne 1993). For instance, during the current interglacial, north and east facing cliffs are cooler and more moist during stressful summer months than other habitats (Nekola 1998). These cliffs provide refuges for glacial relict populations of some boreal species (Stroh 2005). In contrast, nearby exposed rock outcrops often harbor xeric-adapted populations that occur predominately in more arid regions (Hutchison 1997). These community associations suggest that differences along local microclimate gradients in eastern North America are comparable in magnitude to regional climate change since the Last Glacial Maximum (Jackson and Overpeck 2000).

**Taxon and trait:** An excellent group for studying responses to past climate change in eastern North America is *Dodecatheon* (Primulaceae). We will use the traditional taxonomy here, noting that species in this genus were recently transferred to the large genus *Primula* (Mast and Reveal 2007). These plants, commonly known as shooting-stars, are bumble-bee pollinated and their small seeds have no obvious dispersal mechanism. While the genus is very distinctive, infrageneric taxonomy is notoriously complex (Gray 1886, Thomson 1953). Three species are currently recognized in eastern North America (Reveal 2009). Two, *D. frenchii* and *D. amethystinum*, are moist cliff endemics with patchy distributions (Walck *et al.* 1996). The third species, *D. meadia,*

has a much broader distribution that spans diverse habitats including forests, prairies and rock outcrops. The range of *D. meadia* almost completely encompasses the ranges of both rare species (Figure 3.1). Populations of widespread and rare species often grow within the foraging range of shared pollinators (Macior 1970). In some areas, rare and widespread species have parapatric distributions across local microclimate gradients, with the rare species growing on the sheltered cliff face while *D. meadia* grows in the exposed habitat at the summit of the same cliff.

Despite taxonomic complexity, these species differ with respect to an important functional trait. *D. meadia* has thick leaves, while both rare species have thin leaves (Fassett 1944). Leaf thickness mediates a tradeoff between light capture and water loss that is important for photosynthetic performance among habitats that differ in light availability and water stress (Westoby *et al.* 2002). A reciprocal transplant between *D. frenchii* and *D. meadia* demonstrated that the difference in leaf thickness among taxa had a genetic basis (Voigt and Swayne 1955). Thirty years later, the transplanted colony of *D. meadia* had gone extinct. This result suggests that leaf thickness may mediate divergent local adaptation to microclimatic differences between cliff and exposed habitats (Mohlenbrock 1987). Another reciprocal transplant experiment between glade and forest subspecies of *D. meadia* also demonstrated local adaptation to microclimate (Turner and Quarterman 1968). Together the results of these reciprocal transplant experiments suggest that leaf thickness contributes to pervasive local microclimate adaptation within and among eastern North American *Dodecatheon* taxa.

**Responses to climate change:** Two scenarios could explain the fit between traits and habitats in eastern North American *Dodecatheon*. The first is consistent with a

primary role for migration. The rare species may be glacial relicts that diverged from the widespread species before the last glacial maximum (Swayne 1973, Ugent *et al.* 1982). When conditions that typify their current habitat prevailed across the region, these species were widespread. However, due to a lack of adaptive genetic variation, they could not evolve in response to warming and became restricted to moist cliff refugia while *D. meadia* migrated into the region. The second scenario is consistent with a primary role for adaptation. The rare species may represent extreme ecotypes of a highly polymorphic lineage (Fassett 1944). In this lineage, ongoing selection across local microclimate gradients promotes trait differentiation among populations.

These extreme scenarios differ with respect to the timing of the evolution of climate tolerance, the distribution of genetic variation within and among taxa and the spatial scale of processes that match traits to habitats. As such, they make specific predictions for the relationships between trait variation, genetic variation and geographic distributions. If rare species are glacial relicts, they should be deeply diverged from the widespread species, with most genetic differences occurring among three distinct genetic groups that correspond to recognized taxa. Furthermore population genetic distance, as a measure of relatedness, should correlate more strongly with traits that determine regional habitat sorting than with geographic distance among populations of all three distinct species.

However, if rare species are ecotypes, then eastern *Dodecatheon* should represent a single genetically cohesive group. Because trait differences among ecotypes are due to local divergent selection on adaptive alleles (Wu 2001) and *Dodecatheon* are predominately outcrossing (Macior 1964) neutral and adaptive alleles should be unlinked.

Across the entire group, neutral population genetic distance should correlate weakly with morphological distance (McKay and Latta 2002). Furthermore, adaptive evolution of climate tolerance in this highly polymorphic group may have mitigated pressure for climate forced migration. Therefore, we would expect a relatively stronger correlation between neutral population genetic distance and geographic distance as populations remain closer to drift gene flow equilibrium across many habitat types (Hutchison and Templeton 1999).

## MATERIALS AND METHODS

**Collections:** During Spring 2007 and 2008, we sampled populations from across the ranges of all three species (Fig. 3.1). We based taxonomic determinations on the most recent key (Reveal 2009), by conferring with local botanists and by referencing previous determinations at the same localities in museum collections or natural history databases. After referencing the geographic coordinates with a GPS, we made a qualitative assessment of habitat type. If the majority of individuals occurred within two meters of the top or bottom of a vertical rock face, we identified the habitat as "Cliff." For populations not near cliffs, we identified the habitat as "Forested" if the population occurred under a continuous forest canopy or "Open" if tree cover was less than 50%. For a representative sample of plants at each locality, we determined the orientation of the longest transect through the population. Every three meters along that transect, we sampled the closest reproductive individual until we had sampled ten individuals. If the longest extent of the population was less than 30m, we initiated additional transects three meters displaced from the previous transect until we completed the sample. Given the

limited ability for vegetative propagation by these plants (Sørensen 1992), this sampling strategy should reduce the likelihood of sampling ramets from the same genet.

We collected the largest undamaged leaf from each sampled individual for ecophysiological analysis and an additional two grams of fresh leaf tissue from other leaves for genetic analysis. For genetic analysis, we preserved leaves in silica gel and then stored samples at -20°C prior to DNA extraction. We extracted DNA using Viogene plant DNA miniprep kits. Finally, to assess ploidy level, we collected developing buds or pollen from a randomly selected plant. Vouchers are deposited at the Missouri Botanical Garden herbarium along with detailed notes on morphology.

**Ecophysiology:** In order to quantify patterns of variation for a trait that influences fitness, we measured Specific Leaf Area (SLA). SLA is defined as the ratio of fresh leaf area to dry leaf mass. This ratio is closely related to leaf thickness (Vile *et al.* 2005). To quantify SLA, we pressed the largest undamaged leaf from each plant against a laminated grid inside a modified picture frame. We then took a digital photograph of the pressed leaves in the field. To ensure that the image was horizontal, we squared the image of the frame to a rectilinear grid using the lens distortion tool in Adobe Photoshop CS (Adobe Systems Incorporated). We then measured the area of each leaf by taking the average of three independent mearuements in ImageJ v 1.37 (National Institutes of Health). After taking the digital photograph, we dried the leaves in a plant press and weighed them to a precision of 0.1 mg using a Mettler Toledo XSG4 electronic balance.

We tested for differences in log-transformed SLA among taxa and among habitats as fixed effects with mixed model ANOVAs, treating populations as nested random effects using the package 'nlme' (Pinhiero *et al.* 2009) in R v 9.0 (R Development Core

Team). In order to corroborate differences in performance due to SLA, we analyzed carbon isotope ratios in a subset of plants. High carbon isotope ratios are associated with water use efficiency (Farquar *et al.* 1989). In order to determine carbon isotope ratios, we submitted a sample from one leaf per population to the UC Davis Stable Isotope Facility. Because light intensity may influence carbon isotope ratios (Yu *et al.* 2005), and light intensity varied among habitats, we use these values as a simple qualitative assessment that variation in SLA may reflect physiological performance.

**Historical differentiation:** To test for evidence of historical differentiation among taxa, we sequenced and analyzed a non-coding cpDNA spacer. Preliminary analyses of several regions identified polymorphism at *trnH*$^{GUG}$—*psbA*. We amplified this region using the protocol described by Shaw *et al*. (2005). We then purified PCR products using GeneAid kits and sequenced purified templates at the Genome Sequencing Center at Washington University. We aligned sequences by hand, and reconstructed a haplotype network under statistical parsimony (Templeton *et al.* 1992). Our original reconstruction produced two loops (Figure 3.2: haplotypes A-B-C-G and haplotypes G-J-C), involving a substitution and an insertion-deletion polymorphism at a polynucleotide repeat. Because polynucleotide repeats are prone to length variation homoplasy (Ortí *et al.* 1997), we broke these loops (GxE, JxG) by allowing multiple changes in the indel characters (Templeton *et al.* 2000).

Long-term isolation among taxa can produce hierarchical structure in haplotype networks (Templeton *et al.* 1995). In order to test support for historical isolation among taxa, we converted our haplotype network into a series of nested clades following the nesting rules of Templeton and Sing (1993). We then applied a series of contingency

tests for differentiation among taxa at each level of haplotype nesting (Matos and Schaal 2000). Because we found little variation within populations at this locus, we treated each population haplotype as an observation, with fractional observations representing the relative proportion of each haplotype found in the few polymorphic populations. We evaluated the significance of differentiation among taxa at each nesting level by testing the observed chi-squared statistic against a reference distribution generated through $10^5$ replicates of Monte Carlo simulation as implemented in R v 9.0 (package "stats").

**Variation among taxa and populations:** To examine the distribution of genetic variation among taxa and populations we collected an Amplified Fragment Length Polymorphism (AFLP) dataset. We checked DNA concentration and quality by agarose gel electrophoresis. We then generated our AFLP profiles using a protocol optimized for automated scoring (Trybush *et al.* 2006). For every sample, we analyzed variation at four different primer combinations that had been previously used to detect genetic structure among closely related *Primula* (Kelso *et al.* 2009). The primers began with the preselective sequences EcoRI 5'-GAC TGC GTA CCA ATT C XXX, MseI 5'-GAT GAG TCC TGA GTA A XXX and involved the following 5' fluorescent dyes: (1) Mse CTC, Eco ACT, 6-Fam; (2) Mse CTC, Eco AAG HEX; (3) Mse CAG, Eco ACT, 6-Fam; (4) Mse CAG, Eco ACT HEX. We conducted selective amplifications for each Mse primer in multiplex PCR with both dye-labeled Eco primers and generated AFLP profiles using an ABI 3130xl Genetic Analyzer. We scored alleles using GeneMapper 3.7 (Applied Biosystems) with the following peak-detection parameters: peak height threshold=160, bin-width=1.0 bp, peak half width=4 pts, polynomial degree=5, window size=9. These parameters produced allele calls that were similar to manual calls (data not

shown). To estimate error due to our laboratory techniques, we selected one individual from every other population by ascending collection number and generated a second AFLP profile starting with a second DNA extraction. We excluded all individuals that failed for one or more AFLP primer combination from all subsequent analyses.

We evaluated the relationship between taxonomic identity and population genetic variation in two different ways. First, we quantified the proportion of variation in band presences attributable to within population, among population and among taxon components with an Analysis of Molecular Variance (AMOVA) as implemented in Arlequin v. 3.1 (Excoffier *et al.* 1992, Excoffier *et al.* 2005). If the distribution of traits among environments is attributable to habitat tracking by taxa as currently defined, then most variation should occur among taxa, and little within populations. However, alternative responses to climate change could produce genetic clusters that are only weakly associated with taxonomic determinations. In order to identify genetic groups without reference to taxonomy, we applied a nonparametric clustering algorithm to a genetic space defined by variation among individuals. This approach, Principal Coordinate – Modal Clustering, performs well with dominant marker data when groups are recently diverged or potentially obscured by hybridization (Reeves and Richards 2007). It begins with a principal coordinate analysis of pairwise Jaccard distances among all samples. Jaccard distances are advantageous in this application, because they exclude shared absences, which are especially prone to homoplasy in AFLP data (Bonin *et al.* 2007). Following three dimensional ordination, Modal Clustering identifies the number of groups and assigns individuals to those groups with reference to valleys in the point-density landscape across the ordination space. The sensitivity of the approach (the

number of groups identified) depends on the radius of the sphere (a smoothing parameter R) used to estimate local density relative to the extent of the overall ordination. We are interested in the correspondence between taxonomic determinations and membership into three groups. To assign individuals to three groups, we tested a range of smoothing parameters. After finding the largest smoothing parameter that assigned all individuals into at least three groups, we constrained the algorithm to assign individuals to only three groups. Because all individuals from each population were assigned to the same group, we tested the correspondence between taxonomic determination and group membership with a 3x3 exact test (Freeman-Halton extension). We conducted the Principal Coordinate Analysis and the exact test in R v 9.0 (package "stats"). We implemented the Modal Clustering with PROC MODECLUS (Sarle and Kuo 1993) in SAS v. 9.1 (SAS Institute), using the following parameters STANDARD; METHOD=6; CASCADE=1 and MAXCLUSTERS=3 (Reeves and Richards 2007).

**Spatial scale of trait-habitat matching:** To assess the relative roles of regional processes (migration and ecological sorting) versus local processes (gene flow and natural selection) for explaining the match between traits and habitats in eastern North American *Dodecatheon*, we tested whether trait differences or geographic distance explained genetic distance among populations. We quantified trait differences as the pairwise Euclidean distance in mean ln(SLA) among populations. For geographic distance, we projected the coordinates of all localities onto the North American Equidistant Conic projection with ArcGIS v. 9.0 (ESRI) and computed pairwise distances in meters. Finally, we estimated population genetic distances as the mean pairwise Jaccard distance among individuals in each population. To test whether population

genetic distance correlated more strongly with ecophysiological differences or geographic distance, we conducted a multiple matrix regression. We tested for the significance of regression coefficients for both parameters against $10^4$ permutations of the response matrix as a one-tailed test with a significance threshold of 0.05 following the procedure of Legendre *et al.* 1994 as implemented in the R package 'ecodist' (Goslee and Urban 2007). Based on this specification, regression coefficients should be positive and significant if the effect they represent influences the distribution of traits among habitats.

