

FRUIT AND SEED CHARACTERS HELP DISTINGUISH
SOUTHERN ILLINOIS *DODECATHEON* (PRIMULACEAE)
SPECIES AND HIGHLIGHT UNUSUAL
INTERGRADING POPULATIONS

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ABSTRACT. In taxa with few diagnostic characters, highly variable populations pose a major challenge to delimiting species. This is especially true in *Dodecatheon* (Primulaceae), which has a notoriously complex taxonomic history. Previous biosystematic studies of *Dodecatheon* in southern Illinois support the recognition of two species: *D. frenchii*, a diploid sandstone cliff endemic, and *D. meadia*, a widespread tetraploid. However, only one morphological character distinguishes them and some experimental evidence suggests that this character may be environmentally induced. Furthermore, authors have disagreed about the nature of morphologically intergrading populations. In this study, we document patterns of variation for fruit and seed characters among populations in southern Illinois, including one atypical, morphologically intergrading population. Different seed shapes help distinguish these taxa, both in southern Illinois and across their larger ranges. We also show that the intergrading population is significantly more variable than others, and describe the occurrence of such populations in other regions where these two taxa co-occur. These two results suggest that localized hybridization between these species may contribute to their complex taxonomic history.

Key Words: *Dodecatheon*, morphometrics, southern Illinois, hybridization

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, hybridization, and polyploidy. An excellent example of such a group is *Dodecatheon* L., a clade of buzz-pollinated species nested in the large genus *Primula* L. (Mast et al.

2004). Because this paper focuses on closely related species within the *Dodecatheon* clade, we will use the traditional names throughout the paper, although it should be noted that combinations under *Primula* are available (Mast and Reveal 2007). *Dodecatheon* has challenged botanists since the first plants were imported to Europe in the 18th century. The first American botanist to address its diversity, Raffinesque (1833), described over a dozen taxa in eastern North America based on characters such as leaf shape and margin form. In contrast, Asa Gray (1886) noted a lack of diagnostic characters in this “baffling” group and accepted only a single species, albeit with several infraspecific taxa.

The tension between taxonomic lumpers and splitters produced hundreds of names before the first attempt was made to experimentally demonstrate a genetic basis for morphological differences among *Dodecatheon* taxa. In 1944, Norman Fassett reported the results from a series of common garden experiments on material collected from two taxa in southern Illinois. *Dodecatheon meadia* L., the type for the genus, ranges from the eastern Piedmont to the eastern margins of the Great Plains. In southern Illinois, it tends to inhabit relatively exposed sites, such as dry forests and rocky glades. *Dodecatheon frenchii* (Vasey) Rydb., which was first collected in southern Illinois in 1870 but only elevated to species rank sixty years later (Rydberg 1932), is endemic to moist sandstone cliffs, especially below overhanging rocks in areas known as “rockhouses.” The majority of occurrences for this rare species are in the Shawneetown ridge of southern Illinois and adjacent states, with a few outlying occurrences in northwestern Arkansas, northwestern Alabama, and eastern Kentucky. These two taxa differ in leaf shape and thickness, with *D. meadia* being characterized by relatively thick, oblanceolate leaves that gradually taper to a petiole at the base and *D. frenchii* being characterized by relatively thin leaves that abruptly taper to a petiole at the base. Leaf characters usually distinguish typical populations of each species that grow in parapatry either on cliff tops (*D. meadia*) or in the rockhouses below (*D. frenchii*). However, highly variable populations with plants that are morphologically intermediate sometimes occur on cliff faces.

Suspecting that leaf shape differences were environmentally induced, Fassett collected resting plants, consisting of roots with dormant buds, from populations of both taxa and exposed them to varying light intensities in the greenhouse. Whereas *Dodecatheon 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 *D. frenchii*. For this reason, he concluded that *D. frenchii* should be considered a variety of *D. meadia*.

The conclusion that *Dodecatheon frenchii* did not merit species recognition was strongly criticized in a series of publications documenting observations and experiments during the following 35 years. 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 tapering, leaves typical of *D. frenchii*, naturally occurred in relatively exposed sites that are typical habitat for *D. meadia*. Subsequent common garden and reciprocal transplant experiments demonstrated little plasticity in leaf shape. Moreover, observations by Mohlenbrock (1987) indicated that after 30 years, *D. frenchii* plants that Voigt and Swayne (1955) had transplanted into *D. meadia* habitat still retained their characteristic leaf shapes.