**Ploidy level:** Ploidy level varies among populations of Eastern North American *Dodecatheon* (Olah and Defilipps 1968). Changes in ploidy level can cause difference in AFLP profiles (Fay *et al*. 2005) and they can produce transgressive difference in traits (Levin 1983). To examine how variation in ploidy level may have influenced our analysis, we inferred ploidy level from two different kinds of data. We obtained direct chromosome counts from two populations of *D. frenchii* (Oberle 300, Oberle 335) by fixing developing flower buds from those populations in Carnoy's Solution, staining anthers with acetocarmine and counting chromosomes under a phase contrast microscope.

We also inferred ploidy level in 33 populations from measurements of pollen diameter. Pollen diameter correlates with ploidy level in many plants (Muller 1979), including *Dodecatheon* (Suttill and Allen 1992). We coated pollen from one individual from each population with 200 A of gold using a SPI gold sputter coater and took a digital photograph of gold coated pollen with an ISI-SX40 Scanning Electron Microscope run at an emission of 10KV. We measured the longest diameter of several fully developed pollen grains per individual using the Feret's diameter tool in ImageJ v1.37.

We then assigned each individual to one of three pollen size categories using a K-means cluster analysis of mean pollen diameter in R v 9.0 (package "stats"). The K-means algorithm assigns each individual to a group such that the within group variance in mean pollen diameter is minimized. Previous cytological work on Dodecathon had identified three common ploidy levels (diploid, tetraploid and hexaploid) (Suttill and Allen 1992), so we applied the algorithm with a K=3. We validated associating pollen diameter clusters with ploidy levels in two ways. First, we obtained a direct diploid chromosome count for an individual that was assigned to the smallest pollen diameter cluster (Oberle 335). Second, we compared the difference in the mean pollen diameters for each cluster to the reported differences in pollen diameters produced by known diploid, tetraploid and hexaploid plants of *Dodecatheon* taxa from the same section (Suttill and Allen 1992).

For this subset of our original sample, we quantified the amount of variation in AFLP profiles among populations attributable to ploidy level with an AMOVA. If ploidy level influences AFLP band presence and absence then ploidy level should explain significant variation among populations. We also investigated whether differences in ploidy level influenced pairwise population genetic differentiation in the context of spatial and ecological differences among populations by including a matrix of pairwise differences in ploidy level among populations (0 = same ploidy, 1 = different ploidy) in the multiple matrix regression described above. If ploidy level differences promote differentiation in AFLP profiles then the regression coefficient associated with this matrix should be significantly positive.

## RESULTS

**Collections:** Our final dataset consisted of 400 plants from 40 populations spanning over 1800 kilometers (Fig. 3.1). It included 9 populations of *D. frenchii*, 8 populations of *D. amethystinum* and 23 populations of *D. meadia*. All populations of each rare taxon occurred in typical moist cliff habitats. Populations of *D. meadia* occurred in Open (eleven populations), Forested (ten populations) and Cliff (two populations) habitats (Table 3.1).

**Ecophysiology:** Range-wide ecophysiological analysis supports appropriate matching between traits and habitats in eastern North American *Dodecatheon* (Figure 3.3). SLA was higher among populations in more sheltered environments (Mixed Model ANOVA, numDF=2, denDF=37, F=19.38, $p<0.001$). Correspondingly both cliff endemic taxa had leaves with higher SLA than *D. meadia* (Mixed Model ANOVA, numDF=2, denDF=37, F=18.30, $p<0.001$). Based on one individual per population, plants with higher SLA had higher carbon isotope ratios. This is consistent with poor water use efficiency among plants with relatively thin leaves.

**Historical differentiation:** Although cpDNA polymorphism was limited, it was sufficient to test for hierarchical differentiation among taxa. Among all 400 plants we identified 10 haplotypes at the *trnH*$^{(GUG)}$*—psbA* locus (aligned length = 463 bp). Three *D. meadia* populations included two haplotypes, while every other sample was monomorphic (Table 3.1). After resolving ambiguity due to homoplasy (see materials and methods), we inferred the relationship among haplotypes depicted in Figure 3.2. Two common, highly connected haplotypes occurred in populations of all three species (haplotypes C and G). In contrast, 6 out of 7 tip haplotypes occurred exclusively in one

taxon or the other. When taking the hierarchical structure of the network into account with a nested design, only a single one-step clade showed evidence for differentiation among taxa. Haplotype frequencies differed among *D. meadia* and *D. amethystinum* in clade 1-1 ($\chi^2 = 0.67$, simulated $p<0.001$), which occurs only at the northeastern extreme of the range of *Dodecatheon*.

**Variation among taxa and populations:** We detected much more polymorphism with genome wide dominant markers than with non-coding cpDNA sequences. Our AFLP analysis included 383 plants (8-10 plants per population, mean = 9.525, Table 3.1). Each plant was scored at 1182 AFLP loci across all four primer combinations. Based on 5% of the dataset reanalyzed from independent DNA extractions, the error rate across all loci was 0.045. Given these parameters, 1110 of these loci were polymorphic. An Analysis of Molecular Variance detected significant variation among taxa (Table 3.2). However, differences among taxa accounted for only 2.49% of the variation in the dataset. Most of the variation occurred within populations (73.58%), with an intermediate amount occurring among populations (23.92%).

Principal coordinate analysis suggests some genetic structure among groups of populations (Figure 3.4). The first principal coordinate axis largely distinguishes four populations of *D. amethystinum* from all other individuals. Populations with low scores along this principal component axis tend to occur at the margins of the range of *Dodecatheon* in eastern North America (Fig 3.1). The third principal coordinate axis distinguishes among groups of *D. amethystinum* populations: two populations from the northeast and two from the upper Midwest. Nonparametric modal clustering supports these groupings. At a smoothing parameter value of 0.8, individuals are assigned to three

groups corresponding to Northeastern *D. amethystinum*, two upper Midwestern *D. amethystinum* and everything else. Because of the two distinct groups of *D. amethystinum* populations, taxonomic determination is associated with group membership (exact test, $p<0.001$). However, *D. frenchii* and *D. meadia* broadly overlap in genetic space.

**Spatial scale of trait-habitat matching:** In the context of a multiple matrix regression, pairs of populations which are geographically distant tend to be more genetically differentiated, but pairs of populations which are more ecologically different are not (Multiple matrix regression, $R^2=0.174$, mean pairwise jaccard distance = $4.16 \times 10^{-8}$ geographic distance ($p<0.001$) - $3.36 \times 10^{-3}$ mean pairwise difference in ln(SLA) ($p=0.35$)). This result is the same among *D. frenchii* and *D. meadia* excluding *D. amethystinum* (Multiple matrix regression, $R^2=0.074$, mean pairwise jaccard distance = $2.94 \times 10^{-8}$ geographic distance ($p<0.001$) – $2.19 \times 10^{-3}$ mean pairwise difference in ln(SLA) ($p=0.64$)).

**Ploidy level:** We obtained chromosome counts from two populations using standard cytological techniques (Figure 3.5). One mitotic count from a *D. frenchii* population in northwest Arkansas suggests 44 chromosomes. A meiotic count from a population of *D. frenchii* in southern Missouri clearly shows 22 chromosomes. Given a base chromosome number of 22, these counts are consistent with diploids (Thompson 1953). To infer ploidy from pollen diameter, we measured 6-21 pollen grains from a single individual in 31 populations, for a total dataset of 453 measured pollen grains. When clustered into three categories, mean pollen diameter of the smallest category, 10.4 microns, does not differ from the mean pollen diameter of pollen from an individual in

population 335 known to be diploid (two-sample t-test, p = 0.06). The mean difference between adjacent categories was 2.0 microns compared to 2.1 microns between known known diploid and tetraploid *Dodecatheon pulchellum* (Suttill and Allen 1992).

Interpreting these categories as ploidy levels, all taxa show variation among populations (Table 1). Band presence and absence does not vary among ploidy levels (AMOVA, source: among ploidy level, d.f = 2, s.s. =595.5, % variation = 0.44, p = 0.17). Furthermore, the regression coefficient for a matrix of differences in ploidy level on pairwise population genetic difference is not significant in the context of differences in SLA and geographic distance (Multiple matrix regression, regression coefficient p=0.66).

## DISCUSSION

The relationships between genetic variation, traits and geographic distributions in eastern North American *Dodecatheon* indicate that migration and adaptation played different roles in the post-glacial survival of each cliff endemic taxon. We predicted that if cliff endemic species were glacial relicts that responded to warming through range dynamics, then they would show evidence for historical isolation from *D. meadia. D. amethystinum* showed haplotype frequency differentiation from other eastern *Dodecatheon* at a chloroplast DNA locus, supporting an independent origin. *D. frenchii* did not. We also predicted that if cliff endemic species were glacial relicts, then considerable genetic variation would occur among taxa. Some geographically distant populations of *D. amethystinum* shared distinguishing variation at genome-wide dominant markers. This result suggests that *D. amethystinum* had a widespread distribution that has recently become fragmented. However, populations of *D. frenchii*

tended to share more variation with nearby populations of *D. meadia*, suggesting a close evolutionary relationship between these taxa.

These phylogeographic results suggest that range dynamics contributed more to the response of *D. amethystinum*, while adaptive differentiation dominated the relationship between *D. frenchii* and *D. meadia*. However, integrating these data with ecophysiological information in an explicit geographic framework suggested that similar processes contributed to the match between traits and habitats across all eastern North American *Dodecatheon.* Specifically, we predicted that if regional-scale ecological sorting among species matches traits to habitats, then pairwise population genetic distance should correlate strongly with ecophysiological difference, whereas if local natural selection among alternative genes predominated, then population genetic distance should correlate strongly with geographic distance. We found that *Dodecatheon* taxa endemic to cliffs, where glacial relict taxa often occur, had leaves with higher SLA that are appropriate for this moist but light-limited habitat. However, genetic distance between populations correlated strongly with geographic distance and not with differences in SLA. This result suggests that local processes contributed more to the match between traits and environments.

Overall, our results suggest a prominent role for gene flow during the response to warming since the last glacial maximum among eastern North American *Dodecatheon*. We will discuss the roles for gene flow during the response of each rare taxon in the context of other data. Then we will discuss whether considering gene flow can improve understanding of responses to climate change more generally. Finally we will discuss

how identifying a role for gene flow can improve conservation strategies for rare *Dodecatheon* taxa.

**Gene flow in responses to climate change:** Given the evidence that *D. amethystinum* has a glacial relict origin, what process fragmented its distribution following the last glacial maximum if not failure to adapt to warming conditions? The results we present, along with results from complimentary studies, strongly suggest that hybridization with *D. meadia* is responsible. Populations of *D. amethystinum* from cliffs in the Susquehanna River watershed in Pennsylvania are the only populations of either rare taxon that occur outside the range of *D. meadia*. A recent morphometric study of Pennsylvania populations showed no overlap between taxa in multivariate morphological space (Klotz and Loeffler 2006). We found significant haplotype differentiation between taxa in this region, although limited polymorphism makes this inference relatively weak. The signal for genetic distinction was much stronger in the more polymorphic AFLP dataset. Populations of *D. amethystinum* from the Susquehanna River watershed were the most genetically distinctive eastern North American *Dodecatheon* in a multivariate genetic ordination space. A complimentary molecular phylogenetic analysis of other species in *Dodecatheon* sect. *Dodecatheon* frames the distinctiveness of these populations in a larger geographic and historical context (Chapter 2). Haplotypes of a low-copy nuclear gene from an individual collected along the Susquehanna River were more closely related to haplotypes from a western species, *D. pulchellum,* than they were to sequences from *D. meadia*. This phylogenetic relationship is consistent with a hypothesis for the origin of *D. amethystinum*. Ugent *et al.* (1982) suggested that *D.*

*amethystinum* originated as a post-glacial migrant of *D. pulchellum* into eastern North America.

While Pennsylvania populations of *D. amethystinum* showed strong genetic evidence for a glacial relict origin, the genetic distinctiveness of other populations of *D. amethystinum* depended on how far inside the range of *D. meadia* they occurred. Populations of *D. amethystinum* in the upper Midwest, which occurred just within the northern range limit of *D. meadia* were also genetically distinct from other eastern *Dodecatheon* but less so than allopatric populations in Pennsylvania. In our molecular phylogenetic study, an accession from an upper Midwestern population of *D. amethystinum* retained two divergent nuclear haplotypes. One was more closely related to haplotypes from *D. pulchellum* and another that was more closely related to haplotypes from *D. meadia.* Populations of *D. amethystinum* that occured further south overlapped broadly with *D. meadia* in multivariate genetic space. Accessions from these populations had only *D. meadia*-related haplotypes at this nuclear locus.

The correspondence between geographic patterns of overlap in multivariate genetic space, and allele sharing at a low copy nuclear gene are consistent with spatially mediated hybridization (Schaal *et al.* 1998, Joly *et al.* 2009). As such, regional processes of migration and ecological sorting may have played somewhat different roles for these taxa than described for the glacial relict scenario above. Our results suggest that migration has been important because *D. amethystinum* may migrate northward more slowly than *D. meadia*. Where *D. meadia* has overtaken *D. amethystinum* these species hybridize. Because *D. meadia* appears to maintain higher local population abundance, introgression would have occurred disproportionately into *D. amethystinum* when these

species meet (Ellstrand and Elam 1992). As such hybridization may be gradually erasing the signature of climate-change forced migration from western into eastern North America.

Gene flow and natural selection may have played an even more prominent role in the relationship between *D. frenchii* and *D. meadia.* These taxa have been considered separate species on the basis of research from Southern Illinois (Reveal 2009). Reciprocal transplant experiments there demonstrated that leaf thickness differences between these taxa are genetically determined (Voigt and Swayne 1955). Cytological investigations showed different ploidy levels (Olah and DeFilipps 1968). Together these data would indicate that *D. meadia* and *D. frenchii* are ecologically and genetically distinct. Our range-wide results suggest a more complicated picture. Consistent with findings from Southern Illinois, *D. frenchii* had the most extreme ecophysiological traits across all eastern *Dodecatheon* taxa (Fig. 3.3b). Furthermore, *D. frenchii* populations tended to be diploid and *D. meadia* populations polyploid. Despite these ecological and cytological differences, we found no discernable range-wide genetic distinction between *D. frenchii* and *D. meadia*. Inferred ploidy level had no effect on genetic variation at AFLP loci across the dataset. This result differs from theoretical expectations and empirical analyses of other polyploid plants. Ploidy level differences should limit gene flow (Coyne and Orr 2003) and polyploids often form distinct genetic clusters (Guo *et al.* 2005). *Dodecatheon* may differ because of exceptionally dynamic ploidy evolution. We identified multiple ploidy levels within all eastern North American *Dodecatheon* taxa suggesting that ploidy changes may occur frequently in the group. Moreover, in a fine scale study of differentiation between *D. frenchii* and *D. meadia* in Southern Illinois we

found evidence for local intraspecific gene flow mediated by neo-autotetraploids (Chapter 4).