In addition to morphological and ecological differences, cytological data indicated genetic differentiation between these taxa. Meiotic chromosome counts from across southern Illinois demonstrated that *Dodecatheon frenchii* is diploid ($2n = 44$) relative to the tetraploid *D. meadia* ($2n = 88$; Olah and DeFilipps 1968). Ploidy differences reproductively isolate populations (Coyne and Orr 2004; Stebbins 1950), reinforcing the conclusion that these taxa are evolutionarily divergent. However, Olah and DeFilipps (1968) 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). Although no naturally occurring polyhaploid taxon has been conclusively demonstrated (Ramsey and Schemske 2002), all of these characteristics distinguish *D. frenchii* from its proposed parent, *D. meadia* (Olah and DeFillips 1968). 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 *Dodecatheon frenchii* and *D. meadia* as distinct species (Reveal 2009). However, both the discrepancies among previous experiments and the differences among authors in the interpretation of unusual populations raise two outstanding questions about patterns of morphological variation in *Dodecatheon* from southern Illinois: (1) do characters other than leaf shape consistently distinguish these taxa and (2) do intergrading populations differ qualitatively from typical populations? To address these questions we targeted characters that have been applied to taxonomic questions elsewhere in the group and measured these characters on plants from across southern Illinois. We also examined whether the pattern of morphological variation among populations in southern Illinois represented range-wide patterns of variation by evaluating key characters and the occurrence of intergrading populations across the coextensive ranges of these two species in the southeastern United States. Resolving these questions is relevant to correctly identifying and managing populations of *D. frenchii*, which has conservation status in every state where it occurs (Hill 2002).

MATERIALS AND METHODS

Field sampling and morphological characters. To assess morphological variation in southern Illinois we used the traditional taxonomic character of leaf shape to identify six “typical” populations at four different localities in July, 2005 (Table 1). We selected these localities to represent both the geographic extent of *Dodecatheon frenchii* in southern Illinois and parapatric occurrences where accurate taxonomic determinations are especially important. Bear Creek and Lusk Creek are the westernmost and easternmost known occurrences of *D. frenchii* in southern Illinois (Swayne 1972). Populations of both species occur in parapatry in both Jackson Hollow and Jackson Falls. All four typical populations of *D. frenchii* had leaves that abruptly tapered to petioles (e.g., Figure 1A) and occurred in flat areas at the bases of overhanging sandstone cliffs. Both typical populations of *D. meadia* had

Table 1. Collection information and sample sizes of *Dodecatheon* material examined in this study. Coll. # = collection numbers of B. Oberle. Vouchers are deposited at the Missouri Botanical Garden (MO). Species: "Intergrading" and "Intermediate" refer to leaf morphologies in two kinds of atypical populations where the taxa co-occur. St. = State. Location: N.F. = National Forest; Nat. Pres. = Nature Preserve; Nat. Area = Natural Area. Infr. = the number of infructescences analyzed. Seed shape: R = rounded, with only a single visible face; A = angular, with multiple visible faces separated by edges.

Sample Group	Coll. #	Species	St.	County	Location	Lat.	Long.	Infr.	Seed Shape
S. Illinois	247	<i>D. frenchii</i>	IL	Jackson	Jackson Falls, Shawnee N.F.	37.508	-88.687	38	6R
S. Illinois	249	<i>D. frenchii</i>	IL	Jackson	Jackson Hollow, Shawnee N.F.	37.514	-88.703	40	-
S. Illinois	314	<i>D. frenchii</i>	IL	Jackson	Lusk Creek, Shawnee N.F.	37.515	-88.543	48	5R
S. Illinois	315	<i>D. frenchii</i>	IL	Pope	Bear Creek, Shawnee N.F.	37.668	-89.363	38	-
S. Illinois	248	<i>D. meadia</i>	IL	Jackson	Jackson Falls, Shawnee N.F.	37.508	-88.684	40	6A
S. Illinois	250	<i>D. meadia</i>	IL	Jackson	Jackson Hollow, Shawnee N.F.	37.515	-88.703	39	5A
S. Illinois	251	Intergrading	IL	Johnson	Ferne Clyffe St. Park	37.533	-88.989	35	9R:6A
Range Wide	294	<i>D. frenchii</i>	AL	Colbert	Cane Creek Canyon	34.640	-87.008	-	R
Range Wide	300	<i>D. frenchii</i>	AR	Newton	Ozark N.F.	35.847	-93.295	-	R
Range Wide	312	<i>D. frenchii</i>	IN	Perry	Hooster N.F.	38.199	-86.568	-	R
Range Wide	305	<i>D. frenchii</i>	KY	Grayson	Stones Creek Hollow	37.532	-86.407	-	R
Range Wide	335	<i>D. frenchii</i>	MO	Douglas	Mark Twain N.F.	36.992	-92.094	-	R
Range Wide	307	<i>D. meadia</i>	IL	Johnson	Ferne Clyffe St. Park	37.533	-88.989	-	A
Range Wide	313	<i>D. meadia</i>	IL	Gallatin	Shawnee N.F.	37.604	-88.282	-	A
Range Wide	306	<i>D. meadia</i>	KY	Logan	Logan Co. Glades St. Nat. Pres.	36.847	-86.874	-	A
Range Wide	318	<i>D. meadia</i>	MO	St. Louis	LaSalle Farms	38.560	-90.626	-	A
Range Wide	334	<i>D. meadia</i>	MO	St. Clair	Taberville Prairie St. Nat. Area	38.050	-92.764	-	A
Range Wide	336	<i>D. meadia</i>	MO	Taney	Mark Twain N.F.	36.731	-92.848	-	A
Range Wide	302	<i>D. meadia</i>	NC	Buncombe	Pisgah N.F.	35.661	-82.432	-	A
Range Wide	297	<i>D. meadia</i>	OK	Pontotoc	Pontotoc Ridge Nat. Pres.	34.522	-96.609	-	A
Range Wide	301	<i>D. meadia</i>	TN	Mauzy	Duck River Complex Nat. Area	35.567	-86.888	-	A

Table 1. Continued.