Evidence for dynamic ploidy and gene flow among ploidy levels, may explain why *D. meadia* and *D. frenchii* share so much genetic variation. It is also relevant to understanding the origins and maintenance of ecophysiological variation in the group. Several early workers suggested that plants with *D. frenchii* morphology evolved independently in multiple places as a consequence of polyploid evolution (Olah and DeFilipps 1968, Swayne 1973, Levin 2001). Alternatively *D. frenchii* may have had a single ancient origin that has been obscured by pervasive gene flow with *D. meadia.* Distinguishing between these alternatives would require a range-wide comparison of genes responsible for adaptation to *D. frenchii* habitat. Either way, contemporary patterns of natural selection appear to play the predominate role in maintaining adaptive variation among habitats.

Overall, our results suggest that genetic variation and gene flow may play central roles in mediating responses to climate change. This conclusion is consistent with other studies (Jump and Peñuelas 2005). In a recent review Davis *et al.* (2005) argue that migration, adaptation and extinction each reflect fundamental evolutionary processes. Their argument implies that studies on responses to climate change can benefit from focusing on the basis of evolutionary change: genetic variation for traits that confer adaptation to climate. From this perspective, the distinction between migration and adaptation becomes largely a question of linkage. During migration, genes that confer adaptation to climate increase in frequency or shift their geographic distribution in the context of a moving population. Adaptive and neutral variation may be linked by these

demographic events. During adaptation, genes that confer adaptation to climate may increase in frequency independent from unlinked neutral variation. When reproductive barriers are weak or taxonomic determinations poor, this genic distinction between migration and adaptation blurs. In *Dodecatheon,* adaptive genes appear to have moved with taxa as they migrated, and among taxa following hybridization. A complimentary situation has been reported among English Birches. Kelley *et al.* (2003) identified genetically distinct subpopulations within a single stand of birch that germinated during years with different temperatures. In their study, as in ours, taxonomic designations corresponded weakly to ecophysiologically distinct groups. Plants in general show weak correspondence between ecological and genetic distinction and abundant evidence for hybridization (Whittemore 1993, Whittemore and Schaal 1991). Our results suggest that weak reproductive barriers among these sedentary species may improve their abilities to respond to environmental change by allowing new genes to enter through hybridization with ecologically distinct groups.

Finally, our study of responses to historical climate change provides concrete recommendations to improve conservation strategies for this group. The two rare *Dodecatheon* taxa have conservation status in 12 States. However, neither of these taxa meets basic biological criteria for species recognition. Despite claims that rare taxa should be reproductively isolated from *D. meadia* (Olah and Defilips 1968, Iltis and Shaughnessy 1960), neither reproductive barrier is associated with range-wide genetic distinctions. There is no evidence that *D. frenchii* has an evolutionary history that is distinct from *D. meadia.* While some populations of *D. amethystinum* are distinct from *D. meadia,* these taxa appear to hybridize as *D. meadia* naturally

expands its range. Given that the subtle morphological differences that distinguish the rare taxa make taxonomic determinations difficult (Hill 2002, Klotz and Loeffler 2007), we would recommend that conservation agencies devote their limited resources to tracking more distinctive taxa. Moreover, the entire group shows considerable genetic variation within and among adaptively divergent populations. While global warming poses a serious threat to other species (Pounds *et al.* 2006), we would expect this group to have great potential to evolve in response to anthropogenic global warming especially at the northern extent of its range provided that landscape alteration does not dramatically reduce gene flow among populations.

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**TABLE 3.1:** Summary of material collected. Criteria for habitat designation and ploidy level inference are described in the materials and methods. cpDNA refers to haplotypes present in the populations as labeled in Figure 3.2.

| Collection | Taxon | Locality | Habitat | Latitude | Longitude | SLA | cpDNA | # AFLPs | Ploidy |
|---|---|---|---|---|---|---|---|---|---|
| 292 | *D. meadia* | Oconee N.F. Green Co., GA | Forest | 33.596 | -83.263 | 289.87 | G | 10 | 4x |
| 294 | *D. frenchii* | Cane Creek Canyon. Colbert Co., AL | Cliff | 34.640 | -87.808 | 657.07 | G | 8 | 2x |
| 295 | *D. meadia* | Kitsatchie N.F. Natchitoches Pa., LA | Forest | 31.419 | -93.053 | 447.23 | C | 9 | 6x |
| 297 | *D. meadia* | Pontotoc Ridge N.P. Pontotoc Co., OK. | Open | 34.522 | -96.609 | 198.10 | D | 10 | 4x |
| 298 | *D. meadia* | Beaver's Bend S.R.P. McCurtain Co. OK | Forest | 34.137 | -94.696 | 431.65 | F | 9 | . |
| 299 | *D. meadia* | Middle Fork Barrens N.P. Saline Co., AR. | Forest | 34.640 | -92.840 | 335.46 | C | 10 | . |
| 300 | *D. frenchii* | Ozark N.F. Newton Co., AR. | Cliff | 35.847 | -93.294 | 399.11 | C | 10 | 2x |
| 301 | *D. meadia* | Duck River Complex N.A. Maury Co., TN. | Open | 35.567 | -86.888 | 188.64 | G | 10 | . |
| 302 | *D. meadia* | Pisgah N.F. Buncombe Co., NC. | Forest | 35.661 | -82.432 | 242.14 | C | 9 | 2x |
| 304 | *D. meadia* | Standing Stone S.P.. Overton Co., TN. | Forest | 36.470 | -85.415 | 220.51 | C | 10 | 2x |
| 305 | *D. frenchii* | Stones Creek Hollow. Grayson Co., KY. | Cliff | 37.532 | -86.407 | 309.31 | G | 9 | 4x |
| 306 | *D. meadia* | Logan County Glades S.N.P. Logan Co., KY. | Open | 36.847 | -86.874 | 184.42 | I | 10 | . |
| 307 | *D. meadia* | Ferne Clyffe S.P. Johnson Co., IL. | Forest | 37.532 | -88.989 | 229.88 | C | 10 | 4x |
| 308 | *D. meadia* | Portland Arch N.P. Fountain Co., IN. | Forest | 40.211 | -87.332 | 228.27 | I | 8 | . |
| 310 | *D. frenchii* | Carter Caves S.R.P. Carter Co., KY. | Cliff | 38.368 | -83.121 | 489.30 | C | 8 | 4x |
| 311 | *D. meadia* | FloraCliff N.P. Fayette Co., KY. | Cliff | 37.905 | -84.364 | 287.06 | C | 10 | . |
| 312 | *D. frenchii* | Hoosier N.F. Perry Co., IN. | Cliff | 38.199 | -86.568 | 524.43 | H | 10 | 6x |
| 313 | *D. meadia* | Shawnee N.F. Gallatin Co., IL. | Open | 37.604 | -88.282 | 227.11 | I | 9 | 6x |
| 314 | *D. frenchii* | Shawnee N.F. Jackson Co. IL. | Cliff | 37.515 | -88.543 | 465.10 | C | 9 | 2x |
| 315 | *D. frenchii* | Shawnee N.F. Pope Co. IL. | Cliff | 37.668 | -89.363 | 531.70 | G | 9 | 2x |
| 316 | *D. meadia* | Perry County, MO. | Cliff | 37.708 | -89.583 | 319.02 | C | 10 | 6x |
| 317 | *D. frenchii* | Hickory Canyons N.A. Ste. Genevieve Co., MO. | Cliff | 37.870 | -90.307 | 394.37 | G | 10 | 2x |
| 318 | *D. meadia* | St. Louis Co., MO | Forest | 38.559 | -90.626 | 250.24 | C | 8 | . |
| 332 | *D. amethystinum* | Clark's Hill Norton S.H.S. Osage Co., MO | Cliff | 38.561 | -92.026 | 323.13 | G | 10 | 2x |
| 334 | *D. meadia* | Taberville Prairie S.N.A. St. Clair Co., MO. | Open | 38.050 | -93.993 | 185.53 | C, G | 10 | |
| 335 | *D. frenchii* | Mark Twain N.F. Douglas Co., MO. | Cliff | 36.992 | -92.094 | 397.67 | C | 9 | 2x |
| 336 | *D. meadia* | Mark Twain N.F. Taney Co., MO. | Open | 36.731 | -92.848 | 185.83 | G, J | 10 | 4x |
| 337 | *D. meadia* | Naked Mountain N.P. Nelson Co., VA. | Open | 37.749 | -78.833 | 235.68 | C, A | 10 | 4x |
| 338 | *D. meadia* | Franklin Co., PA. | Forest | 39.727 | -78.062 | 323.13 | B | 10 | 2x |
| 339 | *D. amethystinum* | Lancaster Central Park. Lancaster Co., PA | Cliff | 40.021 | -76.285 | 405.52 | B | 10 | 2x |
| 340 | *D. amethystinum* | Columbia Co., PA. | Cliff | 40.949 | -76.483 | 344.35 | B | 10 | 2x |
| 341 | *D. amethystinum* | Ray Norbut S.F.W.A. Pike Co., IL. | Cliff | 39.662 | -90.642 | 252.31 | G | 10 | 4x |
| 343 | *D. meadia* | Freeport Prairie N.P.. Stephenson Co., IL. | Open | 42.277 | -89.622 | 151.01 | C | 10 | 6x |
| 346 | *D. amethystinum* | Mississippi Palisades S.P. Carroll Co., IL. | Cliff | 42.129 | -90.158 | 275.49 | G | 10 | 6x |
| 348 | *D. amethystinum* | Grant Co., WI. | Cliff | 42.852 | -91.072 | 318.27 | D | 9 | 6x |
| 349 | *D. meadia* | Hogback Prairies S.N.A. Crawford, Co., WI | Open | 43.213 | -90.870 | 144.08 | D | 10 | 2x |
| 350 | *D. amethystinum* | North Bear W.M.A, Winneshiek Co., IA. | Cliff | 43.447 | -91.622 | 395.58 | C | 10 | 4x |
| 351 | *D. amethystinum* | Perrot S.P.. Trempleau Co., WI | Cliff | 44.016 | -91.480 | 256.90 | E | 10 | 4x |
| 352 | *D. meadia* | Hayden Prairie S.N.P. Howard Co., IA. | Open | 43.438 | -92.386 | 147.11 | I | 8 | 4x |
| 353 | *D. meadia* | Cedar Co., IA. | Open | 41.665 | -91.140 | 282.82 | I | 10 | 4x |

**TABLE 3.2:** Analysis of Molecular Variance among eastern *Dodecatheon* taxa. All sources of variation are significant at $p<0.0001$.

| Source | D.F. | S. S. | % of variation |
|---|---|---|---|
| Among Taxa | 2 | 947.2 | 2.64 |
| Among populations within taxa | 37 | 9000.6 | 23.56 |
| Within populations | 343 | 20575.8 | 73.81 |

**FIGURE 3.1:** Range map, collection localities and multilocus genetic differentiation for eastern North American *Dodecatheon.* The dashed line represents the approximate extent of the distribution of *D. meadia.* The ranges of the other taxa are represented by the sampling localities. AFLP ordination scores refers to the mean score for each population along the first principal coordinate axis of pairwise Jaccard distances as a proportion of the range between the highest and lowest scores (additional methods in text). Geographic coordinates projected.

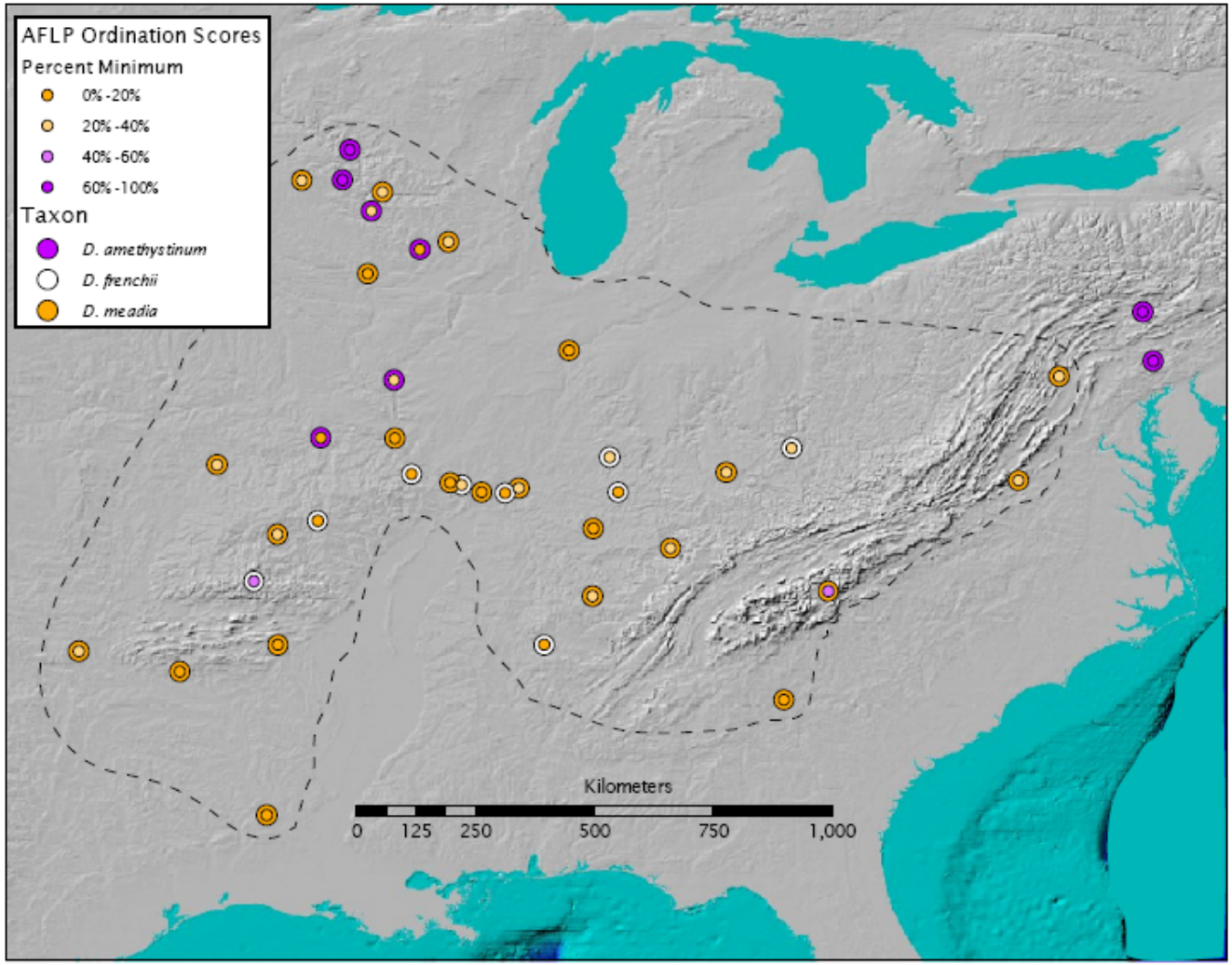

AFLP Ordination Scores
Percent Minimum
0% - 20%
20% - 40%
40% - 60%
60% - 100%
Taxon
D. amethystinum
D. frenchii
D. meadia
Kilometers
0
125
250
500
750
1,000

**FIGURE 3.2:** Nested haplotype network representing inferred relationships among *trnH*$^{GUG}$*-psbA* sequences from all samples. Circle size is proportional to abundance of each haplotype. Lines connecting haplotypes represent inferred mutational differences. Hatches across connecting lines represent unobserved haplotypes. P-values refer to the significance of the association between haplotype (or haplotype clade) and taxonomic determination (additional methods in text).

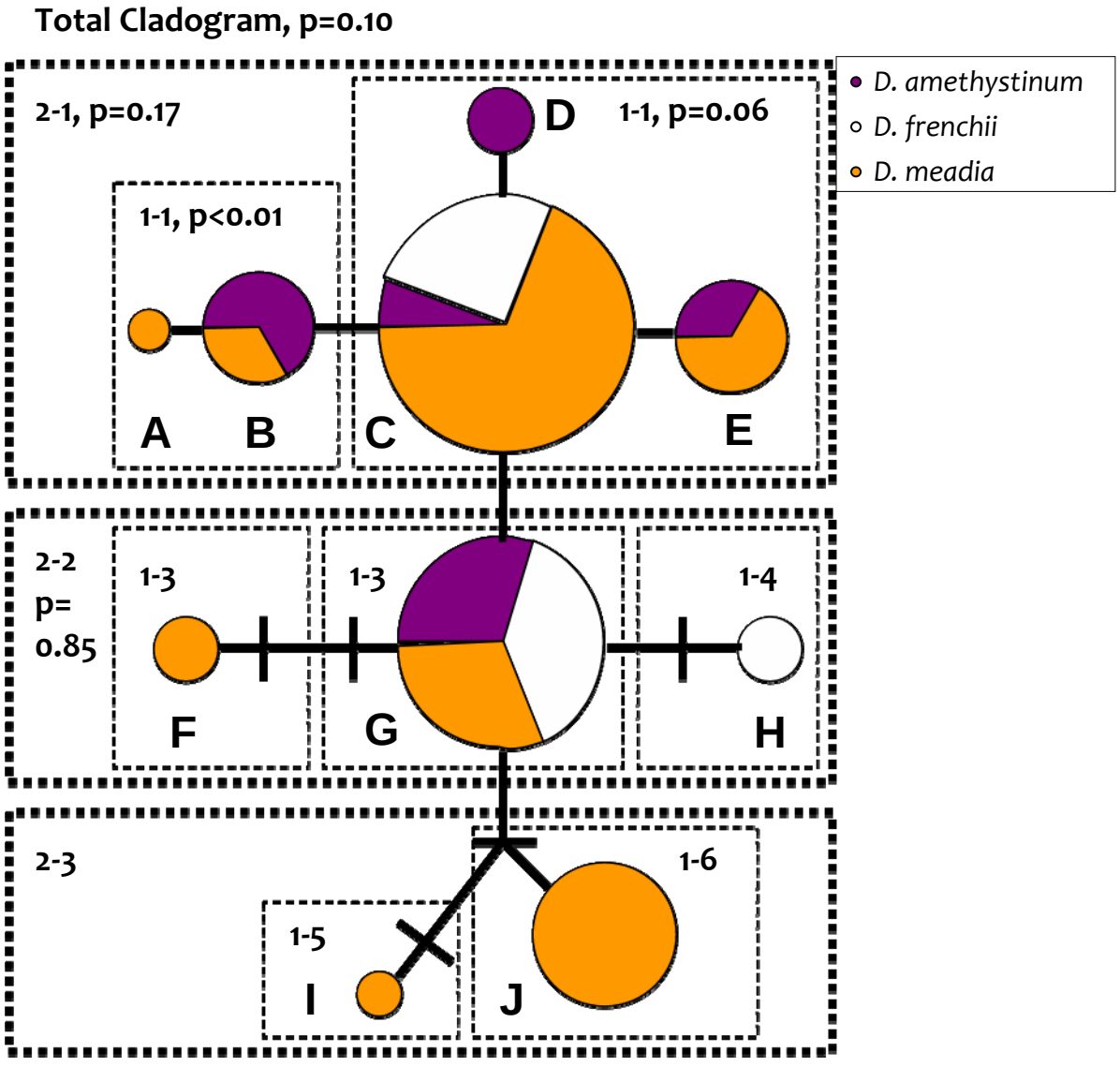


**FIGURE 3.3:** Relationships between Specific Leaf Area (natural log transformed) among Eastern North American *Dodecatheon* by habitat type (a) taxon (b) and carbon isotope ratios (c). Error bars in panels a and b represent standard errors for all samples. Line in panel c represents a least-squares linear regression between ln(SLA) and carbon isotope ratio for one sample per population used to illustrate that the correlation between these variables is consistent with lower water use efficiency among thinner leaves.

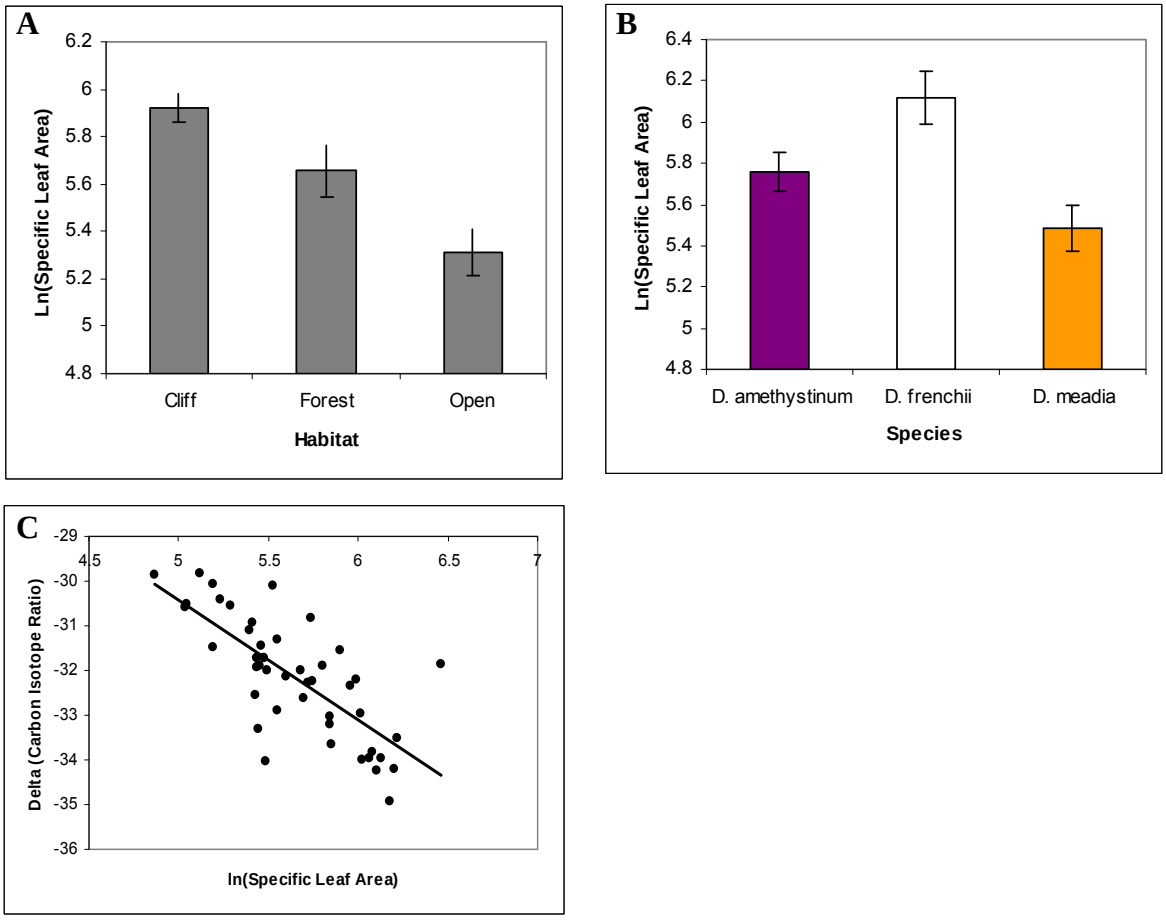


**FIGURE 3.4:** Principal Coordinate analysis of pairwise Jaccard distances between AFLP profiles for 383 plants. Colors correspond to taxa. The individuals in the cluster in the upper left corner of the ordination space are from populations at the northeastern limit of the range (Oberle 339, and Oberle 340). Other individuals with lower scores along the first principal coordinate axis are from the northwestern limit of the range (Oberle 350, Oberle 351).

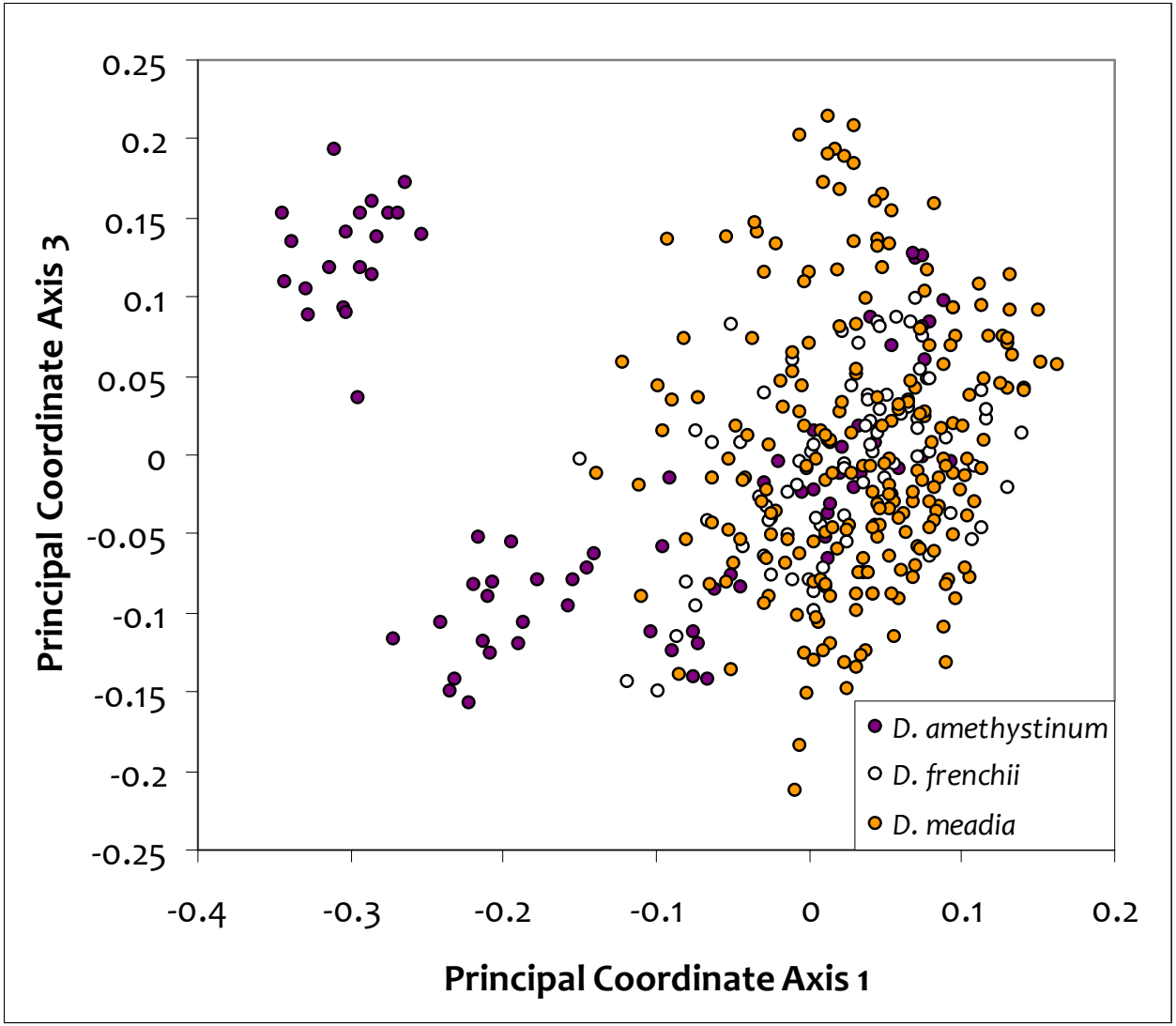
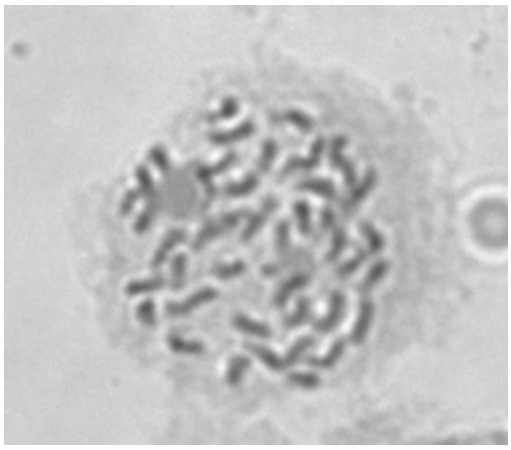
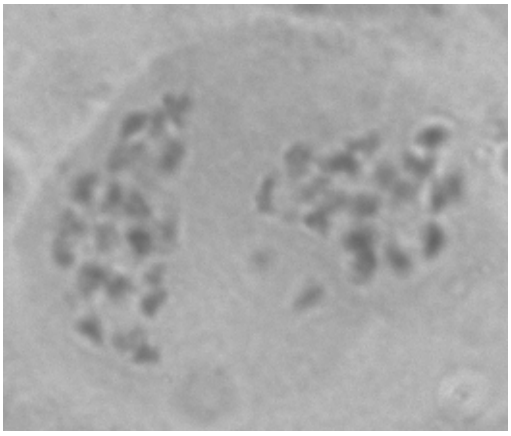