Sample Group	Coll. #	Species	St.	County	Location	Lat.	Long.	Infr.	Seed Shape
Range Wide	337	<i>D. meadia</i>	VA	Nelson	Naked Mountain Nat. Pres.	37.749	-78.833	-	A
Range Wide	300a	Intergrading	AR	Newton	Ozark N.F.	35.857	-93.121	-	-
Range Wide	310a	Intergrading	KY	Carter	Carter Caves St. Resort Park	38.368	-83.121	-	-
Range Wide	317a	Intergrading	MO	Ste. Genevieve	Hickory Canyons Nat. Area	37.873	-90.308	-	-
Range Wide	305a	Intermediate	KY	Grayson	Stones Creek Hollow	37.529	-86.410	-	-
Range Wide	335a	Intermediate	MO	Douglas	Mark Twain N.F.	36.992	-92.094	-	-

uniformly oblanceolate leaves (e.g., Figure 1B) and occurred in forested margins of cliff-top glades.

We also identified one atypical population of plants that spanned the range of leaf shapes between taxa (e.g., Figure 1C). This population occurred in a small rockhouse, approximately five meters long, two meters deep, and one meter tall, near the top of the south-facing bluffline of Happy Hollow at Ferne Clyffe St. Park, Jackson County. This was the only atypical population that we encountered during five field seasons in the region, including several failed attempts to relocate the atypical population of *Dodecatheon frenchii* reported by Fassett (1944) at Giant City State Park.

In order to quantify morphological variation within and among these populations we collected two morphometric datasets. The first focused on infructescence characters that had been proven useful for distinguishing *Dodecatheon meadia* from another closely related rare species, *D. amethystinum* (Fassett) Fassett, in Pennsylvania (Klotz and Loeffler 2007). For this dataset, we randomly selected 38–48 plants from each of the typical populations and all 35 reproductive plants in the atypical population. On every plant, we measured three characters in the field: (1) scape length from the ground level to the base of the involucre bracts, (2) scape width at its midpoint, and (3) the number of developed capsules. We also collected one undamaged capsule from each plant and measured two more characters based on digital photographs taken in the lab: (4) capsule length, and (5) capsule width.

Our second morphometric dataset focused on microscopic seed characters that had recently been described in a related species from western North America, *Dodecatheon austrofrigidum* K.L. Chambers (Chambers 2006). Based on an ordination of the infructescence characters (see Statistical Analysis section below), we selected a capsule from each of a relatively large and a relatively small plant from typical populations of each taxon, as well as capsules from large, small, and intermediate-sized plants in the atypical population. We coated eight to ten seeds from each capsule with 200 Å of gold, using an SPI gold sputter coater (SPI Supplies/Structure Probe, Inc., West Chester, PA), and viewed them with an ISI-SX40 scanning electron microscope (ISI International Scientific Instruments, Inc., Pleasanton, CA) run at an emission of 10KV. We photographed five to six seeds at three different magnifications (50×, 100×, and 1000×) and measured aspects of seed size and shape, as well as the size and shape of features on the seed coat,

using ImageJ 1.37 (National Institutes of Health, Bethesda, MD). For seed size, we measured the longest axis of each seed using the Feret's diameter tool. We also measured the total area and two aspects of the shape of the two-dimensional image of the seed. First we measured each seed's circularity, defined by 4π (area/perimeter²). 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 50 \times . A face was defined as a planar surface separated from another face by an edge where the two faces meet. A spherical or rounded shape would have one face, while a polyhedral or angular shape could have more than one face. We measured the diameter, area, and circularity of the characteristic scales that composed the testa, based on pictures taken at 1000 \times . To standardize for the orientation of the seed surface, we measured three scales from each seed whose orientations were parallel to the viewing plane.

Statistical analyses. To test whether these characters could distinguish taxa, we first analyzed plants from only the six typical populations. We tested for differences in infructescence characters among taxa with linear, mixed effects models, treating taxon as a fixed effect, with 'population' as a random effect nested within taxon, using the package 'nlme' (Pinheiro et al. 2009) in R version 2.11.0 (R Development Core Team 2010). We square root-transformed capsule number, and natural log-transformed scape length and scape width measurements to meet the assumptions of the tests. Because seeds were selected from an ordination across all populations, we tested for differences in seed characters in the same manner, but with 'individuals' treated as random effects nested within taxon. For tests of seed characters, we arcsine-square root-transformed circularity and square root-transformed face numbers.