0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
Principal Coordinate Axis 3
Principal Coordinate Axis 1
D. amethystinum
D. frenchii
D. meadia

**FIGURE 3.5:** Ploidy levels in Eastern North American *Dodecatheon*. Panel A shows a mitotic cell in late prophase from with 44 chromosomes *D. frenchii* in Arkansas (Oberle 300). Panel B shows a meiotic cell in late anaphase I with 22 pairs of chromosomes in the left daughter cell from *D. frenchii* in south-central Missouri (Oberle 335). Panel C shows a scanning electron micrograph of pollen from the same individual counted in Panel B. Panel D shows a scanning electron micrograph of pollen from from *D. amethystinum* in Northern Illinois inferred to be a hexaploid (6x=132) for comparison.

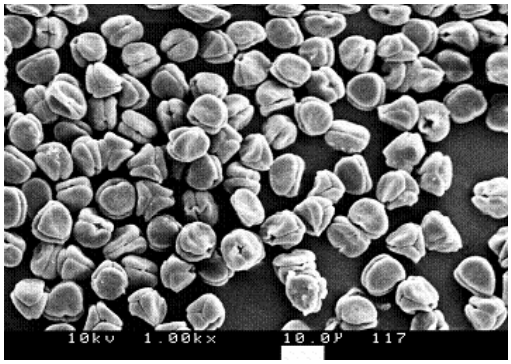
**A.**



**C.**



**C.**




**D.**

# CHAPTER 4

## Fertile neoautotetraploids in a morphologically intergrading population facilitated local gene flow between ecologically and cytologically distinct *Dodecatheon* taxa in Southern Illinois.

## INTRODUCTION

Populations with highly variable morphology pose problems for taxonomists and raise questions about the integrity of species as lineages. This is especially true for groups characterized by complex evolutionary processes such as phenotypic plasticity, convergent local adaptation, polyploidy and hybridization. An excellent example of such a group is *Dodecatheon*, a clade of buzz-pollinated plants nested in the large genus *Primula* (Mast et. al 2004). For consistency with the historical literature, we will use the traditional taxonomy here, despite the fact that recognizing this rank renders *Primula* paraphyletic (Mast and Reveal 2007). *Dodecatheon* has challenged botanists since the first plants were imported to Europe in the 18$^{th}$ century. After Linnaeus (1751) typified the genus, the first American botanist to address its diversity, Raffinesque (1833), described more than a dozen taxa in eastern North America based on relatively fickle characters such as leaf shape and margin form. Later, a more sober Asa Grey (1866) declared the group "baffling" for a lack of reliable characters and identified only a single species, albeit with several infraspecific taxa.

The tension between taxonomic lumpers and splitters produced hundreds of names during nearly a century before the first attempt to experimentally demonstrate a genetic basis for morphological differences among taxa. In 1944, Norman Fassett conducted a series of common garden experiments on material collected from two taxa in Southern Illinois. *D. meadia*, the type for the genus, is a widespread plant that tends to inhabit relatively exposed sites such as dry forests and rocky glades. *D. frenchii*, which was first collected in 1870 but only elevated to species rank in 1932, is endemic to moist sandstone cliffs. These two taxa differ in leaf shape and thickness, with *D. meadia*

having relatively thick, oblanceolate leaves and *D. frenchii* having relatively thin, cordate leaves. They also occur parapatrically across local microclimate gradients, and appear to intergrade in several localities. Suspecting that leaf shape differences were environmentally induced, Fassett collected resting plants consisting of roots with dormant buds from both typical taxa near an intergrading population and exposed them to varying light intensities in the greenhouse. While *D. meadia* plants maintained their typical leaf shape over the range of experimental conditions, leaves from *D. frenchii* became more oblanceolate under the high light conditions that characterize the habitat of *D. meadia*. Fassett (1944) inferred that naturally occurring intergrading populations reflected phenotypic plasticity in leaf shape in *D. frenchii*. For this reason, he concluded that *D. frenchii* should be considered a variety of *D. meadia*.

The conclusion that *D. frenchii* did not merit species recognition was strongly refuted by a series of observations and experiments published during the following 35 years. The first of these (Voigt and Swayne 1955), argued that the apparent intergradation between these taxa reflected genetic variation within and among colonies of *D. frenchii*, rather than environmental effects. They reported several localities in southern Illinois where plants with cordate leaves typical of *D. frenchii* naturally occur in more exposed sites. Further common garden experiments and a reciprocal transplant experiment showed little plasticity in leaf shape. Moreover, after thirty years the colony of *D. frenchii* that had been transplanted into *D. meadia* habitat still retained their characteristic cordate leaves, while the transplanted *D. meadia* colony had gone extinct (Mohlenbrock 1987). These results indicate not only that leaf shape differences are

genetically fixed among taxa, but also that these populations are locally adapted to their respective habitats.

In addition to morphological and ecological differences, cytological data indicated genetic differentiation between these taxa (Olah and DeFilipps 1968). Meiotic chromosome counts from across southern Illinois demonstrated that *D. frenchii* is diploid (n=44) relative to tetraploid *D. meadia* (n=88). Ploidy differences reproductively isolate populations (Stebbins 1950, Coyne and Orr 2004), reinforcing the conclusion that these taxa are evolutionarily distinct. However, these authors proposed an unconventional hypothesis for the evolutionary relationship among them: that *D. frenchii* is a polyhaploid derivative of an autotetraploid *D. meadia*. Polyhaploids are diploids secondarily derived from tetraploid parents. Both spontaneous and experimentally induced polyhaploids have low fitness, disrupted meiosis, unusual leaf shapes and poor competitive ability compared to their parents (Magoon and Khanna 1963). All of these characteristics distinguish *D. frenchii* from *D. meadia*. Despite the fact that no naturally occurring polyhaploid taxon has been conclusively demonstrated (Ramsey and Schemske 2002), Olah and Defillips (1968) suggested this evolutionary origin for *D. frenchii*. This form of catastrophic speciation could explain why *D. frenchii* is rare and why it is restricted to marginal habitats (Raven and Thompson 1964). After additional populations of *D. frenchii* were discovered far from southern Illinois, later authors claimed that independent polyhaploid events in different regions could explain its disjunct distribution (Swayne 1973).

Consistent with the conclusions of Voigt and Swayne (1955) and Olah and Defillips (1968) current taxonomy recognizes both *D. frenchii* and *D. meadia* as distinct species (Reveal 2009). However, discrepancies among the results of reciprocal transplant

experiments and differences among authors in the interpretation of observed patterns of morphological variation raise two outstanding questions: (1) **are these taxa morphologically distinguishable relative to characters besides leaf shape**? and (2) **what role do morphologically intergrading populations play in the evolutionary relationship between taxa**? To address the first question, we identified new characters that are taxonomically useful elsewhere in the genus (Klotz and Loeffler 2007, Chambers 2006) and quantified patterns of variation for these characters across populations of both taxa across southern Illinois. To address the second question, we identified a population of plants that span the morphological differences between these taxa. We compared the mean character values and the variation in character values in this population to typical populations of each taxon. After identifying how this population differs morphologically from typical populations, we began to address its role in the evolution of the group by comparing fitness of plants in this population to plants in nearby typical populations of each taxon. Then, to identify whether plants in the morphologically intergrading population might facilitate local gene flow among taxa, we conducted a population genetic survey. We compared levels of genetic differentiation between allopatric populations of each taxon, parapatric populations where no populations of intergrading morphology occur and parapatric populations near the intergrading population. Finally, to relate morphological, fitness and allele frequency differences among populations to polyploid dynamics, and conducted a limited cytological survey of plants in the intergrading population.

## MATERIALS AND METHODS

**Morphometrics**: To assess whether typical populations of these taxa are morphologically distinguishable we collected two morphometric datasets. For the first dataset we measured mature infructescences of 38-48 randomly selected plants from each of six populations across southern Illinois in July, 2005 (four typical *D. frenchii*, two typical *D. meadia*). For each plant, we measured five characters that have proven useful for distinguishing *D. meadia* from another closely related rare taxon in Pennsylvania (Klotz and Loeffler 2006). We measured scape length (1) from the ground level to the base of the involucral bracts using a measuring tape. We measured scape width (2) at the mid-point of scape length using calipers. We also counted the number of developed capsules (3). We square root transformed capsule number prior to all analyses to meet the assumptions of the statistical methods. We then collected one fully developed capsule from each plant. We used ImageJ 1.37 (National Institutes of Health) to measure capsule length (4) and capsule width (5) based on a digital photographs taken in the lab. We tested for differences among taxa with respect to each character, treating populations as a nested random effect.

Our second morphometric dataset focused on microscopic seed characters. Seeds of another *Dodecatheon* species are small (< 1 mm), irregularly shaped and have a highly textured seed coat (Chambers 2006). We collected seeds from a relatively large and small plant of each taxon. We coated eight to ten seeds from each individual with 200 A° of gold using a SPI gold sputter coater and viewed them with an ISI-SX40 SEM run at an emission of 10KV. We photographed five seeds at three different magnifications 50, 100 and 1000X. Using ImageJ, we measured aspects of seed size and shape as well as the

size and shape of features on the seed coat. For seed size, we measured the longest diameter of each seed using the Feret's Diameter tool. We also measured the total area of the two dimensional image of the seed. For seed shape we measured each seed's circularity defined by $4\pi$ (area / perimeter$^2$). This measurement equals one for a perfect circle and approaches zero for increasingly elongated shapes. We also counted the number of visible faces for each seed at 50X. A face was defined as a flat plane separated from another face by an edge where the two meet. A spherical shape would have one face, while a polyhedral shape could have more than one face. We measured the size and shape of the characteristic scales that compose the testa in the same way that we measured those aspects of entire seeds based on pictures taken at 1000X. To standardize for the orientation of the seed surface, we measured three scales from each seed whose orientations were parallel to the viewing plane. We tested for differences among seeds from individuals in typical populations with mixed-model ANOVAs with individuals treated as random effects nested within taxon (fixed effect). Values for circularity and number of faces per seed image were transformed to normalize the data.

We then identified a population with characters that varied continuously among individuals from a morphology typical of one taxon to a morphology typical of the other. This intergrading population occurred in a small sandstone rockhouse near the top of the south facing bluff line in Happy Hollow at Ferne Clyffe State Park in Johnson Co., IL. We measured the same five infructescence characters on all 35 fruiting plants from this population in July, 2005. For each character, we compared the means and interquartile ranges of plants in the intergrading population to the means and interquartile ranges of the typical taxa. We also compared plants from the intergrading population to plants

from typical populations in multivariate space based on a principal components analysis of all characters and all plants. Finally, we collected seeds from a large, small and intermediate sized plant from the intergrading population, measured them in the same way as we measured seeds from typical populations and compared them to typical taxa.

**Relative fitness:** To begin to address the evolutionary role of the intergrading population, we compared female fitness of plants in this population to female fitness of plants in nearby populations with typical morphologies. We selected the nearest large colony of each species growing at least 500 meters away for comparison. The *D. meadia* population was growing in its typical dry cliff-top habit on the south-east facing rim of the valley, and the *D. frenchii* population was growing in a moist sandstone rockhouse along the west-facing side of the valley. Previous cytological work reported diploid chromosome counts for typical *D. frenchii* and tetraploid counts for typical *D. meadia* at this locality (Olah and Defilips 1968).

To estimate female fitness we collected every mature fruit from up to 21 randomly selected individuals per population in June, 2007. We estimated three components of fitness: fertility (ability to produce a seed), fecundity (number of seeds produced) and viability (germination rate). We determined the first two components from simple seed counts performed under a dissecting microscope. We determined viability of all seeds from a randomly selected subset of fertile capsules in a green house germination trial. Preliminary experiments indicated that *Dodecatheon* seeds have an after-ripening effect that requires time and stratification to break dormancy. Accordingly, we maintained the seeds at room temperature in sterile eppendorf tubes until March, 2008. We then stratified all seeds on moist filter paper in sterile Petri

dishes for three weeks at 4°C. Following stratification, we planted seeds into flats containing REDI-EARTH Plug and Seedling mix. We placed the flats on a mist bench until net germination rates slowed to less than 5%. We randomized the location of flats every two days during the course of the experiment.

To test for fitness differences among these populations, we conducted two different analyses. We first tested for differences in fertility and fecundity using Zero-Inflated Negative Binomial (ZINB) regression models. This approach assumes two data generation processes: one producing zeros (i.e. infertility) and another producing over-dispersed counts (i.e. fecundity). We fit models to counts for both capsules and plants. We also tested whether capsule level fecundity followed a normal distribution in each population using a Shapiro-Wilk W test. We then tested for differences in viability using a mixed-model ANOVA on arcsine square-root transformed germination proportions with capsule treated as a random effect.

**Population genetics:** To see whether intergrading plants might facilitate gene flow between taxa we conducted a population genetic survey across southern Illinois. We had three goals: (1) to identify whether these taxa are genetically differentiated in the region (2) to assess whether the geographic configuration of taxa influences genetic differentiation and (3) to test whether allele frequencies are more similar among typical populations of each taxon near the intergrading population than they are among typical populations separated by similar geographic and environmental distances in an area with no reported intergrading populations. In order to accomplish these goals, in Spring 2008 we randomly sampled 20 individuals from six different populations at four different localities:

1. Parapatric populations of *D. frenchii* and *D. meadia* where intergrading plants are present. (Ferne Clyffe State Park, IL)

2. Parapatric populations of *D. frenchii* and *D. meadia* where intergrading plants are NOT present. (Jackson Hollow, IL)

3. A population of *D. frenchii* that grows in isolation. (Bear Creek, IL)

4. A population of *D. meadia* that grows in isolation. (Pounds Escarpement, IL)

Our total sample consisted of 120 individuals: 60 *D. frenchii* and 60 *D. meadia*. Each of these localities was no closer than 30 kilometers and no further than 40 kilometers away from the neighboring localities. At Ferne Clyffe, we sampled from the same populations of the morphologically typical taxa used in the relative fitness analysis. At Jackson Hollow, we sampled from similarly sized populations that were also approximately 1 km apart. Our determination that intermediates do not occur at Jackson Hollow was based on two results of historical searches (Voigt and Swayne 1955). First they reported no intergrading populations at this locality. They also reported morphologically typical *D. frenchii* growing in habitat typical *D. meadia*. During four consecutive seasons of resurveys (2005-2008) we also failed to find intergrading populations. However, we did relocate several individuals with typical *D. frenchii* morphology growing sympatrically with typical *D. meadia* without any plants of intermediate morphology. Our determination that each taxon grows in isolation at Bear Creek and at the Pounds Escarpment was based on results from earlier surveys (Swayne 1973) and our own resurveys.

We preserved leaf tissue in silica gel and extracted DNA using a modified Viogene DNA extraction protocol. We then PCR amplified a region of the chloroplast

genome between *trnH*$^{GUG}$ and *psbA* using the protocol described by Shaw *et al*. (2005). Preliminary results indicated that this region was polymorphic, yet short enough (459 aligned base pairs) to fully sequence with a single primer. We quantified PCR products via agarose gel electrophoresis and purified them purified the remaining PCR product by adding 3 U Exonuclease I (New England Biolabs) and 0.015 U Shrimp Alkaline Phosphatase (Promega), and then incubating samples at 37° for 30m followed by 80° for 20m. We cycle sequenced each sample using the manufacturer's protocol modified to use less BigDye and reconstructed the sequences using an ABI 3130xl genetic analyzer.

A range-wide population genetic analysis demonstrated no hierarchical structure between these taxa at this locus (Chapter 3). For this reason, we analyzed each haplotype as an independent allele. We tested for allele frequency differentiation in southern Illinois using a series of contingency tests. We tested the null hypothesis of no allele frequency differentiation among taxa across the entire dataset using a log-liklihood G-test with William's correction (Sokal and Rohlf 1995). We then tested the null hypothesis of no difference in allele sharing among both sets of parapatric populations using a three-way log-likelihood G-test. The three-way test evaluates whether two contingency tables, in this case taxa versus alleles in Ferne Clyffe versus Jackson Hollow, differ in their degrees of association. The results of tests for population genetic differentiation based on adjusted $G^2$ values are comparable to results of more commonly used tests (Ryman et al. 2006). We implemented the two-way G-test in R 9.0 using a script written by Peter Hurd. We implemented the three-way G-test using the VassarStats program available from Richard Lowry.

**Cytology:** To relate morphological, fitness and allele frequency differences among populations to polyploid dynamics we conducted a limited cytological analysis. In April 2008, we collected immature flower buds from individuals in the intergrading population that most closely resembled the morphologies of the typical taxa. We fixed those buds in Carnoy's Solution for 24 hours and then stored them at 4° C in 70% ethanol. We removed developing anthers from buds under a dissecting microscope and stained them with acetocarmine. We then squashed the stained anthers and searched for cells with clearly visible chromosomes at 100x under a phase contrast microscope.

## RESULTS

**Morphometrics**: We measured a total of 243 plants across 6 typical populations to identify morphological differences between taxa in Southern Illinois. The two taxa were morphologically distinguishable based on all infructescence characters (Figure 4.1). In each case, *D. meadia* was significantly larger than *D. frenchii* (Mixed-model ANOVAs numDF=1, denDF=4, scape length, $f=138.01$, $p<0.001$; scape width $f=153.00$, $p<0.001$; $\sqrt{}$capsule number, $f=52.19$, $p=0.002$; capsule length $f=24.60$, $p=0.008$; capsule width $f= 25.39$, $p=0.007$). Also, the interquartile ranges did not overlap among these taxa for any of these characters. By comparison, the mean character value of the intergrading population was usually between the mean character values for the two taxa, the interquartile range was usually larger than the interquartile range of typical populations of either taxon and it overlapped the mean of one taxon or the other (Fig. 4.1). The interquartile range in the intergrading population overlapped the mean of *D. frenchii*

populations for all characters except capsule width. Mean capsule width was slightly larger in the intergrading population than in *D. meadia*.

A multivariate ordination of all infructescence characters in all 278 plants reinforced the morphological distinction between the two taxa and the morphological intermediacy of the intergrading population (Figure 4.2). Individuals from typical populations showed little overlap with respect to the first principal component axis. In comparison, individuals from the intergrading population occurred in regions of the ordination space occupied only by typical *D. frenchii*, only by typical *D. meadia*, the narrow region occupied by both taxa and a region occupied by neither taxon.

The two taxa were also morphologically distinguishable based on microscopic seed characters. In general, the seeds from large and small *D. frenchii* plants were more spherical (Figure 4.3), while seeds from large and small *D. meadia* plants were more polyhedral (Figure 4.4). All 11 *D. frenchii* seeds had only a single visible face, while all 11 *D. meadia* seeds had more than one visible face (Mixed-Model ANOVA, √face number, numDF=1, denDF=2, f=109.05, p=0.009). The perimeters of *D. frenchii* seeds tended to be more rounded than the perimeters of *D. meadia* seeds, although the difference was only marginally significant (Mixed-Model ANOVA, arcsin-square root transformed circularity, numDF=1, denDF=2, f=14.37, p=0.063). No other measurements, including seed size, scale size or shape differed significantly between species. In contrast to individuals from typical populations of either species, individuals in the intergrading population produce seeds with both one and more than one face.

**Relative Fitness:** We counted over 4000 seeds in 167 capsules from 59 plants to compare fitness across populations of *D. frenchii, D. meadia* and an intergrading

population that co-occur in Ferne Clyffe State Park. Populations differed in capsule fertility (ZINB, zero inflation model, $p<0.001$, Table 4.1). Capsules from the intergrading population showed the highest fertility rates with three out of four capsules producing at least one seed. Fertility rates among capsules from the *D. frenchii* population were significantly lower than among capsules from either the *D. meadia* population or the intergrading population (ZINB, zero inflation model coefficients, *D. frenchii* versus intergrading, $z=2.93$, $p=0.003$, *D. frenchii* versus *D. meadia* $z=1.97$ $p=0.049$). Plant-level fertility showed a similar pattern, but the differences among populations were only marginally significant (ZINB zero inflation model, $p=0.064$).

Fecundity also differed among the three populations (ZINB count model, $p<0.001$). The number of seeds per fertile capsule did not differ between the *D. frenchii* population and the intergrading population (ZINB count model coefficients, *D. frenchii* versus intergrading, $z= 0.79$, $p=0.49$). However, fecundity in these populations was significantly lower than was fecundity in the *D. meadia* population (ZINB count model coefficients, *D. frenchii* versus *D. meadia*, $z=4.08$, $p<0.001$, intergrading versus *D. meadia* $z=5.19$, $p<0.001$). Fertile capsules from *D. meadia* plants produced over twice as many seeds as did fertile capsules from the other two populations. The shape of the distribution of capsule fecundity also differed among populations (Figure 4.5). The number of seeds per fertile fruit did not differ from a normal distribution in either the *D. meadia* population (Shapiro-Wilk W test, $p=0.49$) or in the *D. frenchii* population (Shapiro-Wilk W test, $p=0.09$). However, the distribution of capsule fecundity in the intergrading population was positively skewed and strongly non-normal (Shapiro-Wilk W test, $p<0.001$). Plant level fecundity differed among populations in a similar way

(ZINB count model, $p<0.001$), with the greater number of capsules per plant exaggerating the overall difference in fecundity between plants in the *D. meadia* population and plants in the other two populations.

Seed viability, as measured in a common garden germination trial, also differed among populations (Mixed Model ANOVA, arcsine square root transformed germination proportion, numDF=2, denDF=80, f=5.01, p=0.009). As with differences among populations in fecundity, the per-capsule germination rate of seeds from the intergrading population did not differ from the per-capsule germination rate of seeds from the *D. frenchii* population (p=0.13). Less than 1/3 of the seeds from each capsule in these populations had germinated by the end of the trial. By comparison, the germination rate of seeds from capsules collected in the *D. meadia* population was over 50%. Viability from capsules collected in the *D. meadia* was significantly higher than in the *D. frenchii* population (Mixed Model ANOVA coefficients test, t=5.01, p=0.009).

**Population genetics:** Among all 120 samples from six populations at four locations across southern Illinois, we identified six haplotypes at trnH-psbA (Table 4.2). The number of haplotypes varied among populations, from one in the *D. meadia* population at Pounds Escarpment to five in the *D. frenchii* population at Jackson Hollow. Haplotype frequencies differed among taxa across Southern Illinois (Adjusted Log-likelihood ratio statistic (G) = 58.48, $\chi^2$ df = 5, $p<0.001$). Comparing differentiation among populations of different geographic configuration, the isolated populations of each taxon shared no haplotypes, while both pairs of parapatric populations shared more than one haplotype. Excluding the allopatric populations, the amount of haplotype sharing differed among parapatric pairs of populations (Adjusted Log-likelihood ratio statistic

(G) for three way interaction = 80.44, $\chi^2$ df = 13, $p<0.001$). The parapatric populations at Ferne Clyffe shared more haplotypes because that population of *D. meadia* contained haplotypes that only occur in *D. frenchii* elsewhere in southern Illinois.

**Cytology:** We obtained one unambiguous meiotic chromosome count at late prophase from an individual with a *D. frenchii* morphology growing in the population of intergrading plants at Ferne Clyffe. In contrast to every other *D. frenchii* counted in southern Illinois, including individuals from typical colonies at Ferne Clyffe (Olah and Defilips 1968), this individual was tetraploid with one additional unpaired chromosome (2x = 4n = 88 + 1b) (Figure 4.6). In addition to the unpaired chromosome in this cell, two pairs of chromosomes occur as tetrads indicating some degree of quadrivalent formation.

## DISCUSSION

We set out to answer two outstanding questions about *Dodecatheon* in southern Illinois: are these taxa morphologically distinguishable and what role do highly intergrading populations play in the evolutionary relationship among them? Our morphometric dataset demonstrates that typical populations *D. frenchii* and *D. meadia* are distinguishable based on infructescence and seed characters. All infructescence characters are significantly smaller for *D. frenchii,* although the ranges for each character overlap (Fig. 4.1). Our multivariate ordination summarizes this pattern. The first principal component separates these taxa, although they overlap at their extremes (Fig. 4.2). While the infructescences of these taxa tend to differ in size, their seeds are discretely different in shape (Figs. 4.3, 4.4). The angular seeds of *D. meadia* are similar

in shape to seeds from another species in *Dodecatheon* sect. *Dodecatheon* (Chambers, 2006), and both differ markedly from the rounded seeds of *D. frenchii.* While our sample size for this comparison was relatively small, observations from other regions and from herbarium collections suggest that rounded seeds, which are visible with a hand lens, could be a useful character for determining *D. frenchii* across its range (data not shown).

These morphological differences apply to typical populations. However, highly variable populations with plants that span the morphological differences among taxa do occur (Fassett 1933). The intergrading population that we analyzed was in fact more variable than and morphologically intermediate to typical populations of either taxon with respect to both infructescence and seed characters. This simple result pertains to the conflicting hypotheses proposed by the taxonomists who worked on the group in the mid 20$^{th}$ century. Fasset (1933) concluded that morphological variation in *D. frenchii* reflects phenotypic plasticity. However, the habitat where this population occurs is not especially heterogeneous. We suspect that some of the morphological variation in this population has a genetic basis. Voigt and Swayne (1955) stated that highly variable populations are simply extremes in the variation of *D. frenchii.* We found similar variation in characters for populations of both *D. frenchii* and *D. meadia*, while the intergrading population was more variable. It also included plants with morphologies that do not occur in typical populations. This population appears to be qualitatively different from *D. frenchii*.

If the atypical population at Ferne Clyffe is neither *D. frenchii* nor *D. meadia,* what is it? It could be both, in the sense that it could represent a sympatric population of typical plants of both species. If so, why do some plants from this population have morphologies that do not occur in typical populations? Given that typical populations at

this locality differ in ploidy level, we will discuss our remaining results with reference to three possible karyotypic origins for unusual plants in this population: triploid hybrids, autotetraploid *D. frenchii* and polyhaploid *D. meadia*.

At Ferne Clyffe, fitness varies among populations based on their taxonomic identity. The relative rank of each population depends on the fitness component compared. The intergrading population shows the highest fertility rates, while the *D. meadia* populations shows the highest rates of fecundity and viability. The population of *D. frenchii* ranks lowest for all fitness components, either by itself (fertility) or tied with the intergrading population (fecundity, viability). These results suggest that the intergrading population may not consist solely of low-fitness triploid hybrids between relatively fit euploid parents (Burton and Husband 2000). Capsules in this population are often fertile, and while *D. meadia* is relatively fit, *D. frenchii* is not.