In order to compare individuals in the atypical population to those in typical populations, we used two approaches. First we used a principal components analysis to show the relative positions of all 278 individuals in multivariate morphospace, based on the five infructescence characters. We then tested for differences in the amount of variation for each infructescence character within each population, using successive Fligner-Killeen tests in R version 2.11.0. Fligner-Killeen tests compare departures from the median among groups. Because this test yields only a summary statistic across all groups compared, we tested the hypothesis that the

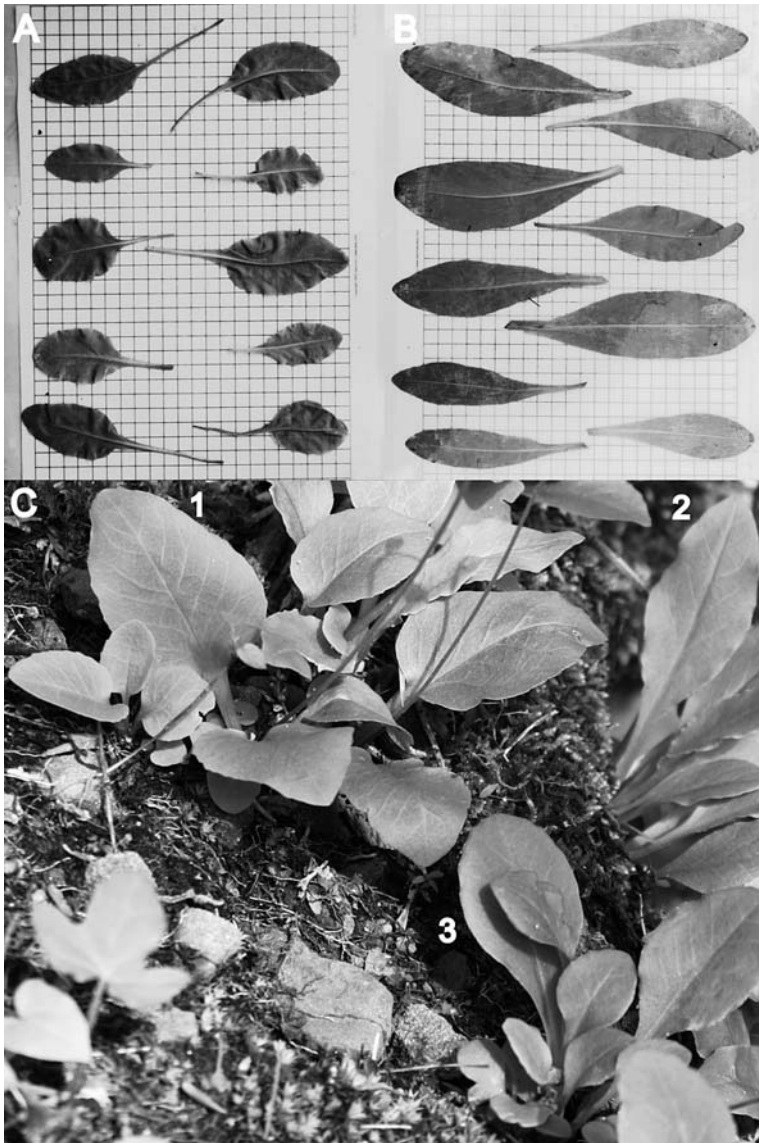


Figure 1. Illustration of variation in leaf characters used to identify populations for further analysis. A. Ten randomly selected leaves from a typical *Dodecatheon frenchii* population (Oberle 314; Table 1). Note the abrupt tapering of the blades at their bases. B. Ten randomly selected leaves from a typical *D. meadia* population (Oberle 313; Table 1). Note the gradual tapering

atypical population was significantly more variable than typical populations by first performing the test on only the six typical populations, and then performing a second test on all seven populations. In cases where the chi-squared statistic for the Fligner-Killeen tests did not meet the critical threshold for typical populations, but exceeded the critical threshold when the atypical population was included, we could reject the null hypothesis that variability in the atypical population is equal to variability in the typical populations. We also compared seeds from plants in typical populations to those from plants in atypical populations.

Regional patterns. In order to evaluate whether patterns of morphological variation in southern Illinois were representative of patterns more generally, we noted seed shape for plants sampled across the coextensive ranges of these species in April 2007 (Table 1). We sampled populations of *Dodecatheon frenchii* in each of the major geographical areas where it has been reported (Hill 2002). We sampled populations representing the portion of *D. meadia*'s geographic range that overlaps with the distribution of *D. frenchii*. At each locality, we searched 20–30 capsules produced during the previous growing season for any remaining seeds. Using a 10× hand lens, we were able to classify all remaining seeds from each population as either rounded or angular. If seed shapes differed within a population, we noted the frequency of each shape. We tested the association between taxonomy and seed shape using a Fisher's exact test.