In addition to the relative ranks of fitness components, the shapes of the distributions for one fitness component differ among taxa in an interesting way. The number of seeds per capsule is normally distributed in both typical populations. Although the means differ, differences among capsules in each typical population could be attributable to random error. However, the number of seeds per capsule is highly skewed in the atypical population. Most capsule produce fewer seeds than the average capsule from the *D. frenchii* population while a few produce as many seeds as the average capsule from the *D. meadia* population. This suggests that more complex processes could contribute to variation in fecundity among capsules in the atypical population. The skewed distribution could result from random pollinator movements and stigmatic occlusion in an admixed population of plants from different ploidy levels

(Husband and Schemske 2000). Specifically, the observed distribution of fecundities could be a composite of three distributions: high fecundity capsules resulting from *D. meadia—D. meadia* movements, lower fecundity capsules resulting from *D. frenchii—D. frenchii* movements and very low fecundity capsules resulting from movements among species. Pollinator observations and experimental pollinations at this population could address this possibility. The shape of the distribution in this population could also reflect the presence of plants with aberrant karyotypes. Triploids, neoautotetraploids and polyhaploids all have lower fitness than their parents (Ramsey and Schemske 2002).

Our population genetic results confirm that cpDNA haplotype frequencies differ among taxa in southern Illinois. This is consistent with the barrier to gene flow among them. Also, the geographic configuration of populations influenced the amount of differentiation. Allopatric populations shared no haplotypes. Both parapatric sets of populations did. Also, the parapatric populations near the intergrading population at Ferne Clyffe shared more variation than the parapatric population at Jackson Hollow, where no intergrading populations have been found. This pattern suggests that the intergrading population may not only consist of an admixture of reproductively isolated individuals of either species and sterile triploid hybrids. Reduced population genetic differentiation at Ferne Clyffe is consistent with local intraspecific gene flow facilitated by plants in the intergrading population population. Not only does this pattern indicate gene flow, but it also suggests the direction and mechanism. We found that the parapatric populations at Ferne Clyffe are similar because *D. meadia* there have haplotypes that only occur in *D. frenchii* elsewhere in southern Illinois. This pattern suggests local gene flow from *D. frenchii* to *D. meadia*. Given that cpDNA is maternally

inherited, the pattern of haplotype sharing suggests that autotetraploid *D. frenchii* in the intergrading population have transferred genes to local populations of tetraploid *D. meadia*.

Finally, our limited cytological analysis identified a tetraploid plant with *D. frenchii* morphology growing in the intergrading population. The tetrads that we observed are consistent with autotetraploid formation. This result demonstrates that morphological differences among species are not simply due to a 'gigas' effect of genome duplication (Levin 1983). In this case, we observed an outwardly typical *D. frenchii* with more than twice as many chromosomes as other plants with that morphology at the same locality. This result also confirms that the skewed distribution of fecundity in the atypical population could reflect the presence of individuals with aberrant karyotypes. Finally this observation supports our hypothesis of local cpDNA gene flow from *D. frenchii* to *D. meadia* through autotetraploids in the intergrading population.

In summary, typical populations of *D. frenchii* and *D. meadia* are morphologically and genetically distinct in southern Illinois. However, intergrading populations can facilitate local gene flow among taxa through dynamic polyploid evolution. These results bear on the evolution of ploidy differences in this group and the taxonomic practice of recognizing groups with different ploidy levels.

The authors who identified the general difference in ploidy level among *Dodecatheon* taxa in southern Illinois proposed two hypotheses for their evolutionary relationship: *D. meadia* as an autotetraploid derived from *D. frenchii* or *D. frenchii* derived as a polyhaploid of *D. meadia* (Olah and Defilips 1968). Our cytological result showed that genome duplication in *D. frenchii* does not automatically generate a *D.*

*meadia* morphology. If *D. meadia* evolved from autotetraploid *D. frenchii* many of the differences that distinguish them evolved after genome duplication. While our results strongly suggest that autotetraploidy in *D. frenchii* contributes to variation in *D. meadia,* one of our most striking results is consistent with a polyhaploid origin for *D. frenchii*. *D. frenchii* has very low fitness. This observation could reflect the low quality of its habitat. Few other plants inhabit sandstone rockhouses, suggesting that environmental conditions in this habitat may limit plant growth (Walck *et al.* 1996). Indeed, our morphometric results show that *D. frenchii* is smaller than *D. meadia*. However, our viability experiment was conducted under common garden conditions and *D. frenchii* still exhibited the lowest fitness. Low fitness is not the only prediction for a taxon with a polyhaploid origin. Polyhaploidy would impose a severe bottleneck, reducing genetic variation. Yet, the most genetically variable population in our survey was *D. frenchii* at Jackson Hollow. Again, if *D. frenchii* evolved via polyhaploidy, this event has either occurred so long ago that new mutations have increased variation in this taxon or so frequently that a several chloroplast types have been introduced from *D. meadia* (Segraves *et al.* 1999). Whether *D. meadia* evolved from *D. frenchii* or vice versa, the evidence for recent gene flow and dynamic ploidy is so strong that definitively excluding either scenario may be very difficult.

Finally, our results raise questions about the taxonomic status of these species and the merit of recognizing polyploid populations in general. In our case, taxa are not reproductively isolated despite a difference in ploidy level. Our results are similar to those of a recent study on interploidy level gene flow among species of *Capsella*. Slotte *et al.* (2008) compared nuclear sequence variation from tetraploid accessions in a region

where a related diploid occurs to variation from accessions where diploids do not occur. They found strong evidence for recent gene flow from diploids into tetraploids where they co-occur. Our population genetic analysis produced a similar result, and our cytological data suggest that neoautotetraploids in a highly-variable population mediate recent gene flow. These results demonstrate that the possibility for dynamic ploidy evolution seriously complicates species delimitation based solely on the identification of different ploidy levels. Contrary to dogma in plant speciation biology, polyploids are not absolutely reproductively isolated from related diploids. Repeated autotetraploidy can introduce genetic variation across this apparent reproductive barrier. For this reason, we disagree with the practice of recognizing autotetraploids based solely on the presumption that polyploidy confers reproductive isolation (Soltis *et al*. 2007). In addition to facilitating gene flow among ploidy levels, dynamic ploidy presents a more basic challenge to identifying species in polyploid complexes. While many species concepts permit some limited gene flow, most require that species represent lineages of a single evolutionary origin (de Quieroz 1999, Coyne and Orr 2004). Dynamic ploidy evolution may greatly complicate identification of a specific origin for a polyploid species (Seagraves *et al.* 1999). The rate of polyploid formation is critically important in determining whether neoplolyploids could evolve cohesive genetic and ecological features that distinguish them from their parents (Thompson and Lumaret 1992, Rodriguez 1996). In order to determine this rate, genomic tools that have been developed to detect ancient polyploid events (Kellis *et al.* 2004) may be adapted to more recent genomic changes. We suspect that in many cases, the events that ultimately distinguish polyploid species from parents may not be genome duplication itself.

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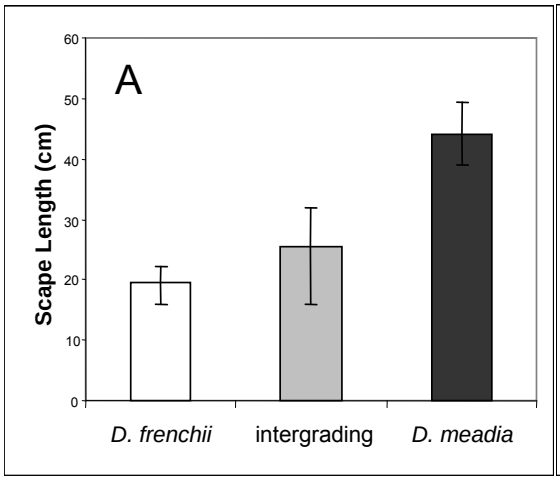
**TABLE 4.1**: Fertility of capsules from different *Dodecatheon* populations at Ferne Clyffe State Park.

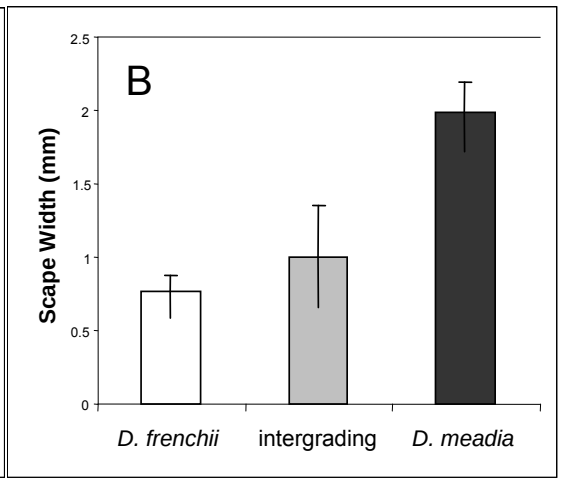
| | *D. frenchii* | Intergrading | *D. meadia* |
|---|---|---|---|
| Fertile | 44% | 75% | 63% |
| Empty | 56% | 25% | 37% |

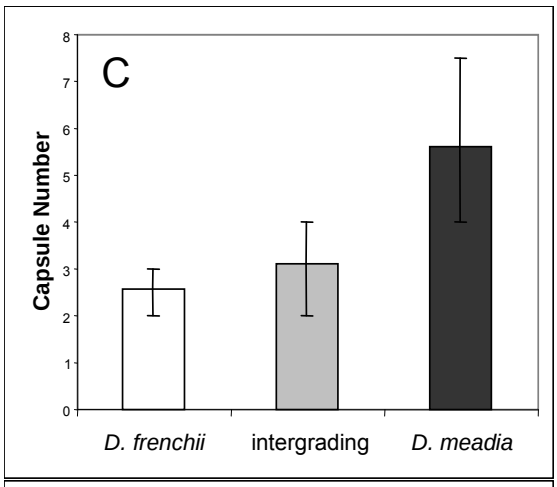
**TABLE 4.2**: Frequency of six haplotypes in six *Dodecatheon* populations across southern Illinois.

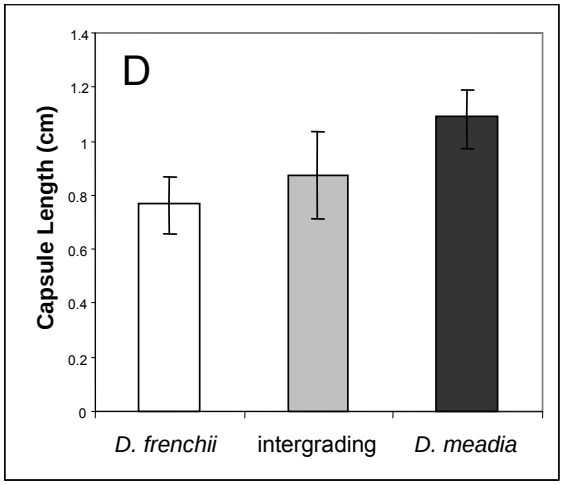
| | | | | HAPLOTYPE | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Locality | Taxon | Configuration | Intergradation | A | B | C | D | E | F |
| Bear Creek | *D. frenchii* | isolated | no | 0 | 0 | 0 | 0 | 6 | 14 |
| Ferne Clyffe | *D. frenchii* | parapatric | yes | 0 | 0 | 0 | 7 | 12 | 1 |
| Ferne Clyffe | *D. meadia* | parapatric | yes | 0 | 0 | 0 | 4 | 13 | 3 |
| Jackson Hollow | *D. frenchii* | parapatric | no | 0 | 3 | 1 | 10 | 5 | 1 |
| Jackson Hollow | *D. meadia* | parapatric | no | 0 | 14 | 6 | 0 | 0 | 0 |
| Pounds Escarpment | *D. meadia* | isolated | no | 20 | 0 | 0 | 0 | 0 | 0 |

**FIGURE 1:** Morphological comparisons of typical populations of both *D. frenchii* and *D. meadia* in Southern Illinois, with an intergrading population. Bars for *D. frenchii* and *D. meadia* in panels A-E represent means over 243 individuals in 6 populations inferred from mixed-model ANOVA with populations as a random effect nested within species. All means are significantly different (A: $t=11.75$, $p<0.001$, B: $t=12.37$, $p<0.001$, C: $t=7.22$, $p=0.002$, D: $t=4.96$ 0.008, E: $t=5.04$, $p=0.007$). The bars for the intergrading population in panels A-E represent the simple mean in that population (35 individuals). Error bars represent the interquartile range for each group.


A
Scape Length (cm)
60
50
40
30
20
10
0
D. frenchii
intergrading
D. meadia


B
Scape Width (mm)
2.5
2
1.5
1
0.5
0
D. frenchii
intergrading
D. meadia


C
Capsule Number
8
7
6
5
4
3
2
1
0
D. frenchii
intergrading
D. meadia


D
Capsule Length (cm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
D. frenchii
intergrading
D. meadia

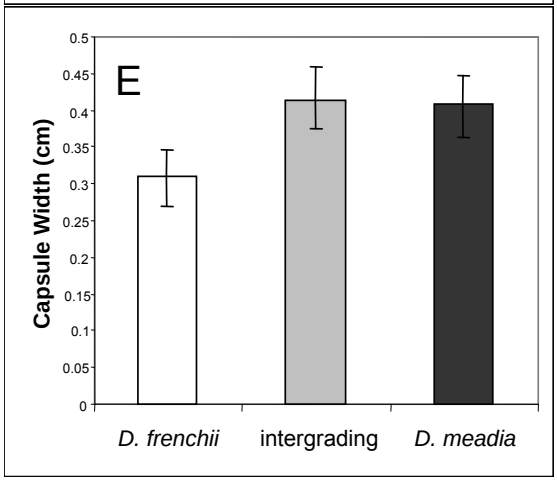

E
Capsule Width (cm)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
D. frenchii
intergrading
D. meadia

**FIGURE 4.2:** Ordination of 278 infructescences from typical populations of both *D. frenchii* and *D. meadia* in Southern Illinois, as well as an intergrading population based on a principal components analysis of five characters. The first principal component axis is horizontal and the second principal component axis is vertical.

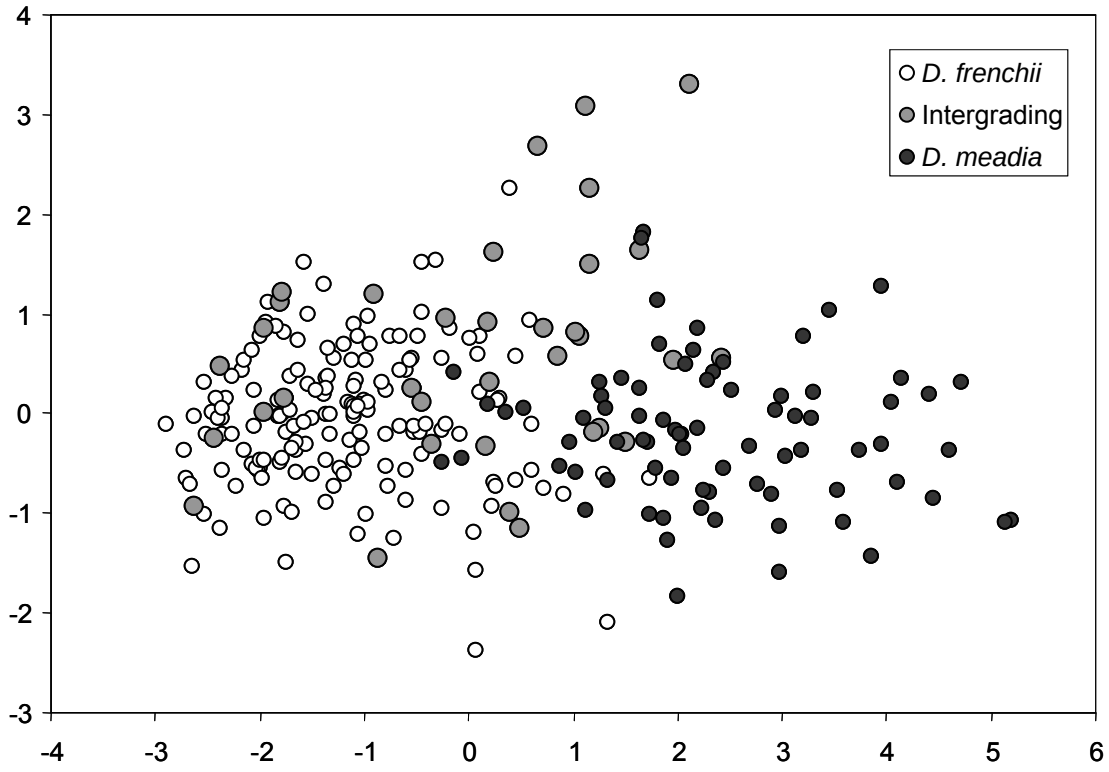


**FIGURE 4.3:** Scanning electron micrograph of a morphologically representative seed from *D. frenchii* in southern Illinois.

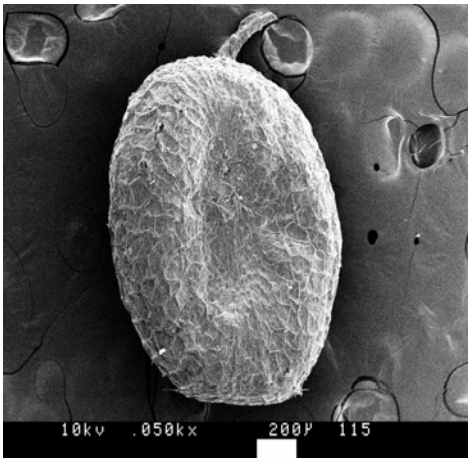



**FIGURE 4.4:** Scanning electron micrograph of a morphologically representative seed from *D. frenchii* in southern Illinois.

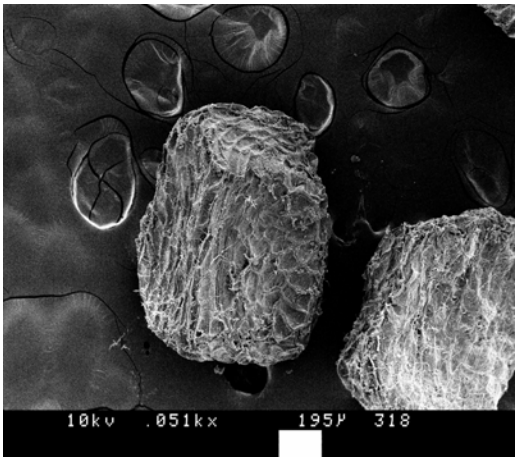


**FIGURE 4.5:** Fecundity of capsules from three populations at Ferne Clyffe State Park as stacked histograms. Dashed lines represent means for each population inferred from mixed-model ANOVA. Arrows around bars represent standard errors.

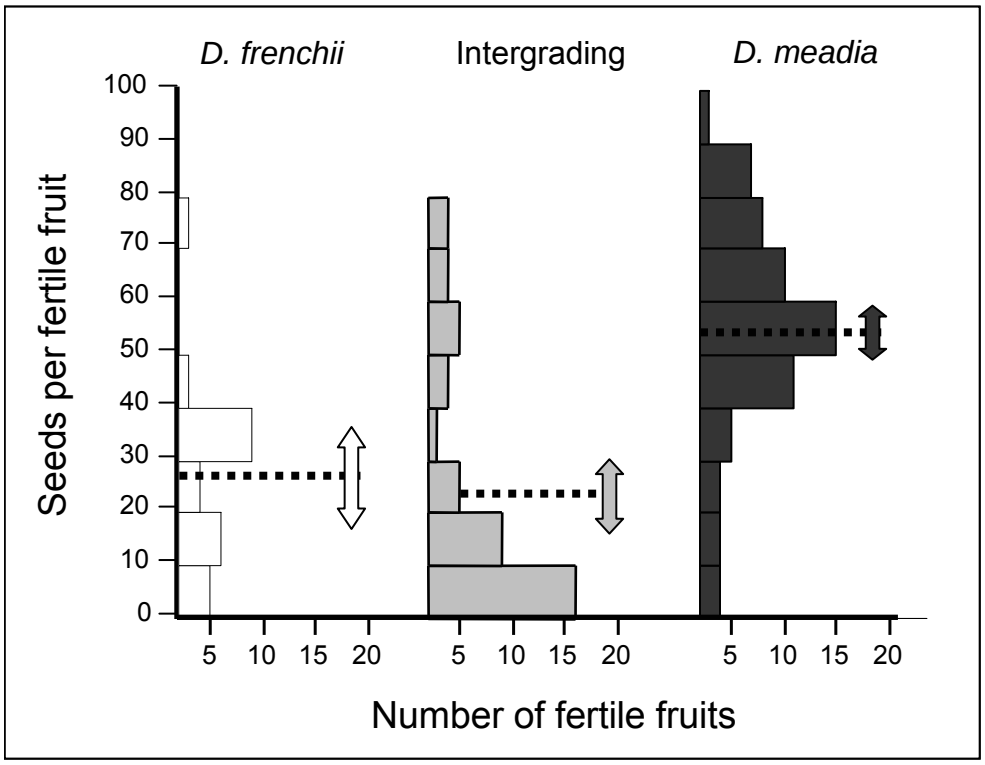
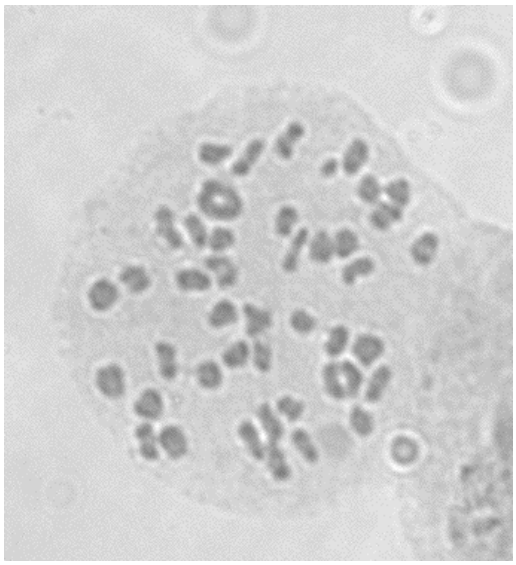


**FIGURE 4.6:** Meiotic chromosome squash from a plant with *D. frenchii* morphology in the intergrading population.



# CONCLUSION OF THE DISSERTATION

Across a variety of taxonomic scales, the analyses presented illustrate a previously unrecognized degree of complexity in the responses of biodiversity to historical climate change. Previous analyses often focused on a dichotomy between adaptation and migration (Davis *et al.* 2005). This polarized view suited categorical assessments of many kinds of data, from fossil pollen to phylogeography. It also reinforced long-standing assumptions of gradualism in the evolutionary processes.

In contrast, I show that the distinction between adaptation and migration is seldom clear. I accomplished this by framing diverse data in appropriate geographic and historical contexts. With a meta-analysis of phylogeographic data, I showed that historical and contemporary factors both contributed to niche breadth in European plants. With a phylogenetic analysis, I showed that geographic heterogeneity interacted with historical climate change to influence apparent differences in physiological tolerances among related species of *Dodecatheon*. With an integrated phylogeographic and ecophysiological analysis of some of those species, I showed that relationships between traits, distributions and genetic variation retain signatures of both historical range dynamics and ongoing adaptive differentiation. Finally, with a fine scale analysis, I showed that dynamic ploidy evolution among parapatric ecologically differentiated taxa greatly complicates distinguishing between range-forced secondary contact and directional adaptive divergence.

At all levels, these results emphasize how migration and adaptation are outcomes of processes that operate on populations. The major process implicated is gene flow. Gene flow plays a multifarious role in responses to climate change. Generally, gene flow

maintains ecological and genetic cohesion within species. When these cohesive effects are strong, species may respond as intact units. However, climate change exposes species to geographic variation that can alter patterns of gene flow through time. As patterns of gene flow shift, diversification may occur within lineages. At this boundary between tokogeny and phylogeny the assumptions of many powerful concepts break down along with interpretations of patterns that are based on them.

One concept that notably losses relevance with decreased gene flow is the ecological niche. The niche concept, as applied in classical ecological theory, assumes that individuals are exchangeable with respect to important ecological interactions (Chase and Leibold 2003). Local adaptation with reduced gene flow violates this assumption. Nevertheless, the niche concept has played a foundational role in understanding responses to climate change. This role is evident in the application and limitations of niche-based species distribution models. Niche-based species distribution models make two key assumptions to infer climate tolerance from occurrence data: that and all individuals have fundamentally similar climate tolerances and that these tolerances are stable through time (Pearman *et al*. 2008). While simple to implement and remarkably accurate for contemporary distributions, their predictions of habitat tracking reflect untested assumptions that preclude the possibility for evolutionary change. For groups with prevalent local adaptation, and over time scales where evolutionary diversification can occur, the predictions of these models become suspect. Our results show that evolutionary diversification may be more commonplace, and may play a more subtle role than often recognized.

Classical evolutionary approaches make certain practical assumptions that limit their applicability to the study of climate change as well. For instance, classical Fisherian quantitative genetic models estimate generational changes in heritable variation among individuals while treating ecological variation as error (Fisher 1918). More sophisticated models produce more realistic predictions for rates of evolutionary change by allowing spatial variation in fitness (Endler 1986). However, estimating quantitative genetic parameters in more than a handful of populations is extremely difficult (Etterson 2004). Our results show that history and geography condition the relationship between genetic variation and environmental heterogeneity under many circumstances.

Our approach is one among many that attempts a compromise between extreme ecological and evolutionary approaches by incorporating additional sources of data. In a few groups, abundant fossils allow a direct reconstruction of paleodistributions, and associated paleoniches. While these approaches are extremely powerful (Pearman *et al.* 2008) they are limited to the subset of taxa amenable to fossilization. An alternative approach that is gaining popularity relaxes the assumption of stable ecological requirements through time by reconstructing ancestral niches along phylogenies (Hoffman 2005, Yesson and Culham 2006, Evans *et al.* 2009). While this approach is promising, it is limited to assessing cladogenetic variation among lineages (Hardy and Linder 2005). In groups where population processes predominate (such as *Dodecatheon*), phylogenetic approaches have limited utility, especially considering that these processes can generate gene-tree species tree conflicts, greatly complicating accurate phylogeny reconstruction . Some phylogeographic approaches explicitly consider gene-tree species tree conflicts during analyses of niche change (Knowles and

Carstens 2007). However, like phylogenetic approaches, these are limited to basically tree-like reconstructions of population history (Templeton 2008). This representation of population history amounts to a dichotomy between allopatry and sympatry. In this way, the bifurcating population history paradigm is similar to other prominent paradigms in the study of the relationship between biodiversity and climate change. Our results clearly show that gene flow within and among populations varies continuously through space and time as climate changes. For this reason, population-tree methods may suffer from some of the same limitations in inferring complex responses under other absolute paradigms.

**A prognosis for biodiversity during anthropogenic climate change:** The direst predictions for biodiversity as climate changes apply some of the most restrictive assumptions (Thomas *et al.* 2004). If species must migrate to survive, they face a whole suite of new obstacles. Rates of anthropogenic global climate change may exceed those during the Pleistocene. Furthermore, human caused habitat fragmentation may impede migration for many species. These new conditions may interact with other stresses from human activity to make the past a poor model for the future. Among the many species that survived warming since the last glacial maximum, a pithy few may survive into the future.

However, history may prove applicable in more ways than one. With respect to the history of scientific inquiry into climate change, the field has been dominated by gradualist paradigms. Closer and more careful examinations of the data often show that rapid adaptation may play have played a more prevalent role in the response of biodiversity to historical climate change. Given the abundant evidence for local

adaptation to climate that occurred during the Holocene, species may draw on stores of genetic diversity that could not occur if their niches were absolutely evolutionarily conserved. In some cases, local adaptation may impede species responses to climate change. However, if gene flow can marshal some of the genetic variation within species from its geographic garrisons into the fronts represented by range boundaries, biodiversity may not retreat into oblivion.

My study into responses to climate change among temperate plants has given me hope that biodiversity is more resilient than pessimist often claim. It has also given me hope that scientists can push the field to develop more flexible models that improve the precision with which we make predictions. The most critical open question is whether, as a global society, we can make the decisions necessary to act on this information in the best interest of future generations that will inherit the consequences of our inaction.

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