During our searches for typical populations, we also noted the presence of atypical populations based on variation in leaf morphology. We distinguished two different kinds of atypical populations. "Intergrading" populations spanned the range of leaf morphologies like the atypical population studied at Ferne Clyffe (e.g., Figure 1C). "Intermediate" populations showed less variation among individuals, but all individuals had leaves that were intermediate in shape between those characteristic of either taxon.

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of the blades at their bases. Panels A, B background grid is 1 cm². C. Detail of an intergrading population (*Oberle 300a*; Table 1) indicating nearby individuals with (1) *D. frenchii*-like leaf morphology, (2) *D. meadia*-like leaf morphology, and (3) intermediate leaf morphology.

RESULTS

Morphological differences among typical populations. We measured a total of 243 plants across six typical populations to identify morphological differences between taxa in southern Illinois. The two taxa were morphologically distinguishable based on all infructescence characters (Figure 2). In each case, *Dodecatheon meadia* was significantly larger than *D. frenchii* [mixed-effects linear model, numDF = 1, denDF = 4: log(scape length), $t = 9.04$, $p < 0.001$; log(scape width), $t = 7.85$, $p = 0.001$; $\sqrt{\text{capsule number}}$, $t = 7.22$, $p = 0.002$; capsule length, $t = 4.96$, $p = 0.008$; capsule width, $t = 5.03$, $p = 0.007$]. The interquartile ranges did not overlap among these taxa for any of these characters.

The two taxa were also morphologically distinguishable based on seed characters. In general, seeds from large and small *Dodecatheon frenchii* plants were more rounded (Figure 3A), whereas seeds from large and small *D. meadia* plants were more angular (Figure 3B). Both measurements of seed shape reflected this difference. All 11 *D. frenchii* seeds had only a single visible face, whereas all 11 *D. meadia* seeds had more than one visible face (mixed-effects linear model, $\sqrt{\text{face number}}$, numDF = 1, denDF = 2, $t = 10.44$, $p = 0.009$). The perimeters of *D. frenchii* seeds tended to be more circular than the perimeters of *D. meadia* seeds, although the difference was only marginally significant (mixed-effects linear model, arcsin-square root transformed circularity, numDF = 1, denDF = 2, $t = 3.79$, $p = 0.063$). No other measurements, including seed size, scale size, or shape differed significantly between taxa.

Morphological comparison to the atypical population. A multivariate ordination of all infructescence characters in all 278 plants reinforced the morphological distinction between plants from typical populations of both taxa as well as the broad morphological range of plants from the atypical population (Figure 4). Individuals from typical populations showed little overlap with respect to the first principal component axis, which loaded similarly on all five characters (range of loadings = 0.42–0.48). In comparison, individuals from the atypical population occurred in ordination space in (1) regions only occupied by plants from typical populations of *Dodecatheon frenchii*, (2) regions only occupied by plants from typical populations of *D. meadia*, (3) the narrow region occupied by both taxa, and (4) a region occupied by neither taxon. This area,

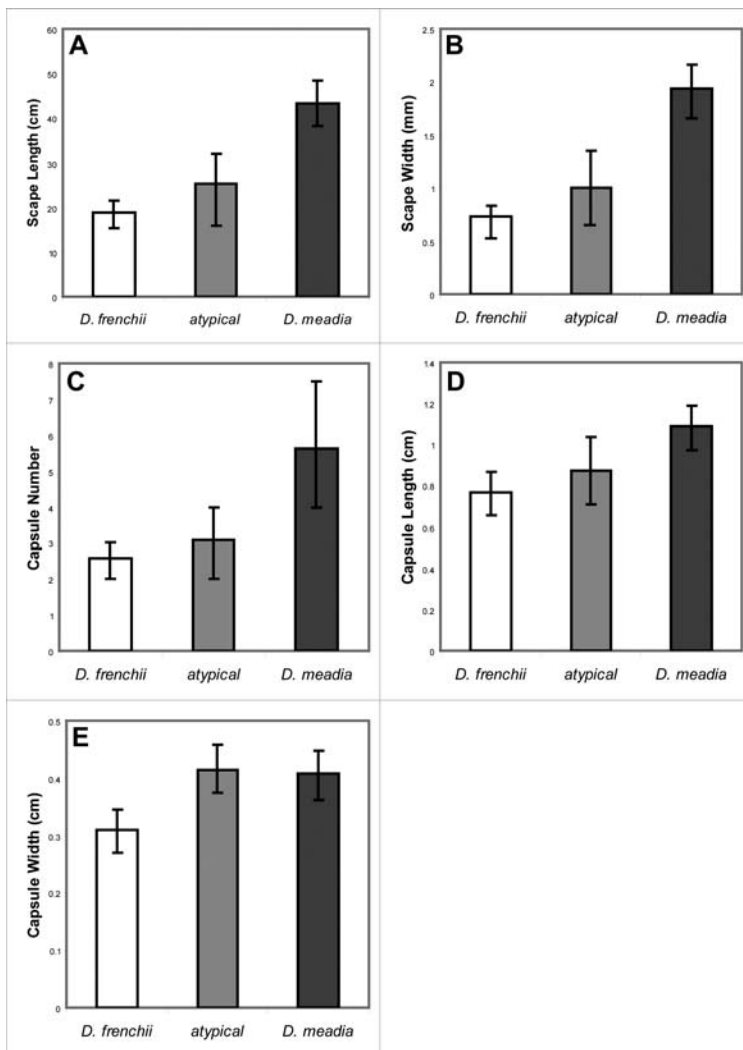


Figure 2. Morphological comparisons of typical populations of both *Dodecatheon frenchii* and *D. meadia* in southern Illinois with an intergrading population. Bars for *D. frenchii* and *D. meadia* in panels A–E represent means over 243 individuals in six populations inferred from mixed-effects linear model with taxon treated as a fixed effect and populations treated as a nested random effect. All means are significantly different (see Results section). 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.

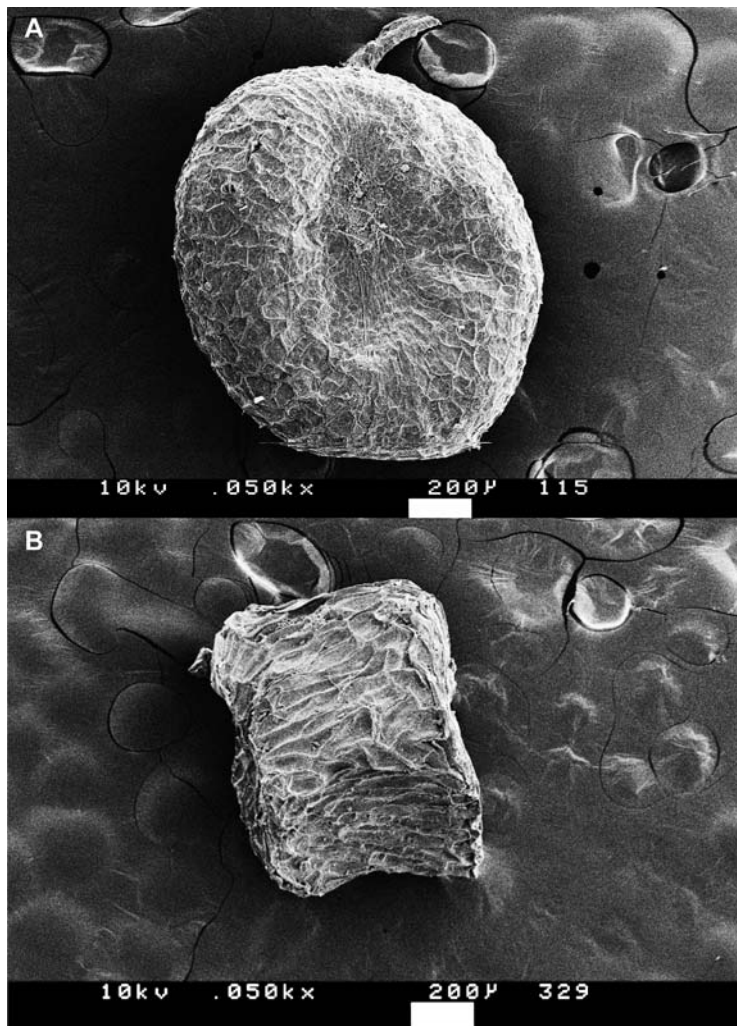


Figure 3. Scanning electron micrographs of a morphologically representative seed from *Dodecatheon* taxa in southern Illinois. A. *D. frenchii*. B. *D. meadia*.

where only plants from the atypical population occurred, was characterized by extreme values along the second principal component axis, representing morphological variation besides that which primarily distinguishes plants from typical populations of *D.*

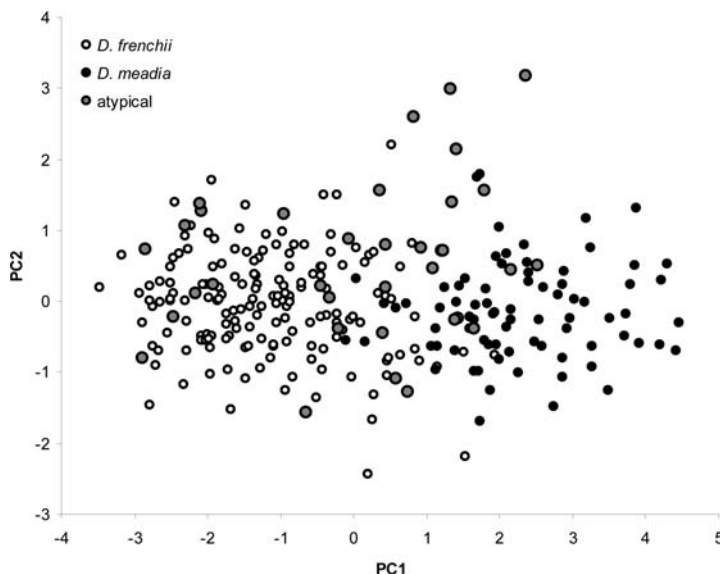


Figure 4. Ordination of 278 infructescences from typical populations of both *Dodecatheon frenchii* and *D. meadia* in southern Illinois, as well as infructescences from an atypical population based on a principal components analysis of all five characters. These first and second principal component axes explain 75.2% and 11.3% of the variation in the dataset, respectively.

frenchii from *D. meadia*. The second principal component axis loaded heavily on capsule number, width, and length (-0.51 , 0.61 , and 0.49 , respectively), with unusual individuals from the intergrading population having relatively few, large capsules.

The mean character value of the atypical population usually fell between the mean character values for typical populations of the two taxa (Figure 2). The interquartile range of the atypical population was usually larger than the interquartile range of typical populations of either taxon and it overlapped the mean of one taxon or the other. The interquartile range in the intergrading population overlapped the mean of *Dodecatheon frenchii* populations for all characters except capsule width. Mean capsule width was slightly larger in the intergrading population than in *D. meadia*.

The atypical population was more variable than typical populations for most morphological characters. For four of the five infructescence characters, Fligner-Killeen tests detected no significant differences in the amount of variation within typical

populations of both taxa, but did detect a significant difference when including the atypical population. Capsule number was an exception, where variation also differed significantly among typical populations.

Plants from the atypical population also produced seeds that varied more in shape than those produced by plants in typical populations. Whereas *Dodecatheon frenchii* plants produced only rounded seeds and *D. meadia* plants produced only angular seeds, plants from the atypical population produced both rounded (9) and angular (6) seeds.

Range-wide patterns. Seed shape differences among taxa observed in southern Illinois were consistent across these species' ranges. All of the seeds examined in ten additional populations of *Dodecatheon meadia* were angular. In contrast, all five populations of *D. frenchii* from outside southern Illinois produced rounded seeds, although one of the fruits examined in a population from Colbert County, Alabama (*Oberle 294*) contained an angular seed. Seed shape and taxonomy were strongly associated regardless of whether this polymorphic population was excluded or considered as producing angular seeds (Fisher's exact tests: *Oberle 294* excluded, $p = 0.001$; *Oberle 294* as angular, $p = 0.004$).

We also noted atypical populations with intergrading or intermediate leaf morphologies in other regions where these two taxa co-occurred (Table 1). In northwestern Arkansas (Newton Co.), a very large intergrading population (thousands of plants) occurred in a northeast-facing moist cliff system spanning approximately 700 meters (Figure 1C). This atypical population was one of several that occurred in this area among many typical populations of either species (Jan Self, Ozark National Forest, pers. comm.). Similar, but smaller intergrading populations occurred in moist cliff complexes in eastern Kentucky (Carter Co.) and Missouri (Ste. Genevieve Co.). Both smaller intergrading populations occurred among many populations of *Dodecatheon meadia* and very few typical populations of *D. frenchii*. Populations with intermediate leaf morphologies, although not intergrading, occurred in western Kentucky and central Missouri (Warren Co. and Douglas Co., respectively). Both morphologically intermediate populations occurred among the ledges of relatively small sandstone canyons with few other *Dodecatheon* populations nearby.

DISCUSSION

Morphological distinctions. Our morphometric analysis of typical populations addressed the question of whether characters other than leaf shape can differentiate the two recognized taxa. We found that a combination of infructescence characters tended to distinguish *Dodecatheon meadia* from *D. frenchii*. However, none of the five characters that we measured discretely differed between species. Based on these characters, the relationship between *D. frenchii* and *D. meadia* in southern Illinois is comparable to the relationship between *D. meadia* and *D. amethystinum* in Pennsylvania. Klotz and Loeffler (2007) found that no single character differed discretely between these species in this portion of their ranges and they advocated treating both as varieties. In their study, as in ours, a multivariate ordination of all characters showed relatively little overlap between taxa, suggesting that combinations of characters may reliably diagnose *Dodecatheon* taxa in eastern North America even when single characters do not.

Although infructescence characters did not differ discretely between these taxa, seed shape did. *Dodecatheon meadia* had angular seeds and *D. frenchii* had rounded seeds. We found that this morphological difference extends across these species' ranges. This diagnostic character should help conservation managers concerned with *D. frenchii*. Its range includes large gaps between known occurrences, such as that between northern Alabama and eastern Kentucky (Timme and Lacefield 1991). These gaps might reflect a truly disjunct distribution, or misidentification of populations in the intervening areas. Because seed shape differences are visible under a regular hand lens (edges present or not), this character will allow for more consistent determinations, which will contribute to a more accurate picture of this rare species' distribution.

Seed shape has limitations as a sole diagnostic character. One population of *Dodecatheon frenchii* in Alabama produced an angular and a rounded seed. A preliminary analysis of seeds preserved with herbarium material suggests that seed shape may change with age (data not shown). In addition, *D. amethystinum* appears to produce rounded seeds in some parts of its range (B. Oberle, pers. obs.). For these reasons, we advocate that *D. frenchii* determinations be based on at least two of three following characters: leaves abruptly tapering to a petiole at the base, rounded seeds present, and diploidy.

Morphological intergradation. Although infructescence and seed characters distinguished the populations of these two taxa that exhibited consistent leaf shape differences (“typical” populations), not every population was typical. Our second question aimed to identify qualitative differences between typical and atypical populations. The atypical population that we analyzed was relatively small (35 reproductive plants). Nevertheless, the atypical population was significantly more variable than typical populations of either taxon. It also included plants with intermediate and completely novel infructescence morphologies. These two characteristics of the atypical population—high variability and morphological intergradation—distinguished it qualitatively from typical populations. They also point to biological mechanisms that might underlie the observed patterns of morphological variation.

A simple description of the habitat where the intermediate population occurred is relevant to alternative theories for its origin. This population occupied a small rockhouse that appeared ecologically intermediate between habitats where typical populations of each taxon occurred. The morphological and ecological intermediacy of this population could support Fassett’s (1944) contention that phenotypic plasticity contributes to morphological variation in the group. However, if phenotypic plasticity were solely responsible for patterns of morphological variation, then all individuals in this intermediate habitat should have the same intermediate morphology. However, the atypical population was highly variable, despite the fact that its habitat did not appear more heterogeneous than habitats where typical populations occurred. This observation supports Voigt and Swayne’s (1955) contention that genetic differences contribute to morphological variation in the group. Yet, Voigt and Swayne (1955) suggested that highly variable populations should be considered to be *Dodecatheon frenchii*. Our results show that “typical” populations of both *D. frenchii* and *D. media* had similar amounts of variation for most morphological characters, and that the atypical population was significantly more variable. The ordination results (Figure 4) also show that some plants in the atypical population had morphologies that did not occur in typical populations. This evidence suggests that the atypical population harbored more genetic variation for morphological traits than typical populations.

The spectrum of morphologies present in the atypical population is particularly suggestive of introgressive hybridization (Anderson

1949). Introgressive hybridization involves the persistence of F_1 hybrids along with more variable F_2 generations and backcrosses to parental types. This kind of admixture can break down genetic correlations between traits that consistently distinguish parents, yielding both morphological intermediates and novel morphologies (Reiseberg and Carney 1998). Many examples of this kind of hybridization have been documented in a wide variety of taxa, including *Primula* (Valentine 1947; Zhu et al. 2009) and *Dodecatheon* (Reveal 2009; Thompson 1953). A recent study in *Primula* shows convincing evidence for ploidy evolution during hybridization. Guggisberg et al. (2008) showed that the tetraploid genome of *P. egalikensis* ($2n = 40$) resulted from a combination of the diploid genomes of *P. mistassinica* Michx. ($2n = 18$) and *P. nutans* Georgi ($2n = 22$) with little subsequent reorganization. Some of the mechanisms involved in the hybridization between these *Primula* could also contribute to the apparent hybridization between diploid *D. frenchii* and tetraploid *D. meadia*. Additional studies combining cytology, relative fitness, and population genetics might illuminate the causes and consequences of apparent hybridization between these taxa.

Whatever underlies morphological intergradation in *Dodecatheon*, this phenomenon is rare enough that populations exhibiting this pattern are clearly distinct from populations with typical morphologies, yet common enough to be observed in many of the regions where these two taxa co-occur. At a practical level, the co-occurrence of intergrading populations with typical populations of both species makes them easy to identify during habitat surveys by comparing the range of variation in seed and leaf characters. At a theoretical level, the prevalence of morphological intergradation may help explain the complex taxonomic history of *Dodecatheon*. These plants may have few diagnostic characters, not simply because they have a simple morphology (Thompson 1953), but because recurrent episodes of interspecific gene flow disrupt the development of highly distinctive lineage-specific morphologies.

ACKNOWLEDGMENTS. The authors would like to thank the Illinois Department of Natural Resources and Shawnee National Forest, along with many other federal, state, and local agencies, for granting collection permits. The authors also thank K. Oberle, A. Greenberg, R. Brammierer, and S. Eder for contributing to data collection. J. Beck, B. Schaal, A. Templeton, P. Raven, P. Hoch,

T. Knight, and two anonymous reviewers provided helpful comments on earlier drafts of this manuscript. This research was supported by the U.S. National Science Foundation (DEB-0608317), the Division of Biology and Biomedical Sciences at Washington University, and the Graduate Program at the Missouri Botanical Garden.

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